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JASMIN AMBER STRICKLAND

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The Role of the GluA1 AMPA Receptor  
Subunit and Hippocampal NMDA Receptors  
in Learning and Memory

Jasmin Amber Strickland

Thesis submitted for the degree  
of Doctor of Philosophy

Department of Psychology  
Durham University

2019

## ABSTRACT

AMPA and NMDA receptors for glutamate are required for long-term potentiation (LTP) of synapses, proposed to be the neural basis of learning, particularly within the hippocampus. Glutamate dysfunction has also been linked to disorders including schizophrenia. The aim of this thesis is to investigate the role of glutamate dysfunction in learning and memory, using two transgenic mouse strains.

*Gria1*<sup>-/-</sup> mice lack the GluA1 subunit of the AMPA receptor and have impaired short-term memory for recently experienced stimuli, but intact long-term memory based on associative retrieval. Contrary to expectation, the experiments in this thesis suggest that the GluA1 subunit is required for cue-competition, but only when dependent on the level of generalisation between the cues. Despite short-term memory being impaired in *Gria1*<sup>-/-</sup> mice, flavour preference learning proposed to be dependent on short-term memory, was found to be intact. Learning about the relative reinforcement rates of levers was also normal, shown in the form of intact matching behaviour. In line with previous findings, mean lick cluster sizes, a measure of palatability, were impaired. *Grin1*<sup>ΔDGCA1</sup> mice lack NMDA receptors specifically within the hippocampus. Flavour preference learning and matching behaviour were found to be normal, but mean lick cluster sizes were impaired. Both the *Gria1*<sup>-/-</sup> and *Grin1*<sup>ΔDGCA1</sup> mice also showed enhanced reversal of matching behaviour compared to the control mice.

The results from this thesis provide further support for glutamate dependent synaptic plasticity not being required for associative learning. Glutamate may however be involved in other aspects of stimulus processing, including perceived hedonic value and sensitivity to the current temporal context. The precise mechanisms for these however remain unclear.

## DECLARATION

I confirm that none of the material presented in this thesis has been submitted elsewhere for any other qualification and is my own work unless referenced otherwise.

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*Dedicated to the memories of Uncle Patrick  
and Justin Browning.*

*Patrick, for helping give me the confidence to do  
this even while struggling to find your own.*

*Justin, for being an amazing teammate and friend  
who reminded me to have some fun along the way,  
and for the project(s) you never got to write.*

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# Chapter 1

## Introduction

Glutamate is a key excitatory neurotransmitter required for long-term potentiation (LTP) of synapses. In the hippocampus, a structure important for learning and memory, LTP has been suggested to provide a potential neural mechanism for learning (Bliss & Collingridge, 1993; Bliss & Lømo, 1973). Two important receptors for glutamate involved in LTP processes, are the AMPA and NMDA receptors. Both of these receptors are tetrameric proteins and have different subunit types. For the AMPA receptor these are the GluA1 – GluA4 subunits, and for the NMDA receptors these include the GluN1 and GluN2 subunits. AMPA receptors are associated with rapid synaptic transmission, mediating excitatory postsynaptic activity by allowing fast inward movement of ions such as sodium ( $\text{Na}^+$ ). If the resulting depolarisation is sufficient, the magnesium ( $\text{Mg}^{2+}$ ) blockade from voltage gated NMDA glutamate receptors is released. This, in turn, is one of the triggers for long-term potentiation of synaptic transmission, through the movement of  $\text{Na}^+$  and  $\text{Ca}^{2+}$  into the dendritic spine, with calcium in particular being important in the signalling cascades and protein synthesis required for LTP (Malenka & Nicoll, 1999).

Evidence for hippocampal synaptic plasticity in particular being important in learning and memory, comes from a range of human and animal studies. In rats for example, lesions of the hippocampus impair the ability to learn the location of the hidden platform in the Morris water-maze task (Morris, Garrud, Rawlins, & O'Keefe, 1982). Furthermore, blocking LTP using glutamate NMDA receptor antagonist AP5, impairs performance in the Morris water-maze (Davis, Butcher, & Morris, 1992; Morris, Anderson, Lynch & Baudry, 1986). Hippocampal damage in human patients has also been found to impair the ability to learn about new information (Scoville & Milner, 1957; Zola-Morgan, Squire, & Amaral, 1986). However, spatial learning in the water maze has also been found to be intact in rats, despite impaired hippocampal LTP as a result of administration of an NMDA receptor antagonist (Bannerman et al, 1995).

In addition, mice lacking NMDA receptors and LTP within the hippocampus also show intact spatial associative learning in the water-maze (Bannerman et al., 2012). Furthermore, despite long-term associative memory being intact in mice lacking the GluA1 subunit of the AMPA receptor, short-term memory is impaired (Sanderson et al., 2010). The precise role of glutamate in learning and memory therefore remains unclear.

Glutamate is also of interest due its association with a range of psychological disorders. Schizophrenia in particular has been linked to altered glutamate signalling and association formation, with glutamatergic dysfunction being proposed to lie upstream of deficits in dopamine and prediction error signalling (Coyle, 2006). Patients with schizophrenia also show altered prediction error learning and aberrant association formation, which have been linked to altered glutamate and dopamine signalling (Corlett et al., 2007; Jensen et al., 2008). The dysregulation of NMDA receptors in particular has been linked to schizophrenia (Javitt & Zukin, 1991), as has the *Grin1* gene that encodes for GluN1 subunit of the receptor (Begni et al., 2003; Qin et al., 2005). Furthermore, mice lacking NMDA receptors have also been found to show some of the negative symptoms associated with schizophrenia, such as impaired social interaction and behavioural inhibition (Halene et al., 2009). The *Gria1* gene, for the GluA1 subunit of the AMPA receptor, has also been identified in genome wide association studies with schizophrenia (Ripke et al., 2013). Mice lacking the GluA1 subunit show reduced palatability, something that is interest due to anhedonia being one of the negative symptoms of schizophrenia (Sanderson et al., 2017). Therefore, although glutamate is linked to schizophrenia and associated symptoms, the precise role(s) it may play are not known.

The main aim of this thesis was to further understand the role of glutamate in learning and memory processes. One way to investigate this is to use transgenic mice with altered glutamatergic signalling. In this thesis, two different transgenic strains of mice are tested, the *Gria1*<sup>-/-</sup> and the *Grin1*<sup>ΔDGCA1</sup> mice. In *Gria1*<sup>-/-</sup> mice the GluA1 subunit of the AMPA receptor is deleted globally, providing a way to investigate the role of this subunit in learning and memory. The GluA1 subunit is of particular interest due to the role it plays in synaptic plasticity (Huganir & Nicoll, 2013).

Furthermore, the GluA1 subunit also seems to play an important role in short-term memory for recently experienced stimuli (Sanderson et al., 2009). The Grin1<sup>ΔDGCA1</sup> knockout mice lack NMDA receptors within the hippocampus, in dorsal CA1 pyramidal cells and dentate gyrus granule cells, resulting in a lack of LTP at CA1-CA3 synapses. These mice therefore provide a way to investigate the role of synaptic plasticity in the hippocampus, a structure important for learning and memory. Interestingly, despite impaired LTP in the hippocampus, the Grin1<sup>ΔDGCA1</sup> mice show intact long-term spatial learning (Bannerman et al., 2012).

To investigate the role of the GluA1 subunit of the AMPA receptor and hippocampal NMDA receptors in learning and memory, Gria1<sup>-/-</sup> and Grin1<sup>ΔDGCA1</sup> mice were tested using various learning procedures. Starting with the GluA1 subunit the following sections of the introduction discuss in greater detail the roles of the GluA1 subunit of the AMPA receptor and the role of the hippocampus and hippocampal NMDA receptors, in learning and memory. More specifically, the GluA1 subunit is discussed in relation to its proposed role in short-term memory (Sanderson et al., 2009) and cue-competition effects. The Grin1<sup>ΔDGCA1</sup> mice are then discussed in relation to the role of hippocampal synaptic plasticity in learning and memory. The association between NMDA receptors and schizophrenia is also outlined. As the hippocampus has also been associated with eating behaviour, this is also briefly discussed. Finally, a microstructural analysis of licking is introduced, as this was used to investigate the role of glutamate dysfunction in hedonic value.

## **1.1 The role of the GluA1 subunit of the AMPA receptor**

The GluA1 subunit of the AMPA receptor is of interest in learning and memory due to the role it plays in synaptic potentiation. For example, LTP seems to be at least partly supported by the addition of GluA1 containing AMPA receptors into synapses (Huganir & Nicoll, 2013; Kessels & Malinow, 2009; Santos, Carvalho, Caldeira, & Duarte, 2009). The subunit is also highly expressed in the hippocampus, a key structure in learning and memory. Furthermore, the Gria1 gene that encodes for the

GluA1 subunit has been linked to schizophrenia in genome wide association studies (Ripke et al., 2013; Ripke et al., 2014).

In *Gria1*<sup>-/-</sup> mice (Zamanillo et al., 1999), the *Gria1* gene encoding for the GluA1 subunit of the AMPA receptor is deleted. Through Immunohistochemistry to verify deletion this has been shown to greatly reduce functional AMPA receptor expression. Reductions were greatest in areas of the brain associated with higher levels of expression of the GluA1 subunit, such as the hippocampus and amygdala, and were lower in areas with lower levels of expression, such as the neocortex. Furthermore, in adult mice, associative LTP induced by tetanic stimulation was absent in the hippocampus at CA3 to CA1 synapses, demonstrating that deletion of the GluA1 subunit impairs hippocampal synaptic plasticity (Zamanillo et al., 1999). There is also evidence that it is the early stages of LTP in particular that are impaired, with a slowly rising form of long-term potentiation in the hippocampus having been observed, that after around 45 minutes, reaches the same normal levels as wild-type control samples (Hoffman, Sprengel, & Sakmann, 2002). The GluA1 subunit of the AMPA receptor therefore appears to be important in the early phases of LTP and for a more short-term form of synaptic potentiation. The life expectancy of these mice is however normal, and they are also indistinguishable from their littermate controls. The fine structure of neuronal dendrites and synapses has also been demonstrated to be normal compared to wild-type control mice. See Zamanillo et al. (1999) for further information on the genetic construction, breeding and genotyping of the *Gria1*<sup>-/-</sup> mice.

Interestingly, *Gria1*<sup>-/-</sup> mice, despite showing impaired hippocampal plasticity, are not impaired on spatial associative memory tasks. In the Morris water-maze task (Morris, 1984), animals are required to learn the location of a hidden escape platform in a pool. Successful performance depends on the ability to learn the location of the platform with respect to the environment and extra-maze cues over successive trials. In this task, *Gria1*<sup>-/-</sup> mice demonstrate learning of the platform location that does not differ from wild-type control mice (Reisel et al., 2002; Zamanillo et al., 1999). The radial arm maze also provides a way to assess spatial learning (Olton, Collison, & Werz, 1977). In this task animals are released into the maze in the central area and

can enter the arms radiating from the centre. Over successive trials, animals learn which of the arms are always baited with a food reward, and which are not. Schmitt et al., (2003) tested *Gria1<sup>-/-</sup>* mice in the radial arm maze, in which 3 of the 6 arms were consistently rewarded over trials. As with the water-maze, the *Gria1<sup>-/-</sup>* mice were found to show normal, if even slightly enhanced, spatial learning compared to wild-type controls.

Further demonstrations of associative learning and memory for spatial locations being intact in the *Gria1<sup>-/-</sup>* mice come from the T and Y maze tasks. Similar to the radial arm maze, animals have to learn which of the 3 arms are consistently baited with a food reward. Resiel et al., (2002) demonstrated that the *Gria1<sup>-/-</sup>* mice could readily learn which one of the two discriminable arms (the third arm being the start arm) were rewarded. All of these spatial memory tasks that are intact in the *Gria1<sup>-/-</sup>* mice, have also been found to be hippocampus dependent. Lesions of the hippocampus impair learning in the water-maze and Y-maze tasks (Morris et al., 1982; Reisel et al., 2002), as well as performance in the radial arm maze (Olton et al., 1978; Schmitt et al., 2003). These results demonstrate that despite deletion of the GluA1 subunit altering hippocampal plasticity, these hippocampal dependent spatial learning tasks are independent of GluA1 mechanisms. Furthermore, this intact associative memory has also been demonstrated in non-spatial tasks, demonstrating it is not specific to the spatial domain (Sanderson et al., 2011a). Taken together, these results demonstrate that associative learning and retrieval of memory are normal in the *Gria1<sup>-/-</sup>* mice. In at least these tasks, these memory processes therefore seem to be independent of the GluA1 subunit of the AMPA receptor.

In contrast to this intact associative retrieval, *Gria1<sup>-/-</sup>* mice do show impaired performance on tasks requiring the use of short-term memory for recently experienced stimuli. Unlike procedures that require learning an association between a place and reward over successive trials, successful performance in spatial working-memory tasks require short-term memory for the recently experienced spatial location(s). The same spatial tasks used to assess spatial learning can also be used to assess spatial short-term memory. In the radial arm maze, this is done by changing which arms are baited over trials. Successful performance therefore depends not on

an association between the place and reward, but memory of which arms have recently been experienced. This allows the animal to explore optimally all the arms for potential food rewards, without re-entering an arm that has already been visited within that trial. In contrast to learning the spatial long-term memory version of the task normally, the *Gria1*<sup>-/-</sup> mice continued to show errors within-trials by visiting arms in which they had already consumed the reward (Schmitt et al., 2003). The *Gria1*<sup>-/-</sup> mice were therefore unable to express a memory for the recently visited arms of the maze, i.e. which arms they had and had not visited. In the Y and T mazes, short-term spatial memory is assessed by testing rewarded alternation behaviour. In this procedure, the arm that is baited is alternated over trials. Successful performance depends on short-term memory for the arm recently rewarded, and in each trial choosing to enter the arm that was non-rewarded in the previous trial. Again, the *Gria1*<sup>-/-</sup> mice are impaired at showing rewarded alternation behaviour, failing to adopt the same win-shift strategy used in the wild-type mice (Reisel et al., 2002). Further evidence for the role of the GluA1 subunit specifically in short-term spatial memory comes from the finding that partially restoring expression of the subunit also partially recovers performance in the radial arm maze (Schmitt et al., 2005).

The impairment in short-term memory has also been seen in spontaneous alternation in the Y-maze and radial arm maze tasks. In these, animals alternate as a result of novelty preference for the locations experienced less recently. This therefore also requires short-term memory for the recently visited locations and has been found to be impaired in the *Gria1*<sup>-/-</sup> mice (Sanderson et al., 2007). Together, these results demonstrate that spatial memory for recently experienced stimuli is impaired in *Gria1*<sup>-/-</sup> mice, resulting in the failure to show a novelty preference for the arm/spatial location less recently experienced. These short-term memory tasks, as with the associative learning spatial tasks, have been found to be hippocampus dependent. In the Y-maze for example, hippocampal lesions result in impaired rewarded alternation behaviour (Reisel et al., 2002). In the radial-arm maze hippocampal lesions similarly result in an impaired ability to preferentially visit the arms not currently visited within that trial (i.e. adopt win-shift behaviour). This shows

that short-term spatial memory is hippocampus dependent. The *Gria1*<sup>-/-</sup> mice therefore show a dissociation between hippocampus-dependent memory tasks. Whereas short-term spatial memory seems to be dependent on the GluA1 subunit, long-term associative memory is independent of GluA1 mechanisms.

The impaired short but not long-term memory in *Gria1*<sup>-/-</sup> mice was further demonstrated by Sanderson et al. (2009). In these experiments *Gria1*<sup>-/-</sup> and wild-type mice were assessed for both short and long-term spatial memory on the same novelty preference Y-maze task. Mice were exposed over repeated trials to one arm of the maze, before being assessed for a novelty preference towards the arm not previously visited. When the interval between the exposure trials was short (1-minute) testing short-term memory, the *Gria1*<sup>-/-</sup> mice were impaired at showing a novelty preference for the unvisited arm of the maze. When the interval between the trials was long (24hr) however, the *Gria1*<sup>-/-</sup> mice showed an enhanced novelty preference compared to the wild-type mice. Hippocampal lesioned mice were impaired in both the short and long-term spatial memory versions of the tasks. These results further demonstrate that hippocampal dependent short-term memory is impaired in *Gria1*<sup>-/-</sup> mice, but that long-term associative memory is intact and may even be enhanced. The results also provide evidence for two memory processes, one short-term and one long-term. Although both are dependent on the hippocampus, they are dissociable in *Gria1*<sup>-/-</sup> mice. Deletion of the GluA1 subunit impairs short-term memory based on recent exposure; but spares long-term memory based on association formation and retrieval.

The impaired short-term memory has been explained as a result of a selective impairment in habituation, which is the decline in responding to recently experienced stimuli (e.g., Sanderson et al., 2009; Sanderson et al., 2011b). This can explain the observed failures in spatial short-term memory tasks, such as the T-maze, Y-maze and radial arm maze, as a consequence of failing to reduce responding to recently experienced locations. The *Gria1*<sup>-/-</sup> mice are therefore unable to express a novelty preference based on having a short-term memory for the recently experienced location. Failure to habituate to recently experienced stimuli in the *Gria1*<sup>-/-</sup> mice has also been demonstrated in non-spatial procedures, showing that

this impairment in short-term habituation is not only selective to the spatial domain (Sanderson et al., 2011a).

The impaired short-term memory, in the form of impaired habituation and reductions in responding, suggests that *Gria1*<sup>-/-</sup> mice attribute aberrant amounts of salience to recently experience stimuli. As stimulus salience is altered, associative learning about stimuli may also be affected. Traditional learning theories (Rescorla & Wagner, 1972; Wagner, 1981), predict that this enhanced salience will increase the ability of the stimulus to enter into associations. Rather than the ability to learn about stimuli decreasing as habituation occurs, the *Gria1*<sup>-/-</sup> mice may instead continue to learn about stimuli that were recently presented. This means that they may form aberrant associations between relatively recently presented stimuli, that would not occur in wild-type mice due to habituation having occurred. Evidence for this was demonstrated by Sanderson et al. (2017) in a trace conditioning procedure, in which the cue and reward are separated by a short interval. The wild-type mice initially learned about the cue but came to inhibit responding during it, with responding increasing instead during the interval preceding the cue. The *Gria1*<sup>-/-</sup> mice in contrast continued to respond to the cue. Whereas the wild-type mice came to show an inhibitory association between the cue and the reward, this was not seen in the *Gria1*<sup>-/-</sup> mice. This demonstrates that the ability for stimuli to enter into either excitatory or inhibitory associations are altered in the *Gria1*<sup>-/-</sup> mice and supports the idea that altered stimulus salience may also result in aberrant association formation. Furthermore, the *Gria1*<sup>-/-</sup> mice have been found to show enhanced associative learning not only in this trace conditioning task, but also during spatial learning. In the radial arm maze when prevented from making working-memory errors during training (i.e. entering arms in which they had already visited), *Gria1*<sup>-/-</sup> mice showed enhanced memory compared to controls (Schmitt et al., 2003). Similarly, in the Y-maze novelty preference task, the *Gria1*<sup>-/-</sup> mice showed an enhanced preference for the novel arm compared to the control mice when the exposure trials were separated by a long (24hr) interval (Sanderson et al., 2009). These enhancements may also be linked to the altered balance between excitatory and inhibitory learning seen in the *Gria1*<sup>-/-</sup> mice, as the increased short-term excitatory stimulus processing may

enhance association formation and resulting memory for the recently experienced locations.

Overall, the *Gria1*<sup>-/-</sup> mice demonstrate that short-term memory for recently experienced stimuli is dissociable from long-term memory based on association formation. This can be seen in the impaired short-term habituation to recently experienced stimuli, but with spared or even enhanced associative memory. These dissociable memory processes also seem to depend on different neural mechanisms. Although they are both dependent on the hippocampus, long-term memory based on associative retrieval seems independent of GluA1 related mechanisms. The expression of short-term memory (habituation to recently experienced stimuli) however requires the GluA1 subunit of the AMPA receptor. This dissociation between short and long-term memory, has led to the role of the GluA1 subunit being explained in relation to Wagner's SOP model (Wagner, 1981), that outlines two separate memory processes.

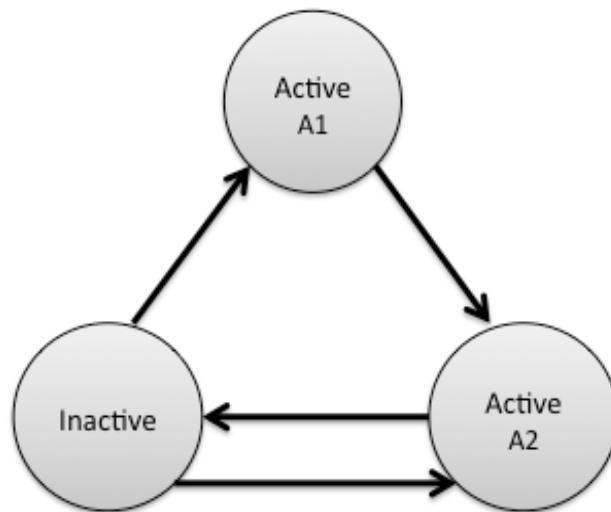
## **1.2 GluA1 and Wagner's SOP model**

The *Gria1*<sup>-/-</sup> mice demonstrate that short-term memory, in the form of habituation to recently experienced stimuli, is dissociable from long-term associative learning and memory. These two memory mechanisms also appear to depend on different hippocampal dependent neural substrates. One model that describes learning and memory using two separate mechanisms is Wagner's SOP (Wagner, 1981), shown in Figure 1.1 This model outlines two separate memory processes, one of which is non-associative and depends on short-term memory decay. The other is associative and dependent on learning associations between stimuli and subsequent associative retrieval of memory. These two separate processes provide a way to explain reductions in responding to stimuli (habituation) occurring through both short and long-term memory.

Short term habituation occurs as a result of recent exposure to stimulus and resulting short-term memory. Long term habituation is the result of associative learning and

retrieval of a memory of the associated stimulus. The model proposes that stimuli are represented by a node containing many representative elements. Each of these elements can be in one of three states of memory at any given time, a primary active state (A1) a secondary active state (A2) and also an inactive (I) state of memory. The primary active state equates to the stimulus representation being at the forefront of attention, resulting in the greatest level of behavioural responding, but has only a limited elemental capacity. The secondary active state relates to the stimulus being at the periphery of attention, with behavioural responding therefore low. The capacity of this secondary active state is, however, greater than the capacity of the primary active state. In contrast to these two active states, elements in the inactive state cannot influence behaviour and can be related to a long-term memory store. The level of behavioural responding to a stimulus is therefore dependent on the state in which the representation is currently held, with greatest levels of responding when in the primary active state of memory.

Importantly, representative elements are limited in the ways in which they can transfer between the three states of memory. Upon presentation of a stimulus its representative elements are able to enter the primary active state (A1) of memory and therefore generate maximum levels of behavioural responding. However, these elements quickly decay from the A1 state to the secondary active state (A2) of memory, resulting in a corresponding reduction of responding to the presented stimulus. This mechanism of decay, from the primary to secondary active states of memory, is therefore critically dependent on the time since presentation, with the elements only being held in the A1 state for a short period of time. Furthermore, elements are only able to further decay into an inactive state from the secondary active state, a process which occurs at a more gradual rate than A1-A2 decay. Elements are unable to return directly into the primary active state from a secondary active state of memory. This process can explain the short-term habituation of behavioural responding, with stimuli entering a secondary active state and not then able to move back to a primary active state until full inactive decay occurs. If the stimulus is presented again shortly after, responding will therefore remain reduced as it has not had time to fully decay and will be in a secondary active state of memory.



*Figure 1.1* Wagner's SOP (1981) model showing the three states of memory. The primary active state (A1), the secondary active state (A2), and an inactive state. Stimulus representations enter the primary active state upon presentation. They then rapidly decay to the secondary state before more slowly decaying to an inactive state. Associations can form between representations concurrently active in A1, allowing the subsequent presentation of one of these stimuli to associatively retrieve a representation of the other directly into the secondary active state. Representations cannot move back into A1 once in A2 and can only further decay into an inactive state.

In contrast to this non-associative process of short-term habituation, which is dependent on time since stimulus presentation, the model provides a different explanation for long-term association formation and habituation. Associations can form between representative elements that are concurrently active in the primary active state of memory. This occurs as a result of stimuli being presented in close temporal proximity, for example a recent CS and resulting US. This association formation results in the ability for the presentation of one of these stimuli, e.g. the CS, to retrieve the elements of the other, the US, into an active state of memory. This primed activation however is restricted to only the secondary active state of memory, retrieved directly from an inactive to a secondary active state of memory.

Therefore, the presentation of one cue can prime the representation of another cue, even if this is not subsequently presented, into the A2 state of memory. However, as the secondary active state can only support a limited amount of behavioural responding, this priming results in a reduced level of behavioural responding. This mechanism of associative retrieval therefore results in a reduced level of responding to a stimulus, that may, or may not, be subsequently presented. In this way, retrieval-generated priming can explain long-term reductions in responding, occurring as a result of association formation and subsequent elemental retrieval directly into the secondary active state of memory.

Wagner's SOP model (Wagner, 1981), can therefore account for reductions in responding to stimuli; short-term habituation occurs as a result of a non-associative process (self-generated priming) and long-term habituation is due to associative retrieval (retrieval-generated priming). The dissociation shown by the *Gria1*<sup>-/-</sup> mice, of impaired short-term but intact, or enhanced, long-term memory, provides evidence for such a dual process account of memory. The model can also provide an explanation of the impaired short-term memory seen in *Gria1*<sup>-/-</sup> mice. As *GluA1* deletion impairs this short-term reduction in responding, it has been suggested to slow the decay rate of the stimulus representation between the primary (A1) and secondary (A2) active states of memory (Sanderson et al., 2009; 2010). The impairment shown by *Gria1*<sup>-/-</sup> mice is therefore a result of stimulus elements remaining in the primary active state for a longer period of time, with responding remaining at higher levels for longer than if decay were to occur more rapidly into the A2 state. Such a change in the decay rate can explain the failures to habituate to recently experienced stimuli seen in tasks such as Y-maze novelty preference (e.g. Sanderson et al., 2009) as a result of elements failing to accumulate in the secondary active state over a short period of time.

As well as providing a potential mechanism for the failure to habituate seen in *Gria1*<sup>-/-</sup> mice, Wagner's SOP model (1981) can also account for the intact associative memory in these mice. This is due to the mechanism resulting in long-term reductions in responding based on a separate mechanism. Rather than decay from the primary to secondary active state, long-term habituation occurs through direct

priming of stimulus representations into the secondary active state from an inactive one. If the impaired short-term habituation is due to a slowed rate of decay from A1-A2 states, then retrieval generated priming, from inactive to secondary active states of memory, should be intact in *Gria1<sup>-/-</sup>* mice. Associative retrieval may even be enhanced in the *Gria1<sup>-/-</sup>* mice compared to the wild-type controls. This is due to the slowed decay rates between the primary and secondary active states of memory resulting in stimuli being maintained in a primary active state of memory for longer. As associations form between stimuli concurrently active in the primary state, association formation and subsequent associative retrieval may therefore be enhanced in the *Gria1<sup>-/-</sup>* mice.

Wagner's SOP (1981) model can also account for another feature of learning, that stimulus associability seems to decline with exposure. Furthermore, the proposed role of the GluA1 subunit in short-term memory (Sanderson et al., 2009) predicts this process of reduced associability should be impaired in the *Gria1<sup>-/-</sup>* mice. Best and Gemberling (1977) for example, observed that recent presentation of a taste impaired learning a taste aversion. This impairment was not seen when longer intervals were used between the pre-exposure to the taste and subsequent taste aversion training. Having a short-term memory for a stimulus therefore reduces the ability for it to enter into an association with another stimulus. Wagner's SOP model can explain this reduction as a result of the representative elements having decayed from a primary to secondary active state of memory. When in this secondary state, the representation is far less able to influence behaviour and is no longer able to enter into associations with other stimuli active in the primary state. In the *Gria1<sup>-/-</sup>* mice, if the decay between the primary and active states is slowed (Sanderson et al., 2009) then short-term memory effects on learning should also be affected. In particular, the slowed decay process means that the reduced stimulus associability seen as a result of this decay should also be slowed in the *Gria1<sup>-/-</sup>* mice. The *Gria1<sup>-/-</sup>* mice may therefore be more likely to form excitatory associations between recently presented stimuli, as representations will be more likely to have decayed to a secondary state in the wild-type mice, but remain in a primary active state in the *Gria1<sup>-/-</sup>* mice. This increased excitatory processing in the *Gria1<sup>-/-</sup>* mice, also provides

a mechanism for the findings of enhanced long-term associative memory (Sanderson et al., 2009). The role of the GluA1 subunit in short-term memory related learning was tested in this thesis, using flavour preference learning based on having a short-term memory for recently presented sucrose.

Overall the dissociation shown by the *Gria1*<sup>-/-</sup> mice, between short and long-term memory processes, supports a dual process account of memory, such as Wagner's SOP model (Wagner, 1981). This model has also been used to explain the impaired short-term memory in the *Gria1*<sup>-/-</sup> mice, by a slowed decay rate between the primary and secondary active states of memory (Sanderson et al., 2009).

### **1.3 GluA1 deletion and cue-competition**

The GluA1 subunit has been proposed to selectively slow the decay rate between the primary and secondary active states of memory in Wagner's SOP model (Sanderson et al., 2009). Associative retrieval mechanisms should nonetheless be intact in the *Gria1*<sup>-/-</sup> mice. In Wagner's SOP (1981) model, associations form between representative elements that are concurrently in a primary active state (e.g., a CS and a following US), resulting in the stimuli acquiring associative strength. This associative strength means the stimulus (the CS), when presented, can directly prime a representation of the associated US directly into A2 from an inactive state (retrieval-generated priming). In this secondary active state, the representative elements are unable to enter back into the primary active state, without first decaying to an inactive state. Learning is therefore the result of prediction error mechanisms, with unexpected events being learned about compared to those that are already well predicted.

This proposed role of the GluA1 subunit in short-term memory (Sanderson et al., 2009), predicts that associative retrieval and prediction error mechanisms will be intact in *Gria1*<sup>-/-</sup> mice. This is due to the pathway between the inactive state and the secondary active state not being altered by deletion of the GluA1 subunit. Stimuli are therefore still able to enter the primary active state from an inactive one, meaning

the ability to form excitatory associations about stimuli not currently predicted will also be intact. Therefore, In  $Gria1^{-/-}$  mice, both excitatory learning and subsequent retrieval generated priming processes are predicted to be intact. If prediction error learning processes are not affected by deletion of the GluA1 subunit, then cue-competition effects, such as blocking (Kamin, 1969; Mackintosh, 1971) and overshadowing (Kamin, 1969; Mackintosh, 1971, 1976; Pavlov, 1927) should also be intact in  $Gria1^{-/-}$  mice. Cue-competition effects occur when stimuli are concurrently presented and affect the learning about each of the individual stimuli as a result. Associative learning theories, such as Rescorla and Wagner (1972) and Wagner's SOP model (Wagner, 1981), explain cue-competition effects as a result of prediction error mechanisms and stimuli being less able to individually acquire associative strength to predict the outcome.

The Rescorla-Wagner model is shown in equation 1. The associative strength ( $V$ ) of a stimulus, ( $A$ ) on any given trial changes ( $\Delta V_A$ ) as a function of the discrepancy between the summed associative strength of the cues present, such as  $A$  and  $X$ , ( $\sum V_{AX}$ ) and the total amount of associative strength that can be supported by the particular outcome, the US, ( $\lambda$ ). Learning therefore occurs as a result of the prediction error between what is already expected to occur ( $\sum V$ ) and what does occur ( $\lambda$ ). Alpha and beta represent learning rate parameters linked to the CS and the US, respectively. Importantly, as the total amount of associative strength available in relation to an outcome is limited ( $\lambda$ ), concurrently presented cues will each gain associative strength, that will then sum together and reduce the error term, lowering the amount of further associative strength each cue can gain. Similarly, if a cue already has a high amount of associative strength, the outcome will be well predicted and other cues that may be presented will be unable to gain much, if any, associative strength.

$$\Delta V_A = \alpha_A \beta (\lambda - (\sum V_{AX})) \quad 1)$$

Wagner's SOP model (1981) also explains cue-competition as a result of cues being individually able to acquire associative strength and a reduction in the subsequent prediction error term. In SOP, prediction error learning occurs as a result of the

discrepancy between the degree of stimulus activation in the primary and secondary active states of memory. More specifically, Stimuli that are expected to occur are primed directly into the secondary active state and unable to form excitatory associations with other stimulus representations. However, if a stimulus occurs, but was not predicted, it is instead able to enter the primary active state of memory. When in this primary state, the representation is able to form excitatory associations with other representations concurrently active in this state. In this way, the model formalises prediction error learning as a result of the discrepancy between what is expected to occur and what does occur. In terms of cue-competition effects, as with the Rescorla and Wagner model (1972), it can similarly explain these as the result of the division of associative strength between concurrently presented cues. When cues are concurrently presented, each will individually gain associative strength, that when summed together acts to increase the degree of self-generated priming directly into the A2 state. This priming reduces the ability for the cues to gain further associative strength, as fewer elements representing the outcome are available in the primary active state to enter into excitatory associations. As a result, when one of the cues is subsequently presented alone, it will be less able to predict the outcome and generate conditioned responding than if it had been trained alone.

Two key cue-competition procedures are blocking and overshadowing. During a blocking procedure (Kamin, 1969; Mackintosh, 1971), in the first stage of training one cue is pre-trained to predict the outcome. This cue is then paired with another cue during the second stage of training, leading to the same outcome. This acts to prevent much, if any, learning about the second cue, seen as low levels of conditioned responding when this cue is presented alone during the test sessions. In terms of prediction error learning, the pre-training of one cue results in this cue gaining high levels of associative strength and being able to predict the outcome well. When this cue is then subsequently paired with another cue in the second stage of training, the outcome is already well predicted, meaning there is little or no prediction error. The new cue is therefore unable to gain any associative strength and enter into an excitatory association with the outcome. Therefore, when this cue

is presented alone at test it will be unable to generate much, if any, conditioned responding, causing the blocking effect.

During overshadowing (Mackintosh, 1971, 1976), two stimuli are concurrently presented during training leading to an outcome. When one of these cues is subsequently presented alone during the test sessions, conditioned responding is reduced compared to if it had been previously trained alone. This can be explained as a result of the two stimuli each being able to gain associative strength and predict the outcome to some degree, with this associative strength summing together to predict the outcome and reduce the error term. At the point when the outcome is fully predicted, each cue will therefore only have half (provided the stimuli saliences are the same) of the total associative strength that the outcome can support. When one of the cues is then presented alone during the test sessions, the outcome will be less well predicted and conditioned responding reduced.

The proposed role of GluA1 deletion, in slowing the decay rates between the primary and secondary active states of memory (Sanderson et al., 2009), predicts that cue-competition effects, including blocking and overshadowing should be intact in the *Gria1<sup>-/-</sup>* mice. This is due to cues still being able to enter into associations with outcomes and subsequently prime them directly into the A2 state, as the pathway between the inactive and A2 states is not proposed to be affected by GluA1 deletion. As a result, fewer elements representing the outcome will be able to enter the A1 state and into associations with cues present and concurrently active in this state. In other words, the summed prediction error is reduced due to the presence of multiple cues acting to prime the outcome directly into the secondary active state. Not only should blocking and overshadowing therefore be intact in the *Gria1<sup>-/-</sup>* mice, but it may even be enhanced compared to the controls. This is due to the slowed decay rate resulting in representations remaining in a primary active state for longer. According to Wagner's SOP (1981), this will also enhance the opportunity for presented cues to enter into excitatory associations with the outcome. Cue-competition effects, occurring as a result of the outcome being predicted and primed by other cues into the secondary active state, preventing further learning, may therefore be enhanced in the *Gria1<sup>-/-</sup>* mice. In this thesis, the prediction that cue-

competition effects would be intact or perhaps even enhanced in the *Gria1*<sup>-/-</sup> mice was directly tested. This was done by assessing blocking and overshadowing of flavour preference learning, as well as with auditory and visual cues.

## 1.4 The role of the hippocampus

The hippocampus has been found to play an important role in the neural and psychological basis of a wide range of cognitive functions. It is widely accepted that the hippocampus is important for memory, and early theories of hippocampal functioning focused on a role for the hippocampus in the encoding of episodic memory (Scoville & Milner, 1957, Squire & Zola-Morgan, 1991). This was supported by evidence from case studies of patients with anterograde amnesia as a result of damage to the hippocampus and medial temporal lobe structures, such as H.M (Scoville & Milner, 1957) and R.B (Zola-Morgan, Squire & Amaral, 1986). Similar findings of impaired episodic type memory have also been observed in hippocampal lesion studies with animals including monkeys (Squire & Zola-Morgan, 1991) and rats (Kim & Fanselow, 1992).

The hippocampus has also been suggested to be an important neural substrate for learning with NMDA receptor dependent LTP, particularly within the CA1 subfield, having been proposed to provide a potential neural basis for associative long-term spatial learning (Bliss & Collingridge, 1993, Martin, Grimwood & Morris, 2000; Tsien, Huerta & Tonegawa, 1996). Hippocampal lesions in rats for example impair performance in the Morris water-maze task (Broadbent, Squire, & Clark, 2006; Reisel et al., 2002). Administration of NMDA receptor antagonist AP5 that blocks LTP, also impairs spatial learning, with the degree of impairment also being found to relate to the level of LTP (Davis et al., 1992). However, although LTP in the hippocampus does seem to play a role in spatial learning and memory, the precise nature of this is unclear. In *Grin1*<sup>ΔGCA1</sup> mice for example, despite deletion of hippocampal NMDAR's (in dorsal CA1 and dentate gyrus) and a lack of LTP at CA3-CA1 synapses, associative spatial learning is intact (Bannerman et al., 2012).

One well established theory of hippocampal functioning is that it may provide a neural representation of the environment in the form of a cognitive map (Hartley, Lever, Burgess & O'Keefe, 2014; O'Keefe & Nadel, 1978). This was proposed as a result of findings of place cells in freely moving rats within the hippocampus and the dentate gyrus, that show spatial receptive fields for a particular location within the environment (O'Keefe & Dostrovsky, 1971). Findings of other cells receptive to spatial properties of the environment and the location of the animal within it; grid cells, head direction cells, and boundary cells, have further supported a role for the hippocampus in spatial cognition (Hartley, Lever, Burgess & O'Keefe, 2014). Other evidence that the hippocampus provides a cognitive map comes from findings that hippocampal lesions impair allocentric spatial memory (encoded with respect to external features of the environment), but not egocentric representations (self-centred) (Eichenbaum, Stewart & Morris, 1990). As well as encoding spatial information, there is also evidence that the hippocampus encodes temporal information. Time cells for example have been identified within the CA1 region, that respond to the temporal structure of events occurring within an environment, such as the temporal order of events and the interval between them (Eichenbaum, 2014). It has therefore been suggested that similar neuronal ensembles within the hippocampus may encode both the spatial and temporal information of events within an environment (Eichenbaum, 2014).

The hippocampus has also been linked to a comparator processes required to detect novelty/uncertainty. The CA1 subfield in particular may be important for this process, comparing current sensory information arriving from the cortex with information about expectation retrieved from dentate gyrus CA3 subfields (Douchamps, Jeewajee, Blundell, Burgess & Lever, 2013; Lisman & Grace, 2005). Theta phase firing of cells in CA1, which is thought to modulate long-term plasticity, has also been found to show a relationship with environmental novelty (Lever, Burton, Jeewajee, Wills, Cacucci et al., 2010). There is also evidence from rodent studies that the CA1 subfield is important in detecting differences between expectation and outcome (Honey, Watt & Good, 1998). Gray (1982) and Gray and McNaughton (2000) suggested that hippocampal structures, including the CA1 subfield, are an important part of a

comparator system related to the detection of uncertainty and conflict. In this case the system mediates anxiety, activated by uncertain situations and generating appropriate responses, such as altered attentional processing and inhibition of ongoing activity. Hippocampal NMDAR receptors, including within CA1, have since been suggested to act as a comparator to resolve uncertainty in the form of disambiguating similar associative long-term spatial memories and between competing behavioural goals (Bannerman, Sprengel, Sanderson, McHugh, Rawlins et al., 2014; Bannerman et al., 2012).

Theories of hippocampal functioning have also looked at the differential roles of dorsal and ventral regions. There is a large body of evidence that whereas the dorsal regions preferentially process spatial information, the ventral region preferentially processes information related to emotion and/or anxiety. Lesions of the ventral hippocampus for example have been shown not to impair spatial learning on a range of spatial tasks, while measures of anxiety across unconditioned tests are reduced (McHugh, Deacon, Rawlins & Bannerman, 2004; Bannerman, Grubb, Deacon, Yee, Feldon & Rawlins, 2003). In contrast, lesions of dorsal hippocampus impair spatial learning, but not measures related to anxiety (Bannerman, Yee, Good, Heupel, Iverson & Rawlins, 1999). Furthermore, whereas the dorsal hippocampus shows connectivity to areas associated with sensory areas and cognition, including the retrosplenial and anterior cingulate cortices, the ventral hippocampus shows connectivity with areas associated with emotion, such as the amygdala and the hypothalamus-pituitary-adrenal axis (Fanselow & Dong, 2010).

The evidence for the ventral and dorsal hippocampus being functionally distinct, despite the consistent anatomical organisation and trisynaptic circuitry along the septotemporal axis, has led to the proposal that both regions may be performing similar comparator calculations, but preferentially related to different information (Bannerman et al., 2014; Fanselow & Dong, 2010). Whereas ventral hippocampus may compare goals and actions required for spatial navigation, dorsal hippocampus may perform similar comparator calculations but related to the processing of more motivational and emotional stimuli and behaviour.

Overall the precise role of the hippocampus remains unclear, particularly in relation to the potential roles of different subfields and regions. The Grin1<sup>ΔDGCA1</sup> mice, that lack NMDA receptors and have impaired synaptic plasticity within the DG and dorsal CA1, provide a way to further investigate these regions more specifically in learning and memory.

## **1.5 The role of hippocampal NMDA receptors in learning and memory**

NMDA receptors, as well as AMPA receptors, are required for LTP in the hippocampus (Bliss & Collingridge, 1993), a structure important for learning and memory. In Grin1<sup>ΔDGCA1</sup> mice, the Grin1 gene encoding for the GluN1 subunit of the NMDA receptor is deleted through doxycycline (dox) sensitive cre-mediated deletion in excitatory hippocampal, but not cortical neurons, of adult mice (Von Engelardt et al., 2008; Shimscheck et al., 2005). This is achieved through Grin1<sup>ΔDGCA1</sup> mice being lox-p tagged for Grin1 alleles and carrying two transgenes, LC1 and CN12. These allow for dox mediated expression of cre and subsequent cre-mediated deletion, by use of a CaMKII promotor fused to a Grin2c silencer element (Suchanek, Seeburg, & Sprengel, 1997). The expression of cre is switched off during embryogenesis by giving the mothers dox that is removed post-natally, with cre-expression detected 4 weeks post-natally (Bannerman et al., 2012). The confirmation of cre-expression using X-gal staining (Bannerman et al., 2012) showed that cre was expressed along the dentate gyrus (DG) and mossy fibres and in dorsal CA1, as well as to a lesser extent in ventral CA1. All the other subfields of the hippocampus were unaffected. Cre expression was however also identified in the olfactory bulb granule cells (Bannerman et al., 2012). In-situ hybridisation also confirmed loss of the GluN1 subunit in dorsal CA1 and DG and that the volume of the DG was also reduced in adult mice. Furthermore, LTP has also been shown to be lacking at the CA3 to CA1 synapses in the dorsal hippocampus in adult Grin1<sup>ΔDGCA1</sup> mice (Bannerman et al., 2012). Therefore, the Grin1<sup>ΔDGCA1</sup> mice show impaired dorsal hippocampal LTP, providing a way to assess the role of

hippocampal NMDA receptors and dependent synaptic plasticity in learning and memory.

Some of the evidence for the importance of the hippocampus in learning comes from studies in which hippocampal lesions impair performance in spatial learning, such as in the Morris water-maze (Broadbent, Squire, & Clark, 2006; Reisel et al., 2002), and radial arm maze tasks (Schmitt et al., 2003). Furthermore, administration of the NMDA receptor antagonist AP5, which blocks NMDA receptors and hippocampal LTP, also impairs performance in the water-maze (Morris et al., 1986). Glutamate dependent hippocampal synaptic plasticity therefore seems important for spatial learning. In addition to this, the impairment in the water-maze as a result of AP5, has been shown to correlate with the degree of hippocampal LTP (Davis et al., 1992). This has led to the suggestion that NMDA receptor activation may be necessary for spatial learning. However, although NMDA receptors are important for memory formation, they do not seem to be necessary for subsequent memory retrieval. For example, Morris (1989) found that infusion of AP5 after training did not affect water maze performance, with rats still able to locate the hidden platform. This suggests that although NMDA receptors may be important in spatial learning, they may not play a role in memory retrieval.

However, if hippocampal synaptic plasticity is required for associative learning, then  $Grin1^{\Delta DGCA1}$  mice, in which hippocampal LTP is impaired, should show impaired learning. Despite this, when tested in the Morris water-maze (Bannerman et al., 2012), the  $Grin1^{\Delta DGCA1}$  mice were able to learn the location of the platform as well as control mice. However, reversal learning, when the location of the platform was moved to the opposite quadrant of the pool, was slightly impaired in the  $Grin1^{\Delta DGCA1}$  mice compared to the control mice. As they were able to learn the location of the platform, spatial learning based on associative retrieval is intact in the  $Grin1^{\Delta DGCA1}$  mice. Contrary to some of the lesion studies, this suggests that hippocampal NMDA receptors are not necessarily required for long-term associative spatial learning. However, although they were normal in this task, spatial learning in the radial arm maze was impaired (Bannerman et al., 2012). In this task mice had to learn, over successive trials, which 3 of 6 arms were always baited with food. Despite being able

to learn the location of the platform in the Morris water-maze, they were impaired in this task, failing to show learning of the baited arms as well as control mice.

In the experiments by Bannerman et al. (2012) however, behavioural inhibition was also found to be impaired in the *Grin1*<sup>ΔDGCA1</sup> mice. This was seen in a variant of the water-maze task, when two visually identical beacons were used, one of which signalled the location of the platform with the other a decoy beacon. Although the *Grin1*<sup>ΔDGCA1</sup> mice were able to learn the location of the platform as well as control mice, they were not able to inhibit responding towards the nearest beacon, even if this beacon did not signal the location of the platform. They were, however, able to swim towards the correct beacon when they were placed equidistance between the two. The *Grin1*<sup>ΔDGCA1</sup> mice were not impaired at inhibiting responding when a similar procedure was carried out using visually distinctive beacons, being able to select the correct beacon as often as controls, even when started near the incorrect beacon. The *Grin1*<sup>ΔDGCA1</sup> mice also showed intact reversal learning in this version of the water-maze task.

These results suggest that NMDA receptors within the hippocampus are not in fact required for spatial associative learning. The role of hippocampal NMDA receptors has therefore been suggested not to relate to associative learning, but instead to a mechanism involved in separating out similar spatial associations (Bannerman et al., 2012; Taylor et al., 2014). This could explain the failure to learn in the radial arm maze, as the *Grin1*<sup>ΔDGCA1</sup> mice would be unable to inhibit responding to the arms not baited, that all look visually similar, despite knowing the actual locations of the food reward. Similarly, the impairment in reversal learning in the water-maze could be explained by an inability to inhibit responding to the original spatial location.

This thesis further investigates the role of hippocampal NMDA receptors in learning and memory using the *Grin1*<sup>ΔDGCA1</sup> mice, in non-spatial procedures. In particular, flavour preference learning is tested, as is the ability to learn about reinforcement rate by assessing matching behaviour.

## 1.6 Hippocampal NMDA receptors and schizophrenia

As well as playing an important role in learning and memory, glutamate has also been implicated in psychiatric disorders including schizophrenia. For example, glutamatergic dysfunction, in areas including the hippocampus, has been associated with the disorder (Javitt & Zukin, 1991; Kantrowitz & Javitt, 2010). For example, a meta-analysis of MRI studies of patients with schizophrenia showed significant bilateral reductions in hippocampal volume (Wright et al., 2000). The NMDA receptor in particular has been linked to the disorder, with administration of NMDA receptor antagonists resulting in both some of the positive and negative symptoms associated with schizophrenia (Javitt, & Zukin, 1991; Moghaddam & Javitt, 2012). Dysregulation of the GluN1 subunit of the NMDA receptor in the hippocampus has also been identified in the brains of people that were diagnosed with the disorder (Vrajová et al., 2010). Further evidence linking the NMDA receptor to schizophrenia comes from genetic association studies linking NMDA receptor subunits, including the GluN1 subunit, to the disorder (Allen et al., 2008; Begni et al., 2003; Qin et al., 2005). These findings have led to the glutamate hypofunction model of schizophrenia, with NMDA receptor dysregulation being a potentially key pathology of the disorder (Kantrowitz & Javitt, 2010). Furthermore, mice lacking NMDA receptor functioning globally have been shown to be a potential model for some of the negative symptoms of schizophrenia, including impaired behavioural inhibition and social interactions (Halene et al., 2009).

The Grin1<sup>ΔDGCA1</sup> mice, that lack NMDAR's specifically in the dorsal CA1 and dentate gyrus subfields, provide a way to further investigate the role of hippocampal NMDA receptors in schizophrenia and associated symptoms. Anhedonia is one of the negative symptoms associated with schizophrenia and has been linked to glutamatergic dysfunction in human and animal studies (Der-Avakian & Markou, 2012). In this thesis, the role of hippocampal NMDA receptors in hedonic value was tested using a microstructural analysis of licking behaviour, discussed in section 1.7, with the Grin1<sup>ΔDGCA1</sup> mice. More specifically palatability, measured by mean lick cluster size, was assessed during consumption of highly palatable sweet sucrose solutions in control and Grin1<sup>ΔDGCA1</sup> mice.

## 1.7 The hippocampus and eating behaviour

The hippocampus has been implicated in eating behaviour, including appetitive behaviour (incentive motivation) in a wide range of studies. This has led to the proposed role of the hippocampus in incentive motivation and behaviour (Jarrard, 1973; Tracy, Jarrard & Davidson, 2001). These include studies looking at the effect of hippocampal function on appetitive and consummatory behaviours. For example, hippocampal lesions have been found to impair changes in appetitive approach behaviour, reduced running speed down a runway, that occurs as a result of a negative contrast in the number of sucrose pellet rewards at the end of the runway. Negative contrast behaviour was however normal in the form of a reduced lick frequency during negative contrast with sucrose solutions (Flaherty, Coppotelli, Hsu & Otto, 1998). As a result of these findings Flaherty et al. (1998) suggested the hippocampus plays an important role in affect approach behaviour, but not necessarily in consummatory behaviour in terms of the amount consumed. Developing on this theory Tracy et al. (2001) proposed that the hippocampus, in its entirety, may be involved in multiple motivational functions related to consummatory behaviour. They noted however that one area of interest could be the difference between appetitive and consummatory behaviour, although the biological and psychological mechanisms for this are not currently clear. It is also not clear the extent that different hippocampal subfields may affect appetitive behaviour, although Jarrard (1973) suggested that the CA1 subfield may be linked to incentive motivation, while CA3-CA4 may be more linked to behavioural inhibition. For example, chemical stimulation of CA1, but not CA3-CA4 subfields induced drinking behaviour in rats (Grant & Jarrard, 1968). The  $\text{Grin1}^{\Delta\text{DGCA1}}$  mice, in which NMDA receptors are deleted in dorsal DG and CA1 of the hippocampus, provide a way to investigate the role of these subregions of the hippocampus in eating behaviour and incentive motivation.

Hippocampal synaptic plasticity has also been implicated in the regulation of eating behaviour and satiety mechanisms. This is therefore worth considering when using consummatory measures to test learning and behaviour in mice with impaired long-term potentiation within the hippocampus. For example, the hippocampus has

populations of receptors for hormones involved in the regulation of appetite and also has connections with other areas linked to energy regulation (Kanoski & Grill, 2017; Tracy, Jarrard, & Davidson, 2001). Furthermore, rats with hippocampal lesions also show an inability to use internal energy signals and regulate eating behaviour, resulting in weight gain (Davidson et al., 2009). However, as well as altering the amount consumed, hippocampal lesions have also been found to affect consummatory patterns. In rats, hippocampal lesions have been found to alter eating in the form of increasing the number of meals, but these are smaller, meaning that there little change in the overall intake. Consummatory patterns were therefore changed from eating more, less often, to less but more often (Clifton, Vickers, & Somerville, 1998). Therefore, although impaired hippocampal functioning may not necessarily alter the total amount consumed, eating patterns may be affected.

As well as potential regulation of eating behaviour through processing of satiety signals, the role of the hippocampus and synaptic plastic in learning and memory may also have an effect on eating behaviour. For example, if mice are unable to learn an association between a taste or flavour cue and the unconditioned stimulus, such as sucrose or nausea, flavour preference and taste aversion learning may be impaired. However, hippocampal lesions have been found not to impair taste aversion learning, although there was some impairment over long trace intervals (Koh et al., 2009). Similarly, the ability to learn to associate a stimulus with a food reward in appetitive magazine conditioning, has also been found to be intact following hippocampal lesions (Davidson & Jarrard, 2004). However, given the link between the hippocampus with learning and memory, and energy regulation, it has been suggested to play a more complex role in regulating consumption. In particular, it has been suggested to integrate aspects of the internal and external environment, including contextual and learned information. The integration of this information may be important in adaptive conditioned responding to food and appropriate regulation of eating behaviour (Davidson et al., 2007; Konoski & Grill, 2017). Importantly, this means that when using procedures such as flavour preference learning, related to consumption, a variety of factors may affect responding. This

could include satiety signals and energy regulation, as well as learning about the conditioned and unconditioned stimuli used.

To assess the role of hippocampal NMDA receptors in hedonic value, a microstructural analysis of licking behaviour is used. In particular, the mean lick cluster size made during consumption has been suggested to provide a measure of palatability (Dwyer, 2012). Learning is also tested in this thesis by assessing flavour preference learning, in which the amount consumed is used as a measure of the preference shown.

## **1.8 Microstructural analysis of licking behaviour**

One way to assess learning about a consummatory conditioned stimulus, such as a flavour cue during flavour preference learning, is to measure the amount consumed. However, intake may be affected by various factors other than what has been learned about it, such as the physical and/or motivational state of the animal. Furthermore, learning may not only alter consumption, but may also change the hedonic value and the way the stimulus is consumed. For example, a taste aversion learned by pairing a taste with nausea-inducing lithium chloride, reduces intake as well as the palatability of the taste (Dwyer, Boakes, & Hayward, 2008). Furthermore, wanting a food reward (incentive motivation) and liking of the same food reward (pleasure, or palatability), have been found to be dissociable at both a neural and psychological level (Berridge, Robinson, & Aldridge, 2009). Pairing a taste solution with an aversive shock for example, does reduce intake but does not alter the palatability of the taste (Pelchat, Grill, Rozin, & Jacobs, 1983). It is therefore important to be able to measure not only the amount consumed, but also any changes in how the stimulus is being perceived in terms of its hedonic value.

Palatability in rodents has typically been measured using taste reactivity analysis, in which orofacial responses are used to determine the hedonic value of a consummatory stimulus. Palatable tastes like sweet sucrose generate appetitive responses, such as rhythmic tongue protrusions. Unpalatable tastes, such as bitter,

instead result in aversive responses including gaping (Grill & Norgren, 1978). That responses are consistent across mammalian species provided further evidence for these orofacial responses to be a valid measure of palatability. They have also been found to be affected by previous experience and learning effects. Pairing a normally liked sweet solution (such as sucrose), with nausea inducing un-palatable lithium, reduces the appetitive responses made during consumption (Breslin, Spector, & Grill, 1992). There are however limitations to using taste reactivity analysis, such as the categorisation of responses into only a small number of categories (often either appetitive or aversive).

A microstructural analysis of licking provides another way to assess palatability in rodents. This analysis is based on the finding that in rodents, licking occurs in rapid runs of licks, made in the form of clusters of licks separated by pauses. Not only does this provide a direct measure of intake, in the form of the total number of licks made during consumption, but the mean lick cluster size made can also be calculated (Davis, 1973; Davis & Smith, 1992). Importantly, these two measures have been found to be dissociable and to represent different aspects of eating behaviour and food reward (Dwyer, 2012). For example, both of these measures have been found to differentially vary as a function of sucrose concentration. Whereas the total number of licks made during consumption shows an inverted-U shaped function with increasing sucrose concentration, the mean lick cluster size instead shows a monotonic increase. This demonstrates that the mean lick cluster size made during consumption can alter independently of intake (Austen, Strickland, & Sanderson, 2016; Dwyer, 2012). Furthermore, as well as lick cluster sizes increasing with sweetness, they also show a corresponding decrease to bitter and unpalatable solutions (Hsiao & Fan, 1993). The mean lick cluster size made during consumption has therefore been suggested to measure the palatability of the solution being consumed (Dwyer, 2012). Using a microstructural analysis of licking allows both the preference for a solution, in terms of intake, as well as the perceived hedonic value of the solution, using mean lick cluster sizes, to be measured.

Using a microstructural analysis of licking in the *Gria1*<sup>-/-</sup> and *Grin1*<sup>ΔDGCA1</sup> mice, allows the effect of altered glutamatergic signalling to be investigated in relation to intake

and potentially changes in hedonic value as well. This is of particular interest due to glutamatergic dysfunction being linked to anhedonia, a negative symptom of schizophrenia (Der-Avakian & Markou, 2012). Furthermore, the genes encoding the GluA1 and the GluN1 subunits have also been linked to schizophrenia in genome wide association studies (Begni et al., 2003; Qin et al., 2005; Ripke et al., 2013). The *Gria1*<sup>-/-</sup> mice have been found to have impaired lick cluster sizes during consumption of palatable solutions. In particular, Austen et al. (2017) showed that although flavour preference learning was intact, mean lick cluster sizes to palatable sucrose solutions were reduced across a range of sucrose concentrations. The *Gria1*<sup>-/-</sup> mice could therefore discriminate between concentrations and prefer the flavour paired with the higher concentration, but the measure of palatability was reduced. It is not yet known however if lick cluster sizes will be altered as a result of deletion of hippocampal NMDA receptors in the *Grin1*<sup>ΔDGCA1</sup> mice.

If mean lick cluster sizes are reduced, this may suggest that hippocampal NMDA receptors in dorsal CA1 and DG could play a role in hedonic value, something not predicted by current theories of hippocampal functioning and appetitive behaviour (Tracy et al., 2001; Jarrard, 1973). Furthermore, the finding may also correspond to the link between dysregulation of NMDA receptors, schizophrenia, and hedonic value, indicating the *Grin1*<sup>ΔDGCA1</sup> mice could provide an animal model of anhedonia. However, the potential for deletion of hippocampal NMDA receptors impairing the expression of mean lick cluster sizes, rather than palatability, would also need to be considered.

## 1.9 Overview of the thesis

In chapter 2, the role of the GluA1 subunit of the AMPA receptor in cue-competition is assessed and the prediction that in *Gria1*<sup>-/-</sup> mice these effects will be intact, or potentially even enhanced compared to wild-type control mice, is tested. This prediction is due to GluA1 deletion being proposed to selectively affect short-term memory decay, but not affecting association formation or retrieval (Sanderson et al., 2009). In particular, the effects of blocking and overshadowing during flavour or taste preference conditioning were tested in experiments 1 – 4. To test the generality of the cue-competition effects seen in these experiments, blocking and overshadowing were then repeated using auditory and visual cues in experiments 5 and 6. Finally, the potential role of within-compound associations during cue-competition are also discussed and tested using a sensory preconditioning procedure in experiments 7 and 8.

Chapter 3 further investigated the role of the GluA1 subunit in learning about recently presented stimuli. *Gria1*<sup>-/-</sup> mice show impaired short-term habituation, something that according to traditional learning theories (Rescorla & Wagner, 1972), should also increase excitatory short-term stimulus processing. This was tested by looking at flavour preference learning based on contrast effects, in which learning the preference depends on having a short-term memory for recently experienced sucrose. An account of the flavour preference result based on increased familiarity is also discussed and ruled out in a follow up experiment.

In chapter 4 the role of hippocampal NMDA receptors in palatability and consummatory behaviour are tested. In experiment 11 the licking behaviours of *Grin1*<sup>ΔDGCA1</sup> mice during consumption of palatable sucrose solutions, of varying concentrations are analysed. Following on from this, the effect of reduced lick cluster sizes on the ability to learn a flavour preference is then tested in experiment 12. In experiment 13, the contribution of flavour-flavour and flavour-nutrient associations during flavour preference learning are also investigated and discussed. Fructose was used to test the role of flavour-flavour associations and maltodextrin to assess flavour-nutrient associations.

Chapter 5 further investigated the role of glutamate in learning and memory by looking at sensitivity to reinforcement rate during an instrumental conditioning procedure. In experiment 14 the matching behaviour of *Gria1*<sup>-/-</sup> mice, using two levers with different reinforcement rates, was tested. Experiment 15 followed a similar design but used the *Grin1*<sup>ΔDGCA1</sup> mice, investigating the role of hippocampal synaptic plasticity in learning about reinforcement rate. In chapter 6, the key findings from the previous four chapters are summarised. The results from the *Gria1*<sup>-/-</sup> mice are firstly discussed together and used to consider the role of the GluA1 subunit in learning and memory. The results from the *Grin1*<sup>ΔDGCA1</sup> mice are then also discussed and used to consider the role of hippocampal NMDA receptors in learning and memory.

## Chapter 2

### The role of the GluA1 subunit in cue-competition

The GluA1 subunit of the AMPA receptor plays an important role in synaptic plasticity as well as in learning and memory processes. For example, long-term potentiation of synapses seems at least partly modulated by the addition of GluA1 containing AMPA receptors into the synapse (Kessels & Malinow, 2009). *Gria1*<sup>-/-</sup> mice also show impaired short-term habituation, but intact or even enhanced associative retrieval. One way in which this impairment in short-term memory has been explained, is through Wagner's SOP model (Wagner, 1981). In particular, GluA1 deletion has been proposed to slow the decay rate of stimulus representations between the primary and secondary active states of memory (Sanderson et al., 2009). This account predicts that associative retrieval should be intact in *Gria1*<sup>-/-</sup> mice, due to only the short-term memory pathway (between the A1 and A2 states) and not the associative retrieval pathway (between the inactive and A2 states), being affected by deletion of the GluA1 subunit. In order to further investigate the role of the GluA1 subunit in cue-competition, *Gria1*<sup>-/-</sup> mice were tested on two different cue-competition procedures, blocking and overshadowing.

During blocking (Kamin, 1969) a cue is associated with an outcome during the first stage of training (CS-US pairings). This cue is then paired in compound with another cue, leading to the same outcome as in the first stage of training. The result of this procedure is that the new cue, added in the second stage, generates minimal levels of conditioned responding when presented during the test stage. The process of pre-training one cue therefore seems to 'block' learning about another cue subsequently paired with it. Traditional learning theories (Rescorla & Wagner, 1972; Wagner, 1981) explain this effect using prediction error mechanisms and competition for associative strength. In the first stage of training, the cue is able to enter into an association with the outcome and acquire substantial amounts of associative strength. If training is complete (the associative strength of the cue is near asymptote) this leads to the

associative strength of the cue nearing, or equalling, the maximum amount that can be supported by the US ( $\lambda$ ). In the second stage of training, the presence of this cue and its high levels of associative strength, mean the outcome is already well predicted. The prediction error generated is therefore minimal, meaning the new cue will be unable to acquire much, if any, associative strength and be able to become associated with the outcome. When it is then presented alone during the test sessions, the low levels of associative strength means it will not predict the outcome well, generating low levels of conditioned responding as a result.

Overshadowing (Mackintosh, 1971, 1976; Pavlov, 1927) occurs when two cues are presented together during training. When one alone is then presented during test sessions, conditioned responding is reduced compared to if the cue were previously trained alone. Similarly to blocking, traditional learning theories explain this effect as a result of competition between the two cues to enter into an association with the US and acquire associative strength. If the saliences of the two cues are equal, then each cue will be able to gain half the amount of the total associative strength available (half  $\lambda$ ).

Both of these cue-competition procedures have been observed in various species and procedures, including the use of flavours and taste stimuli. In one particular series of studies, Dwyer, Haselgrove, & Jones (2011) looked at the effects of blocking and overshadowing of flavour preference learning. During an overshadowing procedure, rats were presented with flavours in a within-subjects design. Compound AB was paired with 8% maltodextrin, flavour C was also paired with 8% maltodextrin, and cue D paired with .1% saccharin. In a two-bottle choice test it was found that rats consumed significantly more of flavour C than B, demonstrating an overshadowing of flavour preference effect. Furthermore, this reduced consumption was not due to enhanced neophobia towards cue B, something that may have occurred as a result of it not previously being presented alone. The blocking procedure they used was similar, with stage 1 training consisting of flavour A paired with 16% maltodextrin and flavour B paired with only 2%. During stage 2 of training, these were each paired in compounds AC and BD with the higher 16% maltodextrin, meaning that learning to flavour C should be blocked compared to the control flavour D. Again, using a two-

bottle testing procedure it was found that rats consumed significantly more of flavour D than flavour C, demonstrating a blocking effect. As well as this finding of blocking of flavour preference using maltodextrin as the reinforcer, it has also been shown in other studies, with rats, when sucrose was used as the reinforcer paired with flavour cues (Balleine, Espinet, & González, 2005; González, Garcia-Burgos, & Hall, 2014). González et al. (2014) for example, used sucrose and kool-aid flavours in a between subjects design. It was found that the blocking group, that had previous training of flavour A being paired with sucrose, showed less of a flavour preference for B, that was presented in compound with A during the second stage of the experiment. This was seen in the form of the blocking group consuming less of flavour B than the control group during the flavour preference test sessions.

These experiments demonstrate that the cue-competition effects of blocking and overshadowing can be seen using flavour preference procedures. The first three experiments in this chapter also used flavour preference procedures, with Kool-Aid flavours and sucrose, to test for blocking and overshadowing in the *Gria1<sup>-/-</sup>* and control wild-type mice. If it is the case that GluA1 deletion selectively impairs short-term memory for recently experienced stimuli, with long-term memory based on associative retrieval intact (Sanderson et al., 2009), then it would be expected that wild-type and *Gria1<sup>-/-</sup>* mice should show blocking and overshadowing. Furthermore, if association formation and associative retrieval are enhanced in the *Gria1<sup>-/-</sup>* mice, as a result of the increased duration of stimulus representations in a primary active state of memory, then cue-competition effects may even be enhanced in the *Gria1<sup>-/-</sup>* mice compared to the wild-type control mice.

## 2.1 Experiment 1

### Blocking of flavour preference learning

In this experiment, blocking of flavour preference learning was tested in *Gria1*<sup>-/-</sup> and control wild-type mice. The proposed role of the GluA1 subunit in slowing the decay rate between the primary and secondary active states of memory (Sanderson et al., 2009), predicts that cue-competition effects should be intact or even enhanced compared to the control mice. Furthermore, the slowed decay rate and resulting increase in the duration of primary state activation, may even enhance stimulus associability and association formation. In this experiment the flavour stimuli used were kool-Aid flavours and sucrose was used as the reinforcer, in a within-subjects design (shown in Table 2.1).

In the first stage of training there were two flavour cues, one paired with the higher 32% sucrose (the CS+) and another paired with the lower concentration of 4% sucrose (the CS-). In the second stage, these two cues were each mixed with a new flavour to form flavour compounds, paired with 32% sucrose. The pre-training of one of these flavours with 32% sucrose should act to block learning about the newly paired flavour cue. This blocking effect was tested by presenting the cues paired in compound, alone, during the test sessions. Any blocking should be seen in the form of a reduced preference to the blocked compared to the control flavour cue. If the role of the GluA1 subunit is selective to short-term memory decay, then this blocking effect should be evident in both *Gria1*<sup>-/-</sup> and control mice, and may even be slightly enhanced in the *Gria1*<sup>-/-</sup> mice. Throughout the experiment three measures were recorded during each testing session. These were: the total number of licks, the mean lick cluster size, and the volume consumed. Both volume and the total numbers of licks provide measures of intake. The total numbers of licks will highly correlate with volume, given that the volume consumed per lick is presumed to be consistent in mice during consumption. Studies investigating flavour preference learning using bottle tests, such as two bottle choice tests, often report only the volume consumed (e.g., González et al., 2014; Dwyer et al., 2011). This is due however to the number

of licks made during consumption being unable to be recorded from these bottles. In the experiments in this thesis, the lickometer attached to the sipper tube means that the numbers of licks can be accurately recorded and time-binned, providing a way to investigate the microstructural analysis of licking behaviour during consumption in the mice. Therefore, although volume provides one way to look at volume and will be reported throughout the thesis, the total number of licks provides a way to assess licking behaviour during consumption across time within a session and will therefore also be reported. It is expected however that these measures will show similar patterns across experiments. In the *Gria1*<sup>-/-</sup> mice, the mean lick cluster sizes made during consumption of sucrose solutions have been previously found to be impaired, although the amount consumed was normal, compared to control mice (Austen et al., 2017).

### **2.1.1 Methods**

#### **Subjects**

25 *Gria1*<sup>-/-</sup> (11 females, 14 males) and 24 wild-type mice (13 females, 11 males) bred in the life sciences support unit at Durham university were used. Mice were bred from heterozygous pairs, resulting in the offspring being either *Gria1*<sup>-/-</sup> knockout mice (approximately 25%), wild-type littermate control mice (approximately 25%), or heterozygous mice (approximately 50%). See Zamanillo et al. (1999) for full details regarding breeding, genetic construction and genotyping, including the PCR methods used that were the same as for the mice in these experiments. The mice were approximately 9-11 months old at the start of testing and caged in groups of 1-5 in a temperature-controlled housing room, with a 12hr light dark cycle (8am-8pm). During testing they were maintained at 85% of their free feeding body weights (85% weights: 17.2g – 28.6g) with ad libitum access to water in their home cages. The mice had also been used in previous appetitive magazine conditioning experiments in similar operant chambers and had previous experience consuming sucrose solutions, but no previous experience with flavour cues, or in the same apparatus as in this experiment.

## **Apparatus**

Eight identical operant chambers (interior dimensions: 21.6 x 17.8 x 12.7 cm; ENV-307W, Med Associates, Inc., Fairfax, VT, USA), enclosed in sound-attenuating cubicles (ENV-022V, Med Associates) were used. The chambers were controlled by Med-PC IV software (Med Associates). The side walls were made from aluminium, and the front and back walls and the ceiling were made from clear Perspex. The chamber floors each comprised a grid of 24 stainless steel rods (0.32 cm diameter), spaced 0.79 cm apart and running perpendicular to the front of the chamber (ENV-307W-GFW, Med Associates). A fan (ENV-025F, Med Associates) was located within each of the cubicles and was turned on during sessions. Retractable sippers (ENV-352AW, Med Associates) and a small hole in one wall of each chamber allowed sipper tubes to be extended into, and retracted from, the chambers. The graduated sipper tubes (10:0.1 ml) allowed measurement of consumption by comparing the volumes before and after testing. Contact lickometer controllers (ENV-250, Med Associates) allowed contacts between the mice and the sipper tubes to be recorded at a resolution of 0.01 s. Sucrose solutions were made weight/volume with commercially available sucrose in distilled water.

## **Procedure**

The design of the experiment is shown in Table 2.1. Each daily testing session lasted for 15-minutes with the sipper tube inserted into the chamber after 5-minutes, giving 10-minutes of access to the sucrose solution. In the first stage of training, mice were given two flavoured sucrose solutions; the CS+ (flavour X) was paired with 32% sucrose and the CS- (flavour Y) with 4% sucrose. These were presented in double alternation for 4 sessions of each cue (resulting in 8 sessions in total during stage 1). For approximately half of the mice, flavours X and Y were cherry and grape Kool Aid (0.05% wt/vol, Kraft Foods., Rye Brook, NY) counterbalanced across mice, with respect to genotype and sex as far as possible given the numbers. These were further counterbalanced so that as far as possible given the numbers, half had flavour X as cherry and Y as grape and vice versa for the remaining mice. For the other half of the mice, flavours X and Y were apple and orange, again counterbalanced so that for half of the mice X was apple and Y orange and vice versa for the remaining mice. The

flavour cues were presented so that for the mice in which X and Y were cherry and grape, the cherry flavour was presented first. For those in which X and Y were apple and orange, the apple flavour was presented first. This meant that approximately half the mice received the CS- (flavour X) first and the other half the CS+ (flavour Y) first.

In the second stage of training the previously trained flavours were presented in compound with a second flavour (XA and YB), both with 32% sucrose. For those mice in which the CS+ (X) and the CS- (Y) were cherry and grape, the new flavours A and B were apple and orange. For half of these, A was apple and for the other half A was orange. For those mice in which X and Y were apple and orange, A and B were cherry and grape. For half of these, A was cherry and the other half A was grape. These compounds were each presented for 4 sessions, in double alternation, with either the cherry compound first (if X and Y were cherry and grape) or the apple compound first (if X and Y were apple and orange). This meant that approximately half the mice received the blocking compound (XA) first and the other half the control compound first (YB).

During the first stage of test, the blocked (A) and control (B) flavours were presented with 4% sucrose for 4 daily sessions, again in a double alternating order. The order of presentations of the control and blocking flavour cues were counterbalanced across subjects as far as possible given the numbers, with respect to flavour allocation in the previous two stages. In the final test stage, the pre-trained flavours, the CS+ (X) and CS- (Y), were presented with 4% sucrose for a further 4 daily sessions. This was counterbalanced with respect to genotype and previous flavour allocation as far as possible, with approximately half the mice given access to the CS- control solution first in the double alternating sequence, the other half the experimental CS+ solution.

Table 2.1

*Design of experiment 1*

Stage 1	Stage 2	Test 1	Test 2
X 32%	XA 32%	A 4%	X 4%
Y 4%	YB 32%	B 4%	Y 4%

**Microstructural analysis of licking behaviour**

For all the experiments in this thesis using a microstructural analysis of licking, the same three measures were taken. The total number of licks, the mean lick cluster size, and the volume consumed. The criteria used to define a completed cluster were the same as those used in previous studies (Austen et al., 2016; Davis & Smith, 1992). Licks that were separated by an interval of less than 500ms were classed as being part of the same cluster, with pauses greater than 500ms defining a separate bout of licking. This time period is used due to the inter-lick interval in rodents being short, around 150-250ms (i.e. the time taken to make a lick itself), whereas bouts are usually separated by intervals ranging from 500ms to many seconds (Davis & Smith, 1992). The 500ms interval, timed from the end of one lick to the start of the next lick, should therefore include all licks within a bout, while preventing another separate cluster being included in the previous. The mean lick cluster size for any given session, was calculated by dividing the total number of licks made by the number of completed lick bouts. In each session, the sipper tube was retracted from the chamber as soon as the specified session duration was reached. This meant that it was possible for the mouse to be licking, within a bout of licks, when the tube was retracted. In this case, the licks were counted and added to the total number, but no completed cluster was counted and added to the total number of bouts. The mean lick cluster size here may therefore differ slightly from if the number of clusters started, rather than completed, had been used to calculate it.

## Statistical analysis

For all stages data were analysed using mixed model ANOVA, with genotype, cue, and session as factors. For the first session of the first test stage, additional mixed model ANOVA were carried out, with genotype and cue as factors. This was due to testing occurring under extinction, as both flavour cues were presented with the same lower sucrose concentration. Any preference effects are therefore likely to diminish over testing sessions, meaning it is possible that effects may only be seen early in testing. Where appropriate, interactions were analysed with simple main effects analysis using the pooled error term from the original error term. Where sphericity of within-subjects variables could not be assumed, a Greenhouse-Geisser correction was applied to produce more conservative p-values. One mouse was removed from the analysis due to a failure to respond throughout the experiment (a male *Gria1*<sup>-/-</sup> mouse), meaning the analysis includes the data from a total of 24 *Gria1*<sup>-/-</sup> mice and 24 wild-type mice.

### 2.1.2 Results

#### Stage 1

##### Total Licks:

The mean total numbers of licks made during consumption of the CS- and CS+ flavour cues, by wild-type and *Gria1*<sup>-/-</sup> mice during training, are shown in Figure 2.1 (upper panel). Both the wild-type and *Gria1*<sup>-/-</sup> mice made greater numbers of licks during consumption of the CS+ than the CS-. Across sessions the number of licks made by the wild-type mice remained relatively stable, whereas the *Gria1*<sup>-/-</sup> mice showed a slight reduction in intake over training. The *Gria1*<sup>-/-</sup> mice also made slightly smaller numbers of licks than the wild-type mice. The ANOVA showed that there was a significant effect of flavour cue,  $F(1,46) = 41.1$ ,  $p < .001$ , and genotype,  $F(1,46) = 5.5$ ,  $p = .024$ , on the mean total number of licks made during consumption. There was no significant interaction between flavour cue and genotype,  $F(1,46) = .24$ ,  $p = .63$ . The effect of session was significant,  $F(3,138) = 29.7$ ,  $p < .001$ , and there was a significant interaction between session and genotype,  $F(3,138) = 23.1$ ,  $p < .001$ , as well as

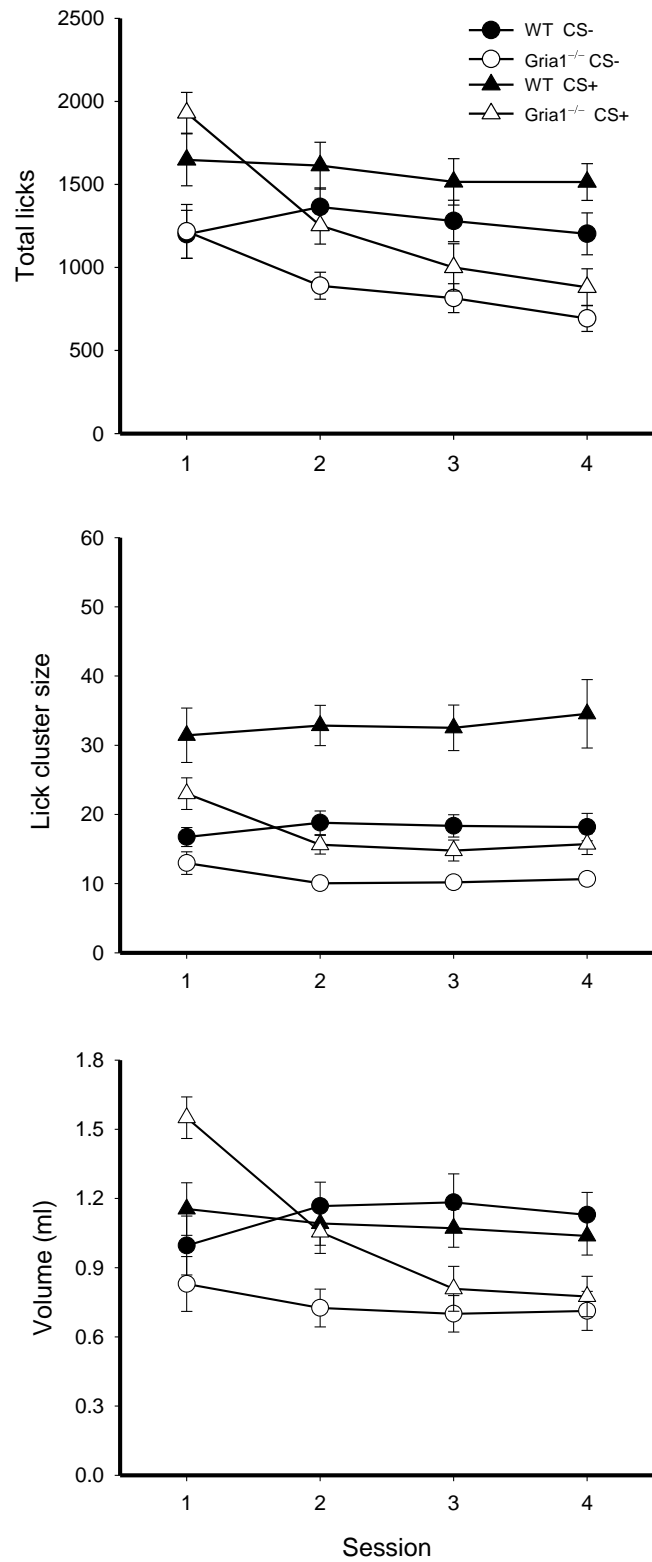
between flavour cue and session,  $F(3,138) = 4.6$ ,  $p = .004$ . There was no interaction between the three factors of flavour cue, session, and genotype,  $F(3,138) = 1.3$ ,  $p = .29$ .

#### Lick Cluster Size:

The mean lick cluster sizes made during consumption of the CS- and CS+ flavour cues, by the wild-type and *Gria1*<sup>-/-</sup> mice during training, are shown in Figure 2.1 (middle panel). Both the wild-type and *Gria1*<sup>-/-</sup> mice made greater lick cluster sizes during consumption of the CS+ compared to the CS-. The *Gria1*<sup>-/-</sup> mice also made smaller lick cluster sizes than the wild-type mice, particularly during consumption of the CS+. The ANOVA showed that there was a significant effect of cue  $F(1,46) = 52.1$ ,  $p < .001$ , and a significant effect of genotype  $F(1,46) = 21.8$ ,  $p < .001$ . There was a significant interaction between flavour cue and genotype,  $F(1,46) = 9.4$ ,  $p = .044$ , and no significant effect of session,  $F(3,138) = 1.78$ ,  $p = .17$ . The interaction between session and genotype was significant,  $F(3,138) = 6.3$ ,  $p = .003$ . The interaction between flavour cue and session was not significant,  $F(3,138) = 1.18$ ,  $p = .32$ , and there was no significant interaction between the three factors of cue, session, and genotype,  $F(3,138) = 1.4$ ,  $p = .24$ .

#### Volume:

The mean volumes consumed of the CS- and CS+ flavour cues, by wild-type and *Gria1*<sup>-/-</sup> mice during training, are shown in Figure 2.1 (lower panel). Consumption of the two flavour cues by the wild-type mice remained stable across sessions. The *Gria1*<sup>-/-</sup> mice generally consumed less of both cues than the wild-type mice, although consumption of the CS+ was higher than the wild-type mice in the first session. The ANOVA showed that there was a significant effect of cue,  $F(1,46) = 13.3$ ,  $p = .001$ , no significant effect of genotype  $F(1,46) = 3.4$ ,  $p = .073$ , and a significant interaction between flavour cue and genotype,  $F(1,46) = 19.8$ ,  $p < .001$ . The effect of session was significant,  $F(3,138) = 10.9$ ,  $p < .001$ , as was the interaction between session and genotype,  $F(3,138) = 14.3$ ,  $p < .001$ , and also between flavour cue and session,  $F(3,138) = 14.0$ ,  $p < .001$ . The interaction between the three factors of cue, session, and genotype was not significant,  $F(3,138) = 2.7$ ,  $p = .063$ .



*Figure 2.1.* Experiment 1, training stage 1. The mean numbers of total licks (upper), lick cluster sizes (middle), and volumes consumed (lower) of the CS- and CS+ flavour cues, by wild-type (WT) and *Gria1*<sup>-/-</sup> mice. Error bars show ± SEM.

## Stage 2

### Total Licks:

The mean numbers of total licks made during consumption of the control and blocking compound flavour cues, by the wild-type and *Gria1*<sup>-/-</sup> mice during stage 2 of training, are shown in Figure 2.2 (upper panel). Both the wild-type and *Gria1*<sup>-/-</sup> mice made similar numbers of licks to the two compound flavour cues, although the *Gria1*<sup>-/-</sup> mice made fewer licks than the wild-type mice. The ANOVA showed that the effect of cue was not significant,  $F(1,46) = .007$ ,  $p = .93$ , but there was a significant effect of genotype  $F(1,46) = 13.2$ ,  $p = .001$ , with no significant interaction between these two factors,  $F(1,46) = .46$ ,  $p = .50$ . There was a significant effect of session,  $F(3,138) = 9.7$ ,  $p < .001$ , which did not interact with genotype,  $F(3,138) = .06$ ,  $p = .96$ . The interaction between flavour cue and session was also not significant,  $F(3,138) = .34$ ,  $p = .71$ , and there was no significant interaction between the three factors of cue, session, and genotype,  $F(3,138) = .18$ ,  $p = .91$ .

### Lick Cluster Size:

The mean lick cluster sizes made during consumption of the two compound flavour cues during stage 2 of training, by the wild-type and *Gria1*<sup>-/-</sup> mice, are shown in Figure 2.2 (middle panel). The *Gria1*<sup>-/-</sup> mice also made smaller cluster sizes than the wild-type mice. The ANOVA showed that there was no significant effect of cue (either control or blocking),  $F(1,46) = .33$ ,  $p = .57$ , but that the effect of genotype was significant,  $F(1,46) = 31.4$ ,  $p < .001$ , and there was no significant interaction between flavour cue and genotype,  $F(1,46) = .31$ ,  $p = .58$ . There was also no significant effect of session,  $F(3,138) = .17$ ,  $p = .89$ , or interaction between session and genotype,  $F(3,138) = .29$ ,  $p = .80$ . The interaction between flavour cue and session was not significant,  $F(3,138) = .82$ ,  $p = .49$ , and there was also no significant interaction between the three factors of cue, session, and genotype,  $F(3,138) = .47$ ,  $p = .70$ .

## Volume:

The mean volumes consumed of the compound flavour cues during stage 2 of training, by the wild-type and *Gria1*<sup>-/-</sup> mice, are shown in Figure 2.2 (lower panel). Consumption remained stable over sessions, although the *Gria1*<sup>-/-</sup> mice consumed less of both cues than the wild-type mice. The ANOVA showed that there was no significant effect of cue,  $F(1,46) = .07$ ,  $p = .79$ , but that there was a significant effect of genotype,  $F(1,46) = 13.1$ ,  $p = .001$ , with no significant interaction between these two factors,  $F(1,46) = .14$ ,  $p = .71$ . The effect of session was significant,  $F(3,138) = 6.5$ ,  $p = .001$ , with no significant interactions between session and genotype,  $F(3,138) = .25$ ,  $p = .86$ , flavour cue and session,  $F(3,138) = .45$ ,  $p = .68$ , or between the three factors of cue, session, and genotype,  $F(3,138) = .52$ ,  $p = .64$ .

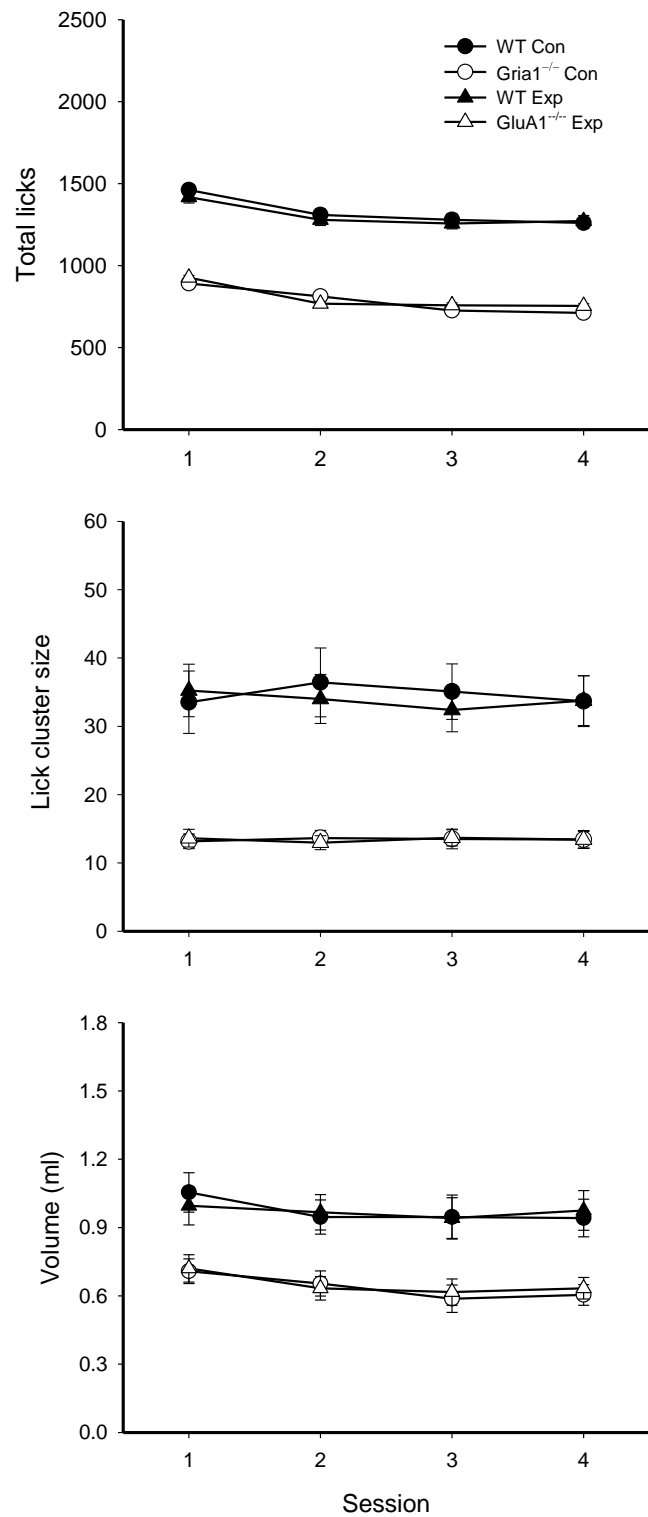


Figure 2.2. Experiment 1, training stage 2. The mean total numbers of licks (upper), lick cluster sizes (middle), and volumes consumed (lower), of the control and experimental compound flavour cues, by the wild-type and *Gria1*<sup>-/-</sup> mice. Error bars show ± SEM.

## Test stage 1

### Total Licks:

The mean numbers of total licks made during consumption of the control and blocked cues, by the wild-type and *Gria1*<sup>-/-</sup> mice, in the first and second test sessions, are shown in Figure 2.3 (upper panel). The wild-type mice made a slightly lower number of licks during consumption of the blocked than the control flavour, whereas the *Gria1*<sup>-/-</sup> mice made similar numbers of licks to both cues. The *Gria1*<sup>-/-</sup> mice also made fewer licks than the wild-type mice. The ANOVA showed that there was no significant effect of cue,  $F(1,46) = .29$ ,  $p = .59$ , but there was a significant effect of genotype,  $F(1,46) = 5.02$ ,  $p = .03$ . There was however a significant effect of test session,  $F(1,46) = 48.1$ ,  $p < .001$ , with a greater numbers of licks made in the second test session, an effect that did not show a significant interaction with genotype,  $F(1,46) = 1.7$ ,  $p = .19$ . There was also no significant interaction between flavour cue and session,  $F(1,46) = 1.2$ ,  $p = .29$ , or between the three factors of flavour cue, session, and genotype,  $F(1,46) = 2.7$ ,  $p = .11$ . As there was a significant effect of test session, and testing occurred under extinction, the results from the first session were analysed using a mixed model ANOVA, with genotype and cue as factors.

The ANOVA on the first test session alone further showed that there was no significant effect of flavour cue,  $F(1,46) = .903$ ,  $p = .35$ , but that the effect of genotype was significant,  $F(1,46) = 4.7$ ,  $p = .035$ . There was also a significant interaction between flavour cue and genotype,  $F(1,46) = 4.2$ ,  $p = .047$ . Simple main effects analysis of this interaction showed that whereas the wild-type mice made a significantly smaller number of licks during consumption of the blocked than control flavour,  $F(1,46) = 4.5$ ,  $p = .04$ , demonstrating blocking, this was not seen in the *Gria1*<sup>-/-</sup> mice  $F(1,46) = .60$ ,  $p = .44$ . Also, during consumption of the control flavour cue, the wild-type mice made a significantly greater number of licks than the *Gria1*<sup>-/-</sup> mice  $F(1,46) = 7.7$ ,  $p = .008$ , but there was no such difference in consumption of the blocked flavour cue,  $F(1,46) = 1.3$ ,  $p = .27$ .

#### Lick Cluster Size:

The mean lick cluster sizes made during consumption of the control and blocked flavour cues in both test sessions, by wild-type and *Gria1*<sup>-/-</sup> mice, are shown in Figure 2.3 (middle panel). Lick cluster sizes increased in the second test session compared to the first and were also reduced in the *Gria1*<sup>-/-</sup> compare to the wild-type mice. The mean lick cluster sizes were however similar during consumption of the two cues (blocked and control) in both test sessions. The ANOVA showed that there was no significant effect of flavour cue,  $F(1,46) = .44$ ,  $p = .51$ , and a significant effect of genotype,  $F(1,46) = 14.4$ ,  $p < .001$ , with no significant interaction between these two factors,  $F(1,46) = 1.3$ ,  $p = .26$ . There was a significant effect of test session,  $F(1,46) = 49.3$ ,  $p < .001$ , with no significant interaction between test session and genotype,  $F(1,46) = 3.2$ ,  $p = .081$ , or between flavour cue and test session,  $F(1,46) = .21$ ,  $p = .65$ . There was also no significant interaction between the three factors of flavour cue, test session, and genotype,  $F(1,46) = .38$ ,  $p = .54$ .

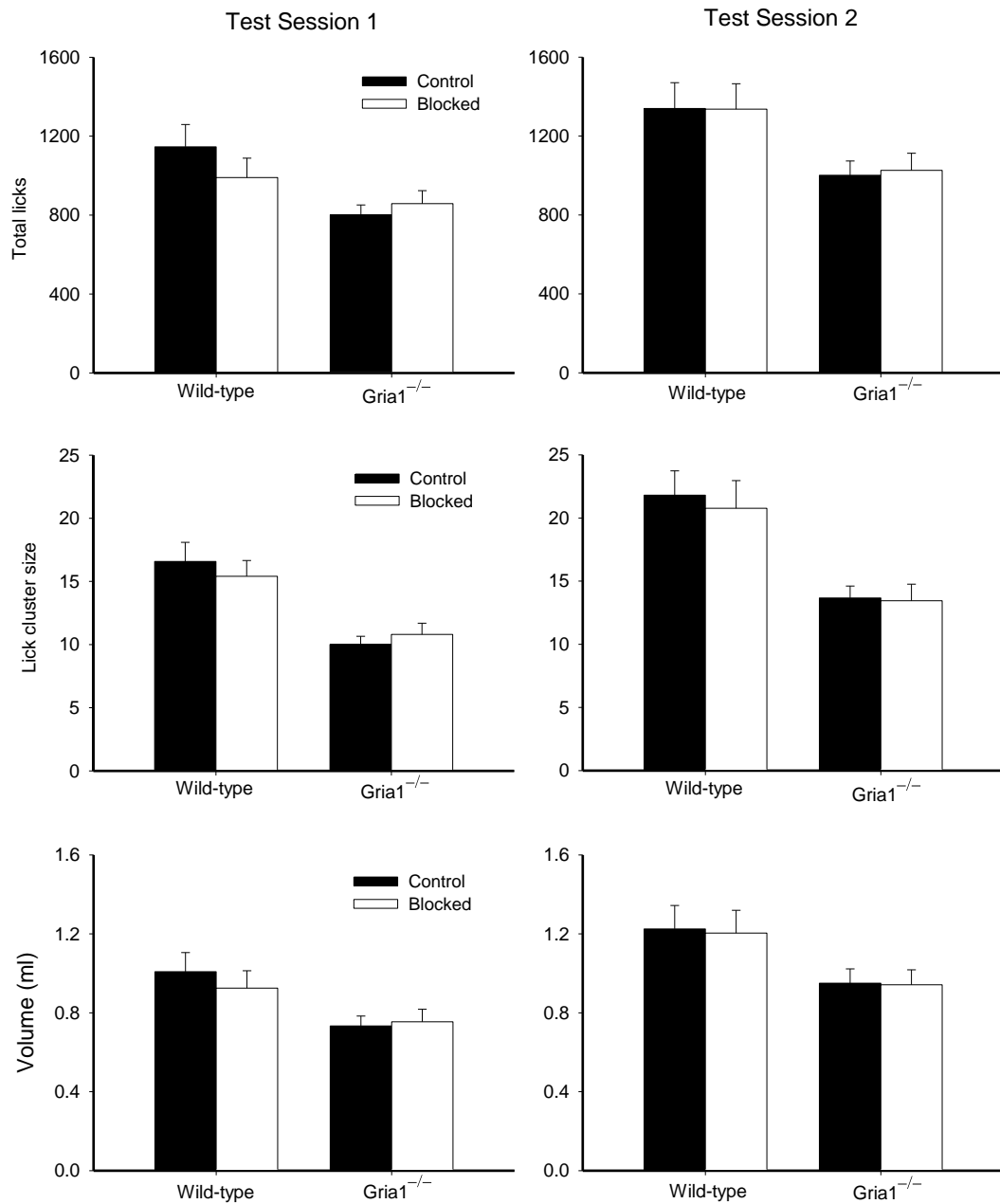
The ANOVA on the first test session alone further showed that there was no significant effect of flavour cue,  $F(1,46) = .082$ ,  $p = .78$ , but that the effect of genotype was significant,  $F(1,46) = 14.9$ ,  $p < .001$ , with no significant interaction between these two factors,  $F(1,46) = 2.2$ ,  $p = .15$ .

#### Volume:

The mean volumes consumed of the control and blocked flavour cues, by wild-type and *Gria1*<sup>-/-</sup> mice, during the two test sessions, are shown in Figure 2.3 (lower panel). The *Gria1*<sup>-/-</sup> mice consumed less than the wild-type mice and also showed little difference in the volume consumed of the blocked and control flavour cues. The wild-type mice however consumed slightly less of the blocked flavour compared to the control mice in the first testing session. The ANOVA showed that there was no significant effect of cue,  $F(1,46) = .57$ ,  $p = .45$ , but that there was a significant effect of genotype,  $F(1,46) = 4.5$ ,  $p = .039$ , and no significant interaction between these two factors,  $F(1,46) = .92$ ,  $p = .34$ . There was a significant effect of test session,  $F(1,46) = 66.4$ ,  $p < .001$ , with no significant interactions between session and genotype,  $F(1,46)$

= .69,  $p = .41$ , cue and session  $F(1,46) = .19$ ,  $p = .66$ , or between the three factors of cue, session, and genotype,  $F(1,46) = 1.5$ ,  $p = .24$ .

The ANOVA on the first test session alone further showed that there was no significant effect of flavour cue within this test session,  $F(1,46) = .58$ ,  $p = .45$ , but there was a significant effect of genotype,  $F(1,46) = 4.9$ ,  $p = .032$ , and no significant interaction between these two factors,  $F(1,46) = 1.6$ ,  $p = .21$ .



*Figure 2.3.* Experiment 1, test stage 1. The mean total numbers of licks (upper), lick cluster sizes (middle), and volumes consumed (lower), of the control and blocked flavour cues, by the wild-type and *Gria1*<sup>-/-</sup> mice in the first (left) and second (right) test sessions. Error bars show ± SEM.

## Test stage 2

### Total Licks:

The mean total numbers of licks made during consumption of the CS- and CS+ (control and blocking flavour cues) by the wild-type and *Gria1*<sup>-/-</sup> mice, in both test sessions, are shown in Figure 2.4 (upper panel). In the first test session, the *Gria1*<sup>-/-</sup> mice showed a slightly greater number of licks during consumption of the blocking than control flavour cue, with the wild-type mice making a similar number of licks during consumption of the two cues. During the second session however the total numbers of licks were similar across the two cues, for both genotypes. The ANOVA showed that there was a significant effect of cue,  $F(1,46) = 10.0$ ,  $p = .003$ , and no significant effect of genotype,  $F(1,46) = 1.4$ ,  $p = .25$ , but there was a significant interaction between these two factors,  $F(1,46) = 4.9$ ,  $p = .031$ . The effect of session was not significant,  $F(1,46) = .62$ ,  $p = .44$ , and there was no significant interaction between session and genotype,  $F(1,46) = 1.1$ ,  $p = .31$ . The interaction between flavour cue and session was significant,  $F(1,46) = 12.6$ ,  $p = .001$ , and there was no significant interaction between the three factors of cue, session, and genotype,  $F(1,46) = 2.8$ ,  $p = .101$ .

Simple main effects analysis of the interaction between flavour cue and genotype, further showed that the *Gria1*<sup>-/-</sup> mice made a significantly smaller number of licks during consumption of the control than blocking flavour cue,  $F(1,46) = 14.5$ ,  $p < .001$ , with no significant difference between the flavour cues in the wild-type mice,  $F(1,46) = .44$ ,  $p = .51$ . For both the control and blocking flavour cues, there were also no significant differences in the numbers of licks between the wild-type and *Gria1*<sup>-/-</sup> mice (control flavour:  $F(1,46) = 2.5$ ,  $p = .12$ ; blocking flavour,  $F(1,46) = .52$ ,  $p = .47$ ).

The same analysis for the interaction between cue and session, showed that in the first test session mice made a significantly smaller number of licks during consumption of the control than blocking flavour cue,  $F(1,46) = 21.8$ ,  $p < .001$ , but there was no significant difference between the flavour cues in the second test session,  $F(1,46) = .002$ ,  $p = .96$ . They also showed that consumption of the control cue was significantly less in the first than in the second test session,  $F(1,46) = 10.5$ ,  $p$

= .002, but there was no significant difference between the sessions for the amount consumed of the blocking cue,  $F(1,46) = 2.4$ ,  $p = .13$ .

#### Lick Cluster Size:

The mean lick cluster sizes made during consumption of the control and blocking flavour cues, in both test sessions, by the wild-type and *Gria1*<sup>-/-</sup> mice, are shown in Figure 2.4 (middle panel). The wild-type mice showed a slightly greater mean lick cluster size during consumption of the control than the blocking cue, in both test sessions. Mean lick cluster sizes were reduced in the *Gria1*<sup>-/-</sup> mice compared to the wild-type control mice and also showed a smaller difference between the cues, in both test sessions. The ANOVA showed that there was no significant effect of cue,  $F(1,46) = 1.5$ ,  $p = .23$ , a significant effect of genotype,  $F(1,46) = 6.2$ ,  $p = .017$ , and a significant interaction between these two factors,  $F(1,46) = 6.2$ ,  $p = .016$ . There was also a significant effect of test session,  $F(1,46) = 20.5$ ,  $p < .001$ , and no significant interaction between session and genotype,  $F(1,46) = .92$ ,  $p = .34$ , or between flavour cue and session,  $F(1,46) = .66$ ,  $p = .42$ . There was also no significant interaction between the three factors of flavour cue, test session, and genotype,  $F(1,46) = .02$ ,  $p = .88$ .

Simple main effects analysis of the interaction between flavour cue and genotype, further showed that the wild-type mice made greater lick cluster sizes during consumption of the control than blocking flavour  $F(1,46) = 6.9$ ,  $p = .012$ , but the *Gria1*<sup>-/-</sup> mice did not show a significant difference between the flavour cues,  $F(1,46) = .82$ ,  $p = .37$ . Furthermore, during consumption of the control cue, the *Gria1*<sup>-/-</sup> mice made significantly smaller lick cluster sizes than the wild-type mice,  $F(1,46) = 7.9$ ,  $p = .007$ , but there was no significant difference in mean lick cluster sizes during consumption of the blocking flavour,  $F(1,46) = 3.7$ ,  $p = .062$ .

## Volume:

The mean volumes consumed of the control and blocking flavour cues, during the two test sessions by the wild-type and *Gria1*<sup>-/-</sup> mice, are shown in Figure 2.4 (lower panel). Both the wild-type and *Gria1*<sup>-/-</sup> mice consumed slightly more of the blocking flavour cue than the control, with the levels of consumption similar across the two test sessions. The ANOVA showed that there was a significant effect of cue,  $F(1,46) = 9.3$ ,  $p = .004$ , with no significant effect of genotype,  $F(1,46) = 1.2$ ,  $p = .27$ , as well as no significant interaction between these two factors,  $F(1,46) = 1.1$ ,  $p = .303$ . The effect of session was also not significant,  $F(1,46) = .21$ ,  $p = .65$ , and there was no significant interaction between session and genotype,  $F(1,46) = .27$ ,  $p = .65$ , or between flavour cue and session,  $F(1,46) = 1.3$ ,  $p = .26$ . The interaction between the three factors of flavour cue, test session, and genotype was also not significant,  $F(1,46) = 1.5$ ,  $p = .22$ .

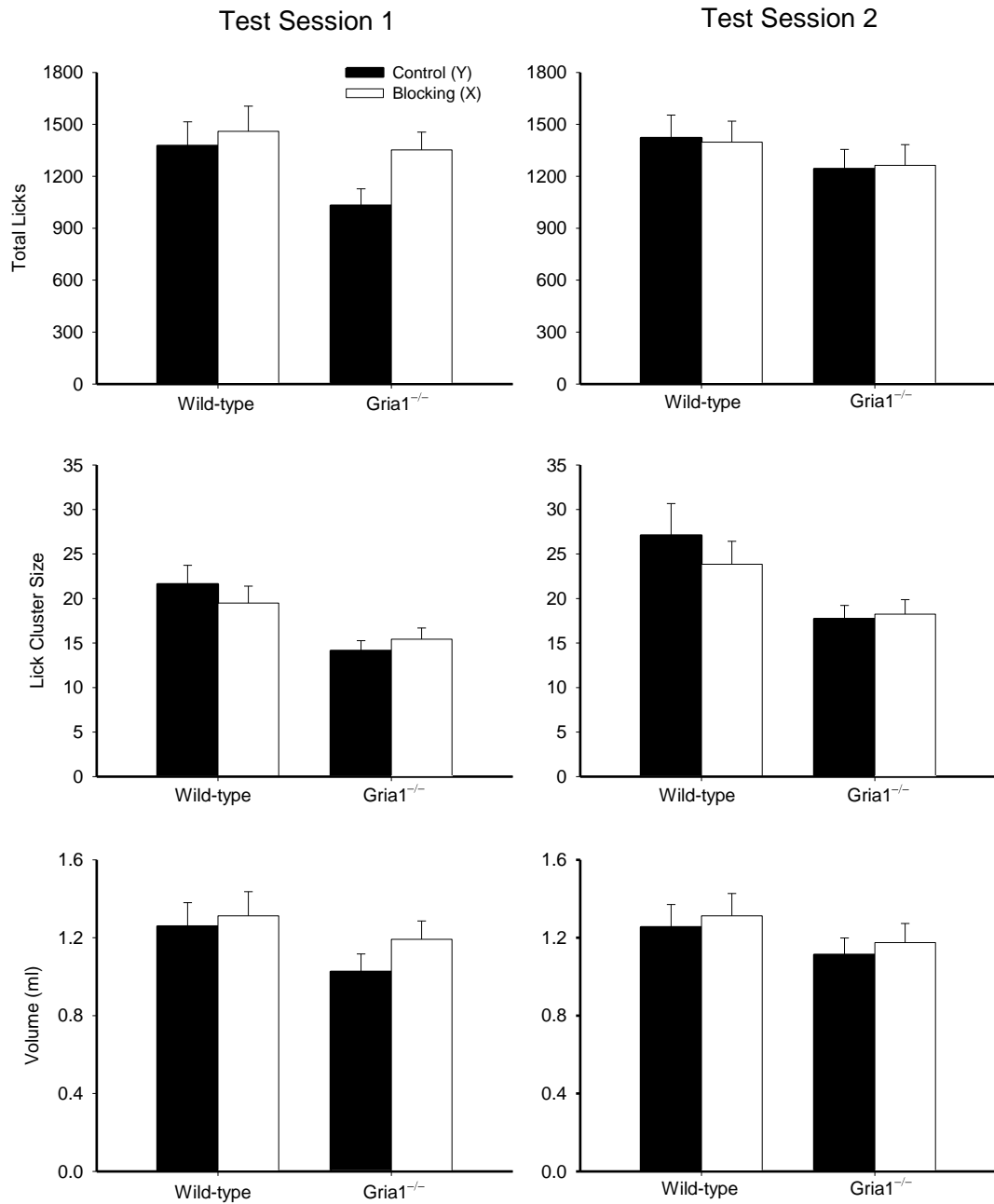


Figure 2.4. Experiment 1, test stage 2. The mean total numbers of total licks (upper), lick cluster sizes (middle) and volumes consumed (lower), of the control and blocking flavour cues, by the wild-type and *Gria1*<sup>-/-</sup> mice in the first (left) and second (right) test sessions. Error bars show  $\pm$  SEM.

### 2.1.3 Discussion

The results showed that although the wild-type mice demonstrated a small blocking effect, this was not seen in the *Gria1*<sup>-/-</sup> mice, despite the prediction that it would be intact, or even enhanced. This was seen in the results of the first test stage, in which the wild-type mice made a smaller number of total licks to the blocked than the control cue. This effect was transient, being evident only in the first test session and not in the second test session. This is likely however to be due to testing occurring under extinction of the flavour preference, as both flavours were paired with the lower sucrose concentration during the test sessions. In terms of the lick cluster sizes, neither genotype showed a blocking effect in the form of reduced mean lick cluster sizes during consumption of the CS- compared to the CS+ flavour. The blocking effect was also not significantly evident in the volumes consumed. This could however be due to the total number of licks perhaps being a more sensitive measure than the volume consumed, with the volume only being measured to .1 of a ml, corresponding to approximately 100 licks. As the differences between flavour cues were generally only of a magnitude of around 100-200 licks, then the volume consumed may have lacked sensitivity in this experiment. In contrast to this blocking effect in the wild-type mice, the *Gria1*<sup>-/-</sup> mice did not demonstrate a reduction in intake of the blocked cue compared to the control. The *Gria1*<sup>-/-</sup> mice therefore seem to fail to show blocking of flavour preference learning, despite the expectation this would be intact or perhaps enhanced.

Furthermore, the second test stage showed that the *Gria1*<sup>-/-</sup> mice did show a flavour preference for the blocking cue, in terms of the total number of licks and the volume consumed. Therefore, despite learning a flavour preference in the first stage of training, this subsequently failed to block learning of the second flavour paired in compound with the first in the *Gria1*<sup>-/-</sup> mice. The results also showed that the *Gria1*<sup>-/-</sup> mice generally consumed less than the wild-type mice. However, this did not seem to affect learning of a flavour preference in the first stage of training, as they still showed flavour preference in the second test stage. This means that blocking of flavour preference should still have been able to occur, to some extent, in the second training stage. Although it is possible that the lower levels of consumption may have

reduced the ability to see any differences between the two cues, in the *Gria1*<sup>-/-</sup> mice, during the first test stage.

Although the wild-type mice showed a blocking effect, the flavour preference effect in the second test stage was far smaller in the wild-type compared to the *Gria1*<sup>-/-</sup> mice. However, seeing as learning of the flavour preference during the first stage is required for blocking to occur, this lack of effect may have been due to testing being carried out in extinction. The blocking effect in the wild-type mice was also transient, only being apparent only in the first test session. Furthermore, the lack of blocking in the *Gria1*<sup>-/-</sup> mice seemed to result from making fewer licks to the control cue, rather than any significant difference in consumption of the blocked flavour cue (as demonstrated by the simple-main effects analysis of the interaction between flavour cue and genotype in this test stage). The lack of blocking effect in the *Gria1*<sup>-/-</sup> mice in this study should therefore be taken with some caution. To further test the role of GluA1 in blocking of flavour preference learning, this experiment was replicated using a slightly different procedure.

## **2.2 Experiment 2**

### **Blocking of flavour preference (repeat)**

The previous experiment demonstrated that contrary to expectation, blocking of flavour preference was impaired in the *Gria1*<sup>-/-</sup> mice compared to the wild-type control mice. However, the blocking effect in the previous experiment was transient, seen in the first but not the second testing session. It was also only evident in differences in consumption of the control cue, rather than differences in consumption of the blocked flavour cue. In order to further assess the role of GluA1 deletion in blocking, the procedure was repeated.

In unpublished observations I have seen that providing mice with two sessions a day, one with each of the two sucrose solutions (e.g. the CS- and CS+), results in a greater flavour preference effect than only having a single presentation per daily session.

Although the precise reason for this is not clear, it may be that when running these procedures in mice the use of two presentations per daily session aids discrimination between the two flavour cues. The blocking procedure in the previous experiment (experiment 2.1) was therefore altered so that in each daily session mice were presented with two periods of access, one with each of the two flavour cues. During each day and throughout the experiment, mice therefore experienced both the flavour cues relevant for the particular stage of the experiment. The rest of the procedure was the same as in the previous blocking experiment (2.1), with kool-Aid flavoured sucrose solutions used. The previous experiment demonstrated that the blocking effect was transient and limited to the first testing session. The analysis was therefore limited to only one test session, corresponding to the analysis carried out on the first test session of the previous blocking of flavour preference experiment.

### **2.2.1 Methods**

#### **Subjects**

27 *Gria1*<sup>-/-</sup> mice (12 females, 15 males) and 40 wild-type (21 female, 19 male) mice, bred and housed in the same way as experiment 2.1, were used. The mice were aged between 11 – 35 weeks at the start of testing and caged in groups of 1-7, with 85% body weights ranging between 15.6g – 31.8g. Approximately half of the mice had previously been used in appetitive magazine conditioning in similar operant chambers, but had no previous experience of experiments involving licking analysis. The remaining mice were naïve.

#### **Apparatus**

The apparatus used was the same as in experiment 2.1. The amount consumed was measured by weighing the sipper tubes before and after each session of access (rather than comparing the gradations before and after each session as in the previous experiment).

#### **Procedure**

The design and procedure was similar to the previous blocking of flavour preference experiment (experiment 1, see Table 2.1), with the alteration of two 15-minute

sessions per daily testing session. Within each 15-minute session mice were placed into the operant chamber, with the sipper tubes available after a period of 5-minutes. Between the two 15-minute sessions, mice were returned to their home cages for a period of approximately 10-minutes, in which time the sipper tubes were weighed and changed to contain the other sucrose solution. The mice were then returned to the chambers for the second session, in which the other flavoured sucrose solution was presented.

### **Statistical analysis**

Statistical analysis were carried out in a similar manner to the previous experiment. Mixed model ANOVA, with genotype, cue and session as factors were carried out on the data from the training stage. For the test stages, genotype, cue, and test order were the factors. When given sessions in quick succession, such as in this experiment with the two flavour cues given one after the other in the same session, I have observed that mice nearly always consume less of the second solution than the first. During the analysis of the test sessions, test order, a factor that was counterbalanced across mice, was added as a factor. This was done in order to account for the variance resulting from the differences in consumption across the two cues given in the test session. Importantly, this allowed other factors of interest to be assessed independently of the variance caused by test order. In the test analysis, test order was therefore ignored, both as a main factor and in terms of any interactions that involved this factor.

The previous experiment demonstrated that the blocking effect was transient, only being seen in the first test session. The analysis of this experiment was therefore limited to the first test session. Where lick cluster sizes could not be calculated for a mouse within a session, the average lick cluster size made during consumption of that cue, by that mouse within the stage, was given for that particular session.

## 2.2.2 Results

### Stage 1

#### Total Licks:

The mean total numbers of licks made during consumption of the CS- and CS+ flavour cues, by the wild-type and *Gria1*<sup>-/-</sup> mice during the first training stage, are shown in Figure 2.5. The wild-type and *Gria1*<sup>-/-</sup> mice both made a greater number of licks during consumption of the CS+ than the CS- flavour. Over sessions the numbers of licks remained similar during consumption of the CS-, although the licks made to the CS+ slightly decreased for the *Gria1*<sup>-/-</sup> mice, whereas for the wild-type mice the numbers of licks slightly increased. The ANOVA showed that there was a significant effect of flavour cue,  $F(1,65) = 319.9$ ,  $p < .001$ , and no significant effect of genotype,  $F(1,65) = .41$ ,  $p = .52$ , as well as no significant interaction between cue and genotype,  $F(1,65) = 2.3$ ,  $p = .13$ . There was a significant effect of session,  $F(3,195) = 5.1$ ,  $p = .01$ , as well as a significant interaction between session and genotype,  $F(3,195) = 7.2$ ,  $p = .002$ , and also between flavour cue and session,  $F(3,195) = 4.7$ ,  $p = .017$ . The interaction between the three factors of cue, session, and genotype was not significant,  $F(3,195) = 3.1$ ,  $p = .06$ .

#### Lick Cluster Size:

The mean lick cluster sizes made during consumption of the CS- and CS+ flavour cues, by the wild-type and *Gria1*<sup>-/-</sup> mice during the first training stage, are shown in Figure 2.5 (middle panel). The mean lick cluster sizes were reduced in the *Gria1*<sup>-/-</sup> mice compared to the wild-type mice during consumption of both flavour cues, but genotypes made larger lick cluster sizes during consumption of the CS+ compared to the CS-. The wild-type mice also showed an increase in the mean lick cluster size over training sessions during consumption of the CS+, whereas they slightly decreased in the *Gria1*<sup>-/-</sup> mice. The ANOVA showed that there were significant effects of flavour cue,  $F(1,65) = 207.5$ ,  $p < .001$ , and genotype,  $F(1,65) = 18.5$ ,  $p < .001$ , as well as a significant interaction between these two factors,  $F(1,65) = 12.3$ ,  $p = .001$ . The effect of session was not significant,  $F(3,195) = 1.3$ ,  $p = .29$ , although there was a significant

interaction between session and genotype,  $F(3,195) = 5.8$ ,  $p = .001$ , as well as between cue and session,  $F(3,195) = 3.2$ ,  $p = .026$ . The interaction between the three factors of cue, session, and genotype was also significant,  $F(3,195) = 4.9$ ,  $p = .003$ .

#### Volume:

The mean volumes consumed of the CS- and CS+ flavour cues, by the wild-type and *Gria1*<sup>-/-</sup> mice in the first training stage, are shown in Figure 2.5 (lower panel). Both the wild-type and *Gria1*<sup>-/-</sup> mice consumed more of the CS+ than the CS-, although the wild-type mice consumed slightly more of the CS- than the *Gria1*<sup>-/-</sup> mice. The ANOVA showed that there was a significant effect of flavour cue,  $F(1,65) = 457.8$ ,  $p < .001$ , no significant effect of genotype,  $F(1,65) = 3.0$ ,  $p = .088$ , and a significant interaction between these two factors,  $F(1,65) = 10.7$ ,  $p = .002$ . The effect of session was not significant,  $F(3,195) = 1.4$ ,  $p = .24$ , and there was a significant interaction between session and genotype,  $F(3,195) = 5.8$ ,  $p = .004$ . The interaction between cue and session was not significant,  $F(3,195) = 1.5$ ,  $p = .22$ , and there was also no significant interaction between the three factors of cue, session, and genotype,  $F(3,195) = .16$ ,  $p = .21$ .

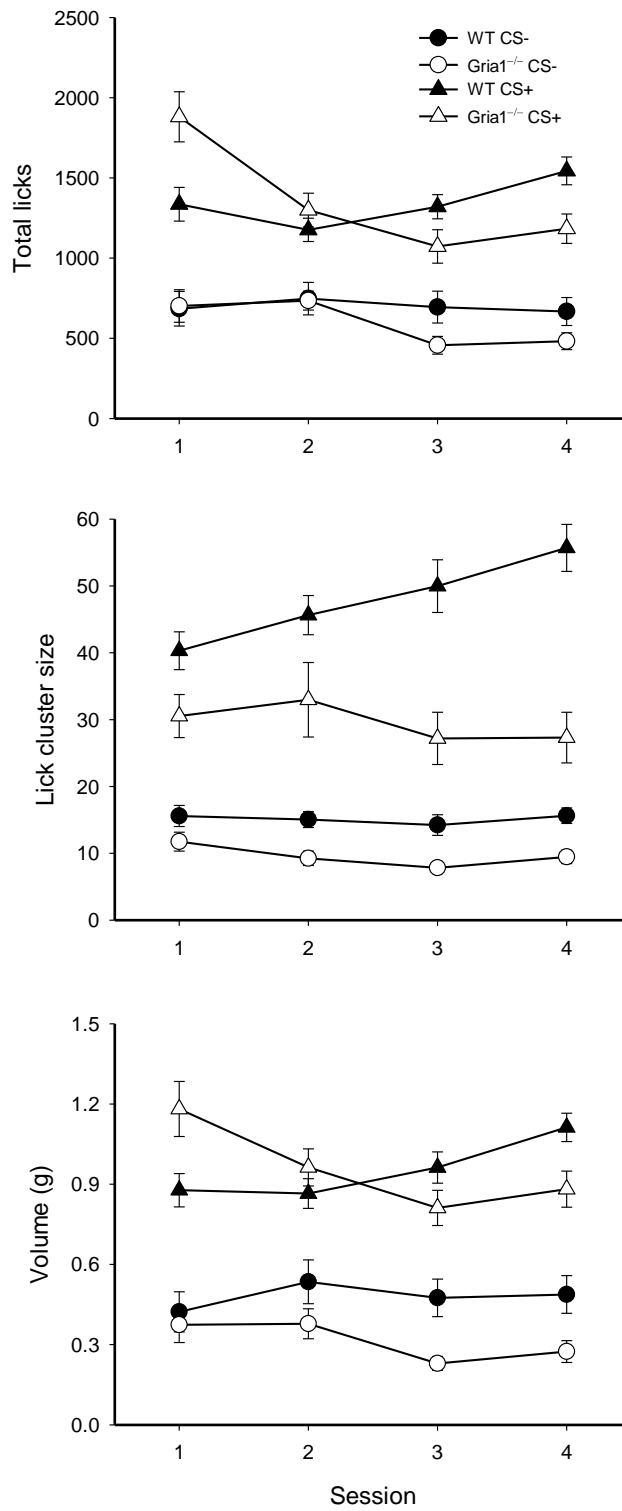


Figure 2.5. Experiment 2, training stage 1. The mean numbers of total licks (upper panel), lick cluster sizes (middle) and volumes consumed (lower) of the CS- and CS+, by the wild-type (WT) and *Gria1*<sup>-/-</sup> mice. Error bars show ± SEM.

## Stage 2

### Total Licks:

The mean total numbers of licks made during consumption of the control and experimental compound flavour cues, by the wild-type and *Gria1*<sup>-/-</sup> mice, are shown in Figure 2.6 (upper panel). Both the *Gria1*<sup>-/-</sup> and wild-type mice made fewer licks during consumption of the experimental than control compound cue, with both genotypes also showing a reduction in the total numbers of licks over sessions. The *Gria1*<sup>-/-</sup> mice also showed smaller numbers of licks, as well as a greater reduction in the numbers of licks over sessions, than the wild-type mice. The ANOVA showed that there was a significant effect of flavour cue,  $F(1,65) = 5.4$ ,  $p = .023$ , and genotype  $F(1,65) = 9.3$ ,  $p = .003$ , with no significant interaction between flavour cue and genotype,  $F(1,65) = .083$ ,  $p = .77$ . The effect of session was also significant,  $F(3,195) = 36.9$ ,  $p < .001$ , as was the interaction between session and genotype,  $F(3,195) = 4.0$ ,  $p = .017$ . The interaction between cue and session was not significant,  $F(3,195) = .36$ ,  $p = .79$ , and there was no significant interaction between the three factors of cue, session, and genotype,  $F(3,195) = .12$ ,  $p = .82$ .

### Lick Cluster Size:

The mean lick cluster sizes made during consumption of the control and experimental compound flavour cues, by the wild-type and *Gria1*<sup>-/-</sup> mice, are shown in Figure 2.6 (middle panel). The mean lick cluster sizes were reduced in the *Gria1*<sup>-/-</sup> mice compared to the wild-type mice, but both genotypes made similar lick cluster sizes during consumption of the two cues. In addition, the wild-type mice showed a slight inverted-U shape function over sessions, whereas cluster sizes remained stable in the *Gria1*<sup>-/-</sup> mice. The ANOVA showed that there was no significant effect of cue (blocked or control compound) on the mean lick cluster size,  $F(1,65) = .23$ ,  $p = .64$ , and the effect of genotype was significant,  $F(1,65) = 18.3$ ,  $p < .001$ , with no significant interaction between these two factors,  $F(1,65) = .48$ ,  $p = .49$ . The effect of session was not significant,  $F(3,195) = 1.4$ ,  $p = .26$ , although there was a significant interaction between session and genotype,  $F(3,195) = 3.7$ ,  $p = .015$ . The interaction between cue and session was not significant,  $F(3,195) = .95$ ,  $p = .40$ , and there was also no

significant interaction between the three factors of cue, session, and genotype,  $F(3,195) = .26, p = .80$ .

#### Volume:

The mean volumes consumed of the control and experimental compound flavour cues, by the wild-type and *Gria1*<sup>-/-</sup> mice, are shown in Figure 2.6 (lower panel). The *Gria1*<sup>-/-</sup> mice consumed slightly less than the wild-type mice, but both genotypes consumed slightly more of the control than experimental compound flavour cue. The ANOVA showed that there was a significant effect of flavour cue,  $F(1,65) = 5.8, p = .019$ , as well as a significant effect of genotype,  $F(1,65) = 8.7, p = .004$ , and no significant interaction between these two factors,  $F(1,65) = 1.5, p = .22$ . The effect of session was also significant,  $F(3,195) = 18.7, p < .001$ , with no significant interaction between session and genotype,  $F(3,195) = .52, p = .65$ , or between cue and session,  $F(3,195) = .21, p = .73$ . The interaction between the three factors of cue, session, and genotype, was also not significant,  $F(3,195) = .16, p = .77$ .

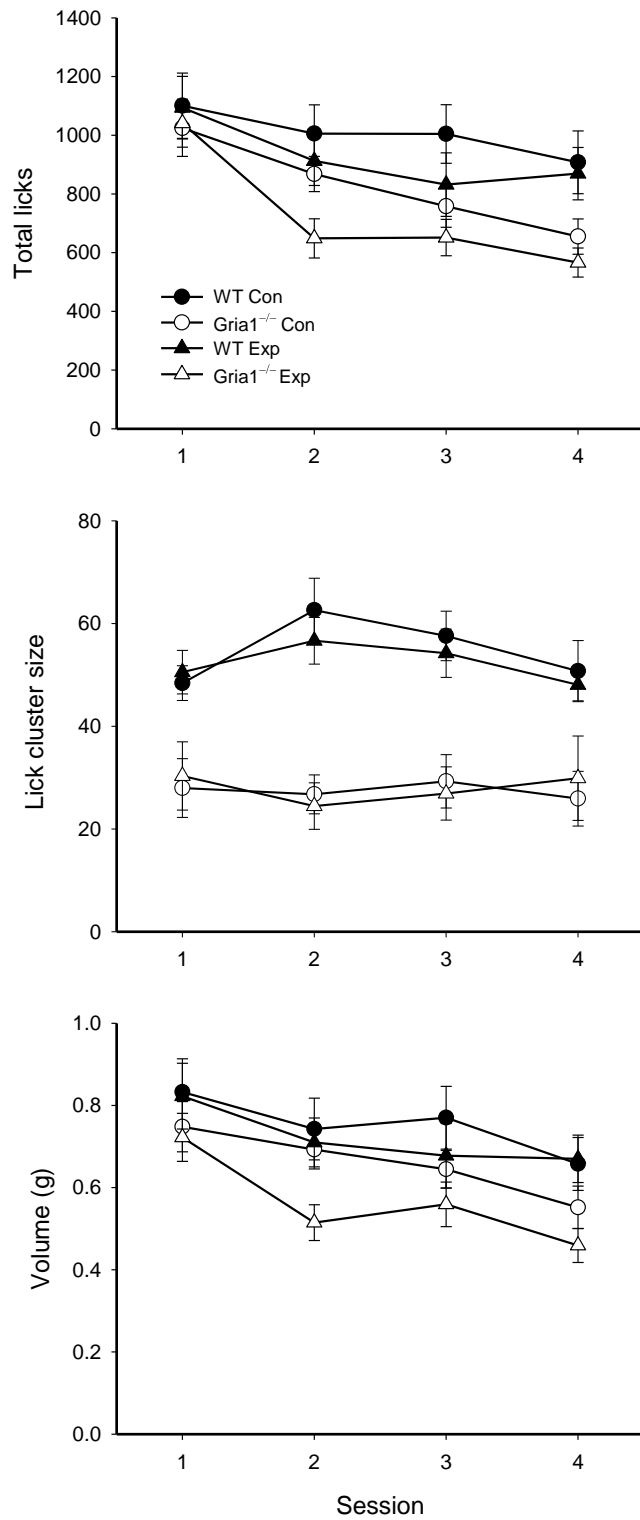


Figure 2.6. Experiment 2, training stage 2. The mean numbers of total licks (upper panel), lick cluster sizes (middle) and volumes consumed (lower), of the control and experimental cues, by the wild-type (WT) and Gria1<sup>-/-</sup> mice. bars show ± SEM

## Test stage 1

### Total Licks:

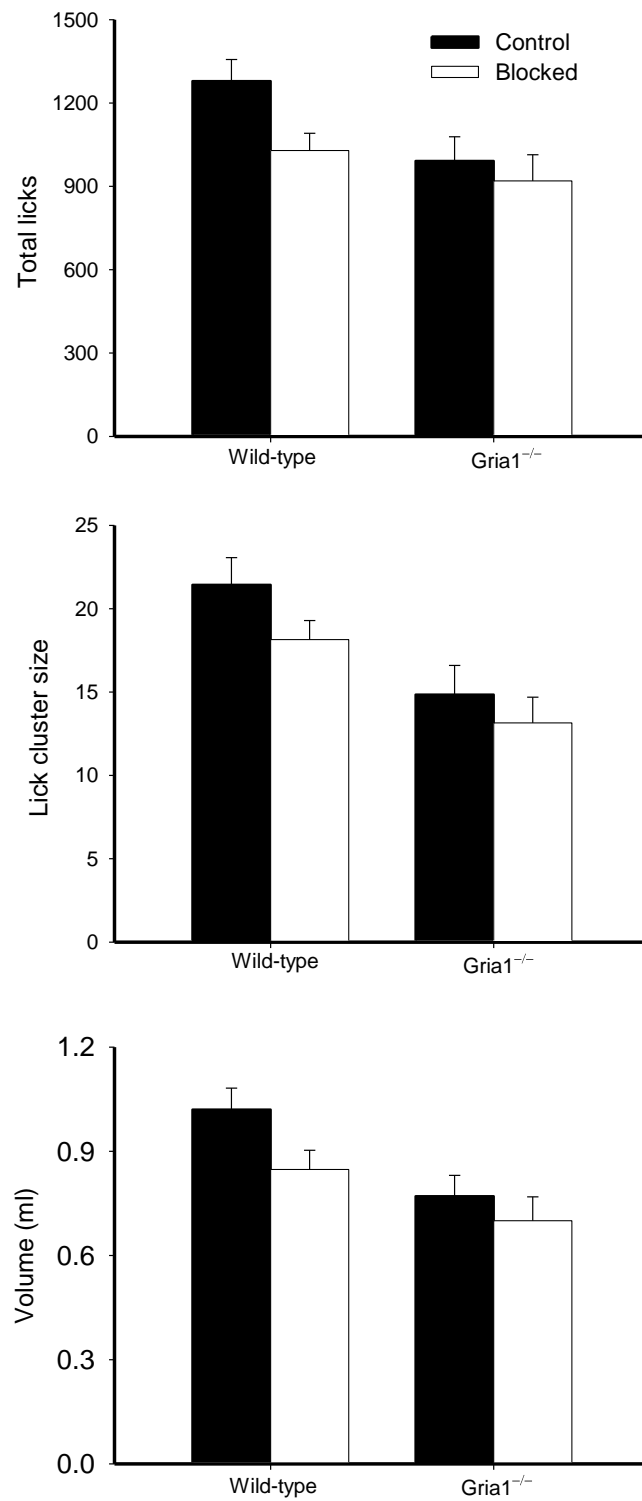
The mean total numbers of licks made during consumption of the control and blocked flavour cues, by the wild-type and *Gria1*<sup>-/-</sup> mice during the first session of the test stage, are shown in Figure 2.7 (upper panel). The wild-type mice made a greater number of licks during consumption of the control than blocked flavour cue, but the *Gria1*<sup>-/-</sup> mice showed little difference between the two cues. The ANOVA showed that the effect of flavour cue was significant,  $F(1,63) = 13.6$ ,  $p < .001$ , with a near significant effect of genotype,  $F(1,63) = 3.7$ ,  $p = .058$ , as well as a significant interaction between these two factors,  $F(1,63) = 4.8$ ,  $p = .033$ . Simple main effects analysis of the interaction between cue and genotype, further showed that the wild-type mice made a significantly greater number of licks during consumption of the control than blocked flavour cue,  $F(1,63) = 21.4$ ,  $p < .001$ , but that this was not seen with the *Gria1*<sup>-/-</sup> mice  $F(1,63) = .95$ ,  $p = .334$ . The wild-type mice also made a greater number of licks during consumption of the control flavour cue than the *Gria1*<sup>-/-</sup> mice,  $F(1,63) = 6.6$ ,  $p = .013$ , but there was no significant difference between the genotypes in the numbers of licks made during consumption of the blocked flavour cue,  $F(1,63) = .95$ ,  $p = .334$ .

### Lick Cluster Size:

The mean lick cluster sizes made during consumption of the control and blocked flavour cues, by the wild-type and *Gria1*<sup>-/-</sup> mice during the first test session, are shown in Figure 2.7 (middle panel). Both the wild-type and the *Gria1*<sup>-/-</sup> mice made greater mean lick cluster sizes during consumption of the control compared to the blocked flavour cue. The mean lick cluster sizes were also reduced in the *Gria1*<sup>-/-</sup> mice compared to the wild-type mice. The ANOVA showed that there was a significant effect of flavour cue,  $F(1,63) = 10.1$ ,  $p = .002$ , a significant effect of genotype,  $F(1,63) = 8.0$ ,  $p = .006$ , and no significant interaction between these two factors,  $F(1,63) = 1.3$ ,  $p = .27$ .

## Volume:

The mean volumes consumed of the control and blocked flavour cues, by the wild-type and *Gria1*<sup>-/-</sup> mice during the first test session of the first test stage, are shown in Figure 2.7 (lower panel). Both genotypes consumed more of the control than the blocked flavour cue, although the wild-type mice consumed slightly more of both cues than the *Gria1*<sup>-/-</sup> mice. The ANOVA showed that there was a significant effect of cue,  $F(1,63) = 10.3$ ,  $p = .002$ , and a significant effect of genotype,  $F(1,63) = 6.2$ ,  $p = .016$ , with no significant interaction between these factors,  $F(1,63) = 2.2$ ,  $p = .15$ .



*Figure 2.7.* Experiment 2, test stage 1. The mean numbers of total licks (upper panel), lick cluster sizes (middle) and volumes consumed (lower), of the control and blocked flavour cues, by wild-type and Gria1<sup>-/-</sup> mice. Error bars show  $\pm$  SEM.

## Test stage 2

### Total Licks:

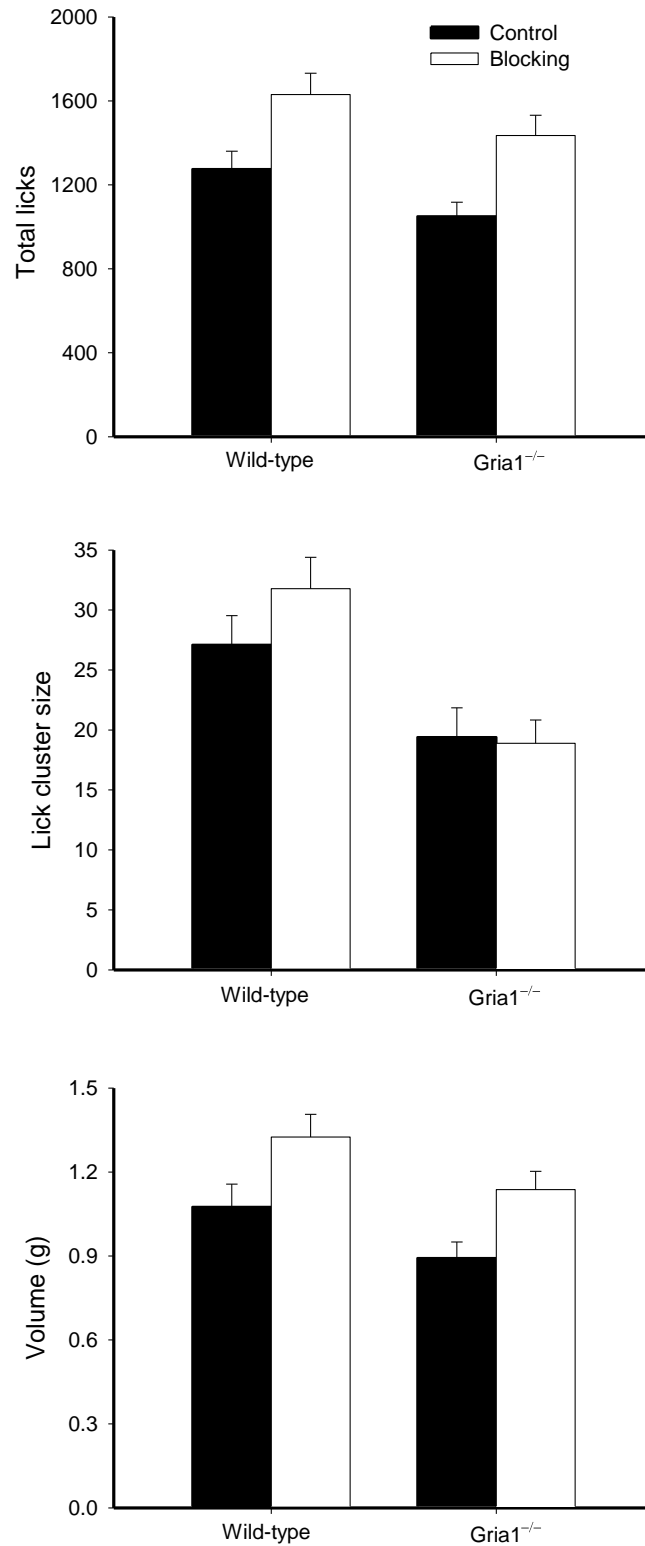
The mean total numbers of licks made during consumption of the control and blocking flavour cues, by the wild-type and *Gria1*<sup>-/-</sup> mice during the first test session of the second test stage, are shown in Figure 2.8 (upper panel). Both genotypes made greater numbers of licks during consumption of the blocking than control flavour cue, with similar numbers of licks made by wild-type and *Gria1*<sup>-/-</sup> mice. The ANOVA showed that there was a significant effect of flavour cue on the total number of licks,  $F(1,63) = 43.5$ ,  $p < .001$ , no significant effect of genotype ( $F(1,63) = 2.9$ ,  $p = .092$ ), and no significant interaction between cue and genotype,  $F(1,63) = .21$ ,  $p = .65$ .

### Lick Cluster Size:

The mean lick cluster sizes made during consumption of the control and blocking flavour cues, by the wild-type and *Gria1*<sup>-/-</sup> mice during the first test session of the second test stage, are shown in Figure 2.8 (middle panel). The mean lick cluster sizes were reduced in the *Gria1*<sup>-/-</sup> mice compared to the wild-type mice during consumption of both flavour cues. The wild-type mice also showed a larger mean lick cluster size during consumption of the blocking compared to the control flavour. The ANOVA showed that there was a marginal significant effect of cue,  $F(1,63) = 4.0$ ,  $p = .050$ , and genotype,  $F(1,63) = 9.4$ ,  $p = .003$ , as well as a significant interaction between these two factors,  $F(1,63) = 5.3$ ,  $p = .025$ . Simple main effects analysis of the interaction between cue and genotype, further showed that only the wild-type mice made significantly greater lick cluster sizes during consumption of the blocking than control flavour, (wild-type mice,  $F(1,63) = 11.5$ ,  $p = .001$ ; *Gria1*<sup>-/-</sup> mice,  $F(1,63) = .037$ ,  $p = .85$ ). They also showed that the mean lick cluster sizes were greater in the wild-type than the *Gria1*<sup>-/-</sup> mice during consumption of both the control,  $F(1,63) = 5.1$ ,  $p = .028$ , and blocking  $F(1,63) = 12.97$ ,  $p = .001$  flavour cues.

## Volume:

The mean volumes consumed of the control and blocking flavour cues, by the wild-type and *Gria1*<sup>-/-</sup> mice during the first test session of the second test stage, are shown in Figure 2.8 (lower panel). Both the wild-type and *Gria1*<sup>-/-</sup> mice consumed more of the blocking than control flavour cue, with slightly lower levels of consumption in the *Gria1*<sup>-/-</sup> mice compared to the wild-type controls. The ANOVA showed that there was a significant effect of cue on consumption,  $F(1,63) = 31.3$ ,  $p < .001$ , and a near significant effect of genotype  $F(1,63) = 3.5$ ,  $p = .067$ . The interaction between cue and genotype was not significant,  $F(1,63) = .032$ ,  $p = .86$ .



*Figure 2.8.* Experiment 2, test stage 2. The mean numbers of total licks (upper panel), lick cluster sizes (middle) and volumes consumed (lower), of the control and blocking flavour cues, by wild-type and Gria1<sup>-/-</sup> mice. Error bars show  $\pm$  SEM.

### 2.2.3 Discussion

The results showed that the wild-type mice demonstrated a blocking effect, evident by a reduction in consumption and mean lick cluster size, for the blocking compared to the control flavour cue. This blocking effect also seemed to be impaired in the *Gria1*<sup>-/-</sup> mice compared to the control mice, as although the wild-type mice demonstrated a blocking effect in the total numbers of licks, there was no significant difference in the *Gria1*<sup>-/-</sup> mice. In terms of the mean lick cluster sizes and the volumes consumed, there was an effect of cue that did not interact with genotype. The failure to see this interaction however likely relates to a lack of sensitivity, as in both measures the difference in the *Gria1*<sup>-/-</sup> mice between the blocked and control cues was small. However, the finding of a lick cluster size effect does suggest that blocking of flavour preference learning may affect the palatability of the flavour cues. In particular, the blocking of flavour preference learning may also prevent an increase in the palatability for the blocked compared to the control flavour.

It was also observed in both experiments that the total numbers of licks were slightly lower in the knockout mice compared to the control mice. However, this reduction during training sessions did not seem to subsequently impair the ability for *Gria1*<sup>-/-</sup> mice to learn a flavour preference, meaning that it would still be expected for blocking of this flavour preference learning to be able to occur. Therefore, this reduction seems unlikely to account for the failure to see blocking in the *Gria1*<sup>-/-</sup> mice in the two experiments, although this is difficult to completely rule out.

The results from this experiment therefore do seem to replicate the findings from the first blocking of flavour preference learning experiment, with impaired blocking in the *Gria1*<sup>-/-</sup> mice compared to the wild-type mice. The results from the second stage, assessing learning in the first stage of training, also found that both wild-type and *Gria1*<sup>-/-</sup> mice showed a preference for the blocking compared to the control flavour cue. This further demonstrates that both genotypes were able to discriminate between and learn a flavour preference in the first stage of training. Therefore, despite learning about the two flavours in the first stage of training, this learning did not block subsequent learning in the *Gria1*<sup>-/-</sup> mice. As with the previous experiment,

this difference was again seen in the consumption of the control rather than the blocked flavour cue. However, as the *Gria1*<sup>-/-</sup> mice failed to show any significant differences between the blocked and control flavour cues, an effect seen in two experiments in the total numbers of licks, they do seem to show impaired blocking of flavour preference.

It was also observed in both experiments that the total numbers of licks were slightly lower in the knockout mice compared to the control mice. However, this reduction during training sessions did not seem to subsequently impair the ability for *Gria1*<sup>-/-</sup> mice to learn a flavour preference, meaning that it would still be expected for blocking of this flavour preference learning to occur in the *Gria1*<sup>-/-</sup>, even if this might be to a slightly lesser extent than the controls. Therefore, this reduction seems unlikely to account for the failure to see blocking in the *Gria1*<sup>-/-</sup> mice seen in both blocking experiments reported here.

This result, of impaired blocking of flavour preference learning in the *Gria1*<sup>-/-</sup> mice, goes against the original prediction that cue-competition effects should be intact, or possibly even enhanced, in the *Gria1*<sup>-/-</sup> mice. This could suggest that the effect of GluA1 deletion may not be as specific to short-term memory decay as proposed (Sanderson et al., 2009) and may extend into prediction error and resulting cue-competition effects. To further investigate cue-competition effects in the *Gria1*<sup>-/-</sup> mice, using flavour preference learning, another cue-competition effect, overshadowing, was tested in the wild-type and *Gria1*<sup>-/-</sup> mice.

## 2.3 Experiment 3

### Overshadowing of flavour preference learning

To further assess the role of the GluA1 subunit in cue-competition, wild-type and *Gria1*<sup>-/-</sup> mice were tested for overshadowing of flavour preference learning. During overshadowing (e.g., Mackintosh, 1971), training a cue in compound reduces learning about that cue compared to if it had been trained alone. During training in

an overshadowing procedure, one cue is therefore presented alone, with another cue presented in compound. Both of these cues, the single and the compound, are paired with the same reinforcer. Test sessions then assess conditioned responding to one of the compound cues (now presented alone) compared to the single cue trained and tested alone. Overshadowing is then seen in the form of reduced conditioned responding to the compound cue compared to the control cue.

In this flavour preference experiment, the single flavour cue (B) and the compound cue (AX), were both paired with the same high sucrose concentration during training. The presence of another flavour cue in compound (X), should result in overshadowing of learning about the first flavour (A), compared to the flavour trained alone (B). During test, overshadowing should result in a reduced flavour preference for the overshadowed flavour (A) compared to the control flavour (B).

The proposed role of GluA1 in short-term memory decay (Sanderson et al., 2009), predicts that  $Gria1^{-/-}$  mice should show intact, or perhaps even enhanced, cue-competition effects such as overshadowing. However, in the previous two experiments blocking of flavour preference was impaired or reduced in the  $Gria1^{-/-}$  mice. It may therefore be the case that overshadowing of flavour preference could also be impaired in the  $Gria1^{-/-}$  compared to the wild-type control mice. This would be seen in the form of similar levels of preference towards the two flavour cues, rather than reduced responding to the overshadowed cue.

### **2.3.1 Methods**

#### **Subjects**

15  $Gria1^{-/-}$  (6 females, 9 males) and 20 wild-type mice (15 females, 5 males) bred and housed in the same way as experiment 2.1, were used. The mice were aged between approximately 2 and 8 months at the start of testing, caged in groups of 1-12, with 85% weights of between 14g – 28g. Mice had been previously used in an appetitive magazine conditioning procedure in similar apparatus, but had no previous experience of experiments involving licking analysis or the apparatus used in this experiment.

## **Apparatus**

The apparatus used were the same as in experiment 2.1.

## **Procedure**

The design of the experiment is shown in Table 2.2. There were 12 daily training sessions, each lasting 15-minutes with the sipper tube being available after 5-minutes in the chamber. In each session they had access to one of three sucrose solutions. The overshadowed cue (AX) and the control cue (B) were both paired with 32% sucrose. There was also an additional control cue (C) that was paired with a lower 4% sucrose, added to aid discrimination between the cues and prevent high levels of generalisation between the flavour cues. These three flavoured sucrose solutions were presented in an intermixed order across training sessions, with four presentations of each cue over the 12 sessions of training. For all mice flavour X was cherry. Flavours A, B, and C were apple, orange, or grape Kool Aid (0.05% wt/vol) counterbalanced across animals, with respect to genotype and sex as far as possible given the numbers. Following the training sessions there were two test sessions, one with each of the overshadowed (A) and control (B) flavour cues, both now paired with 4% sucrose. The order of presentations of these flavour cues were counterbalanced across mice, with respect to genotype and flavour allocation as far as possible. This meant that half of the mice received the overshadowed flavour (A) in the first test session and the other half received the control flavour (B) first.

Table 2.2

*Design of experiment 3*

Training	Test
AX 32%	A 4%
B 32%	B 4%
C 4%	

### Statistical analysis

Statistical analyses were carried out in a similar way to experiment 2.1. For the training data, mixed model ANOVA with cue, session, and genotype as factors were carried out for all three measures. For the test data, mixed model ANOVA with cue and genotype as factors were carried out.

#### 2.3.2 Results

##### Training

##### Total Licks:

The mean total numbers of licks made during consumption of the three flavour cues, by the wild-type and *Gria1*<sup>-/-</sup> mice during the four training sessions, are shown in Figure 2.9 (upper panel). The wild-type mice made a similar number of licks to the three cues in the first three training sessions, although consumption of C was lower in the final training session. The *Gria1*<sup>-/-</sup> mice showed a difference in the first training session, with the total number of licks greater during consumption of flavour B than during AX and C. The ANOVA showed that there was a significant effect of flavour cue,  $F(2,66) = 3.2$ ,  $p = .048$ , and no significant effect of genotype,  $F(1,33) = .308$ ,  $p = .58$ , as well as no significant interaction between these two factors,  $F(2,66) = 2.4$ ,  $p = .097$ . There was also no significant effect of training session,  $F(3,99) = .55$ ,  $p = .59$ , but this did show a significant interaction with genotype,  $F(3,99) = 13.3$ ,  $p < .001$ . The

interaction between cue and session was significant,  $F(6,198) = 4.04$ ,  $p < .001$ , as was the interaction between the three factors of cue, session, and genotype,  $F(6,198) = 5.05$ ,  $p < .001$ .

Lick cluster size:

The mean lick cluster sizes made during consumption of the three flavour cues, by the wild-type and *Gria1*<sup>-/-</sup> mice, are shown in Figure 2.9 (middle panel). Both the wild-type and the *Gria1*<sup>-/-</sup> mice made greater lick cluster sizes during consumption of the cues paired with the higher sucrose concentration (AX and B). The mean lick cluster sizes were also reduced for all cues in the *Gria1*<sup>-/-</sup> compared to the wild-type mice. The mean lick cluster sizes in the wild-type mice also showed a slight increase over sessions during consumption of flavour cues AX and B, but this was not seen with the *Gria1*<sup>-/-</sup> mice. The ANOVA showed that there was a significant effect of flavour cue on the mean lick cluster size,  $F(2,66) = 46.02$ ,  $p < .001$ , as well as a significant effect of genotype,  $F(1,33) = 13.5$ ,  $p = .001$ , and no significant interaction between these two factors,  $F(2,66) = 2.8$ ,  $p = .087$ . The effect of session was not significant,  $F(3,99) = 2.0$ ,  $p = .16$ , but there was a significant interaction between session and genotype,  $F(3,99) = 4.2$ ,  $p = .029$ . There was no significant interaction between cue and session,  $F(6,198) = 1.8$ ,  $p = .14$ , or between the three factors of cue, session, and genotype,  $F(6,198) = 2.02$ ,  $p = .11$ .

Volume:

The mean volumes consumed of the three flavour cues, by both genotypes across training sessions, are shown in Figure 2.9 (lower panel). The volumes consumed by wild-type mice increased slightly over sessions and were also similar for all three cues. For the *Gria1*<sup>-/-</sup> mice consumption differed in the first training session, being greater to B and AX than to C. The ANOVA showed that there was a significant effect of flavour cue on the volumes consumed,  $F(2,66) = 4.1$ ,  $p = .021$ , but no significant effect of genotype,  $F(1,33) = .009$ ,  $p = .93$ , and a significant interaction between these two factors,  $F(2,66) = 3.7$ ,  $p = .03$ . The effect of session was not significant,  $F(3,99) = 1.9$ ,  $p = .16$ , but there was a significant interaction between session and genotype,  $F(3,99) = 9.9$ ,  $p < .001$ . There was no significant interaction between flavour cue and

session,  $F(6,198) = .89$ ,  $p = .477$ , and a near significant interaction between the three factors of cue, session, and genotype,  $F(6,198) = 2.1$ ,  $p = .053$ .

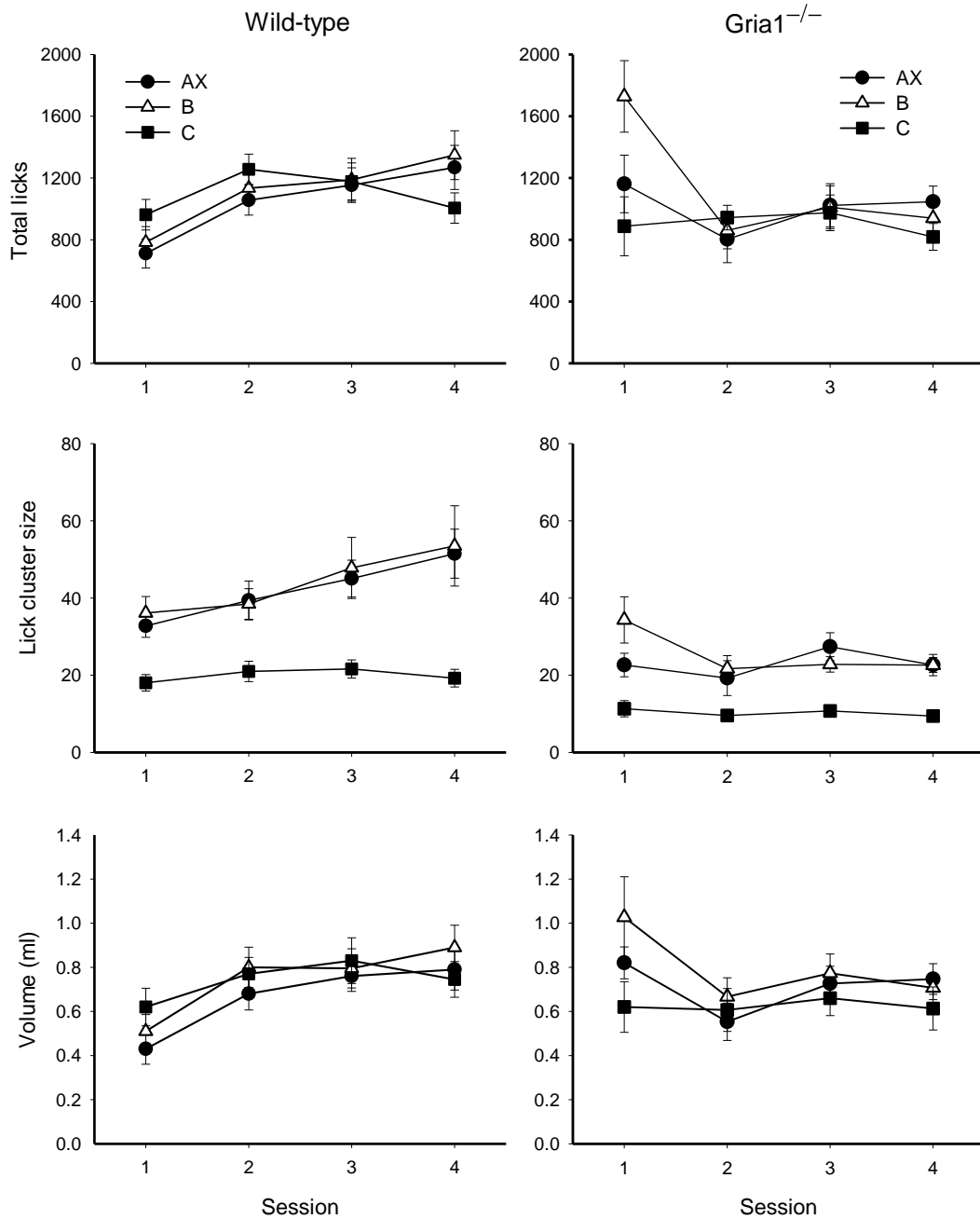


Figure 2.9. Experiment 3, training stage. The mean numbers of total licks (upper panel), lick cluster sizes (middle) and volumes consumed (lower), by the wild-type (left) and *Gria1*<sup>-/-</sup> mice (right). Error bars show  $\pm$  SEM.

## Test

### Total Licks:

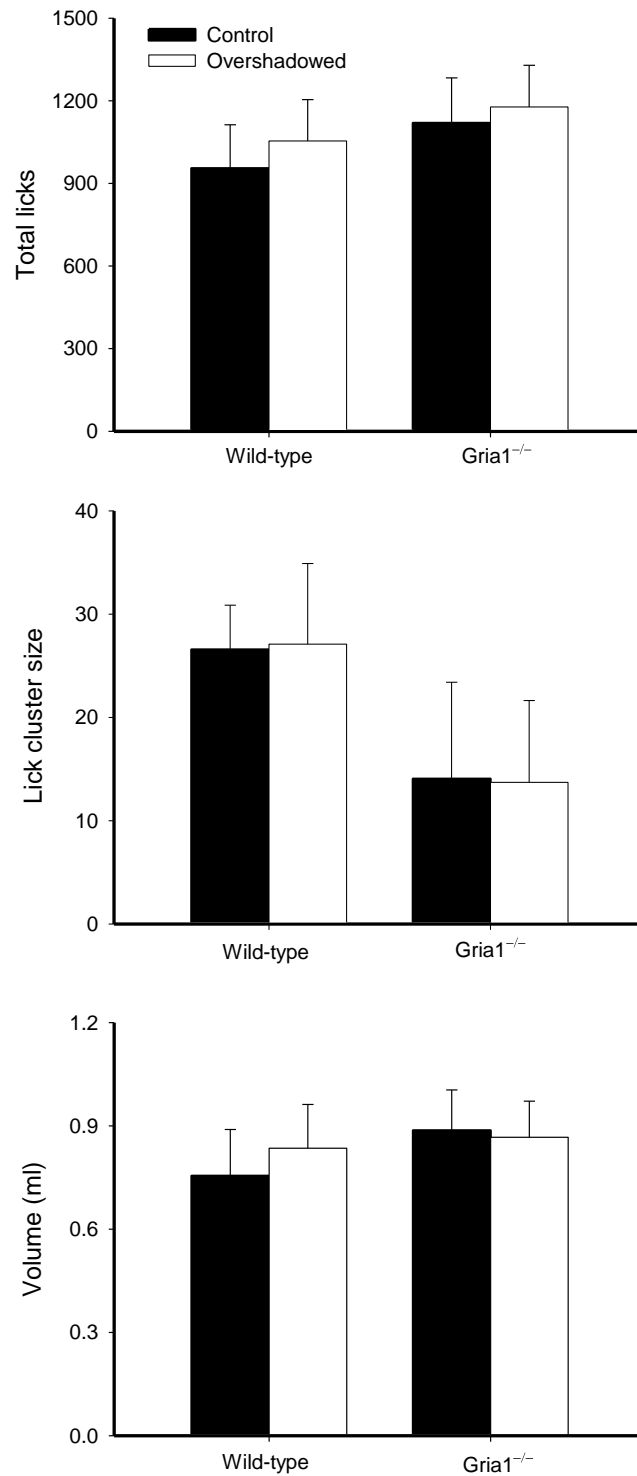
The mean total numbers of licks made during consumption of the overshadowed and blocked flavour cues, by the wild-type and *Gria1*<sup>-/-</sup> mice during the test session, are shown in Figure 2.10 (top panel). Both genotypes made similar numbers of licks during consumption of the control and overshadowed flavour cues. The ANOVA showed that the effect of cue (overshadowed or control) was not significant,  $F(1,33) = 2.2$ ,  $p = .14$ , and there was also no significant effect of genotype,  $F(1,33) = .44$ ,  $p = .51$ . There interaction between flavour cue and genotype was also not significant,  $F(1,33) = 1.5$ ,  $p = .702$ .

### Lick Cluster Size:

The mean lick cluster sizes made during consumption of the control and overshadowed flavour cues, by the wild-type and *Gria1*<sup>-/-</sup> mice during the test session, are shown in Figure 2.10 (middle panel). Both the wild-type and *Gria1*<sup>-/-</sup> mice made similar lick cluster sizes during consumption of the overshadowed and control flavour cues, with lick cluster sizes also reduced in the *Gria1*<sup>-/-</sup> mice compared to the wild-type mice. The ANOVA showed that there was no significant effect of flavour cue on mean lick cluster size,  $F(1,33) = .001$ ,  $p = .98$ , but that there was a near significant effect of genotype,  $F(1,33) = 4.1$ ,  $p = .051$ , and no significant interaction between these two factors,  $F(1,33) = .014$ ,  $p = .91$ .

### Volume:

The mean volumes consumed of the control and overshadowed flavour cues, by the wild-type and *Gria1*<sup>-/-</sup> mice during the test session, are shown in Figure 2.10 (lower panel). For both wild-type and *Gria1*<sup>-/-</sup> mice the volumes consumed of the two cues (overshadowed or control) were similar. The ANOVA showed that there was no significant effect of flavour cue,  $F(1,33) = .49$ ,  $p = .49$ , as well as no significant effect of genotype,  $F(1,33) = .22$ ,  $p = .64$ . The interaction between these two factors was also not significant,  $F(1,33) = 1.4$ ,  $p = .25$ .



*Figure 2.10.* Experiment 3, test stage. The mean numbers of total licks (upper), lick cluster sizes (middle) and volumes consumed (lower), of the control and overshadowed flavour cues by the wild-type and Gria1<sup>-/-</sup> mice. Error bars show  $\pm$  SEM.

### 2.3.3 Discussion

The results of the test session showed that in both the wild-type and *Gria1*<sup>-/-</sup> mice, for all three measures, there was no effect of flavour cue, either overshadowed or control. This means that neither genotype showed any evidence of overshadowing of flavour preference learning. It can therefore not be determined to what extent GluA1 deletion alters cue-competition in the form of overshadowing, as neither the wild-type nor the *Gria1*<sup>-/-</sup> mice seemed to show an effect. This null result is perhaps surprising given the previous demonstrations of overshadowing of flavour preference learning in rats (Dwyer et al., 2011).

One way to explain this failure to observe overshadowing is that the mice may have failed to discriminate between, and learn about, the different flavour cues during training. However, the same Kool-aid flavours as in the previous blocking of flavour preference experiments (2.1 & 2.2) were used, in which there was evidence for flavour preference learning having occurred. The mean lick cluster sizes were also greater to the cues paired with the higher concentrations, demonstrating that they could discriminate between the different sucrose concentration solutions. It also suggests that the mice did seem to find the higher concentration of sucrose more palatable, something that should contribute to learning a flavour preference to the flavour paired with the higher sucrose concentration. Another finding was that mean lick cluster sizes were impaired in the *Gria1*<sup>-/-</sup> mice, corresponding to the results from the previous experiments. This further shows that the measure of palatability, the mean lick cluster size made during consumption, is reduced in the *Gria1*<sup>-/-</sup> mice compared to the control mice. Although the possibility of the impaired mean lick cluster sizes being related to a deficit in the expression of cluster sizes, rather than altered palatability, cannot be ruled out.

Overall GluA1 deletion impaired blocking of flavour preference learning, but neither wild-type nor *Gria1*<sup>-/-</sup> mice showed overshadowing of flavour preference learning. This result could be due to flavours not being susceptible to overshadowing in mice, despite overshadowing having been found in rats (Dwyer et al., 2011). Although the possibility that the mice failed to learn in this experiment is difficult to fully rule out,

despite learning occurring in experiments 2.1 and 2.2, using the same flavours and similar procedures. To further investigate this lack of overshadowing effect, a second overshadowing experiment was run, using a slightly different procedure and stimuli.

## **2.4 Experiment 4**

### **Overshadowing of taste preference**

The previous experiment failed to observe overshadowing of flavour preference learning, in either the wild-type or the *Gria1*<sup>-/-</sup> mice. This is in contrast to the finding of overshadowing of flavour preference in rats (Dwyer et al., 2011), although an absence of overshadowing has been seen in other similar procedures using odour and taste cues (Holder, 1991). The null result in the previous experiment could relate to an absence of overshadowing effect, although it could also have been due to failure to learn a flavour preference in the first stage of the experiment. In the case of the former, mice show equivalent levels of conditioned responding to the overshadowed and control cues as a result of being able to learn about the flavour cues similarly. This is instead of overshadowing occurring between the two flavours presented together in the compound cue.

To further test if stimuli, such as flavours, are not susceptible to overshadowing of learning in mice, a second overshadowing experiment was carried out. The stimuli and procedure were slightly different, with two taste cues, rather than flavour cues used. These taste cues were used due to the mice having previous experience with the flavour cues. Similarly, rather than sucrose, the reinforcer that was used was maltodextrin, a non-sweet polysaccharide. As with sucrose, consumption of maltodextrin in rats follows an inverted-U shape function with concentration, whereas lick cluster sizes show a linear increase with concentration (Dwyer, 2008, 2012). It is also highly effective at supporting flavour preference learning in rats, shown in an increased intake and an increase in mean lick cluster sizes (Dwyer, 2008; Sclafani & Nissenbaum, 1988). Therefore, as with sucrose, flavour preference

learning based on high maltodextrin concentrations also seems to alter the palatability of the cue in a way that is analogous to increasing the actual concentration of maltodextrin (Dwyer, 2008). However, whereas sugars such as sucrose and fructose seem to support preference learning based on flavour-flavour learning (i.e. between the flavour of the cue and highly palatable sweet taste of the reinforcer), carbohydrates such as maltodextrin support preference learning based on the post-ingestive consequences (Dwyer, 2008; Elizalde & Sclafani, 1988). As maltodextrin appears to be effective at supporting flavour preference learning, it was used in this study alongside the tastes in order to further test overshadowing in wild-type and *Gria1*<sup>-/-</sup> mice. The procedure was also altered to a between subjects, rather than the within-subjects design used in the previous experiment (2.3).

#### **2.4.1 Methods**

##### **Subjects**

15 *Gria1*<sup>-/-</sup> mice (12 females, 3 males) and 25 wild-type mice (12 females, 13 males), bred and housed in the same way as experiment 2.1, were used. They were aged between 10-15 months at the start of testing, caged in groups of 1-5, and had 85% weights ranging between 17.6g – 33.1g. Mice had previously been used in appetitive magazine conditioning and flavour preference experiments, but had no experience of taste cues or maltodextrin.

##### **Apparatus**

The apparatus used was the same as in experiment 2.1. The taste stimuli were .0006M quinine, .01 M hydrochloric acid (Sigma-Aldrich, Dorset, UK) and .01M Salt, with solutions made using deionised water. Maltodextrin (Special Ingredients Ltd, Chesterfield) was used in place of sucrose as the reinforcer, made up weight/volume.

##### **Procedure**

The design of the experiment is shown in Table 2.3. Mice were split into two groups, each with as equal numbers as possible in terms of genotype and sex. Group 1 formed the overshadowing group (8 *Gria1*<sup>-/-</sup> mice: 7 females, 1 male. 13 wild-type mice: 7 females, 6 males) and group 2 the control group (7 *Gria1*<sup>-/-</sup> mice: 6 females,

1 male. 12 wild-type mice: 6 females, 6 males). Each daily testing session followed the same procedure as experiment 2, with two periods of access to the maltodextrin solutions per daily testing session. The access periods and interval between the two solutions were also the same as experiment 2, with 10-minutes of access (following 5-minutes in the chamber) to each solution and an approximate 10-minute interval between these presentations. During training mice in group 1 received access to cue B paired with 2% maltodextrin, intermixed with sessions of access to cue A that was presented in compound with X (AX), paired with 16% maltodextrin. In group 2, mice received cue A alone paired with 16% maltodextrin, and cue B paired with 2% maltodextrin.

For all mice, cues A and B were either hydrochloric acid (HCl) or quinine, counterbalanced across subjects as far as possible given the numbers with respect to genotype and sex. For half of the mice cue A was HCl, and B was quinine, and vice versa for the remaining half of the mice. Additionally, for mice in group 1 cue X was salt. The order of presentations of the two cues were counterbalanced so that, as far as possible given the numbers, half received the A (if in group 2) or AX (if in group 1) cue first, the remaining half of the mice received cue B first. These were then presented in double alternation across the four training sessions, resulting in a total of four presentations of each cue. This training order was also counterbalanced, as much as possible, with respect to the counterbalancing of the tastes for cues A and B. Following training, mice in both groups were tested with cues A and B, both paired with 2% maltodextrin. These were given in a single test session, in the same way as the training sessions. Test order was counterbalanced, with respect to previous training order and taste, as far as possible, so that half received cue A first and the other half cue B.

Table 2.3

*Design of experiment 4*

	<b>Training</b>	<b>Test</b>
Overshadowing	AX 16%	A 2%
	B 2%	B 2%
Control	A 16%	A 2%
	B 2%	B 2%

### Statistical analysis

Statistical analyses were carried out in a similar way as experiment 2.1. Mixed model ANOVA were carried out, with group, genotype, cue, and session as factors. For the test data, test order was also added as a factor. As with experiment 2, it will however be ignored as a main effect and in terms of any interactions, due to it being used only to account for the variance caused by two successive periods of access to the test solutions.

For the training data, lick cluster sizes were unable to be calculated for two wild-type mice in group 1 during consumption of cue B for one session. In these instances they were given the average lick cluster size made to that cue by that mouse, over the other 3 sessions with that cue during training. One mouse (control group, *Gria1*<sup>-/-</sup>) failed to make any licks during presentation of cue B during the test session, so was not included in the analysis of the test data.

## 2.4.2 Results

### Training

#### Total licks:

The mean total numbers of licks made during consumption of the two cues during training, by both groups and genotypes, are shown in Figure 2.11. For both groups, control and overshadowing, the number of licks were greater during consumption of the cues paired with 16% (AX for the overshadowing group and A for the control group) than those paired with 2% (cue B). The *Gria1*<sup>-/-</sup> mice in both groups also showed slightly higher levels of intake of the cue paired with 16% than the wild-type mice. The ANOVA showed that there was a significant effect of cue,  $F(1,36) = 381.0$ ,  $p < .001$ , and a significant effect of genotype,  $F(1,36) = 6.1$ ,  $p = .018$ , with a near significant interaction between these two factors,  $F(1,36) = 3.8$ ,  $p = .058$ . There was no significant effect of group,  $F(1,36) = .001$ ,  $p = .98$ , or significant interaction between genotype and group,  $F(1,36) = .082$ ,  $p = .78$ , and also no significant interaction between cue and group,  $F(1,36) = .85$ ,  $p = .36$ . The effect of session was significant,  $F(3,108) = 65.1$ ,  $p < .001$ , as was the interaction between cue and session,  $F(3,108) = 21.6$ ,  $p < .001$ . All other interactions were not significant,  $F$  values  $< 2.1$ ,  $p$  values  $> .13$ .

#### Lick cluster size:

The mean lick cluster sizes made during consumption of the two cues during training, by both groups and genotypes, are shown in Figure 2.11. The mean lick cluster sizes were greater during consumption of the cue paired with 16% maltodextrin (AX for the overshadowing group and A for the control group) than 2% (cue B). The *Gria1*<sup>-/-</sup> mice did however show smaller mean lick cluster sizes than the wild-type mice, although this was not the case by the last session of training in the control group. The ANOVA showed that there was a significant effect of cue,  $F(1,36) = 147.1$ ,  $p < .001$ , and genotype,  $F(1,36) = 6.8$ ,  $p = .013$ , with no significant interaction between these two factors,  $F(1,36) = 2.5$ ,  $p = .13$ . The effect of group was not significant,  $F(1,36) = .086$ ,  $p = .771$ , and there was no significant interaction between group and genotype,

$F(1,36) = .81, p = .38$ . There was also no significant interaction between cue and group,  $F(1,36) = .2, p = .66$ . The effect of session was significant,  $F(3,108) = 18.8, p < .001$ , as was the interaction between cue and session,  $F(3,108) = 8.02, p < .001$ . The interaction between the three factors of session, group, and genotype, was also significant,  $F(3,108) = 4.8, p = .007$ , and the interaction between the four factors of cue, session, group, and genotype, neared significance,  $F(3,108) = 2.8, p = .055$ . All other interactions were not significant,  $F$  values  $< .84$ ,  $p$  values  $> .45$ .

#### Volume:

The mean volumes consumed of the two cues during training, by both groups and genotypes, are shown in Figure 2.11. In both groups, mice consumed more of the cue paired with the higher 16% maltodextrin concentration (AX or A), than cue B paired with 2%. The wild-type mice in the overshadowing group did however consume slightly less of cue AX than the *Gria1*<sup>-/-</sup> mice, with no differences between the genotypes, for either cue, in the control group. The ANOVA showed that there was a significant effect of cue,  $F(1,36) = 323.6, p < .001$ , and no significant effect of genotype,  $F(1,36) = 2.09, p = .16$ , with a significant interaction between these two factors,  $F(1,36) = 4.9, p = .033$ . The effect of group was not significant,  $F(1,36) = 1.3, p = .26$ , and there was no significant interaction between group and genotype,  $F(1,36) = .80, p = .38$ , or between cue and group,  $F(1,36) = 2.4, p = .13$ . The interaction between the three factors of cue, group, and genotype, was significant,  $F(1,36) = 5.1, p = .030$ . There was also a significant effect of session,  $F(3,108) = 80.8, p < .001$ , and a significant interaction between cue and session,  $F(3,108) = 24.8, p < .001$ . All other interactions were not significant,  $F$  values  $< 1.6$ ,  $p$  values  $> .21$ .

To analyse the interaction between the three factors of cue, group, and genotype, repeated measures ANOVA were carried out for each genotype, with cue and group as factors. For the wild-type mice this showed that there was no significant interaction between cue and group,  $F(1,23) = .34, p = .57$ , however this interaction was significant for the *Gria1*<sup>-/-</sup> mice,  $F(1,13) = 159.4, p = .033$ . Simple main effects analysis of this interaction further showed that *Gria1*<sup>-/-</sup> mice in the overshadowing group consumed significantly more of AX than mice in the control group consumed

of A,  $F(1,13) = 4.9$ ,  $p = .045$ , but there was no significant difference in the amount consumed of B,  $F(1,13) = .57$ ,  $p = .46$ . This analysis also showed that in the overshadowing group,  $Gria1^{-/-}$  mice consumed more of AX than B,  $F(1,13) = 120.8$ ,  $p < .001$ , and mice in the control group also consumed more of A than B,  $F(1,13) = 49.07$ ,  $p < .001$ .

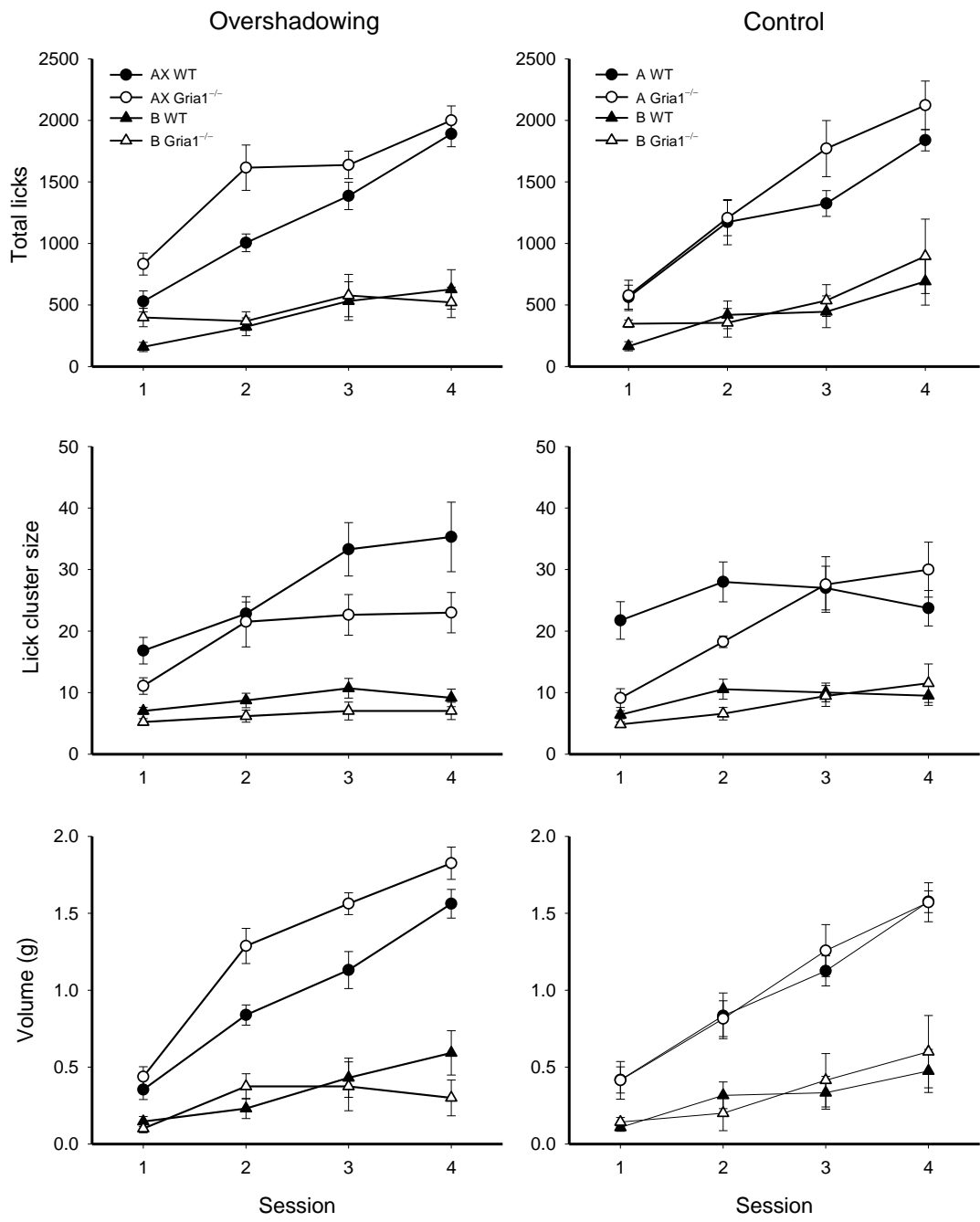


Figure 2.11. Experiment 4, training stage. The mean numbers of total licks (upper), lick cluster sizes (middle) and volumes consumed (lower), by the wild-type (WT) and *Gria1*<sup>-/-</sup> mice. The overshadowing group is shown on the left and the control group on the right. Error bars show ± SEM.

## Test

### Total licks:

The mean numbers of total licks made during consumption of the two cues in the test session, the CS+ (flavour A) and the CS- (flavour B), by wild-type and *Gria1*<sup>-/-</sup> mice in the control and overshadowing groups, are shown in Figure 2.12. Both genotypes consumed more of the CS+ than the CS-, with no difference between the overshadowing and control groups. The *Gria1*<sup>-/-</sup> mice did however make a higher number of licks during consumption of the CS+, and fewer to the CS-, than the wild-type mice. The ANOVA showed that there was a significant effect of cue,  $F(1,31) = 159.1$ ,  $p < .001$ , no significant effect of genotype,  $F(1,31) = .008$ ,  $p = .93$ , and also no significant effect of group,  $F(1,31) = 1.3$ ,  $p = .26$ . The interaction between cue and group was not significant,  $F(1,31) = .58$ ,  $p = .45$ , although the interaction between cue and genotype was significant,  $F(1,31) = 7.7$ ,  $p = .009$ .

Simple main effects analysis of the interaction between cue and genotype further showed that both genotypes consumed more of the CS+ than the CS-, (wild-type mice,  $F(1,31) = 71.2$ ,  $p < .001$ , *Gria1*<sup>-/-</sup> mice  $F(1,31) = 89.6$ ,  $p < .001$ ). The *Gria1*<sup>-/-</sup> mice did however make significantly fewer licks during consumption of the CS- than the wild-type mice,  $F(1,31) = 4.7$ ,  $p = .038$ , with no significant difference between the genotypes during consumption of the CS+,  $F(1,31) = 2.1$ ,  $p = .16$ .

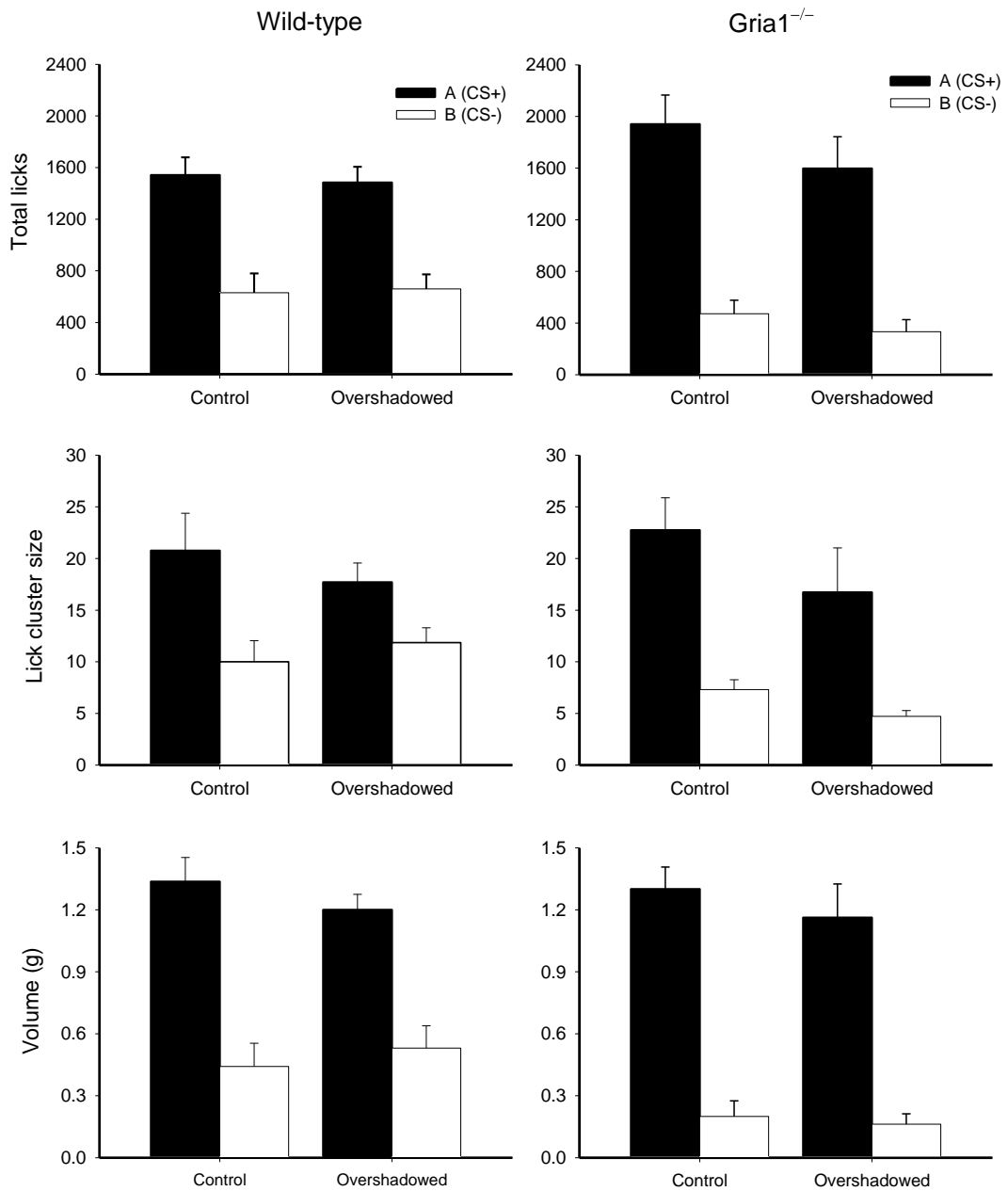
### Lick cluster size:

The mean lick cluster sizes made during consumption of the two cues in the test session, by the wild-type and *Gria1*<sup>-/-</sup> mice in the overshadowing and control groups, are shown in Figure 2.12. Both genotypes made greater lick cluster sizes during consumption of the CS+ (flavour A) than the CS- (flavour B). The *Gria1*<sup>-/-</sup> mice also showed similar lick cluster sizes to the wild-type mice. The ANOVA showed that there was a significant effect of cue,  $F(1,31) = 43.9$ ,  $p < .001$ , with no significant effect of group,  $F(1,31) = 1.3$ ,  $p = .27$ , or genotype,  $F(1,31) = 1.3$ ,  $p = .27$ . The interaction between cue and group was not significant,  $F(1,31) = 1.5$ ,  $p = .23$ , and there was also no significant interaction between cue and genotype,  $F(1,31) = 2.5$ ,  $p = .12$ .

Volume:

The mean volumes consumed of the two cues in the test session, by wild-type and *Gria1*<sup>-/-</sup> mice in the overshadowing and control groups, are shown in Figure 2.12. Both groups and genotypes consumed more of the CS+ (flavour A) than the CS- (flavour B), with little difference between the control and overshadowed groups. The ANOVA showed that there was a significant effect of cue,  $F(1,31) = 174.1$ ,  $p < .001$ , with no significant effect of group,  $F(1,31) = 1.3$ ,  $p = .26$ , or genotype,  $F(1,31) = 1.4$ ,  $p = .24$ . The interaction between cue and group was not significant,  $F(1,31) = 1.8$ ,  $p = .19$ , but the interaction between cue and genotype was significant,  $F(1,31) = 6.8$ ,  $p = .014$ .

Simple main effects analysis of the interaction between cue and genotype further showed that both genotypes consumed more of the CS+ than the CS-, (wild-type mice,  $F(1,31) = 94.5$ ,  $p < .001$ , *Gria1*<sup>-/-</sup> mice,  $F(1,31) = 82.5$ ,  $p < .001$ ). The wild-type mice also consumed significantly more of the CS- than the *Gria1*<sup>-/-</sup> mice,  $F(1,31) = 11.2$ ,  $p = .002$ , but there was no significant difference between the genotypes in the amount consumed of the CS+,  $F(1,31) = .46$ ,  $p = .504$ .



*Figure 2.12.* Experiment 4, test stage. The mean numbers of total licks (upper), lick cluster sizes (middle) and volumes consumed (lower), by the control and overshadowed groups. The wild-type mice are shown on the left side and the *Gria1*<sup>-/-</sup> mice on the right. Error bars show ± SEM.

### 2.4.3 Discussion

The results showed that although both groups demonstrated a taste preference for the CS+ (cue A), an effect seen in all three measures, there was no difference between the overshadowing and control groups. There was therefore no evidence of overshadowing of taste preference, in the form of a reduced preference in the overshadowing compared to the control group. There was also no difference between the wild-type and *Gria1*<sup>-/-</sup> mice in this lack of effect. Both genotypes did however learn a taste preference in the first stage of the experiment, shown as a preference for the CS+ (flavour cue A) compared to the CS- (flavour cue B) during the test session. Therefore, despite learning a taste preference, this did not result in overshadowing of the compound flavour in the overshadowing group compared to the control group.

An additional result was that in contrast to the previous experiments, the *Gria1*<sup>-/-</sup> mice did not show impaired lick cluster sizes to maltodextrin during the test sessions. This reduction in lick cluster size was still generally present however during training, with impaired cluster sizes to the taste cue paired with the higher maltodextrin concentration, compared with the wild-type mice. During the test session, lick cluster sizes in the *Gria1*<sup>-/-</sup> mice were the same during consumption of the CS+ (cue A) as the wild-type mice, while being slightly lower to the CS-(cue B). The *Gria1*<sup>-/-</sup> mice also showed reduced consumption and mean lick cluster sizes for the CS- (cue B) than the wild-type mice. This suggests that the taste preference in this experiment may actually have been slightly enhanced in the knockout mice compared to the controls, as they showed a greater difference between the two taste cues during test. Although, intake to the CS+ (cue A) was not increased, meaning any enhancement was seen as a result of reduced intake of the non-preferred cue, rather than an increase in the preferred one. The lack of lick cluster size impairment during the test session, suggests that when using maltodextrin as the reinforcer, lick cluster sizes, a measure of palatability, may not be impaired in the *Gria1*<sup>-/-</sup> mice. It could for example be the case that palatability is not altered in the same way during a taste preference procedure with maltodextrin, compared to when sucrose is used as the reinforcer. Whereas sucrose seems to support preference learning, at least partly,

through an increase in the perceived palatability of the CS+ (Dwyer, 2012; Dwyer, Pincham, Thein, & Harris, 2009) maltodextrin, that does not have a sweet taste, seems to support preference learning based on its post-ingestive consequences (Dwyer, 2008; Sclafani & Nissenbaum, 1988). GluA1 deletion may therefore not impair an increase in lick cluster sizes, a measure of palatability, occurring as a result of these post-ingestive consequences. However, the effect of GluA1 deletion on mean lick cluster size could also still be related to impairments in the expression of cluster size rather than effects on palatability, but this impaired expression may only be seen in tasks with sufficient sensitivity. It may be that when using maltodextrin, it is not possible to see difference in the mean lick cluster sizes in the *Gria1<sup>-/-</sup>* mice compared to the wild-type controls.

The failure to observe overshadowing in this experiment replicates the previous failure to see overshadowing of flavour preference (experiment 3). This further suggests that when using taste or flavour stimuli neither wild-type or *Gria1<sup>-/-</sup>* mice show cue-competition in the form of overshadowing. There have however been other failures to observe cue-competition effects, some of which have also been in experiments using flavour and/or taste stimuli. Holder (1991) for example, failed to observe overshadowing when using odour and taste cues paired with sucrose, despite a small blocking effect was still observed. Furthermore, Capaldi and Privitera (2008) actually observed potentiation, i.e. an increase in responding to the overshadowed cue when using conditioned flavour preference in rats. In terms of blocking, Capaldi and Hunter (1994) also failed to demonstrate blocking of odors, when using tastes that were pre-trained prior to being paired in compound with the odors.

One way in which these failures to observe cue-competition have been explained, is by considering flavour and taste cues to be processed in a more configural rather than an elemental manner. Traditional learning theories (Rescorla & Wagner, 1972; Wagner, 1981) are based on internal stimulus representations being processed separately. Each of these representations consists of a set of representative 'elements' that can enter into associations. The associative strength a stimulus acquires is therefore the result of the proportion of representative elements that

have entered into an association with another, such as the unconditioned stimulus. Once all the elements constituting a stimulus representation have entered into an association, asymptotic learning is reached. Importantly, each stimulus has its own representation and constituent representative elements, with each of these representations separate and able to gain individual amounts of associative strength.

There is however an alternative account of how stimuli representations may be processed and enter into associations. In configural processing (Pearce, 1987, 1994, 2002) any stimuli present, rather than having separate representations, form a single configural unit that is able to enter into associations. The level of conditioned responding generated when a test stimulus is presented is therefore not dependent on how much associative strength was individually acquired by that cue, but rather how similar the test stimulus is to the previous configuration. The more similar the test stimulus to the configuration, the more conditioned responding will occur. Therefore, it is the level of generalisation between the cues presented during training and test that is important in determining how much an animal will show conditioned responding and learning towards a particular cue (Pearce, 1987, 1994, 2002).

This form of processing has been particularly considered when learning about flavour cues during flavour preference procedures. Pearce (2002) suggested that when flavour cues are presented in compound, they form a single configural representation. When one flavour cue is presented alone it is able to activate, at least partly, the configural unit, thereby also activating a representation of the other cue previously paired in compound with it. If the US in this procedure relates to the sensory properties of the reinforcer (e.g. sweet taste of sucrose), then this enters into the configural unit along with the CS flavour cue. When the flavour cue is subsequently presented alone, it is able to excite, to some degree, the configural unit, activating the representation of the sweet tasting US, generating the relevant conditioned response (e.g., high palatability). There may also however be a secondary association linked to the post-ingestive nutrients of the reinforcer. When the configural unit is activated, it also activates the representation of the US and its associated post-ingestive consequences. Importantly however, flavour preference learning will ultimately depend on the level of generalisation between the training

and test stimuli. Capaldi and Hunter (1994) suggested that within their experiments the compound of a taste and odour may have formed single configuration that was more similar to the odour element than to the taste. There would therefore be only a small amount of generalisation between the pre-trained taste to the compound, allowing learning of the taste/odour compound to occur resulting in little blocking of learning about the odour.

Furthermore, Dwyer, Haselgrove and Jones (2011) proposed an adapted configural model of flavour preference learning, in which they modified the original model proposed by Pearce (1987, 1994, 2002). This model accounted for the fact that generalisation between cues can be variable and also that conditioning to a compound stimulus proceeds more rapidly than to a single cue alone. In this particular configural model, if the generalisation between the flavour cues is assumed to be high, then this could explain the occurrence of blocking while failing to see overshadowing. This is the case as during overshadowing there will be a large amount of generalisation between the compound training cue and the single test cue, generating a high level of conditioned responding and a failure to see an overshadowing effect. For blocking however, if generalisation is high then the associative strength acquired by the pre-trained cue will largely generalise to the compound cue, meaning the new paired cue will be unable to gain much, if any, associative strength.

Therefore in this configural model, the strength of overshadowing is therefore inversely related to the strength of the blocking effect, i.e. weak overshadowing corresponds to strong blocking. This model can explain many of the previous failures to observe overshadowing, such as Holder (1991), in which there may have been a high level of generalisation between the cues. It can also explain findings of potentiation (Capaldi & Privitera, 2008) as a result of conditioning to the compound proceeding more readily than to the single control stimulus, with a high level of generalisation between this compound cue and the test stimulus. Although for this to occur training must have not reached asymptote, as at this point conditioned responding would be similar between the control and overshadowed cues i.e. no overshadowing effect.

This configural model can also explain some of the blocking and overshadowing results from the experiments in this thesis. In particular, it is able to explain an absence of overshadowing while still seeing blocking, as was seen in the wild-type mice. However, it is not able to explain the lack of blocking in the *Gria1*<sup>-/-</sup> mice alongside the lack of overshadowing. This is due to the lack of blocking being explained through low levels of generalisation between the flavour cues, while the lack of overshadowing is explained as a result of high levels of generalisation between the flavour cues. Why generalisation between the flavour cues would be high in one experiment while low in the other is unclear, particularly given the high degree of similarity between the procedures and use of the same flavours. However, it does seem that configural processing can explain the results from the wild-type mice, as well as other previous failures to observe cue-competition in flavour preference procedures.

This therefore raises the question of if the same results would be seen (no overshadowing but blocking only in the wild-type mice) if cues that are not likely to be processed in a configural manner were used. It has been suggested that using cues that are processed in different modalities may increase elemental rather than configural processing, with cues that are highly similar having been linked to more configural processing (Soto, 2018; Soto, Gershman, & Niv, 2014; Wagner, 2003). More direct evidence of this comes from Kehoe, Horne, Horne, and Macrae, (1994) who found that cues from different modalities (auditory and visual) resulted in a summation effect, whereas those from the same auditory modality failed to summate. As summation provides evidence for elemental processing (with the two separately trained cues summing together to enhance conditioned responding) this suggests that cues in separate auditory and visual domains are processed more elementally, whereas cues from the same modality are processed in a more configural manner. In order to assess the role stimulus similarity and configural processing may have played in the previous cue-competition experiments, along with the role of *GluA1* in cue-competition, blocking and overshadowing procedures were therefore repeated using auditory and visual cues. As cues presented in different sensory modalities appear to result in more elemental rather than configural

processing, these procedures may result in different cue-competition effects than previously observed, with the failure to observe overshadowing potentially a result of the configural processing of flavours in the previous experiments.

## **2.5 Experiment 5**

### **Blocking using auditory and visual cues**

The previous blocking of flavour preference experiments (2.1 & 2.2), found that although there was a blocking effect in the wild-type mice, this was impaired in the *Gria1*<sup>-/-</sup> mice. This suggests that rather than GluA1 deletion resulting in intact or enhanced cue-competition, it may instead impair cue-competition effects. However, the lack of overshadowing in either the wild-type or the *Gria1*<sup>-/-</sup> mice also suggested that there may have been more configural, as opposed to elemental processing, of the flavour cues used in the previous experiments. In this instance, the effects of blocking and overshadowing would have been determined by the level of generalisation between the cues. It may therefore be the case that the effects of GluA1 deletion will differ when cues that are likely to be processed more elementally rather than configurally are used.

In this blocking experiment auditory and visual cues, processed in different modalities, were used. This should result in the cues being processed more elementally rather than configurally (Kehoe et al., 1994). The auditory cues were presented in the first stage of training before being paired with visual cues in the second stage of training. Rather than sucrose being used as the reinforcer to form a flavour preference, these cues were presented in an appetitive magazine conditioning procedure, with sucrose pellets used as the unconditioned stimulus. This should result in mice learning about the reinforced cues, demonstrated by an increase in the number of head entries made into the magazine as they increasingly expect delivery of the sucrose pellet reward. As similar procedures have already been used to observe blocking in mice with auditory and visual cues (Sanderson, Jones, &

Austen, 2016) blocking should be evident, at least in the wild-type mice. Although the proposed role of the GluA1 subunit predicts that *Gria1*<sup>-/-</sup> mice should not be impaired at showing cue-competition effects (Sanderson et al., 2009), the previous failure to observe blocking with flavours may suggest that wider cue-competition may in fact be altered. If however the use of flavours and configural processing plays a role, and is affected by GluA1 deletion, then it may be the case that with more elemental processing the *Gria1*<sup>-/-</sup> mice could show different results to those in the previous blocking of flavour preference experiments (2.1 & 2.2).

### **2.5.1 Methods**

#### **Subjects**

15 *Gria1*<sup>-/-</sup> (13 females, 2 males) and 25 wild-type mice (13 females, 12 males), bred and housed in the same way as experiment 2.1, were used. The mice were approximately 5.5 – 11 months old at the start of testing and caged in groups of 1-5 with 85% body weights ranging between 17.2g – 28.6g. Mice had previous experience in similar operant chambers during a flavour preference procedure but had no experience of appetitive magazine conditioning using auditory and visual cues.

#### **Apparatus**

Eight operant chambers (15.9 × 14.0 × 12.7 cm; ENV307A, Med Associates), enclosed in sound-attenuating cubicles (ENV-022V, Med Associates), controlled by Med-PC IV software were used. The front and back walls and the ceiling of each chamber were made from clear Perspex and the sidewalls were made from aluminium. The floor was a grid of stainless steel rods (0.32 cm diameter) each separated by 0.79 cm. Sucrose pellets (14 mg TestDiet, ETH) could be dispensed into a magazine (2.9 × 2.5 × 1.9 cm; ENV-303 M, Med Associates) located in the centre of one of the sidewalls. Breaks in an infrared beam (ENV-303HDM, Med Associates) across the bottom of the entrance to the magazine were used to measure the number of magazine head entries at a resolution of 0.1 s. White noise (ENV-325SM, Med Associates) could be emitted from a speaker (ENV-324 M, Med Associates) located at the top right corner

of the wall opposite the magazine. A clicker (ENV-335 M, Med Associates) was located on the exterior top left corner of the wall opposite the magazine. A28V, 100mA house light (ENV-315 M, Med Associates) was located next to the speaker in the centre of the wall. Presentation of the house light resulted in illumination of the chamber. Two LEDs (ENV-321 M, Med Associates) were positioned to the left and the right, above the magazine. Presentation of the LEDs resulted in limited, localised illumination. A fan (ENV-025AC) was positioned above the left LED and was turned on during sessions.

### **Procedure**

The design of the experiment is shown in Table 2.4. In the first stage of training mice were presented with three 10s auditory cues. Cue A was reinforced with a sucrose pellet delivered at the end of the cue, and cues B and C were non-reinforced. During each daily testing session each cue was presented for 8 times, with 24 trials in total and an inter-trial interval between cue-offset to cue on-set of 120s. The three auditory cues were a white-noise, clicker, and a tone. The allocation of these to the three cues A, B, and C, were counterbalanced across subjects so that for a third of the mice cue A was a tone, cue B was a white-noise, and cue C was the clicker. For another third cue A was the clicker, B the white-noise, and cue C the tone. For the final third, cue A was the white-noise, cue B was a tone, and cue C was a clicker. This was also counterbalanced with respect to sex and genotype as far as possible given the numbers.

During each session the cues were presented randomly with the constraint that within every 12 trials there were 4 presentations of each cue. In the second stage of training, for a further ten sessions, these three auditory cues were paired with 3 visual cues, X, Y, Z, which were either a house-light, LED, or flashing LED to form auditory-visual compound cues. The allocation of these visual cues to the auditory ones, were counterbalanced across mice as far as possible, so that each auditory cue was subsequently paired with all three of the visual cues across mice. For example, for the third of mice that had cue A as the tone, B as the clicker, C as the white-noise, a third of these had X as the house-light, Y as the LED, and Z as the flashing LED. The

other third had X as the LED, Y as the flashing LED, Z as the house-light. The final third had X as the flashing LED, Y as the house-light and Z as the LED. This pattern was followed for the other two-thirds of mice with the two other auditory cue-combinations from the first stage of training. Again, this was also counterbalanced with respect to sex and genotype as far as possible. Compound cues AX and BY were now both reinforced with a sucrose pellet at the end of the 10s compound cue. The pre-training of auditory cue A should result in blocking of learning about the visual cue X, but as auditory cue B was not reinforced in stage 1, mice should still be able to learn about the visual cue Y. Compound cue CZ was non-reinforced to provide a never reinforced control cue. As well as these three compound cues, the auditory cues from stage 1 were also continued to be presented, with the same reinforcement contingencies. The orders of these cue presentations were random, with the constraint that within every 12 trials there were 3 of each compound, and 1 of each auditory cue from stage 1.

In the test stage, for two test sessions, learning about the visual cues was assessed by presenting each of these cues non-reinforced. The compounds from stage 2 also continued to be presented (AX+, BY+ and CZ-), to prevent levels of responding rapidly reducing due to lack of reinforcement. As with the training stages these were presented randomly with the constraint that within every 12 trials there were 2 of each visual cue test trials, and 2 of each of the compounds.

Table 2.4

*Design of experiment 5*

Stage 1	Stage 2	Test
A+	AX+	X
B-	BY+	Y
C-	CZ-	Z
	A+	AX+
	B-	BY+
	C-	CZ-

### **Statistical analysis**

Levels of responding to each cue were measured using the average number of head entries made, per second, during the 10s presentation of each cue. This was calculated for each daily session by averaging over all the presentations of that cue in that particular session. For all the data, the mean rates of responding per second were multiplied by 60 to give the rate of responding per minute (RPM) during the presentation of each of the cues.

Statistical analysis were carried out using mixed-model ANOVA in a similar way as for experiment 2.1. For the first stage of training, mixed model ANOVA on the three auditory cues were carried out, with cue, session, and genotype as factors. For the data from the second stage, mixed model ANOVA were carried out on the three auditory-visual compound cues. This was also done for the 3 auditory cues that had also been presented previously in the first stage of training. For the test stage, the rate of responding to each cue were averaged over the two test sessions, with mixed model ANOVA carried out on the test presentations of the three visual cues. This

analysis allowed a comparison between responding to the blocked and control cues during test presentations. Mixed model ANOVA were also carried out on the three auditory-visual compound cues presented in the second stage, analysed together due to having all been previously presented during training.

## 2.5.2 Results

### Stage 1

The mean rates of responding (head entries into the magazine) per minute (RPM), during the auditory cues in stage 1 of training, by both wild-type and *Gria1*<sup>-/-</sup> mice, are shown in Figure 2.13. The rates of responding to all three cues increased over the first three sessions, with responding continuing to increase and then remaining high to A+, but declining and remaining low to non-reinforced cues B and C. The wild-type mice also showed a more continued increase, up to session 8, than the *Gria1*<sup>-/-</sup> mice, in which responding levelled off after session 4.

The ANOVA showed that there was a significant effect of cue,  $F(2,76) = 243.3$ ,  $p < .001$ , as well as a significant effect of genotype,  $F(1,38) = 14.1$ ,  $p = .001$ , with no significant interaction between these two factors,  $F(2,76) = .41$ ,  $p = .56$ . There was also a significant effect of training session,  $F(9,342) = 6.3$ ,  $p < .001$ , with a significant interaction between session and genotype,  $F(9,342) = 8.4$ ,  $p < .001$ , as well as between cue and session,  $F(18,684) = 59.0$ ,  $p < .001$ . The interaction between the three factors of cue, session, and genotype, neared significance,  $F(18,684) = 2.2$ ,  $p = .050$ .

Post-hoc analysis of the effect of cue further showed that responding to cue A was significantly greater than both B ( $p < .001$ ) and C ( $p < .001$ ), but that there was no significant difference in responding during presentations of cues B and C ( $p = 1.0$ ).

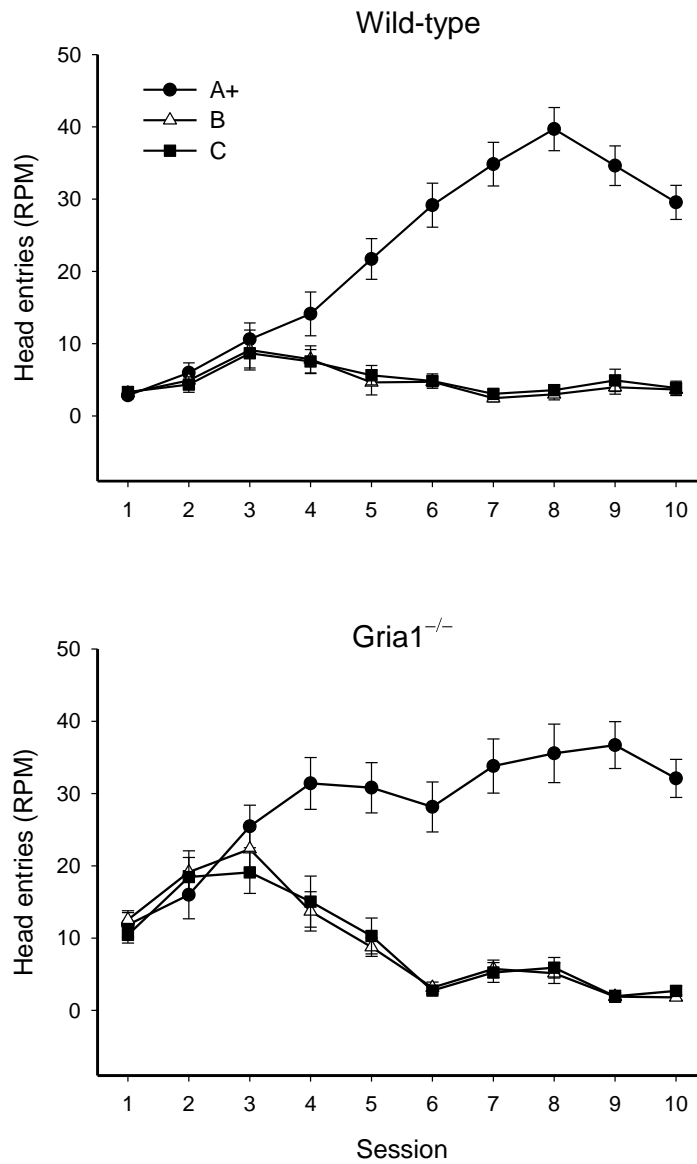


Figure 2.13. Experiment 5, training stage 1. The mean rates of responding per minute (RPM), during presentations of the three auditory cues by the wild-type (upper) and *Gria1*<sup>-/-</sup> mice (lower). Error bars show ± SEM.

## Stage 2

The mean rates of responding (RPM) by wild-type and *Gria1*<sup>-/-</sup> mice, during presentations of the 3 auditory-visual compound cues and the 3 auditory cues alone, in stage 2 of training, are shown in Figure 2.14. For the three compound cues, responding to AX+ remained high across training sessions. Levels of responding to the BY+ compound showed an increase over sessions, with the wild-type mice showing a steeper increase to reach similar levels of responding as the *Gria1*<sup>-/-</sup> mice. For the CZ compound, responding remained low in both wild-type and *Gria1*<sup>-/-</sup> mice across training. For the three auditory cues, responding to A+ remained high, with cue B showing an increase over training, and cue C continuing to show low levels of responding.

The ANOVA on the three compound cues showed that there was a significant effect of cue,  $F(2,76) = 192.9$ ,  $p < .001$ , no significant effect of genotype,  $F(1,38) = .203$ ,  $p = .66$ , as well as no significant interaction between these two factors,  $F(2,76) = 1.6$ ,  $p = .21$ . There was a significant effect of session,  $F(9,342) = 4.0$ ,  $p = .002$ , with no significant interaction between session and genotype,  $F(9,342) = .88$ ,  $p = .49$ . The interaction between cue and session was significant,  $F(18,684) = 21.8$ ,  $p < .001$ , as was the interaction between the three factors of cue, session, and genotype,  $F(18,684) = 2.6$ ,  $p = .006$ .

To further investigate the interaction between these three factors, mixed model ANOVA were carried out on the data from the first and last training sessions in this stage, for both wild-type and *Gria1*<sup>-/-</sup> mice. This allowed to test if cues that were not different at the start of training, differed by the end of this training stage, demonstrating learning to have occurred. This showed that for the wild-type mice, there was a significant effect of cue in the first session,  $F(2,48) = 137$ ,  $p < .001$ , with post-hoc analysis using the Bonferroni correction for multiple comparisons further showing that all three cues significantly differed ( $p$  values  $< .017$ ). For the *Gria1*<sup>-/-</sup> mice in the first session, there was also a significant effect of cue,  $F(2,28) = 48.1$ ,  $p < .001$ , with post-hoc analysis showing that there was no significant difference between compounds BY and CZ ( $p = 1.0$ ), but all other comparisons were significant

( $p$  values  $< .001$ ). During the final session, the wild-type mice showed a significant effect of cue,  $F(2,48) = 54.3$ ,  $p < .001$ , with post-hoc analysis further showing that there was now no significant difference between compound AX and BY ( $p = 1.0$ ), but that all other comparisons were significant ( $p$  values  $< .001$ ). For the  $Gria1^{-/-}$  mice, there was also a significant effect of cue in the last session,  $F(2,28) = 45.2$ ,  $p < .001$ , with post-hoc analysis further showing that compounds AX and BY also did not significantly differ ( $p = 1.0$ ), but that all other comparisons were significant ( $p < .001$ ).

The ANOVA on the three single auditory cues showed that there was a significant effect of cue,  $F(2,76) = 174.5$ ,  $p < .001$ , no significant effect of genotype,  $F(1,38) = .010$ ,  $p = .92$ , and no significant interaction between these two factors,  $F(2,76) = 2.2$ ,  $p = .13$ . The effect of session was significant,  $F(9,342) = 2.6$ ,  $p = .022$ , with no significant interaction between session and genotype,  $F(9,342) = 1.8$ ,  $p = 1.1$ . There was however a significant interaction between cue and session,  $F(18,684) = 11.1$ ,  $p < .001$ , but not between the three factors of cue, session, and genotype,  $F(18,684) = 1.5$ ,  $p = .12$ .

To further investigate the interaction between cue and session, and to see if cues differed by the end of training, mixed model ANOVA were carried out on the first and last training sessions. This showed that for the first session the effect of cue was significant,  $F(2,76) = 121.0$ ,  $p < .001$ , with no significant effect of genotype,  $F(1,38) = 3.0$ ,  $p = .092$ , or significant interaction between cue and genotype,  $F(2,76) = 2.1$ ,  $p = .14$ . Pairwise comparisons for the effect of cue further showed that cues B and C did not significantly differ ( $p = 1.0$ ) but that the other comparisons were all significant ( $p$  values  $< .001$ ). For the final training session, there was again a significant effect of cue,  $F(2,76) = 76.4$ ,  $p < .001$ , no significant effect of genotype,  $F(1,38) = .13$ ,  $p = .73$ , with no significant interaction between these two factors,  $F(2,76) = .12$ ,  $p = .89$ . Pairwise comparisons for the effect of cue further showed that responding to cue B was still significantly lower than to cue A ( $p = .043$ ), with all other comparisons also significant ( $p$  values  $< .001$ ).

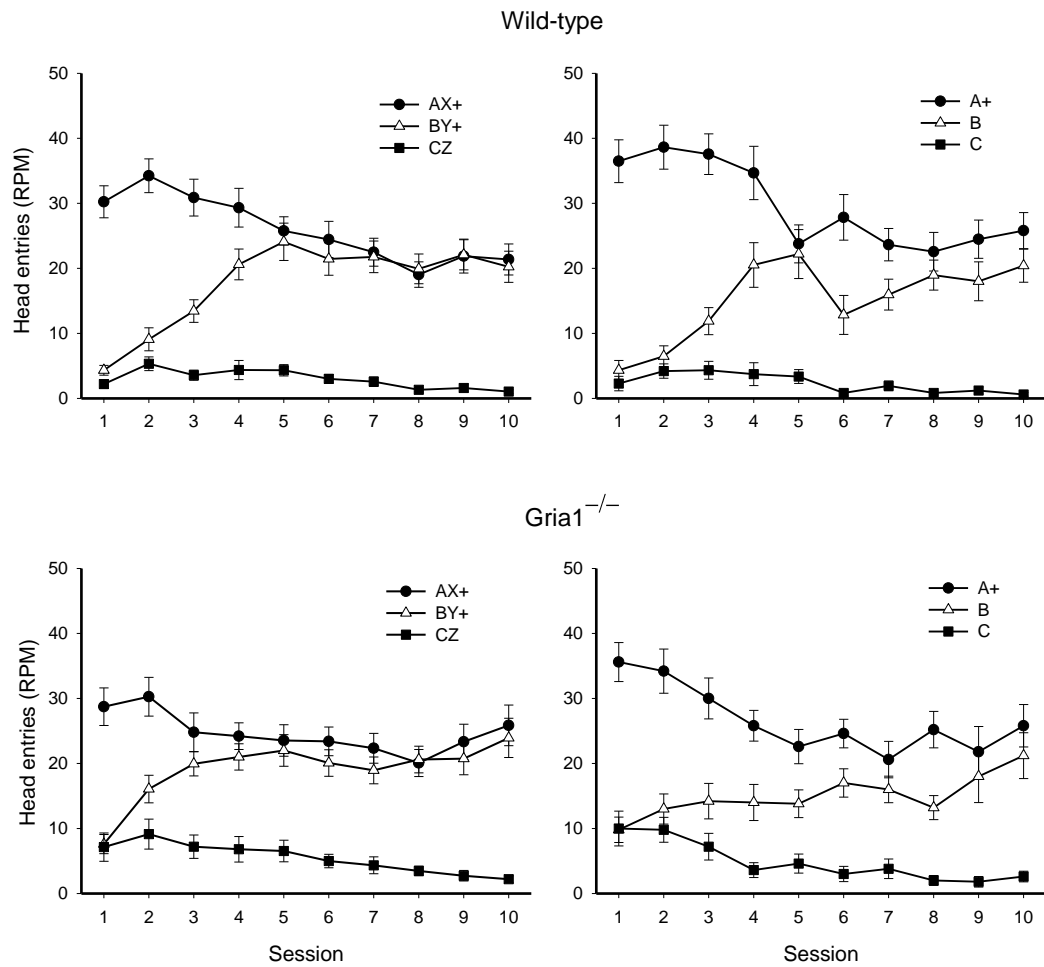


Figure 2.14. Experiment 5, training stage 2. The mean rates per minute (RPM) of responding during presentations of the three auditory-visual compound cues (left) and the three auditory cues alone (right), by the wild-type (upper) and *Gria1*<sup>-/-</sup> mice (lower). Error bars show  $\pm$  SEM.

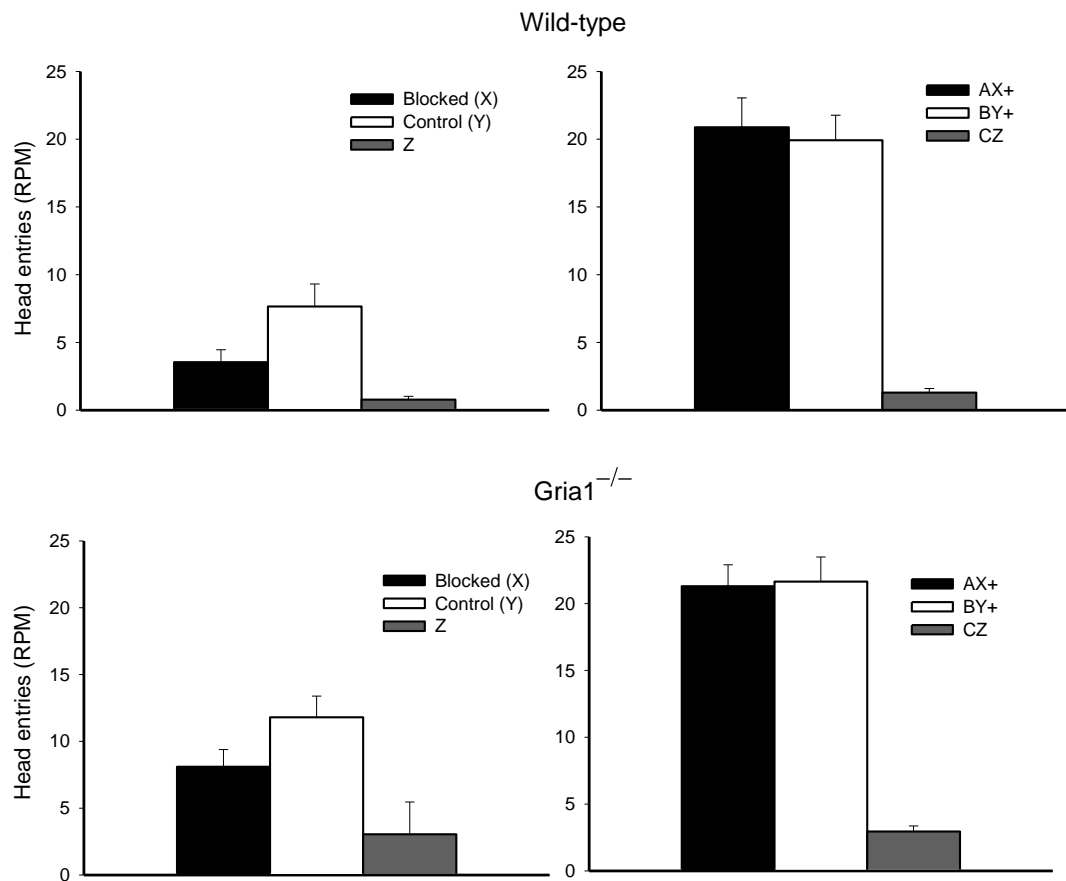
## Test

The mean rates of responding during presentations of the three test cues (left) and the three compound cues (right), averaged over the two test sessions, are shown in Figure 2.15, for both wild-type (upper) and *Gria1*<sup>-/-</sup> mice (lower). During the test presentations of the three visual cues, responding was greater to the control (Y) than the blocked cue (X), with even lower responding to the never reinforced cue (Z). This pattern was the same for both wild-type and *Gria1*<sup>-/-</sup> mice, although rates of responding were slightly higher in the *Gria1*<sup>-/-</sup> than the wild-type mice. When looking at rates of responding to the three compound cues, responding continued to be greater to the two reinforced AX+ and BY+ compounds, with low responding to CZ, and similar levels of responding shown by both genotypes.

The ANOVA on the three visual cues showed that there was a significant effect of cue,  $F(2,76) = 26.5$ ,  $p < .001$ , along with a significant effect of genotype,  $F(1,38) = 9.4$ ,  $p = .004$ . Importantly, there was no significant interaction between cue and genotype,  $F(2,76) = .65$ ,  $p = .53$ . Post-hoc analysis of the effect of cue using pairwise comparisons and Bonferroni corrected for multiple comparisons, further showed that rates of responding significantly differed between all three cues ( $p$  values  $< .004$ ).

The ANOVA on the three compound cues showed that there was again a significant effect of cue,  $F(2,76) = 146.5$ ,  $p < .001$ , no significant effect of genotype,  $F(1,38) = .44$ ,  $p = .51$ , and no significant interaction between these two factors,  $F(2,76) = .17$ ,  $p = .85$ . Post-hoc analysis of the effect of cue further showed that there was no difference between compounds AX+ and BY ( $p = 1.0$ ) but that responding to CZ was significantly lower than both AX and BY ( $p$  values  $< .001$ ).

To further investigate the difference between the blocked and control cues in each of the genotypes, repeated measure ANOVA were carried out for each of the wild-type control mice and the *Gria1*<sup>-/-</sup> mice separately comparing responding during the blocked and control cues. This showed that both the wild-type and the *Gria1*<sup>-/-</sup> responded significantly less during the blocked than the control cue (WT,  $F(1,24) = 8.3$ ,  $p = .008$ ; *Gria1*<sup>-/-</sup> mice,  $F(1,14) = 4.7$ ,  $p = .047$ ).



*Figure 2.15.* Experiment 5, test stage. The mean rates per minute (RPM) of responding during presentations of the three visual cues (left) and the three compound cues (right), by wild-type (upper) and *Gria1*<sup>-/-</sup> (lower) mice. Error bars show  $\pm$  SEM.

### 2.5.3 Discussion

The results showed that both wild-type and *Gria1*<sup>-/-</sup> mice made lower rates of responding to the blocked cue compared to the control cue during the test sessions, demonstrating a significant blocking effect in both genotypes. Furthermore, although the *Gria1*<sup>-/-</sup> mice generally showed higher rates of responding, this was seen across all cues and the lack of an interaction between test cue and genotype demonstrates blocking to a similar extent in both genotypes.

Therefore, although in the previous flavour preference experiment blocking was impaired in the *Gria1*<sup>-/-</sup> mice, this was not the case when auditory and visual cues

were used. The effect of GluA1 deletion therefore seems specific to blocking of flavour preference learning, with no impairment when auditory and visual cues in an appetitive magazine conditioning procedure are used. As cues that are highly similar, such as flavours, have been suggested to be processed more configurally rather than elementally (Dwyer et al., 2011; Soto et al., 2014) it may be that the effect of GluA1 deletion on cue-competition effects is linked to configural processing. In particular, the subunit may play a role when cue-competition depends on the level of generalisation between the cues. It does not however seem to be required for cue-competition when cues are likely processed more elementally rather than configurally. To further test if cue-competition using auditory and visual cues is intact in the *Gria1*<sup>-/-</sup> mice, mice were tested for overshadowing also using these cues. If the effect of GluA1 deletion is specific to cue-competition to configural cues, such as flavours, then it would be expected that overshadowing may also be normal in the *Gria1*<sup>-/-</sup> mice compared to the control mice, when using auditory and visual cues.

## 2.6 Experiment 6

### Overshadowing using auditory and visual cues

In the previous overshadowing of flavour preference experiment (experiment 4), rather than the *Gria1*<sup>-/-</sup> mice showing intact cue-competition, they instead failed to show any overshadowing effect. However, this failure was also seen in the wild-type mice, suggesting that the flavour cues used may have been processed in a more configural as opposed to an elemental manner. In this case, the failure to observe overshadowing could be explained as a result of a high level of generalisation between the training compound and the test cue in both genotypes (Dwyer et al., 2011).

Therefore in this experiment, the same visual and auditory cues as the previous blocking experiment (2.5) were used, but with an overshadowing procedure similar to experiment 2.4. As these cues previously resulted in a blocking effect, in both wild-

type controls and *Gria1*<sup>-/-</sup> mice, it might also be the case that overshadowing is seen here in both genotypes, despite overshadowing of flavour preference learning not previously not being evident. In this experiment, visual cues were presented during training, with one of these cues paired alongside an auditory cue, with this acting to overshadow learning about the visual cue. Any overshadowing effect should therefore be seen as a reduced rate of responding to the overshadowed visual cue compared to the control cue that was presented alone during training.

### **2.6.1 Methods**

#### **Subjects**

14 *Gria1*<sup>-/-</sup> (7 females, 7 males) and 22 wild-type mice (12 females, 10 males) bred and housed in the same way as experiment 2.1 were used. The mice were approximately 6.5-11 months old at the start of testing and caged in groups of 1-7, with 85% body weights between 18.3g – 31.4g. Mice had previous experience of appetitive magazine conditioning using auditory cues (a clicker and a white noise, one of which was reinforced) and also had experience of learning in similar operant chambers about flavour cues. They had no previous experience however learning about visual cues, or of the auditory tone cue.

#### **Apparatus**

The apparatus used were the same as in the previous blocking experiment (2.5) but with only one auditory cue (the tone) that mice had not previously experienced.

#### **Procedure**

The experimental design is shown in Table 2.5. Each daily testing session followed the same procedure as the previous blocking experiment (2.5), with 24 trials containing 10s cue presentations and 120s between cue-offset and next cue-onset. During the first stage of training, for ten daily sessions, mice were presented with three cues, AX, B, and C. Cues A, B and C were all visual cues, either a house-light, LED or flashing LED. These were counterbalanced as far as possible given the numbers with respect to genotype and sex. This meant that for a third of the mice cue A was the house-light, a third it was the LED and the remaining third it was the

flashing LED. This was the same for cues B and C, with all 6 possible combinations of cues used across all mice. Cue A was presented in compound with an auditory cue, the tone, which was the same for all mice, to form compound AX. Cues AX and B were both reinforced with a sucrose pellet at the end of the cue presentation. During this training stage the three cues were presented randomly each session, with the constraint that there were 4 presentations of each cue in every 12 trials.

Following this training phase, for two test sessions, mice were presented with non-reinforced test trials of the three visual cues, A, B, and C. These presentations were intermixed with presentations of the cues given in training, AX, B, and C, with cues AX and B continuing to be reinforced. Again the order of cue presentations was random with the constraint that within every 12 trials there were 2 presentations of each trial type (test trials: A, B, C. training trials: AX, B, C). This meant that cues A, B, AX+, and B+ were presented 4 times each over the 24 trials, and cue C presented 8 times.

Table 2.5  
*Design of experiment 6*

<b>Training</b>	<b>Test</b>
AX+	A
B+	B
C	AX+
	B+
	C

## Statistical analysis

The rates of head entry responding, per minute (RPM) were calculated and analysed using mixed model ANOVA in a similar way as the previous experiment (2.5). For the training data, mixed model ANOVA with cue, session, and genotype as factors were carried out. For the test data, rates were averaged over the two test sessions and two separate analyses done. Mixed model ANOVA with cue and genotype as factors, were carried out on the test presentations of the overshadowed and control visual cues, now presented without reinforcement in the test trials. This allowed to directly test for an overshadowing effect between the overshadowed and control cue. Mixed model ANOVA, with cue and genotype as factors, were also carried out on the three cues that were continued to be presented from training, with the same reinforcement contingencies (cues AX+, B+, and C). As the analysis up to this point does not test if responding to the non-reinforced control (cue C) was lower than to the overshadowed cue (cue A), a mixed model ANOVA was also carried out directly comparing the rates of responding during the test presentations of cues C and B, with genotype and cue as factors.

### 2.6.2 Results

#### Training

The mean rates of responding per minute (number of head entries) during presentations of the three cues, by wild-type and *Gria1*<sup>-/-</sup> mice are shown in Figure 2.16. Both genotypes showed greater rates of responding during presentation of the two rewarded cues (AX+ and B+) than the non-rewarded cue (C), with slightly greater responding to AX+ than B+. The wild-type mice also showed a slight decrease in responding to the rewarded cues over sessions, whereas the *Gria1*<sup>-/-</sup> mice showed more variable rates of responding. The ANOVA showed that there was a significant effect of cue,  $F(2,68) = 157.9$ ,  $p < .001$ , and genotype,  $F(1,34) = 11.2$ ,  $p = .002$ , with *Gria1*<sup>-/-</sup> mice responding more than the wild-type mice, as well as a significant interaction between these two factors,  $F(2,68) = 4.4$ ,  $p = .021$ . There was also a significant effect of session,  $F(9,306) = 3.8$ ,  $p = .004$ , and a significant interaction between session and genotype,  $F(9,306) = 2.9$ ,  $p = .020$ . The interaction between cue

and session was also significant,  $F(18,612) = 10.0$ ,  $p < .001$ , and there was no significant interaction between the three factors of cue, session, and genotype,  $F(18,612) = 1.8$ ,  $p = .072$ . Post-hoc analysis of the effect of cue, using the Bonferroni correction for multiple comparisons, further showed that rates of responding were significantly different to all three cues ( $p$ 's  $< .001$ ).

To further analyse the difference in rates of responding between the two genotypes, an additional repeated measures ANOVA was carried out on the pre-CS rates of responding, with cue, session and genotype as factors. This allows to test if the  $Gria1^{-/-}$  mice respond more than the wild-type control mice, even when cues are not presented. The ANOVA showed that the  $Gria1^{-/-}$  mice did respond significantly more in the pre-CS periods than the control mice,  $F(1,34) = 8.3$ ,  $p = .007$ .

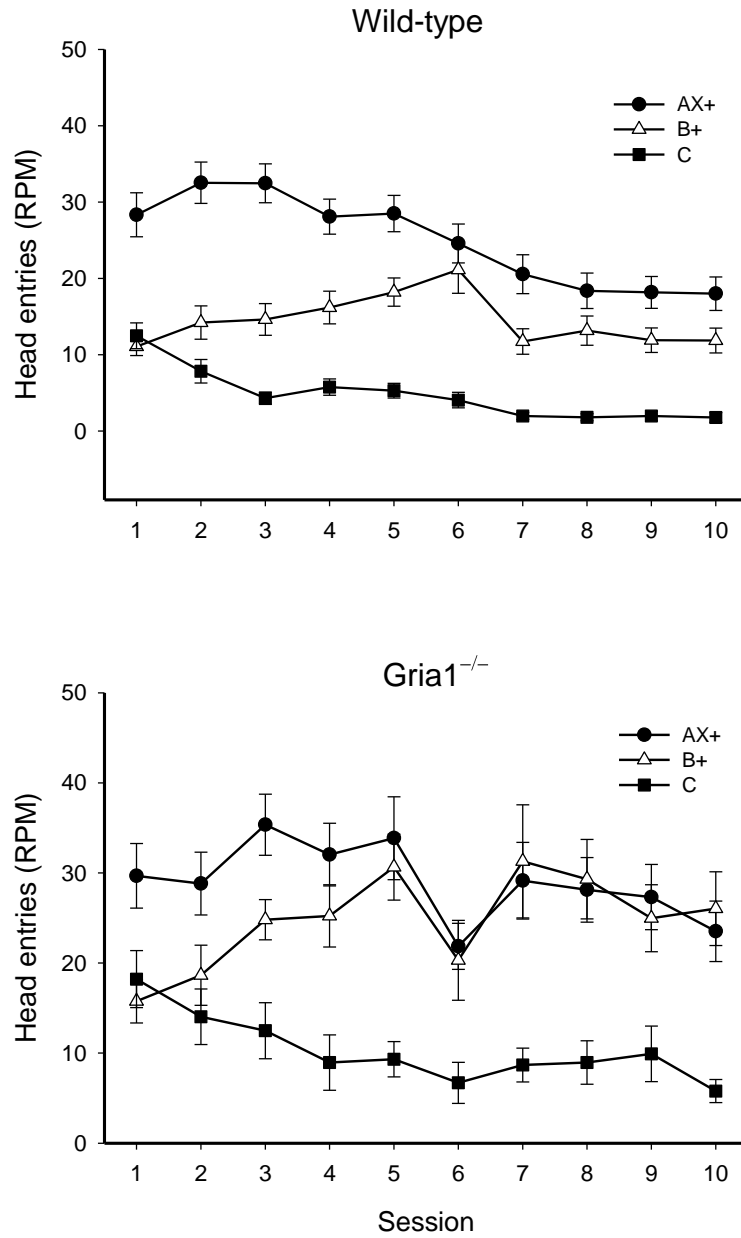


Figure 2.16. Experiment 6, training stage. The mean rates per minute (RPM) of responding during presentations of the three cues, by wild-type (upper) and *Gria1*<sup>-/-</sup> (lower) mice. Error bars show ± SEM.

## Test

The mean rates of responding to the overshadowed and control visual cues, averaged over the two sessions, by wild-type and *Gria1*<sup>-/-</sup> mice are shown in Figure 2.17 (left panel). Both wild-type (upper) and *Gria1*<sup>-/-</sup> mice (lower) made greater rates of responding during presentation of the control compared to the overshadowed cue. The *Gria1*<sup>-/-</sup> mice also generally responded more than the wild-type mice. The rates of responding to cues AX+, B+, and C, are also shown in Figure 2.17 (right panel), with both genotypes responding more to AX+ and B+, than C. The wild-type mice (upper) also showed greater responding to AX+ than B+, with the *Gria1*<sup>-/-</sup> mice (lower) showing a smaller difference between these two cues.

The ANOVA on the two test cues, A and B, showed that responding was significantly lower to the overshadowed than control cue,  $F(13.8)$ ,  $p = .001$ , along with a significant effect of genotype, demonstrating the wild-type mice responded significantly less than the *Gria1*<sup>-/-</sup> mice,  $F(1,34) = 11.7$ ,  $p = .002$ . However, the interaction between these two factors was not significant,  $F(1,34) = .43$ ,  $p = .52$ . To further test the degree of overshadowing in each of the genotypes, repeated measures ANOVA comparing the effect of cue (overshadowed or control) were carried out for each genotype. This showed that for both the *Gria1*<sup>-/-</sup> and wild type mice responding was significantly lower during the overshadowed than the control cue (WT,  $F(1,21) = 8.0$ ,  $p = .01$ ; *Gria1*<sup>-/-</sup> mice,  $F(1,13) = 5.6$ ,  $p = .034$ ).

From this analysis it is not possible however to see if responding to the overshadowed cue (cue A) was statistically greater than to the non-reinforced control (cue C). An additional ANOVA was therefore carried out comparing these two cues, with genotype as a factor. This further showed that responding to the control cue was significantly lower,  $F(1,34) = 64.5$ ,  $p < .001$ , but that there was also significant effect of genotype,  $F(1,34) = 12.5$ ,  $p = .001$ , as well as a significant interaction between cue and genotype,  $F(1,34) = 7.1$ ,  $p = .012$ . Simple main effects analysis of this interaction showed that during both the overshadowed and control cues the *Gria1*<sup>-/-</sup> mice responded significantly more than the wild-type mice (overshadowed cue,  $F(1,34) = 12.4$ ,  $p = .001$ ; control cue,  $F(1,34) = 6.4$ ,  $p = .016$ ). They also showed

that both genotypes made significantly greater rates of responding during the overshadowed than control cue (wild-type mice,  $F(1,34) = 18.5$ ,  $p < .001$ ;  $Gria1^{-/-}$  mice,  $F(1,34) = 46.9$ ,  $p < .001$ ).

The ANOVA on cues AX+, B+ and C, also showed a significant effect of cue,  $F(2,68) = 69.04$ ,  $p < .001$ , and genotype,  $F(1,34) = .006$ , as well as a significant interaction between these two factors,  $F(2,68) = 3.5$ ,  $p = .043$ .

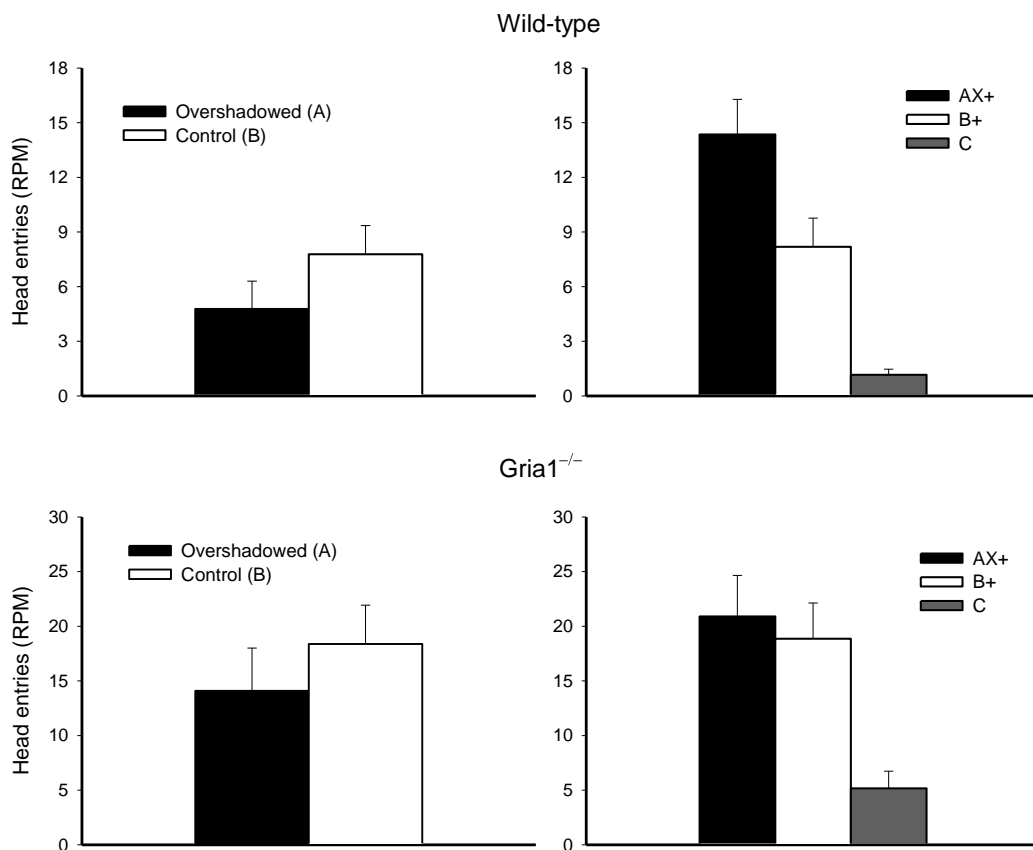


Figure 2.17. Experiment 6, test stage. The mean rates per minute (RPM) of responding during presentations of the overshadowed and control visual cues (left) and the three other cues (right), by the wild-type (upper) and  $Gria1^{-/-}$  (lower) mice. Error bars show  $\pm$  SEM.

### 2.6.3 Discussion

The results showed that the wild-type mice and the *Gria1*<sup>-/-</sup> mice made greater rates of responding during presentations of the control than the overshadowed cue. Both genotypes therefore showed an overshadowing effect when using auditory and visual cues, despite neither the wild-type nor the *Gria1*<sup>-/-</sup> mice previously showing overshadowing of flavour preference learning. As with the previous blocking experiment (2.5), the *Gria1*<sup>-/-</sup> mice did however show higher rates of responding during the cue presentations than the wild-type mice, although as this did not interact with test cue, it does seem that they show overshadowing to a similar extent as the wild-type mice. Responding was also particularly high in both the wild-type and the *Gria1*<sup>-/-</sup> mice during the early training sessions to the auditory visual compound. This was however likely the result of the previous experience the mice had in learning about auditory cues (see methods). As mice had previously learned that an auditory cue was reinforced, some of this associative strength may have initially generalised to the new auditory cue, the tone. This would account for the high rates of responding to the auditory visual compound cue that was particularly evident early in training.

The intact overshadowing in this experiment is in line with the proposed role of the GluA1 subunit in short-term memory (Sanderson et al., 2009) and the prediction that cue-competition effects would be intact in the *Gria1*<sup>-/-</sup> mice. This intact overshadowing and cue-competition also corresponds to the previous blocking experiment (2.5), in which auditory and visual cues were used and both genotypes showed a blocking effect. It therefore seems that, when using auditory and visual cues, cue-competition effects are intact in *Gria1*<sup>-/-</sup> mice, as both overshadowing and blocking effects did not significantly differ from the wild-type controls. When using flavours however this effect is less clear, with the *Gria1*<sup>-/-</sup> mice showing impaired blocking and neither genotype showing overshadowing. The role of the GluA1 subunit may therefore be specific to cue-competition when it is dependent on configural processing and the lever of generalisation between the stimuli.

However, as well as through configural processing, previous failures to observe cue-competition effects have been explained as a result of the formation of within-compound associations, (Durlach & Rescorla, 1980; Speers, Gillan, & Rescorla, 1980). These occur when an association forms between the two elements of a compound, something that could potentially explain the failures to see overshadowing and blocking. This is due to the presentation of one element alone then being able to retrieve a representation of the other cue that was previously paired with it, as a result of the within-compound association. Both the cue itself, and the retrieved representation of its associate, can therefore act to directly and indirectly predict the outcome and generate conditioned responding. In the case of overshadowing, an association between the two elements of the compound cue means that when one alone is presented during the test session, not only does it directly predict the occurrence of the outcome, but it also retrieves a memory of the other cue to indirectly predict the outcome. Taken together, the sum of these direct and indirect associations with the outcome may result in higher conditioned responding, resulting in the failure to see overshadowing. In the case of blocking, an association between the two elements of the compound presented in the second stage of training may similarly act to provide both direct and indirect associations with the outcome when the single element is presented alone during test. Again, this sum of associations, if great enough, may result in the subsequent failure to see blocking.

Within-compound associations have particularly been suggested to form when using flavour or taste cues presented together in compound (Rescorla & Cunningham, 1978). Furthermore, Durlach and Rescorla (1980) suggested their observation of potentiation, in which the presence of a taste potentiated learning about an odour rather than overshadowed it, could have occurred as a result of within-compound associations. In terms of blocking, Speers, Gillan and Rescorla (1980) also suggested that failures to observe blocking, particularly in flavour aversion paradigms, could be due to the formation of within-compound associations. It may therefore be the case that during the flavour preference conditioning procedures used in this chapter (experiments 1 - 4), within-compound associations formed between the cues used (either flavour or taste cues), resulting in the failure to see overshadowing. The

failure to see blocking in the *Gria1*<sup>-/-</sup> mice, but not the wild-type mice, could also be explained by an enhanced tendency for the knockout mice to form within-compound associations. In order to further investigate the role of within-compound associations within flavour preference learning, wild-type and *Gria1*<sup>-/-</sup> mice were run through a sensory preconditioning procedure. This provides a demonstration of the formation of within-compound associations between cues trained in compound, by assessing if subsequent learning about one of these cues results in conditioned responding to the other cue that has never directly been paired with the outcome.

## **2.7 Experiment 7**

### **Sensory preconditioning**

One way in which the previous impairment in blocking of flavour preference learning in the *Gria1*<sup>-/-</sup> mice (experiments 2.1 & 2.2) could be explained, is through an enhanced tendency to form within-compound associations. Sensory preconditioning provides a way to assess the formation of within-compound associations between cues previously presented as part of a compound. During this procedure (Rescorla & Cunningham, 1978; Ward-Robinson et al., 2002), cues are presented in compound during the first stage of training, before one stimulus from the compound is paired alone with an outcome in the second training stage. The formation of within-compound associations is then tested by presenting alone the other compound cue during the test stage. Any conditioned responding to this cue should reflect the formation of within-compound associations, as the test cue itself has never been directly paired with the unconditioned stimulus in the second stage of training. Rather, the responding can be explained through the test cue retrieving a representation of the associated cue that was directly paired with the outcome in the second stage of training. This retrieved representation is then able to indirectly predict the outcome, resulting in the conditioned responding to the test cue.

To test the prediction that the previous failure to observe blocking during flavour preference learning in the *Gria1*<sup>-/-</sup> mice was the result of enhanced within-compound associations, sensory preconditioning was carried out in both wild-type and the *Gria1*<sup>-/-</sup> mice. Although this procedure is generally conducted using a taste aversion paradigm, such as in Rescorla & Cunningham (1978) and Ward-Robinson et al. (2002), this was modified here to test for the formation of within-compound associations during flavour preference learning. Rather than the cue being paired with sickness during the second stage, the flavour cue was instead paired with a reduction in sucrose concentration. This results in a negative contrast from the first stage in which the compounds are paired with the higher concentration of 32%, generating negative prediction error which should act to reduce the preference to this flavour cue. The formation of within-compound associations should then be evident during the test sessions, in the form of a reduced preference to the cue previously paired with the devalued flavour, compared to the control flavour that was not paired with a devalued cue in stage 2. Furthermore, if GluA1 deletion enhances the tendency to form within-compound associations, then this sensory preconditioning effect would be predicted to be greater in the *Gria1*<sup>-/-</sup> mice compared to the wild-type controls.

### **2.7.1 Methods**

#### **Subjects**

17 *Gria1*<sup>-/-</sup> (6 female, 11 male) and 21 wild-type mice (8 female, 13 male), bred and housed in the same way as in experiment 2.1 were used. Mice were approximately 2-7 months old at the start of testing and caged in groups of 1-8, with 85% weights ranging between 19.8g – 21.3g. None of the mice had any previous experimental experience.

#### **Apparatus**

The apparatus used were the same as in blocking of flavour preference experiments (2.1 & 2.2). Volume was measured by comparing the graduated pipettes before and after each testing session.

## Procedure

The design of the experiment is shown in Table 2.6. Each daily testing session consisted of the same 15-minute duration as previous flavour preference experiments (1 & 3), with the sipper tube containing the sucrose solution available after the first 5-minutes. During the first training stage mice were presented with two compound flavour cues, cues AX and BY, both paired with 32% sucrose. These were presented in a double alternating order over sessions, so that over the course of 8 daily testing sessions mice had 4 presentations of each compound flavour cue. These flavours were counterbalanced with respect to genotype and sex as far as possible given the numbers, so that for half of the mice A and B were either cherry or grape Kool-Aid flavours, and for the other half these were either apple or orange. The allocation of these two flavours within these two groups was also counterbalanced as far as possible. For example, for those mice in which A and B were cherry or grape, for half A was cherry and B was grape, for the other half A was grape and B was cherry. This was the same for mice in which A and B were apple and orange. For flavours X and Y, for mice in which A and B were cherry or grape, these were either apple or orange. Those in which A and B were apple or orange, X and Y were cherry or grape. These were also counterbalanced in the same way as flavours A and B, so that for those in which X and Y were cherry and grape, half had cherry as X and grape as Y, with the same for those in which X and Y were apple and orange.

The compounds were presented so that half received AX first and for the remaining half BY was given first. For mice in which flavours A and B were apple and orange, they were presented with the apple containing compound first. Mice in which A and B were cherry and grape received the cherry containing compound first. During the second stage of training mice were then presented with flavour A paired with 32% sucrose and flavour B paired with 4% sucrose, also for four sessions each with 8 sessions in total. These were again presented in double alternation, continuing the same pattern as during stage 1, so that mice had either the apple or cherry flavour first in the double alternating sequence. This meant that half received flavour cue A first and the remaining half flavour cue B. In the first test stage, flavours X and Y were both presented alone, paired with 4% sucrose, to test for a sensory preconditioning

effect. These were each presented for two test sessions, in double alternation. The test order was counterbalanced with respect to the flavours from the first and second stage of training, with half of the mice receiving cue X first and half cue Y. In the second test stage there were two further test sessions with each of the cues A and B, to test for learning in the second training stage. These were also each presented in double alternation and counterbalanced with respect to training and test order as well as to flavour allocation as far as possible given the numbers.

Table 2.6

*Design of experiment 7*

	Stage 1	Stage 2	Test Stage 1	Test Stage 2
Control	AX-32%	A-32%	X-4%	A-4%
Experimental	BY-32%	B-4%	Y-4%	B-4%

**Statistical Analysis**

Results were analysed in a similar way as experiment 2.1. Mixed model ANOVA, with genotype, cue, and session, were carried out on the training data. For the test stage, as with the previous flavour preference experiments (1, 2 & 3), although two test sessions were run only the data from the first test session were analysed. This was due to the flavour preference effect, as seen in experiment 1, only being evident in the first test session. Mixed model ANOVA, with genotype and cue as factors, were carried out on the data from the test session. For one mouse (wild-type) during the second training stage, lick cluster sizes were unable to be calculated for two sessions, due to insufficient licks or bouts, so was excluded from the analysis, of lick cluster size, for the second training stage.

## 2.7.2 Results

### Stage 1

#### Total Licks:

The mean total numbers of licks made during consumption of the two compound flavour cues, by the wild-type and *Gria1*<sup>-/-</sup> mice during stage 1 of training, are shown in Figure 2.18 (upper panel). For the wild-type mice the numbers of licks made were similar during consumption of the two compound cues and showed an increase over sessions. For the *Gria1*<sup>-/-</sup> mice however, the number of licks showed a slight decrease over sessions and they also made fewer licks during consumption of AX than BY in sessions 2 and 3. The ANOVA showed that there was no significant effect of cue,  $F(1,36) = 1.3$ ,  $p = .25$ , but that the effect of genotype was significant,  $F(1,36) = 6.5$ ,  $p = .015$ , and there was no significant interaction between these two factors,  $F(1,36) = 2.8$ ,  $p = .102$ . There was also a significant effect of session,  $F(3,108) = 12.6$ ,  $p < .001$ , as well as a significant interaction between genotype and session,  $F(3,108) = 40.8$ ,  $p < .001$ . The interaction between cue and session was not significant,  $F(3,108) = 1.04$ ,  $p = .36$ , and there was no significant interaction between the three factors of cue, session, and genotype,  $F(3,108) = .85$ ,  $p = .43$ .

#### Lick Cluster Size:

The mean lick cluster sizes made during consumption of the two compound cues, by both genotypes during the first stage of training, are shown in Figure 2.18 (middle panel). The *Gria1*<sup>-/-</sup> mice made smaller mean lick cluster sizes than the wild-type mice, but for both wild-type and *Gria1*<sup>-/-</sup> mice there was little difference in cluster sizes made during consumption of the two compound cues. The ANOVA showed that there was no significant effect of cue,  $F(1,36) = 1.9$ ,  $p = .18$ , a significant effect of genotype,  $F(1,36) = 15.5$ ,  $p < .001$ , and no significant interaction between these two factors,  $F(1,36) = .82$ ,  $p = .37$ . There was a significant effect of session,  $F(3,108) = 12.4$ ,  $p < .001$ , as well as a significant interaction between session and genotype,  $F(3,108) = 6.7$ ,  $p = .001$ . The interaction between cue and session was not significant,  $F(3,108)$

= .13,  $p = .90$ , as was the interaction between the three factors of cue, session, and genotype,  $F(3,108) = 1.6$ ,  $p = .21$ .

#### Volume:

The mean volumes consumed of the two compound flavour cues during stage 1 of training, by both the wild-type and *Gria1*<sup>-/-</sup> mice, are shown in Figure 2.18 (lower panel). The wild-type mice consumed more of both flavour cues than the *Gria1*<sup>-/-</sup> mice. Although the volumes of the two cues were generally similar, the *Gria1*<sup>-/-</sup> mice consumed slightly less of AX in sessions 2 and 3. The wild-type mice also showed an increase in the amount consumed over sessions, of both cues, whereas the *Gria1*<sup>-/-</sup> mice showed a slight decline. The ANOVA showed that there was no significant effect of cue,  $F(1,36) = .98$ ,  $p = .33$ , but that the effect of genotype was significant,  $F(1,36) = 5.1$ ,  $p = .03$ , and there was no significant interaction between these two factors,  $F(1,36) = 2.2$ ,  $p = .14$ . There was a significant effect of session,  $F(3,108) = 13.8$ ,  $p < .001$ , as well as significant interaction between session and genotype,  $F(3,108) = 26.1$ ,  $p < .001$ . The interaction between cue and session was not significant,  $F(3,108) = .65$ ,  $p = .55$ , with no significant interaction between the three factors of cue, session, and genotype,  $F(3,108) = .38$ ,  $p = .72$ .

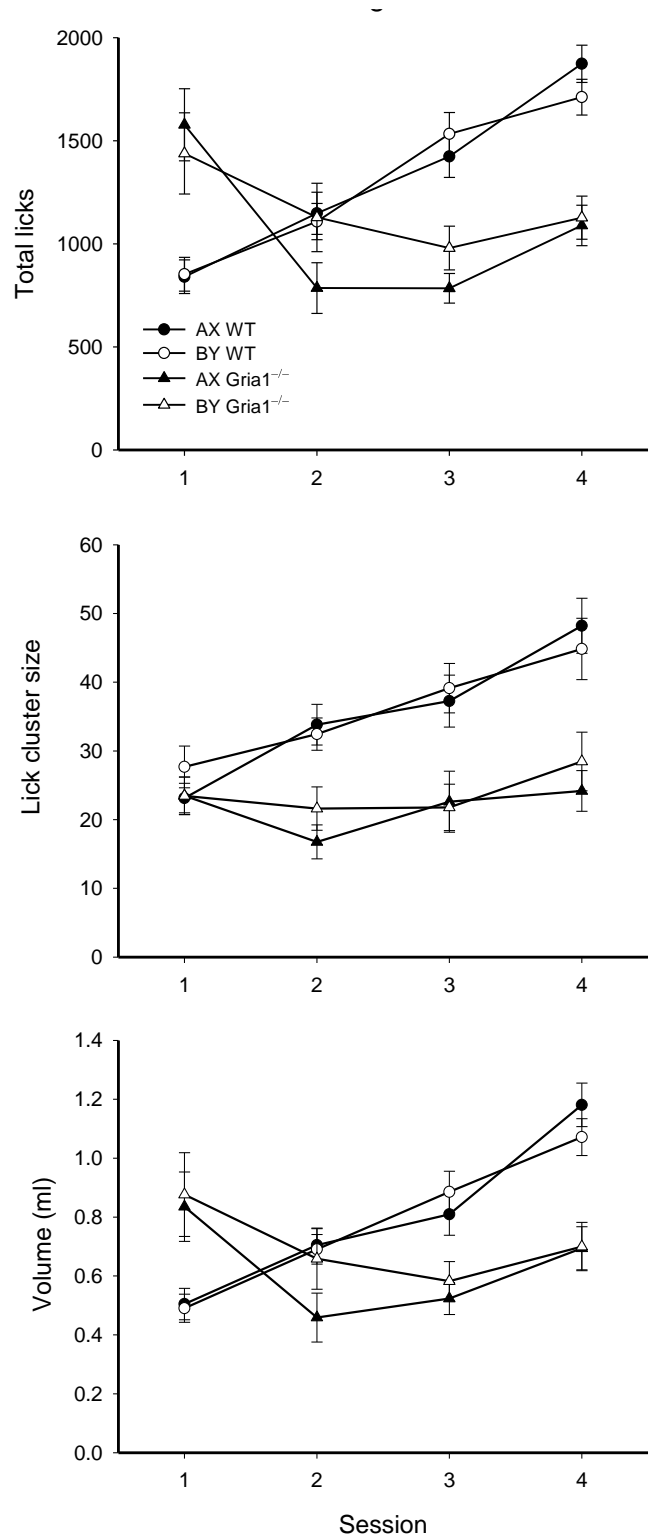


Figure 2.18. Experiment 7, training stage 1. The mean numbers of total licks (upper), lick cluster sizes (middle) and volumes (lower) consumed, by wild-type (WT) and Gria1<sup>-/-</sup> mice. Error bars show ± SEM.

## Stage 2

### Total Licks:

The mean total numbers of licks made during consumption of the two flavour cues (A32% and B4%), by the wild-type and *Gria1*<sup>-/-</sup> mice during the second training stage, are shown in Figure 2.19 (upper panel). Both genotypes made a greater number of licks during consumption of cue A, paired with 32% sucrose, than B, now paired with the lower 4% sucrose. The wild-type mice also made a greater number of licks than the *Gria1*<sup>-/-</sup> mice during consumption of both flavour cues. There was also a slight decrease in the numbers of licks over training, with this effect greatest in the wild-type mice during consumption of flavour A. The ANOVA showed that there was a significant effect of flavour cue,  $F(1,36) = 51.6$ ,  $p < .001$ , and genotype  $F(1,36) = 16.4$ ,  $p < .001$ , as well as a significant interaction between these two factors,  $F(1,36) = 8.5$ ,  $p = .006$ . The effect of session was also significant,  $F(3,108) = 30.5$ ,  $p < .001$ , and there was a significant interaction between session and genotype,  $F(3,108) = 3.1$ ,  $p = .031$ . The interaction between cue and session was not significant,  $F(3,108) = 2.4$ ,  $p = .069$ , and there was also no significant interaction between the three factors of cue, session, and genotype,  $F(3,108) = 2.0$ ,  $p = .12$ .

### Lick Cluster Size:

The mean lick cluster sizes made during consumption of the two flavour cues in the second training stage, by wild-type and *Gria1*<sup>-/-</sup> mice, are shown in Figure 2.19 (middle panel). Both the wild-type and *Gria1*<sup>-/-</sup> mice made larger mean lick cluster sizes during consumption of flavour A, paired with 32% sucrose, than flavour B, paired with 4%, although this difference was smaller in the *Gria1*<sup>-/-</sup> mice than the wild-type mice. The *Gria1*<sup>-/-</sup> mice also showed reduced mean lick cluster sizes compared to the wild-type mice. The ANOVA showed that there was a significant effect of flavour cue,  $F(1,35) = 108.1$ ,  $p < .001$ , and genotype,  $F(1,35) = 34.4$ ,  $p < .001$ , as well as a significant interaction between these two factors,  $F(1,35) = 17.7$ ,  $p < .001$ . There was no significant effect of session,  $F(3,105) = 1.3$ ,  $p = .28$ , or significant interactions between session and genotype,  $F(3,105) = 2.4$ ,  $p = .094$ , cue and session,

$F(3,105) = 1.8, p = .16$ , or between the three factors of cue, session, and genotype,  $F(3,105) = 1.5, p = .22$ .

Volume:

The mean volumes consumed of the two flavour cues, by the wild-type and *Gria1*<sup>-/-</sup> mice, are shown in Figure 2.19 (lower panel). The wild-type mice consumed more than the *Gria1*<sup>-/-</sup> mice, but both genotypes consumed more of flavour A (paired with 32%) than B (paired with 4%). There was also a gradual reduction in consumption over sessions, for both genotypes and cues, although this was less so for the *Gria1*<sup>-/-</sup> mice during consumption of flavour A. The ANOVA showed that there was a significant effect of flavour cue,  $F(1,36) = 42.5, p < .001$ , and a significant effect of genotype,  $F(1,36) = 15.1, p < .001$ , with no significant interaction between these two factors,  $F(1,36) = .84, p = .37$ . The effect of session was also significant,  $F(3,108) = 24.2, p < .001$ , as were the interactions between session and genotype,  $F(3,108) = 3.6, p = .019$ , cue and session,  $F(3,108) = 3.0, p = .04$ , and between the three factors of cue, session, and genotype,  $F(3,108) = 1.2, p = .31$ .

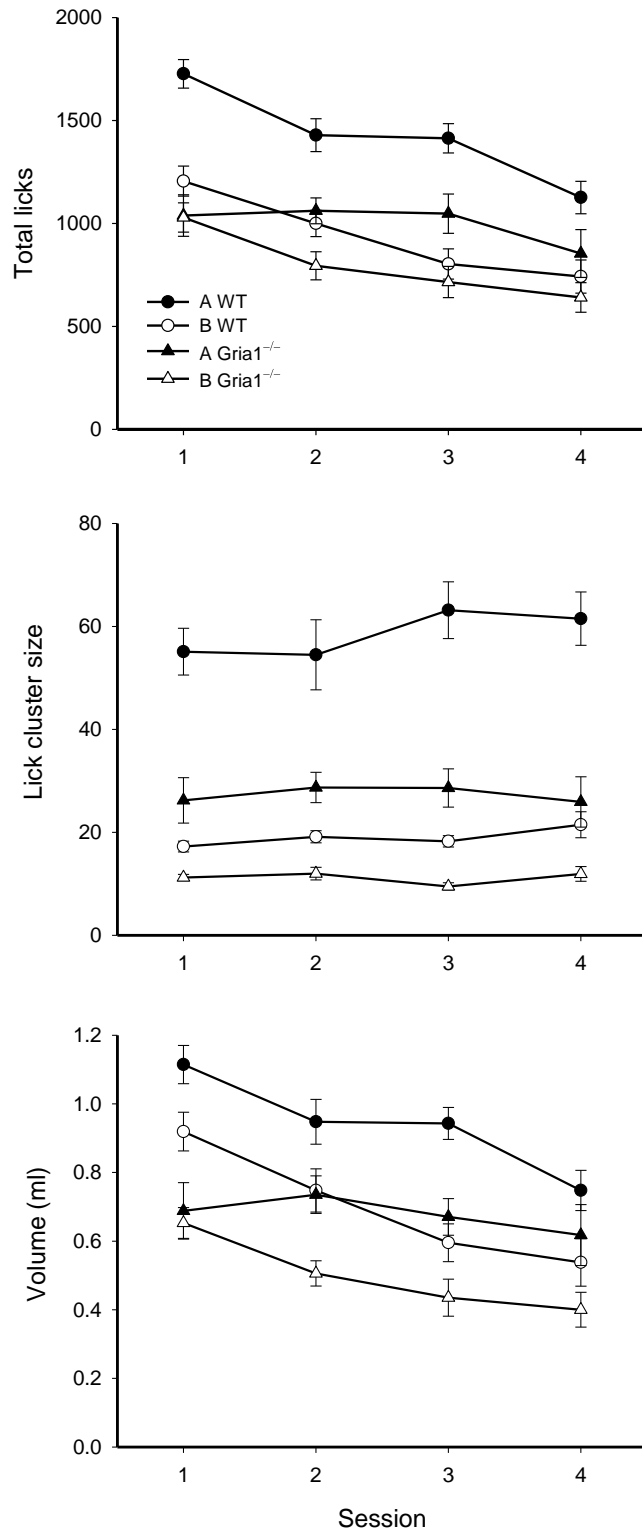


Figure 2.19. Experiment 7, training stage 2. The mean numbers of total licks (upper), lick cluster sizes (middle) and volumes (lower) consumed, by wild-type (WT) and Gria1<sup>-/-</sup> mice. Error bars show ± SEM.

## Test Stage 1

### Total Licks:

The mean total numbers of licks made during consumption of the control flavour cue (previously paired with the non-devalued flavour in stage 2 of training) and the experimental flavour cue (previously paired with the devalued flavour in stage 2) flavour cues during the first test stage, by wild-type and *Gria1*<sup>-/-</sup> mice, are shown in Figure 2.20 (upper panel). The wild-type and *Gria1*<sup>-/-</sup> mice both made similar numbers of licks during consumption of the control and experimental flavour cues. The *Gria1*<sup>-/-</sup> mice did however consume less of both cues than the wild-type control mice. The ANOVA showed that the effect of cue (control or experimental) was not significant,  $F(1,36) = .33$ ,  $p = .57$ , but there was a significant effect of genotype,  $F(1,36) = 4.6$ ,  $p = .038$ , and there was no significant interaction between cue and genotype,  $F(1,36) = .23$ ,  $p = .63$ .

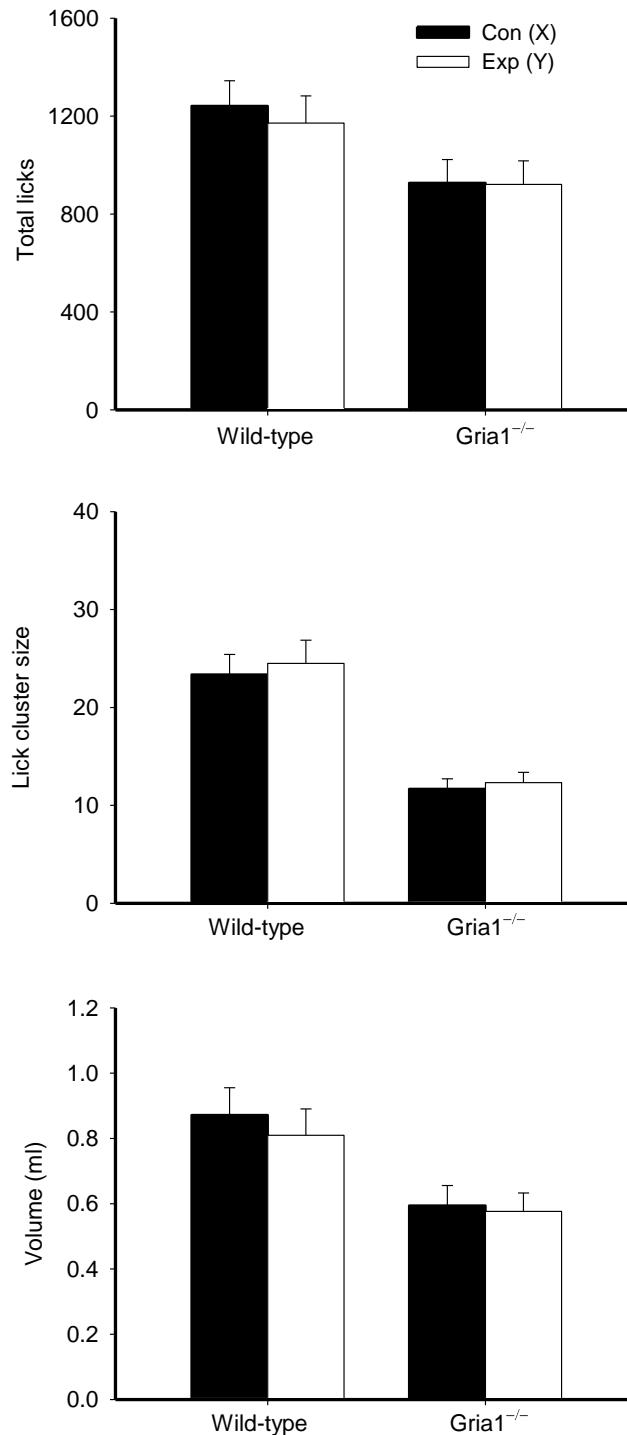
### Lick Cluster Size:

The mean lick cluster sizes made during consumption of the control and experimental flavour cues, by wild-type and *Gria1*<sup>-/-</sup> mice during the first test stage, are shown in Figure 2.20 (middle panel). The *Gria1*<sup>-/-</sup> mice made smaller mean lick cluster sizes during consumption of both cues than the wild-type mice. For both wild-type and *Gria1*<sup>-/-</sup> mice however, the mean lick cluster sizes were similar during consumption of the two flavour cues (experimental or control). The ANOVA showed that there was no significant effect of flavour cue,  $F(1,36) = .49$ ,  $p = .49$ , a significant effect of genotype,  $F(1,36) = 26.6$ ,  $p < .001$ , and no significant interaction between these two factors,  $F(1,36) = .035$ ,  $p = .85$ .

### Volume:

The mean volumes consumed of the control and experimental flavour cues, by the wild-type and *Gria1*<sup>-/-</sup> mice during the first test stage, are shown in Figure 2.20 (lower panel). The *Gria1*<sup>-/-</sup> consumed less than the wild-type mice of both the control and experimental flavour cues, with neither genotype showing a difference in consumption between the two cues. The ANOVA showed the effect of flavour cue on

consumption was not significant,  $F(1,36) = .64$ ,  $p = .43$ , but that there was a significant effect of genotype,  $F(1,36) = 7.3$ ,  $p = .011$ , and no significant interaction between these two factors,  $F(1,36) = .20$ ,  $p = .66$ .



*Figure 2.20.* Experiment 7, test stage 1. The mean numbers of total licks (upper), lick cluster sizes (middle) and volumes (lower) consumed, of the control (X) and experimental (Y) flavour cues, by wild-type (WT) and *Gria1*<sup>-/-</sup> mice. Error bars show  $\pm$  SEM.

## Test Stage 2

### Total Licks:

The mean numbers of total licks made during consumption of the two flavour cues, A (control flavour, previously paired with 32%) and B (experimental flavour, previously paired with 4%), by the wild-type and *Gria1*<sup>-/-</sup> mice during the second test stage, are shown in Figure 2.21. Both genotypes made a greater number of licks during consumption of the control (A) than the experimental (B) flavour cue. The *Gria1*<sup>-/-</sup> mice also made fewer licks than the wild-type mice during consumption of both flavour cues. The ANOVA showed that the effect of cue was significant,  $F(1,36) = 38.7$ ,  $p < .001$ , as was the effect of genotype,  $F(1,36) = 10.8$ ,  $p = .002$ , and there was no significant interaction between these two factors,  $F(1,36) = .28$ ,  $p = .603$ .

### Lick Cluster Size:

The mean lick cluster sizes made during consumption of the control and experimental flavour cues, by wild-type and *Gria1*<sup>-/-</sup> mice in the second test stage, are shown in Figure 2.21 (middle panel). The *Gria1*<sup>-/-</sup> made smaller mean lick cluster sizes during consumption of both cues than the wild-type mice. There was also little difference in mean lick cluster sizes made during consumption of the control compared to the experimental flavour cue for either genotype. The ANOVA showed that there was no significant effect of cue,  $F(1,36) = .00$ ,  $p = .98$ , a significant effect of genotype,  $F(1,36) = 22.4$ ,  $p < .001$ , and also no significant interaction between these two factors,  $F(1,36) = .16$ ,  $p = .69$ .

### Volume:

The mean volumes consumed of the control and experimental flavour cues, by wild-type and *Gria1*<sup>-/-</sup> mice in the second test stage, are shown in Figure 2.21 (lower panel). The *Gria1*<sup>-/-</sup> mice consumed less than the wild-type mice, although both genotypes showed slightly greater consumption of the control than the experimental flavour cue. The ANOVA showed that there was a significant effect of cue,  $F(1,36) = 9.1$ ,  $p = .005$ , and genotype,  $F(1,36) = 7.2$ ,  $p = .011$ , with no significant interaction between these two factors,  $F(1,36) = 1.3$ ,  $p = .26$ .

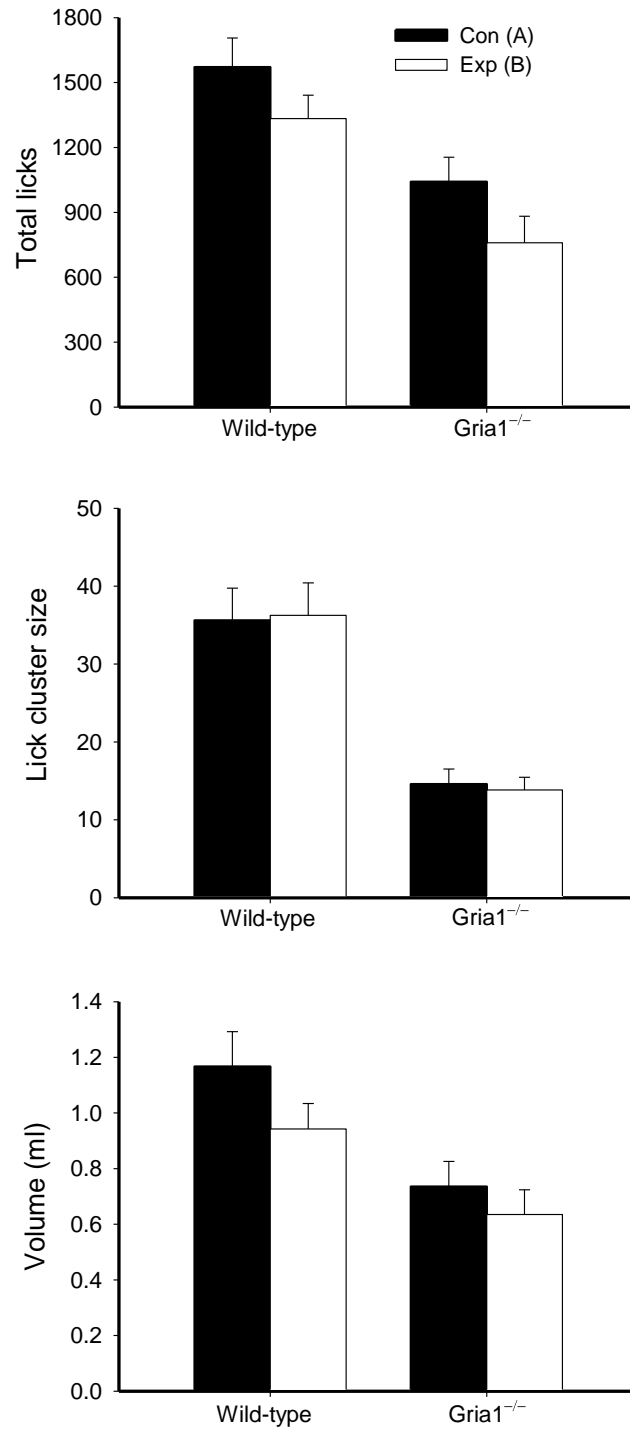


Figure 2.21. Experiment 7, test stage 2. The mean numbers of total licks (upper), lick cluster sizes (middle) and volumes (lower) consumed, of the control (A) and experimental (B) flavour cues, by wild-type (WT) and Gria1<sup>-/-</sup> mice. Error bars show  $\pm$  SEM.

### 2.7.3 Discussion

The results demonstrate that there was no sensory preconditioning effect, in either the wild-type or the *Gria1*<sup>-/-</sup> mice. As rather than showing a reduced preference for the experimental flavour cue, the total numbers of licks, mean lick cluster sizes, and volume consumed, were all similar during consumption of the control and experimental flavour cues in the first test stage. This shows that pairing the experimental cue with a flavour that was subsequently devalued (by pairing this flavour with a lower sucrose concentration) did not act to reduce the flavour preference for the experimental cue compared to the control.

The results also showed that the *Gria1*<sup>-/-</sup> mice did generally consume less than the wild-type mice, however this effect did not interact with flavour cue, supporting the absence of a sensory preconditioning effect in both genotypes. It was also not the case that the failure to see an effect was due to a failure to learn in the second test stage, as both wild-type and *Gria1*<sup>-/-</sup> mice showed a flavour preference for the control cue that was not devalued in the second stage of training. Although this effect wasn't seen in the mean lick cluster sizes made during consumption, it was seen in both measures of intake (total licks and volumes consumed). This shows that mice did learn the devaluation in the second stage of training, but this did not result in subsequent sensory preconditioning towards the experimental flavour cue in the first test stage. The lack of sensory preconditioning, in either genotype, suggests that within-compound associations did not form in this experiment, as the learning about the devalued flavour did not result in indirect conditioned responding to the cue previously paired with it (the experimental flavour). However, it could also be the case that the procedure used was not sensitive enough to see any evidence of within-compound associations that may have formed during compound training.

It cannot therefore be seen to what extent *GluA1* deletion may enhance the formation of within-compound associations, something that could explain the impaired blocking in the *Gria1*<sup>-/-</sup> mice. The lack of effect in this study is despite previous findings of sensory preconditioning in taste aversion studies in rats (Rescorla & Cunningham, 1978; Ward-Robinson et al., 2002). In these previous

experiments, the cue presented in the second stage was paired with a sickness inducing substance, rather than a reduced sucrose concentration as used in this experiment. It may be the case that this procedure is more optimal for sensory preconditioning to be seen, with the US of sickness potentially being more motivationally salient than a decrease in sucrose concentration. In order to further test the role of GluA1 deletion on the formation of within-compound associations, sensory preconditioning was carried out again, but this time using a taste aversion procedure.

## **2.8 Experiment 8**

### **Sensory preconditioning of taste aversion**

To further assess the formation of within-compound associations in wild-type and *Gria1*<sup>-/-</sup> mice, mice were tested for sensory preconditioning of taste aversion. Whereas in the previous experiment flavour cues paired with sucrose were used, in this experiment taste cues were used, with injections of sickness inducing lithium chloride (LiCl) as the unconditioned stimulus. This sickness was used to devalue the cue in the second stage, whereas the previous experiment used a reduction in the concentration of sucrose. This experiment also differed to the previous experiment in being a between-subjects design. For the experimental group, the test cue was indirectly paired with sickness, as a result of its associated cue being directly paired with sickness. For the control group, the test cue was not indirectly paired with sickness, rather a separate cue, never presented in compound, was consumed before the LiCl injection. Any sensory preconditioning effect should therefore be seen in a reduced intake of the first test cue in the experimental group compared to the control group. To ensure the mice were sufficiently motivated to consume the taste solutions, which are not paired with palatable sucrose, mice were placed on a water restriction schedule. This sensory preconditioning procedure is similar to previous sensory preconditioning studies (Rescorla & Cunningham, 1978; Ward-Robinson et

al., 2002) in which taste aversion with LiCl and water restriction were also used. If GluA1 deletion enhances the formation of within-compound associations, then it would be predicted that sensory preconditioning may also be enhanced in the *Gria1*<sup>-/-</sup> mice compared to the wild-type mice. This would be due to the taste cue presented in the test session being able to retrieve a representation of its associated compound cue to a greater extent in the *Gria1*<sup>-/-</sup> mice, resulting in greater taste aversion to this cue in the *Gria1*<sup>-/-</sup> mice.

### **2.8.1 Methods**

#### **Subjects**

33 *Gria1*<sup>-/-</sup> (21 female, 12 male) and 27 wild-type mice (14 female, 13 male), bred and housed in the same way as experiment 2.1 were used. They were caged in groups of 1- 9 and ranged between approximately 2.5 - 9 months old at the start of testing. Mice were placed on a water restriction schedule with bottles returned to the home cages for one hour a day, given after each daily testing session, with ad libitum access to food (lab chow pellets) and free-feeding weights of between 15.6g – 30.5g. All mice had previous experience of appetitive magazine conditioning in the same apparatus as used in this experiment, as well as learning about flavour cues in similar operant chambers.

#### **Apparatus**

In addition to the same operant boxes and pipettes as experiment 2.1, the taste solutions were either salt (0.1M); quinine (.00006M) or hydrochloric acid (.01M). Lithium chloride was also used (0.2M) given via intraperitoneal injection (either 20ml/kg in the first run or 40ml/kg in the second run of the experiment).

#### **Procedure**

The design of the experiment is shown in Table 2.7. Mice were allocated to one of two groups of 30 animals, with respect to genotype and sex as far as was possible. The experimental group (17 *Gria1*<sup>-/-</sup> mice: 11 females, 6 males. 13 wild-type mice: 7 females, 6 males) or the control group (16 *Gria1*<sup>-/-</sup> mice: 10 females, 6 males. 14 wild-type mice: 7 females, 7 males). The experiment was carried out in two separate runs,

with 38 mice in the first run (20 wild-type mice, 18 *Gria1*<sup>-/-</sup> mice) and the remaining 22 in the second run (7 wild-type mice, 15 *Gria1*<sup>-/-</sup> mice). The procedures for both runs were the same, with the exception of a higher dose of LiCl and the addition of recovery days in the second run of the experiment. During the first stage of training both groups (experimental and control) were presented with a compound taste solution (AB) and another single taste (C). The three tastes used, salt (0.1M), bitter (.0006M quinine), and sour (.01M HCl), were counterbalanced across mice with respect to genotype, as far as possible given the number of mice per group. In each daily session the sipper tube was presented after 5-minutes in the testing chamber, which remained accessible for 40-minutes. During training, the two taste solutions were presented in double alternation, starting with the compound (AB) cue for all mice.

In the second stage mice were again presented with daily testing sessions although the time of access to the sipper tube was reduced to 10-minutes. This was done to reduce the time between consuming the taste solution and receiving the LiCl injection, to try and maximise the association between the taste solution and the sickness effect. All mice received one session with taste cue A and another with taste cue C. The order of presentations was counterbalanced within groups and as far as possible with respect to flavour combination, given the numbers of mice. This meant that on each daily session within this stage, approximately half received a LiCl injection and the remaining half did not. For mice in the experimental group, taste A was followed by sickness inducing lithium chloride, those in the control group cue C was instead paired with LiCl. The LiCl (0.2M, either 20ml/kg or 40ml/kg) was given via IP injection as soon as possible after the 10-minute session ended, with all mice initially returned to their home cages before those to be injected individually removed and the IP injection given. Mice in the first run received the lower dose of LiCl, and those in the second run the higher dose. The mice with the higher dose also received two recovery days, one between each of the two days of this stage of training. During these recovery days the water bottles were returned to the home cages immediately following the training session, before being removed the subsequent day at the same time their hour of access would otherwise have ended.

Three daily test sessions were then carried out, with both groups receiving taste cues B, A, and C, in that order respectively, with 40-minutes of access to the sipper tube, the same as during the first training stage.

Table 2.7  
*Design of experiment 8*

	<b>S1</b>	<b>S2</b>	<b>Test</b>
<b>Control</b>	AB	A	B, A, C
	C	C+	
<b>Experimental</b>	AB	A+	B, A, C
	C	C	

### **Statistical analysis**

Statistical analysis were carried out in a similar way to experiment 1. For the data from the first training stage, mixed model ANOVA were carried out, with genotype, replication, group, cue, and session as factors. Similar ANOVA, without session, were carried out for the data from the second stage of training. For the test stage data, a one-way ANOVA was carried out for each of the three taste cues that were tested in succession (B, A, and C), with group, genotype, and replication as factors. Replication was added as a factor to account for any variance resulting from the slight differences in procedure between the first and second run of the experiment, such as altered dose of lithium chloride. One volume measurement was lost during testing of taste cue B, meaning the analysis of volume contains 59 rather than 60 volumes.

## 2.8.2 Results

### Stage 1:

#### Total Licks:

The mean total numbers of licks made during consumption of the compound AB and single taste cue C during stage 1 of training, by wild-type and *Gria1*<sup>-/-</sup> mice in the control and experimental groups, are shown in Figure 2.22 (upper panel). The numbers of licks did show some variation across sessions, although the *Gria1*<sup>-/-</sup> mice generally made slightly fewer licks during consumption of the two cues (AB and C) than the wild-type mice. The ANOVA showed that there was no significant effect of cue,  $F(1,52) = .31$ ,  $p = .58$ , and there were no significant interactions between cue and group  $F(1,52) = 2.1$ ,  $p = .15$ , cue and genotype,  $F(1,52) = .89$ ,  $p = .35$ , or cue and replication,  $F(1,52) = .45$ ,  $p = .51$ . There was however a significant effect of session,  $F(3,156) = 6.7$ ,  $p = .001$ , with no significant interaction between session and group  $F(3,156) = 1.1$ ,  $p = .37$ , session and genotype,  $F(3,156) = 2.6$ ,  $p = .068$ , or session and replication,  $F(3,156) = 1.04$ ,  $p = .37$ . Although there was a significant interaction between cue and session,  $F(3,156) = 10.4$ ,  $p < .001$ . There was a significant effect of genotype, with *Gria1*<sup>-/-</sup> mice making a significantly smaller number of licks than the wild-type mice,  $F(1,52) = 6.2$ ,  $p = .016$ . The effect of group however was not significant,  $F(1,52) = 1.1$ ,  $p = .29$ , and there was no significant effect of replication,  $F(1,52) = 1.7$ ,  $p = .19$ . All other interactions were also not significant,  $F$  values  $< 2.7$ ,  $p$  values  $> .054$ .

#### Lick Cluster Size:

The mean lick cluster sizes made during consumption of the two cues, by both groups and genotypes during the first stage of training, are shown in Figure 2.22 (middle panel). For both groups and cues, the *Gria1*<sup>-/-</sup> mice made smaller mean lick cluster sizes during consumption than the wild-type mice. The mean lick cluster sizes were also generally slightly greater during consumption of cue C than the compound cue AB. The ANOVA showed that there was a significant effect of cue, making significantly smaller lick cluster sizes during consumption of cue C than the compound AB,  $F(1,52)$

= 5.5,  $p = .023$ , and no significant interactions between cue and group  $F(1,52) = 1.9$ ,  $p = .17$ , cue and genotype,  $F(1,52) = .039$ ,  $p = .84$ , or between cue and replication,  $F(1,52) = .17$ ,  $p = .68$ . There was also a significant effect of session,  $F(3,156) = 8.03$ ,  $p = .001$ , with no significant interactions between session and group,  $F(3,156) = .01$ ,  $p = .99$ , session and genotype,  $F(3,156) = 2.4$ ,  $p = .104$ , or session and replication,  $F(3,156) = 1.5$ ,  $p = .23$ . There was a significant interaction between cue and session,  $F(3,156) = 6.4$ ,  $p < .002$ . There was a significant effect of genotype,  $F(1,52) = 9.6$ ,  $p = .003$ , and no significant effect of group  $F(1,52) = .24$ ,  $p = .63$ , or replication,  $F(1,52) = .52$ ,  $p = .47$ . All other interactions were not significant,  $F$  values  $< 2.5$ ,  $p$  values  $> .098$ .

#### Volume:

The mean volumes consumed of the two cues during the first training stage, by both groups and genotypes, are shown in Figure 2.22 (lower panel). Consumption was slightly greater for cue C than the compound AB, and the wild-type mice also showed slightly higher levels of consumption than the *Gria1*<sup>-/-</sup> mice. The ANOVA showed that there was no significant effect of cue,  $F(1,52) = .59$ ,  $p = .45$ , as well as no significant interactions between cue and group,  $F(1,52) = 1.4$ ,  $p = .24$ , cue and genotype,  $F(1,52) = 1.7$ ,  $p = .198$ , or cue and replication,  $F(1,52) = .57$ ,  $p = .45$ . The effect of session was also not significant,  $F(3,156) = 2.01$ ,  $p = .123$ , and did not interact with either group,  $F(3,156) = 1.6$ ,  $p = .19$ , genotype,  $F(3,156) = 1.03$ ,  $p = .37$ , or replication,  $F(3,156) = 1.2$ ,  $p = .31$ . There was a significant interaction between cue and session,  $F(3,156) = 7.4$ ,  $p < .001$ , as well as a significant interaction between the three factors of cue, session, and replication,  $F(3,156) = 3.3$ ,  $p = .023$ . The effect of genotype was significant,  $F(1,52) = 8.6$ ,  $p = .005$ , with no significant effect of replication,  $F(1,52) = 2.6$ ,  $p = .12$ , or group,  $F(1,52) = .22$ ,  $p = .64$ . All other interactions were not significant,  $F$  values  $< 2.6$ ,  $p$  values  $> .056$ .

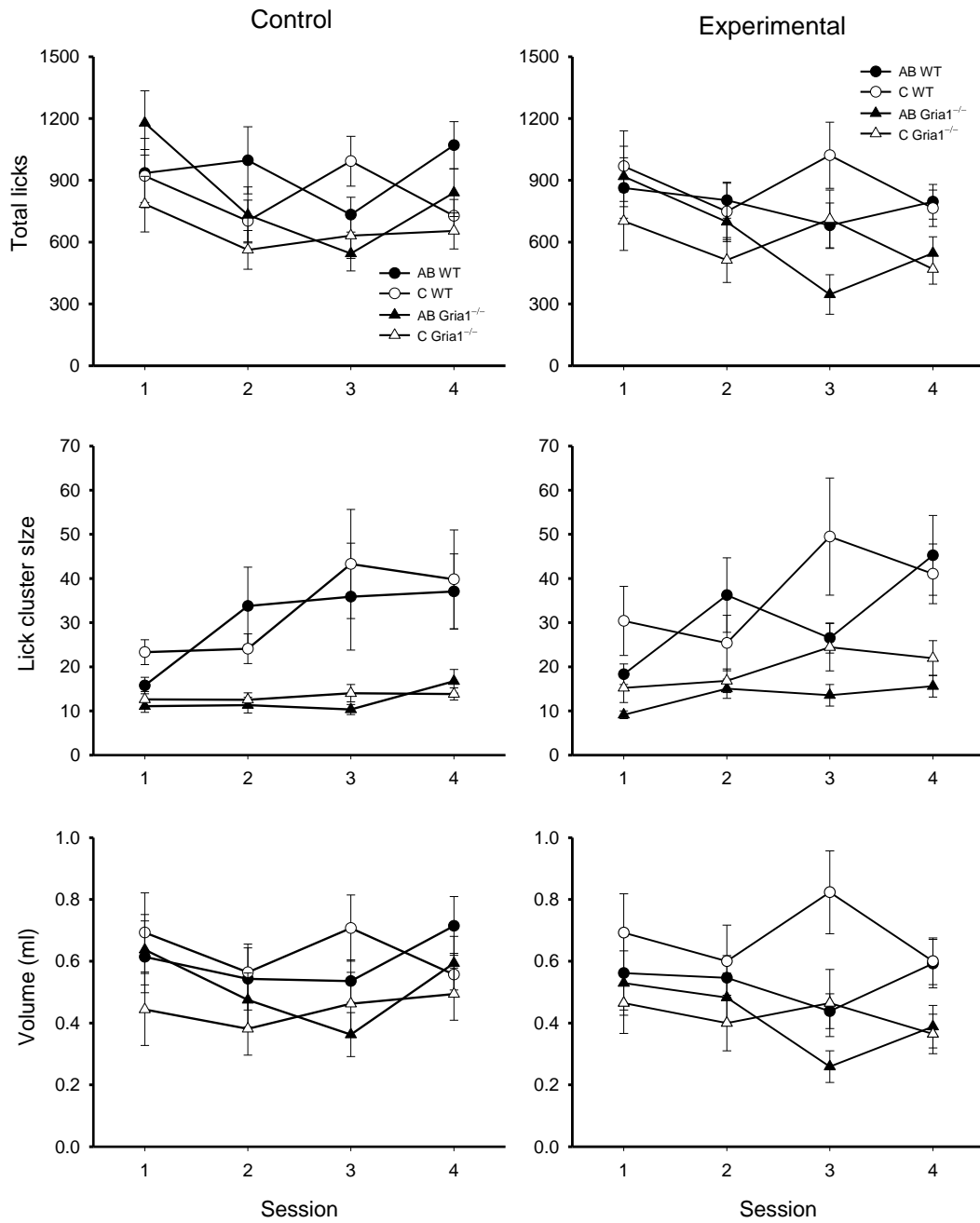


Figure 2.22. Experiment 8, training stage. The mean numbers of total licks (upper), lick cluster sizes (middle) and volumes consumed (lower) of the two taste cues (AB and C), by wild-type (WT) and *Gria1*<sup>-/-</sup> mice. The control group are shown on the left and the experimental group on the right. Error bars show  $\pm$  SEM.

## Stage 2

### Total Licks:

The mean total numbers of licks made during consumption of the two taste cues, A (followed by LiCl injection in the experimental group) and C (followed by LiCl injection in the control group), by both groups and genotypes in the second training stage, are shown in Figure 2.23 (upper panel). Both genotypes showed similar levels of consumption of the two taste cues, although the *Gria1*<sup>-/-</sup> mice generally made a smaller number of licks than the wild-type mice. The ANOVA showed that there was no significant effect of cue (A or C),  $F(1,52) = 1.1$ ,  $p = .30$ , and that this did not interact with either group,  $F(1,52) = .07$ ,  $p = .80$ , or genotype  $F(1,52) = .46$ ,  $p = .50$ . There was a near significant interaction between taste cue and replication,  $F(1,52) = 4.0$ ,  $p = .05$ . There was also no significant effect of group,  $F(1,52) = .078$ ,  $p = .78$ , although the effect of replication was significant, with mice in the second replication making a smaller number of licks than those in the first run,  $F(1,52) = 17.5$ ,  $p < .001$ . There was also a significant effect of genotype,  $F(1,52) = 19.8$ ,  $p < .001$ . All other interactions were non-significant,  $F$  values  $< 2.3$ ,  $p$  values  $> .13$ .

### Lick Cluster Size:

The mean lick cluster sizes made during consumption of the two taste cues, by both groups and genotypes in the second training stage, are shown in Figure 2.23 (middle panel). For both groups the *Gria1*<sup>-/-</sup> mice made smaller mean lick cluster sizes than the wild-type mice, but mean lick cluster sizes were similar during consumption of the two cues A and C. For the wild-type mice, the experimental group showed similar cluster sizes during consumption of the two taste cues, although in the control group the mean lick cluster size was slightly greater during consumption of cue A than C. The ANOVA showed that there was no significant effect of cue,  $F(1,52) = .03$ ,  $p = .86$ , which did also not significantly interact with either group,  $F(1,52) = 1.4$ ,  $p = .24$ , or genotype,  $F(1,52) = .92$ ,  $p = .34$ . The interaction between cue and replication was significant,  $F(1,52) = 5.01$ ,  $p = .029$ . There was no significant effect of group,  $F(1,52) = .13$ ,  $p = .72$ , but the effect of genotype was significant,  $F(1,52) = 16.6$ ,  $p < .001$ . There was also a significant effect of replication, with mice in the first experimental

run making larger lick cluster sizes than those in the second run,  $F(1,52) = 4.5$ ,  $p = .038$ . All other interactions were not significant,  $F$  values  $< 1.7$ ,  $p$  values  $> .20$ .

#### Volume:

The mean volumes consumed, by both groups and genotypes during the second training stage, are shown in Figure 2.23 (lower panel). Both genotypes consumed similar amounts of the two taste cues (A and C), although the wild-type mice generally consumed more than the *Gria1*<sup>-/-</sup> mice, an effect that was seen in both groups. The ANOVA showed that there was no significant effect of cue,  $F(1,52) = 1.03$ ,  $p = .314$ , and that this did not interact with either group,  $F(1,52) = .035$ ,  $p = .85$ , genotype,  $F(1,52) = .14$ ,  $p = .71$ , or replication  $F(1,52) = 1.5$ ,  $p = .25$ . There was also no significant effect of group,  $F(1,52) = .20$ ,  $p = .65$ , with a significant effect of genotype,  $F(1,52) = 10.8$ ,  $p = .002$ . The effect of replication was significant, with mice in the first run of the experiment consuming less than those in the second run,  $F(1,52) = 21.3$ ,  $p < .001$ . All other interactions were not significant,  $F$  values  $< 2.7$ ,  $p$  values  $> .10$ .

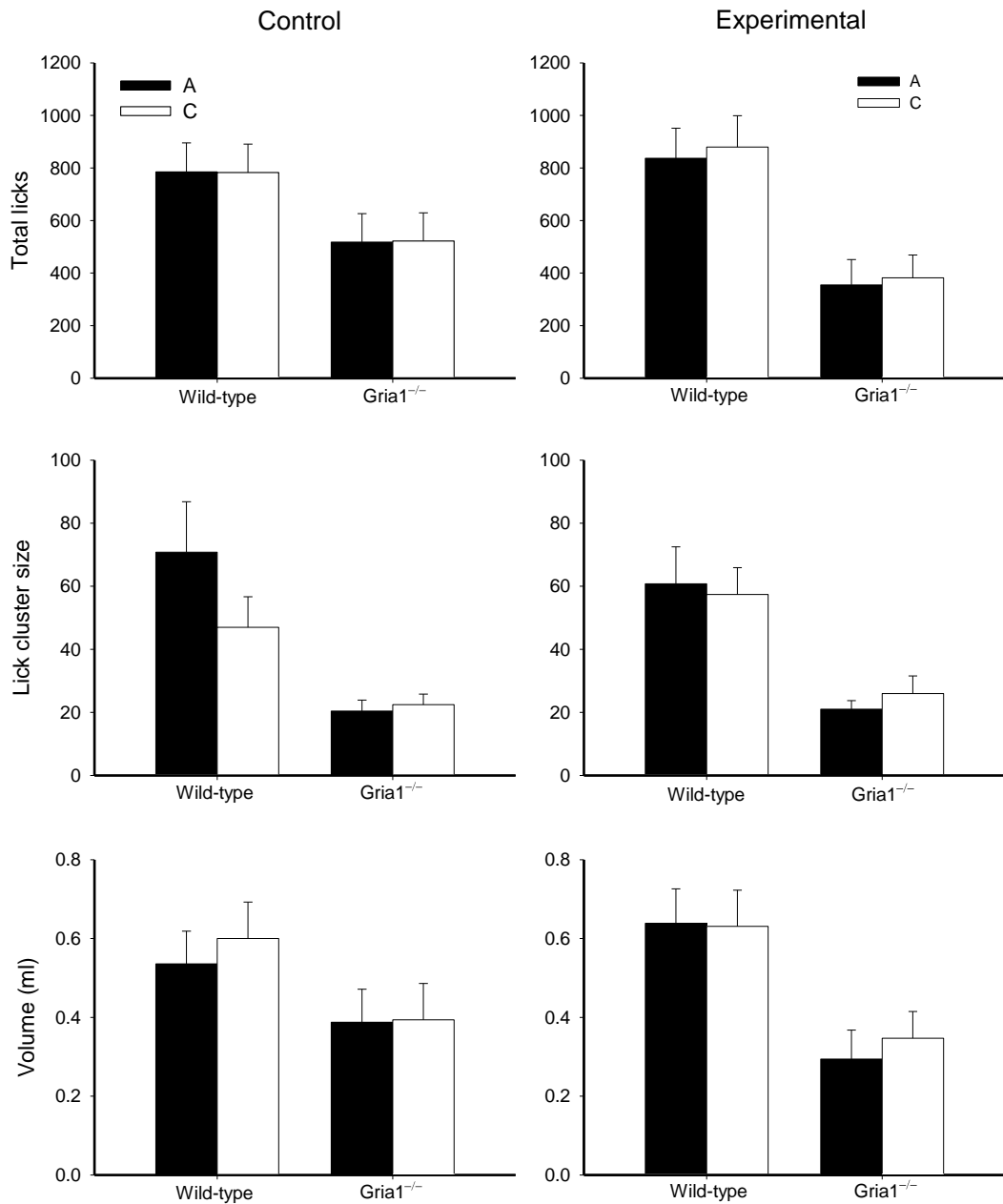


Figure 2.23. Experiment 8, training stage 2. The mean numbers of total licks (upper), lick cluster sizes (middle) and volumes consumed (lower) by the wild-type (WT) and *Gria1*<sup>-/-</sup> mice. Control group are shown on the left, experimental group on the right. Error bars show ± SEM.

## Test - Cue B

### Total Licks:

The mean total numbers of licks made during consumption of taste cue B, by both genotypes and groups, are shown in Figure 2.24 (upper panel). The *Gria1*<sup>-/-</sup> mice made a smaller number of licks than the wild-type mice, in both the experimental and control groups. The experimental group also showed a slightly smaller number of licks than the control group, for both genotypes. The ANOVA however showed that the effect of group was not significant, although there was a near significant trend,  $F(1,52) = 3.3, p = .073$ . The effect of genotype also showed a near significant trend,  $F(1,52) = 3.7, p = .062$ . There was no significant effect of replication on the mean total number of licks,  $F(1,52) = .19, p = .67$ , and all interactions were not significant,  $F$  values  $< .37$ ,  $p$  values  $> .55$ .

### Lick Cluster Size:

The mean lick cluster sizes made during consumption of cue B, by both genotypes and groups, are shown in Figure 2.24 (middle panel). The control group wild-type mice made smaller mean lick cluster sizes during consumption than those in the experimental group, although this was not seen in the *Gria1*<sup>-/-</sup> mice. The mean lick cluster sizes were also reduced in the *Gria1*<sup>-/-</sup> mice compared to the wild-type control mice, in both the control and experimental groups. The ANOVA showed that there was no significant effect of group  $F(1,52) = .35, p = .56$ , or replication  $F(1,52) = .97, p = .33$ . The effect of genotype was significant,  $F(1,52) = 7.8, p = .007$ , and the interaction between genotype and group was not significant,  $F(1,52) = .23, p = .63$ . All other interactions were also not significant,  $F$  values  $< 3.2$ ,  $p$  values  $> .081$ .

### Volume:

The mean volumes consumed of cue B during test, by both genotypes and groups, are shown in Figure 2.24 (lower panel). Mice in the experimental group consumed less than those in the control group, with the *Gria1*<sup>-/-</sup> mice also consuming less of cue B than the wild-type mice. The ANOVA showed that there was a significant effect of group,  $F(1,51) = 5.3, p = .026$ , and genotype,  $F(1,51) = 6.99, p = .011$ . There was no

significant effect of replication,  $F(1,51) = .37$ ,  $p = .55$ , and all interactions were also not significant,  $F$  values  $< .08$ ,  $p$  values  $> .77$ .

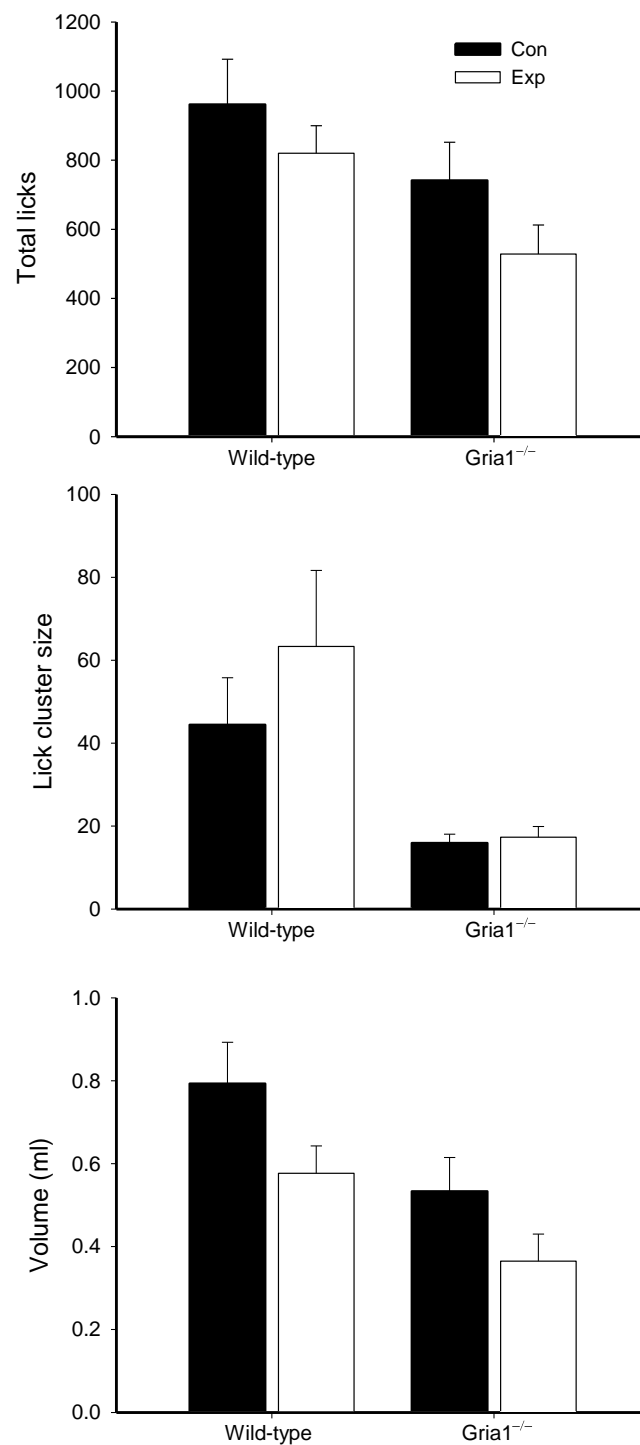


Figure 2.24. Experiment 8, test of cue B. The mean numbers of total licks (upper), lick cluster sizes (middle) and volumes consumed (lower), by control and experimental group, wild-type and Gria1<sup>-/-</sup> mice. Error bars show ± SEM.

## Test - cue A

### Total Licks:

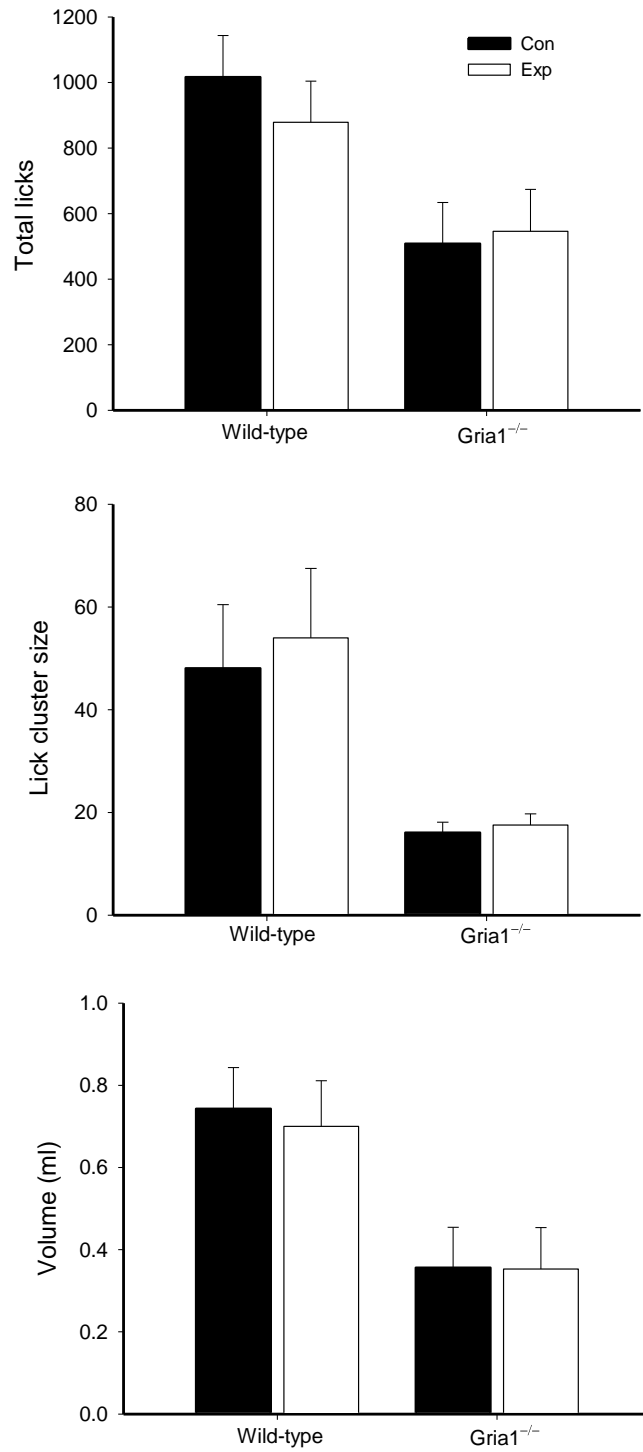
The mean total numbers of licks made during consumption of cue A during test, by both genotypes and groups, are shown in Figure 2.25 (upper panel). The experimental and control groups both made similar numbers of total licks, although the *Gria1*<sup>-/-</sup> mice made a smaller number of licks than the wild-types in both groups. The ANOVA showed that there was no significant effect of group,  $F(1,52) = .12$ ,  $P = .73$ , and a significant effect of genotype,  $F(1,52) = 7.5$ ,  $p = .009$ . There was no significant effect of replication  $F(1,52) = .67$ ,  $p = .42$ , and all interactions were also not significant,  $F$  values  $< 2.3$ ,  $p$  values  $> .13$ .

### Lick Cluster Size:

The mean lick cluster sizes made during consumption of cue A, by both groups and genotypes, are shown in Figure 2.25 (middle panel). The mean lick cluster sizes made during consumption were similar in both groups and mean lick cluster sizes were reduced in the *Gria1*<sup>-/-</sup> mice compared to the wild-type control mice. The ANOVA showed that there was no significant effect of group,  $F(1,52) = .046$ ,  $p = .83$ , or replication,  $F(1,52) = 2.3$ ,  $p = .13$ , but that there was a significant effect of genotype,  $F(1,52) = 7.9$ ,  $p = .007$ . There was also a significant interaction between genotype and replication,  $F(1,52) = 6.7$ ,  $p = .013$ . All other interactions were not significant,  $F$  values  $< .038$   $p$  values  $> .85$ .

### Volume:

The mean volumes consumed of cue A, by both groups and genotypes, are shown in Figure 2.25 (lower panel). The *Gria1*<sup>-/-</sup> mice consumed less than the wild-type mice, but both groups showed similar levels of consumption. The ANOVA showed that there was no significant effect of group,  $F(1,52) = .23$ ,  $p = .64$ , a significant effect of genotype,  $F(1,52) = 8.6$ ,  $p = .005$ , and no significant effect of replication  $F(1,52) = 3.3$ ,  $p = .075$ . All interactions were also not significant,  $F$  values  $< 3.3$ ,  $p$  values  $> .075$ .



*Figure 2.25.* Experiment 8, test of cue A. The mean numbers of total licks (upper), lick cluster sizes (middle) and volumes consumed (lower), by control and experimental group, wild-type and *Gria1*<sup>-/-</sup> mice. Error bars show ± SEM.

## Test - Cue C

### Total Licks:

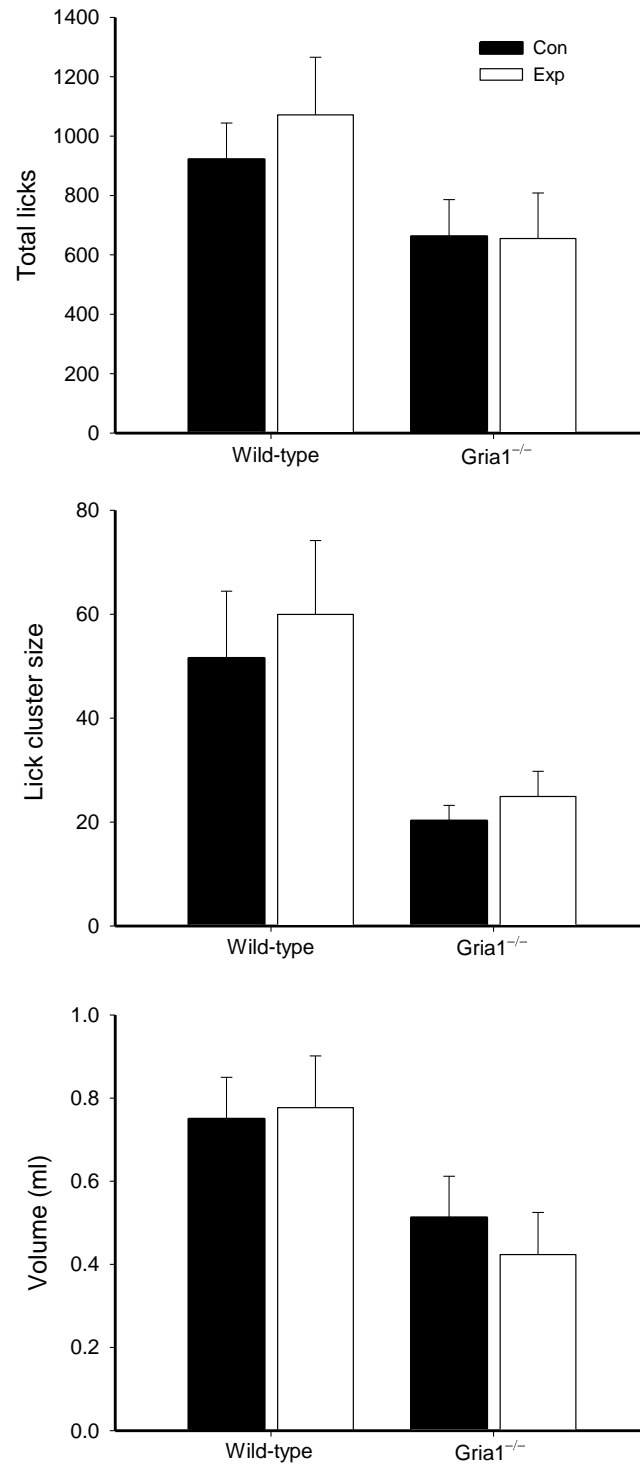
The mean total numbers of licks made during consumption of taste C, for both groups and genotypes, are shown in Figure 2.26 (upper panel). Both groups made similar numbers of total licks, with the *Gria1*<sup>-/-</sup> mice also making a smaller number of licks than the wild-type mice. The ANOVA showed that there was no significant effect of group,  $F(1,52) = .50$ ,  $p = .48$ , a significant effect of genotype,  $F(1,52) = 4.4$ ,  $p = .04$ , and no significant effect of replication  $F(1,52) = .58$ ,  $p = .45$ . All interactions were also not significant,  $F$  values  $< .78$ ,  $p$  values  $> .38$ .

### Lick Cluster Size:

The mean lick cluster sizes made during consumption of cue C, by both groups and genotypes, are shown in Figure 2.26 (middle panel). The mean lick cluster sizes were similar across the two groups, with reduced lick cluster sizes in the *Gria1*<sup>-/-</sup> mice compared to the wild-type mice. The ANOVA showed that there was no significant effect of group,  $F(1,52) = .42$ ,  $p = .52$ , or replication,  $F(1,52) = 2.3$ ,  $p = .13$ , with a significant effect of genotype,  $F(1,52) = 5.9$ ,  $p = .019$ . There was a significant interaction between genotype and replication,  $F(1,52) = 5.9$ ,  $p = .018$ , and all other interactions were not significant,  $F$  values  $< .081$   $p$  values  $> .78$ .

### Volume:

The mean volumes consumed of cue C during test, by both genotypes and groups, are shown in Figure 2.26 (lower panel). Both groups consumed similar amounts, although the *Gria1*<sup>-/-</sup> mice consumed less than the wild-type mice. The ANOVA showed that there was no significant effect of group,  $F(1,52) = .017$ ,  $p = .90$ , a significant effect of genotype,  $F(1,52) = 6.9$ ,  $p = .011$ , and no significant effect of replication,  $F(1,52) = .61$ ,  $p = .44$ . All interactions were not significant,  $F$  values  $< 1.0$ ,  $p$  values  $> .32$ .



*Figure 2.26.* Experiment 8, test of cue C. The mean numbers of total licks (upper), lick cluster sizes (middle) and volumes consumed (lower), by control and experimental group, wild-type and Gria1<sup>-/-</sup> mice. Error bars  $\pm$  SEM.

### 2.8.3 Discussion

The results from this sensory preconditioning experiment show that there was a small sensory preconditioning effect, with no difference in the extent of this effect between the two genotypes. This was seen in the results from the first test session, in which the cue indirectly paired with sickness for the experimental group, but not the control group was tested. Sensory preconditioning was seen in the experimental group consuming less than the control group, demonstrating a greater taste aversion in the experimental group. This effect was significant in terms of the volume consumed, although it neared significance in the total numbers of licks and was not seen in the mean lick cluster sizes. The sensory preconditioning effect in this experiment does therefore seem to be small, resulting in only a slight reduction in intake and not affecting the palatability of the cue indirectly paired with sickness in this experiment. There was also no significant difference between the wild-type and *Gria1*<sup>-/-</sup> mice, demonstrated by no significant interactions between group and genotype. If GluA1 deletion enhances the formation of within-compound associations, it would be expected that the sensory preconditioning effect would be greater in the *Gria1*<sup>-/-</sup> mice. The experiment here does therefore not provide any evidence for such an enhancement, with the same small sensory preconditioning effect seen in both genotypes. It is possible however that as the effect was small, the procedure was not sensitive enough to see any differences between the genotypes.

In the second test session of cue A, that had been directly paired with sickness in the experimental group but not the control group, there was no significant effect of group, either in terms of intake or mean lick cluster size. There is therefore no evidence for mice forming a specific taste aversion to this cue in the experimental group. However, it is also the case that testing was carried out in extinction without any subsequent injections of lithium chloride. This may have meant that the taste aversion effect was no longer apparent by the second test session, as they had already had one test session without any LiCl being administered. In the test session of cue C, which was previously directly paired with sickness in the control, but not the experimental group, there was again no effect of group. The lack of a taste aversion effect in this test may also have been due to extinction effects, having had

two previous test sessions without further lithium chloride and sickness. It does still remain however that there is no direct evidence for specific taste aversions having formed in this experiment. However, the sensory preconditioning effect in the first test session, although small, does suggest that mice were able to learn the taste aversion in the second training stage. As the reduced intake in the experimental group mice compared to the controls demonstrates that the indirect pairing of this cue with the sickness resulted in some aversive conditioned responding to this cue. This also provides evidence for within-compound associations forming between the taste cues. This would suggest that the test cue, when presented, was able to retrieve a representation of its associated cue that was previously paired with sickness, generate conditioned responding and taste aversion to the indirectly paired cue.

During the two training phases, both groups consumed similar amounts of the taste cues, although mice in the first run of the experiment (with the lower lithium chloride dose and lack of recovery days) did consume less than those in the second run during the second stage of training. This indicates that the addition of recovery days and/or the higher dose of lithium chloride, acted to reduce consumption, perhaps due to reduced thirst as a result of the recovery time with free-access to water in the home cages. It was also the case that the *Gria1*<sup>-/-</sup> mice generally consumed less than the wild-type mice, although this reduction in consumption was consistent across training and test sessions. The *Gria1*<sup>-/-</sup> mice also continued to show the reduction in mean lick cluster sizes throughout the experiment, as was also seen in the previous experiments in this chapter. This further demonstrates that GluA1 deletion impairs the mean lick cluster sizes, a measure of palatability, during consumption. This was the case even though there was no palatable sucrose used in this experiment. The greater mean lick cluster sizes in the wild-type mice may have been due to the use of water deprivation, with thirst resulting in the solutions themselves becoming palatable. The effect of GluA1 deletion on this measure of palatability does therefore not seem to be specific to sweet solutions, but may be seen with other factors that would otherwise act to increase the hedonic value of the solution, such as thirst. Although, it cannot be ruled out that the reduced lick cluster sizes resulted from an impairment in the expression of palatability, rather than hedonic value.

Overall, the results from this experiment support the presence of within-compound associations during taste aversion conditioning. However, there was no evidence for these associations being enhanced in the *Gria1*<sup>-/-</sup> mice compared to the wild-type controls. This finding fails to provide support for the idea that the previous failure to see blocking of flavour preference learning, in the *Gria1*<sup>-/-</sup> mice, was due to an enhanced tendency to form within-compound associations. However, the sensory preconditioning effect shown in this experiment was small, with the taste aversion effect itself also seeming to be transient. It may therefore be the case that this procedure was not sensitive enough to show any differences in the formation of within-compound associations between the two genotypes.

## 2.9 General discussion

The overall aim of the experiments within this chapter was to investigate the role of GluA1 deletion in cue-competition effects. In particular, testing the prediction that in *Gria1*<sup>-/-</sup> mice the effects of blocking and overshadowing would be intact or perhaps even enhanced compared to the control wild-type mice. This prediction was the result of the proposed selective role of the GluA1 subunit in short-term memory for recently experienced stimuli, slowing the decay rate between the primary and secondary active states of memory in Wagner's SOP model (Wagner, 1981) (Sanderson et al., 2009). This proposed role of the GluA1 subunit is able to explain both the impaired short-term memory for recently experienced stimuli (impaired habituation), and the enhanced long-term memory based on associative retrieval also observed in these mice (Sanderson et al., 2009; Schmitt et al., 2003). However, as this role of GluA1 is specific to the short-term memory decay pathway, not affecting associative retrieval of memory, then cue-competition effects that are dependent on association formation and subsequent retrieval should also be intact. Furthermore, the increased duration of primary state activation of stimulus representations resulting from the reduced decay rate, predicts that these effects may even be enhanced compared to the wild-type control mice.

In contrast to this prediction however, blocking of flavour preference learning was found to be impaired in the *Gria1*<sup>-/-</sup> mice. This result suggested that GluA1 deletion may in fact alter association formation and retrieval mechanisms. However, it was also subsequently found that there was no overshadowing in either the wild-type or the *Gria1*<sup>-/-</sup> mice. One way in which this failure to see overshadowing could be explained is through flavours being processed more configurally rather than elementally (Dwyer et al., 2011; Pearce, 2002). Such an account can explain the failure to observe overshadowing, as a result of high levels of generalisation between the training compound and test cue. In this case, the learning about the compound cue largely generalises to the test cue, resulting in little or no overshadowing effect.

This account can also explain the presence of blocking in the wild-type mice. If generalisation is presumed to be high between the flavour cues, then the learning in the first training stage generalises well to the compound cue. The prediction error generated is therefore small, meaning little or no learning is able to occur about the new cue now presented in compound with the first, resulting in a blocking effect. In repeat blocking and overshadowing experiments designed to encourage elemental rather than configural processing, by using auditory and visual cues, it was further found that GluA1 deletion did not impair cue-competition in the form of either blocking or overshadowing. Together, the blocking and overshadowing experiments suggest that GluA1 deletion does play a role in cue-competition, impairing blocking, but only when this effect is dependent on the level of generalisation between the cues. This seems to be the case during cue-competition between flavour cues, with the configural model of flavour preference learning proposed by Dwyer et al. (2011) explaining the failure to see overshadowing, while also still seeing a blocking effect in the wild-type mice, as a result of high levels of generalisation between the flavours.

However, exactly how GluA1 deletion may alter generalisation between cues is not clear from the results in this chapter. If GluA1 deletion prevents the otherwise high levels of generalisation between flavour cues, proposed by Dwyer et al. (2011), this could explain the failure to see blocking. This is the case as little stage one learning will generalise to the compound cue in stage 2, allowing learning to occur about the new flavour presented in compound with the previously trained flavour cue. In this

case however, the failure to see overshadowing is difficult to explain. This is the case as with overshadowing the effect is dependent on the level of generalisation between the training compound and the single test cue. In this procedure, low levels of generalisation would actually result in a greater overshadowing effect, as little learning about the compound cue generalises to the test cue. The configural model of Dwyer et al. (2011) therefore predicts an inverted relationship between blocking and overshadowing, with weak blocking corresponding to strong overshadowing. The *Gria1*<sup>-/-</sup> mice do therefore not show this pattern, rather they showed impaired or weak blocking and impaired or weak overshadowing. It therefore seems that the *Gria1*<sup>-/-</sup> mice fail to correspond to expected blocking and overshadowing effects for either high or low levels of generalisation between the cues. This suggests that in instances when the wild-type mice seem to be processing cues in a more configural manner, the *Gria1*<sup>-/-</sup> mice are processing the stimuli in a qualitatively different way, resulting in the failure to see blocking of flavour preference learning.

One possibility is that rather than processing flavour cues in a more configural manner (Dwyer et al., 2011; Pearce, 2002) the *Gria1*<sup>-/-</sup> mice continue to process stimuli in a more elemental manner, as modelled by traditional learning theories (e.g., Rescorla & Wagner, 1972). An enhanced tendency to process elementally, with little generalisation therefore occurring between cues, could also explain the previous findings of enhanced long-term discrimination learning in the *Gria1*<sup>-/-</sup> mice (Sanderson et al., 2009; Schmitt et al., 2003). This is because any associative strength and resulting conditioned responding acquired by one cue is less likely to generalise to another, resulting in greater discrimination learning being evident between the cues. There was no evidence in these experiments however for GluA1 deletion enhancing discrimination and learning, as flavour preference effects were similar in the two genotypes. Although in general the conditioning effects observed in these experiments, particularly when flavours and tastes were used, were small. It may therefore not have been possible to observe enhanced discrimination effects in the *Gria1*<sup>-/-</sup> mice using these stimuli and procedures. With elemental processing, cue-competition effects depend on the amount of associative strength that is able to be gained by each individual stimulus representation. Although this means that

overshadowing and blocking should both be evident, there may also be within-compound associations forming. These would act against the cue-competition effects that would otherwise be seen.

Within-compound associations have particularly been suggested to form between cues that are highly similar, such as when flavour/taste stimuli are used (Durlach & Rescorla, 1980; Speers et al., 1980). The flavour stimuli being processed more elementally, with enhanced within-compound association formation, could therefore explain the failures to see blocking and overshadowing in the *Gria1<sup>-/-</sup>* mice. In the case of overshadowing this would be due to the test flavour cue retrieving a representation of the other cue associated with it. The combined direct and indirect associations with the US could then sum to increase conditioned responding and preventing overshadowing from being seen in these experiments. Similarly, in the case of blocking, the test flavour may retrieve a representation of the flavour previously presented in compound with it and that had previously been trained with the unconditioned stimulus. Again, this could result in greater levels of conditioned responding due to both direct and indirect associations with the outcome, preventing any blocking effect being seen in the *Gria1<sup>-/-</sup>* mice. However, it was found that in sensory preconditioning of taste aversion, both wild-type and *Gria1<sup>-/-</sup>* mice showed evidence for within-compound associations, with no enhancement in the *Gria1<sup>-/-</sup>* mice. This fails to support an enhancement in the tendency to form within-compound associations explaining the impairment in blocking of flavour preference learning. However, it could be the case that this sensory preconditioning procedure was not sensitive enough to show any differences in the tendency to form within-compound associations in *Gria1<sup>-/-</sup>* mice.

Another finding from the experiments within this chapter was that the mean lick cluster sizes, a measure of palatability (Dwyer, 2012), were generally reduced in the *Gria1<sup>-/-</sup>* mice compared to the control wild-type mice. This is in line with the previous findings of Austen et al. (2017) and further supports a role for the GluA1 subunit in perceiving hedonic value, with the *Gria1<sup>-/-</sup>* mice therefore providing a potential model of anhedonia. This impairment did not affect the ability to learn about the solutions, as both wild-type and *Gria1<sup>-/-</sup>* mice showed flavour preference learning.

Again, this replicates the previous findings in the *Gria1<sup>-/-</sup>* mice of intact flavour conditioning (Austen et al., 2017).

An additional finding was that the mean lick cluster sizes were not impaired in the test sessions when maltodextrin was used to support flavour preference learning. The mean lick cluster sizes were however impaired during the training stage with maltodextrin, showing palatability to the sensory properties of the solutions were still reduced in the *Gria1<sup>-/-</sup>* mice. These results suggest that although GluA1 deletion affects palatability based on the perceived sensory properties of a solution (the sweet taste of sucrose), it may not play a role in increased palatability based on the post-ingestive consequences of the reinforcer. This also supports the findings of normal satiety responses in the *Gria1<sup>-/-</sup>* mice, with higher concentrations of sucrose resulting in reduced intake during an extended hour long period of access (Austen et al., 2017). The role of the GluA1 subunit in perceived palatability and hedonic value may therefore be specific to the sensory properties of solutions, not extending to hedonic value based on other factors, such as post-ingestive consequences. However, it could also be that the impairment in lick cluster size is not due to altered palatability, but to a reduced ability to express lick cluster size effects. In this case, the *Gria1<sup>-/-</sup>* mice may not be impaired at perceiving palatability and hedonic value, but are unable to express changes in palatability, through altered lick cluster sizes, to the same extent as the wild-type mice.

In summary, it does seem that when using auditory and visual cues GluA1 deletion does not impair cue-competition in the form of blocking and overshadowing of learning. This is in line with the proposed role of the GluA1 subunit in short-term memory processes (Sanderson et al., 2009), with associative retrieval and cue-competition effects intact. However, when blocking seems to be dependent on the level of generalisation between the cues, GluA1 deletion does seem to have an effect. It therefore seems that the GluA1 subunit plays a role when cue-competition is normally dependent on the level of generalisation between the cues, such as when cues that are highly similar, such as flavours, are used. One way in which this could be explained is through GluA1 deletion altering the tendency to process cues in a configural manner. As while the wild-type mice seem to show configural processing

of the highly similar flavour cues (Dwyer et al., 2011; Pearce, 2002), it may be that the *Gria1*<sup>-/-</sup> mice process in a more elemental manner (e.g., Rescorla & Wagner, 1972). The effect of GluA1 deletion may therefore be related to the likelihood of processing in a configural or elemental manner, with *Gria1*<sup>-/-</sup> mice processing stimuli elementally when wild-type mice process in a configural way. Although from the results in this thesis, it is not clear the extent to which elemental processing and the formation of within-compound associations could explain the failure to see cue-competition with flavour cues. Further research, such as additional tests of within-compound association formation, are therefore required to test the balance of elemental and configural processing in the *Gria1*<sup>-/-</sup> mice.

## Chapter 3

### **GluA1 deletion and contrast dependent flavour preference learning**

Deletion of the GluA1 subunit impairs short-term memory for recently experienced stimuli, but spares or enhances long-term memory based on associative retrieval. The short-term memory impairment is seen in the form of impaired habituation, a reduction in responding, to recently experienced stimuli (Sanderson et al., 2009). The *Gria1*<sup>-/-</sup> mice therefore fail to reduce attention and responding to stimuli as a result of recent experience. One way in which the effect of GluA1 deletion has been explained is through Wagner's SOP model (Wagner, 1981), slowing the decay rate between the primary and secondary active states of memory (Sanderson et al., 2009).

One feature of the account of short-term memory given by Wagner's SOP model, is that once a stimulus representation has decayed into a secondary active state, it will be unable to form excitatory associations with other stimuli active in a primary state. Only once the stimulus representation has decayed fully into an inactive state, would it then be able to re-enter the primary active state and into excitatory associations with other stimuli. Best & Gemberling (1977) for example, found that learning about a stimulus was reduced after recent exposure to this same stimulus, but not if the stimulus presentations were separated by longer intervals. This demonstrates that having a short-term memory for a recently presented stimulus impairs the ability to learn about it. In Wagner's SOP model (Wagner, 1981) this is due to representations in a secondary active state being unable to enter back into the primary active state, until having fully decayed back into an inactive state. Short-term memory and habituation to stimuli will therefore correspond to the associability of a stimulus, reducing as a function of memory decay.

The account of GluA1 deletion proposed by Sanderson et al. (2009), of a slowed decay rate between the primary and secondary active states, predicts that the process of reduced associability will also be slowed. This is due to stimulus

representations remaining in a primary active state for longer, preventing decay into a secondary active state of memory. Therefore, the *Gria1*<sup>-/-</sup> mice should not show the same short-term reductions in associability as the control wild-type mice. As a result, the *Gria1*<sup>-/-</sup> mice may form aberrant associations between recently experienced stimuli that are temporally separate and would not otherwise become associated in control mice.

Altered stimulus associability in *Gria1*<sup>-/-</sup> mice was shown by Sanderson et al. (2017) in a trace conditioning procedure, in which there was an interval between an auditory cue and food reward. Whereas the wild-type mice initially showed excitatory learning about the cue, that became inhibitory with extended training, the *Gria1*<sup>-/-</sup> mice continued to show high levels of conditioned responding to the cue. Therefore, in the *Gria1*<sup>-/-</sup> mice, the predictive cue continued to remain excitatory despite the gap between the cues, whereas the trace procedure resulted in inhibitory learning in the wild-type mice. This suggests that short-term stimulus associability is enhanced in the *Gria1*<sup>-/-</sup> mice compared to the control mice, as representations are more likely to form excitatory associations.

The first experiment in this chapter tested the effect of GluA1 deletion on stimulus associability. In this experiment, *Gria1*<sup>-/-</sup> and wild-type control mice were tested on a procedure in which flavour preference learning is dependent on having a short-term memory for recently experienced sucrose. In particular, the procedure used was similar to that of Dwyer, Figueroa, Gasalla, & López, (2018). In this, rats learned a flavour preference based not on the actual concentration of sucrose currently paired with that flavour, but rather the perceived concentration. This perception was altered by pre-exposing rats to low 2%, or high 32%, concentration sucrose solutions, before one of two flavour cues were presented. The CS- flavour was presented following the 32% sucrose, the CS+ following the 2% sucrose, but both the CS- and the CS+ flavours were paired with 8% sucrose. The initial pre-exposure, to either the high or low sucrose solutions, should result in a short-term memory for this sucrose concentration. When the flavour cue is then consumed, paired with 8% sucrose, the CS+ flavour will have a positive contrast compared to the short-term memory of 2% sucrose. The CS- flavour however will have a negative contrast compared to the

representation of 32% sucrose currently in short-term memory. When the flavour preference was tested, the rats consumed more of the CS+ than the CS-, showing a preference for the CS+. The mean lick cluster size was also greater during consumption of the CS+ compared to the CS-, suggesting that the palatability of the CS+ also increased.

The authors explained these results in terms of Wagner's SOP (Wagner, 1981) model, occurring due to differential levels of habituation to sucrose. In particular, the pre-exposure to 32% sucrose should result in a large amount of initial A1 processing of the elements representing sucrose. There will therefore also be a large number of elements decaying to a secondary active state. During presentations of 2% sucrose, the lower concentration results in fewer elements representing the sweet taste of sucrose entering the A1 state, meaning fewer elements will subsequently decay to a secondary active state of memory. These differential levels of decay mean that the ability of sucrose elements to be activated by the 8% concentration paired with the flavour cue, and able to enter the A1 state, will differ for the CS+ and the CS- flavour cues. For the CS- flavour cue, as this was preceded by the higher concentration, more elements representing sucrose will be in the secondary active state during its consumption. There will therefore be less of a flavour preference, as fewer elements will be in the A1 state to form an association with the flavour cue. For the CS+ flavour cue, as this was preceded by the lower concentration, the 8% sucrose paired with the flavour, will be more able enter the primary active state of memory. This should increase the ability to learn an association between the representation of sucrose and the flavour cue, generating a greater flavour preference for the CS+ compared to the CS-.

Therefore, this procedure provides a way to assess altered learning occurring as a result of short-term habituation, with the flavour preference only being able to occur as result of the pre-exposure to different sucrose concentrations. GluA1 deletion, that impairs short-term memory decay (Sanderson et al., 2009), should therefore also impair this flavour preference learning. As the elements representing sucrose will be unable to decay, into the secondary active state, to the same extent as the wild-type control mice. This means that when the flavour cues are presented, there

will not be the differential levels of A2 processing to generate the flavour preference for the CS+ compared to the CS- flavour cue. In order to test this, the first experiment in this chapter ran *Gria1*<sup>-/-</sup> and control mice through a similar procedure to Dwyer et al. (2018).

### 3.1 Experiment 9

#### Flavour preference based on contrast effects

This experiment tested the prediction that GluA1 deletion will impair the reduced stimulus associability caused by short-term memory decay, proposed to be slowed in *Gria1*<sup>-/-</sup> mice (Sanderson et al., 2009). In a similar procedure to Dwyer et al. (2018), low or high sucrose solutions were presented in pre-exposure trials, either 1% or 16% sucrose concentrations. These were followed by presentations of the flavour cues, both paired with 8% sucrose. The CS+ was presented following trials with 1% sucrose, and the CS- presented following trials with 16% sucrose. After this training, all mice were then tested for a flavour preference towards CS+ compared to the CS- flavour cue. If it is the case that GluA1 deletion impairs short-term habituation and decay of elements into a secondary active state, *Gria1*<sup>-/-</sup> mice may not show the same flavour preference effect as wild-type mice. This is due to the stimulus elements representing sucrose failing to decay to a secondary active state. The pre-exposure to the higher 16% sucrose would therefore not have the same effect of increasing short-term memory decay and A2 processing of sucrose compared to the 1%. This would mean that both the CS+ and CS- flavour cues are able to form an association with the 8% sucrose paired with these flavours, impairing the ability to learn a flavour preference towards the CS+ compared to the CS-, as would be expected to occur in the wild-type mice. A failure to see flavour preference learning in the *Gria1*<sup>-/-</sup> mice would therefore provide support for slowed short-term memory decay and enhanced stimulus associability.

### **3.3.1 Methods**

#### **Subjects**

15 *Gria1*<sup>-/-</sup> mice (2 male, 13 female) and 25 wild-type (12 male, 13 female), bred and housed in the same way as experiment 2.1 were used. Mice were 17-38 weeks old at the start of testing with 85% weights ranging from 16.7g – 32.2g. Mice were naïve and had no previous experience within the apparatus.

#### **Apparatus**

The apparatus used was the same as the flavour preference experiments in chapter 2 (e.g. experiment 2.1). The amount consumed was calculated by weighing the pipettes before and after each session (to .1g accuracy).

#### **Procedure**

The design of the experiment is shown in Table 1. In the training stage, there were 8 daily sessions, each consisting of two 10-minute trials and an inter-trial interval of approximately 10-minutes. During this interval mice were removed from the chambers and returned to their home cages. The sipper tubes were available from the start of each trial. The first trial in each session gave access to either the 1% or 16% sucrose solution, with the second trial giving access to one of the flavoured 8% sucrose solutions. The CS+ flavour always followed trials with 1% sucrose, whereas the CS- flavour followed trials with 16% sucrose. The flavours were cherry and grape Kool-Aid, counterbalanced with respect to genotype and sex as far as possible given the numbers of mice. This meant that for half of the mice the CS- was cherry and the CS+ grape, and vice versa for the remaining half of the mice. The trial orders were counterbalanced so that approximately half of the mice received 1% and then the CS+ first, the other half 16% and the CS- first, with these sessions presented in a double alternating sequence (e.g. 1% CS-, 16 % CS+, 16 % CS+, 1% CS-).

After these training sessions mice were tested with the CS+ and CS- flavour cues, both paired with 8% sucrose. These were given for two test sessions, with one presentation of flavour per session and the same trial duration and inter-trial interval as training sessions. The test order was counterbalanced with respect to genotype

and previous training order, as far as possible given the numbers. Half of the mice were therefore presented with the CS+ before the CS- and the remaining half the CS- before the CS+ flavour cue in the first test session. The test sessions were presented in double alternation across the two test sessions (e.g. CS- CS+, CS+ CS-).

Table 3.1

*Design of experiment 9*

Training	Test
1% → CS+ (8%)	CS+ (8%)
16% → CS- (8%)	CS- (8%)

### Statistical analysis

Statistical analysis were similar to experiment 2.1, using mixed model ANOVA. For the training data, genotype, cue, and session, were added as factors. This analysis was carried out separately for the two preceding sucrose solutions (1% and 16%) and for the two following flavour cues (the CS+ and the CS). For the test data, mixed model ANOVA with genotype, flavour cue, and test session, were carried out.

### 3.1.2 Results

#### Training

#### Total Licks:

The mean total numbers of licks during consumption of the 1% and 16% sucrose solutions are shown in Figure 3.1 (top panel, left). The *Gria1*<sup>-/-</sup> and wild-type mice made a greater number of licks during consumption of the 16% sucrose solution compared to the 1%. There was also an increase over sessions in the total number of licks made during consumption of the 16% sucrose, but not for the 1% sucrose. The ANOVA showed that there was a significant effect of sucrose concentration,  $F(1,38)$

= 652.2,  $p < .001$ , and no significant effect of genotype,  $F(1,38) = .44$ ,  $p = .51$ . The interaction between sucrose concentration and genotype was also not significant,  $F(1,38) = 2.5$ ,  $p = .12$ . There was a significant effect of session,  $F(3,114) = 16.7$ ,  $p < .001$ , and no significant interaction between session and genotype  $F(3,114) = 1.1$ ,  $p = .37$ . The interaction between sucrose concentration and session was significant,  $F(3,114) = 8.7$ ,  $p < .001$ , and there was no significant interaction between the three factors of flavour cue, session, and genotype,  $F(3,114) = 1.8$ ,  $p = .15$ .

For the two flavoured solutions that followed, the CS+ and the CS-, shown in Figure 3.1 (top panel, right), both wild-type and *Gria1*<sup>-/-</sup> mice made a greater number of licks during consumption of the CS+ than the CS-. There was also an increase in the mean total number of licks to the CS+ over sessions, but not for the CS-. The ANOVA showed that there was a significant effect of cue, CS- or CS+,  $F(1,38) = 379.2$ ,  $p < .001$ , and no significant effect of genotype,  $F(1,38) = .26$ ,  $p = .62$ , as well as no significant interaction between cue and genotype,  $F(1,38) = 1.9$ ,  $p = .17$ . The effect of session was not significant,  $F(3,114) = 1.95$ ,  $p = .13$ , and there was no significant interaction between session and genotype,  $F(3,114) = .72$ ,  $p = .54$ . There was a significant interaction between cue and session,  $F(3,114) = 36.8$ ,  $p < .001$ , but no significant interaction between the three factors of cue, session, and genotype,  $F(3,114) = 1.5$ ,  $p = .22$ .

Lick cluster size:

The mean lick cluster sizes made during consumption of the 1% and 16% sucrose solutions, by the wild-type and *Gria1*<sup>-/-</sup> mice, are shown in Figure 3.1 (middle panel, left). For both genotypes the mean lick cluster sizes were greater during consumption of 16% than 1% sucrose, but they were reduced in the *Gria1*<sup>-/-</sup> mice compared to the wild-type control mice, particularly during consumption of the 16% sucrose. There was also an increase in the mean lick cluster sizes over sessions during consumption of the 16% sucrose, but not for the 1%. The ANOVA showed that there was a significant effect of sucrose concentration  $F(1,38) = 140.2$ ,  $p < .001$ , and genotype,  $F(1,38) = 12.9$ ,  $p = .001$ , as well as a significant interaction between sucrose concentration and genotype,  $F(1,38) = 8.9$ ,  $p = .005$ . The effect of session was also significant  $F(3,114) = 10.8$ ,  $p < .001$ , and there was no significant interaction between

genotype and session,  $F(3,114) = .69$ ,  $p = .50$ . There was a significant interaction between sucrose concentration and session,  $F(3,114) = 11.7$ ,  $p < .001$ , and no significant interaction between the three factors of cue, session, and genotype  $F(3,114) = .53$ ,  $p = .58$ .

The mean lick cluster sizes made during consumption of the two flavour cues are shown in Figure 3.1 (middle panel, right). The mean lick cluster sizes were greater during consumption of the CS+ than the CS- for both genotypes, although cluster sizes were reduced in the *Gria1*<sup>-/-</sup> mice compared to the controls. The mean lick cluster sizes also showed a slight increase over sessions during consumption of the CS+, but not during consumption of the CS-. The ANOVA showed that there was a significant effect of cue,  $F(1,38) = 149.6$ ,  $p < .001$ , and a significant effect of genotype,  $F(1,38) = 17.2$ ,  $p < .001$ , as well as a significant interaction between cue and genotype,  $F(1,38) = 8.4$ ,  $p = .006$ . There was also a significant effect of session,  $F(3,114) = 4.9$ ,  $p = .003$ , that did not significantly interact with genotype,  $F(3,114) = .88$ ,  $p = .45$ . There was however a significant interaction between cue and session,  $F(3,114) = 12.9$ ,  $p < .001$ , with no significant interaction between the three factors of cue, session, and genotype  $F(3,114) = 1.4$ ,  $p = .263$ .

#### Volume:

The mean volumes consumed by wild-type and *Gria1*<sup>-/-</sup> mice, of the 1% and 16% sucrose solutions, are shown in Figure 3.1 (lower panel, left). Both genotypes consumed more of the 16% than 1% sucrose solution, with a slight increase in consumption over sessions for the 16% sucrose but not for the 1%. The ANOVA showed that there was a significant effect of sucrose concentration,  $F(1,38) = 598.2$ ,  $p < .001$ , no significant effect of genotype,  $F(1,38) = .13$ ,  $p = .73$ , and no significant interaction between sucrose concentration and genotype,  $F(1,38) = 1.7$ ,  $p = .203$ . There was a significant effect of session,  $F(3,114) = 15.9$ ,  $p < .001$ , with no significant interaction between session and genotype,  $F(3,114) = .16$ ,  $p = .93$ . The interaction between flavour cue and session was significant,  $F(3,114) = 7.6$ ,  $p < .001$ , and there was no significant interaction between the three factors of cue, session, and genotype,  $F(3,114) = 1.1$ ,  $p = .36$ .

For the two flavour cues, shown in Figure 3.1 (lower panel, right), both wild-type and *Gria1*<sup>-/-</sup> mice showed greater consumption of the CS+ than the CS-, with the amount consumed increasing over sessions to the CS+ but for the CS-. The ANOVA showed that there was a significant effect of cue,  $F(1,38) = 326.1$ ,  $p < .001$ , no significant effect of genotype,  $F(1,38) = .36$ ,  $p = .55$ , and no significant interaction between cue and genotype,  $F(1,38) = .42$ ,  $p = .52$ . There was a significant effect of session,  $F(3,114) = 11.9$ ,  $p < .001$ , with no significant interaction between session and genotype,  $F(3,114) = .17$ ,  $p = .92$ . The interaction between cue and session was significant,  $F(3,114) = 36.6$ ,  $p < .001$ , and there was no significant interaction between the three factors of cue, session, and genotype,  $F(3,114) = 1.2$ ,  $p = .31$ .

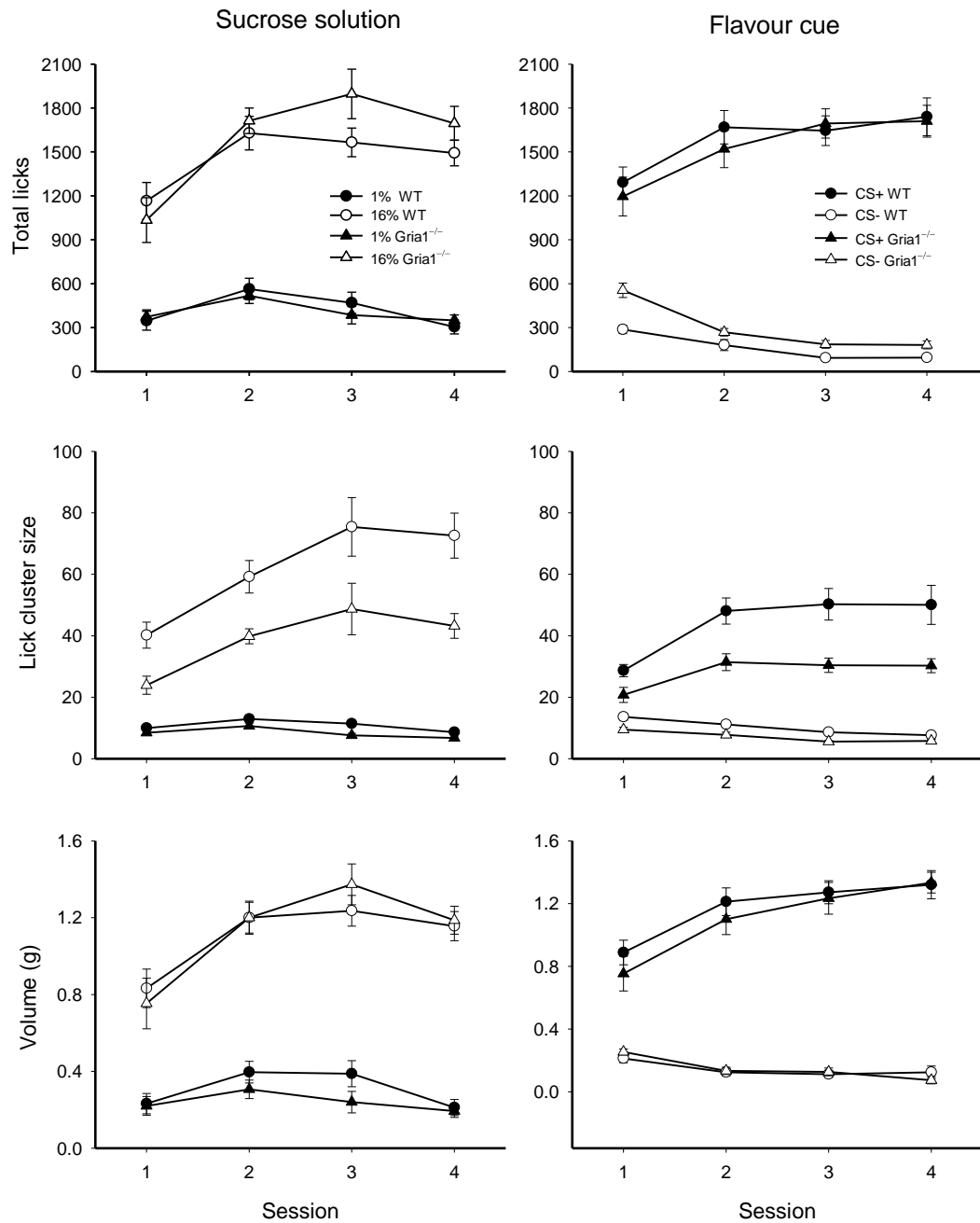


Figure 3.1. Experiment 9, training stage. The mean total numbers of licks (upper panel), lick cluster sizes (middle panel), and volumes consumed (lower panel), by wild-type (WT) and Gri1<sup>-/-</sup> mice of the 1% and 16% sucrose solutions (left side) and the following flavour CS+ and CS- flavour cues (right side). Error bars show  $\pm$  SEM.

## Test

### Total Licks:

The mean numbers of total licks made during consumption of the CS- and CS+ flavour cues, in both the first and second test sessions, are shown in Figure 3.2 (upper panel, left and right respectively). Both the wild-type and the *Gria1*<sup>-/-</sup> mice made similar numbers of licks, that were greater during consumption of the CS+ than the CS-, demonstrating a flavour preference for the CS+. In the second test session the number of licks remained greater to the CS+ in both genotypes, but with a slight increase in the number of licks to the CS-. The ANOVA showed that there was a significant effect of cue,  $F(1,38) = 15.9$ ,  $p < .001$ , and no significant effect of genotype,  $F(1,38) = .49$ ,  $p = .49$ , as well as no significant interaction between cue and genotype,  $F(1,38) = 1.3$ ,  $p = .26$ . There was a significant effect of test session  $F(1,38) = 3.2$ ,  $p = .081$ , and no significant interaction between test session and genotype,  $F(1,38) = 1.5$ ,  $p = .23$ . Test session did also not significantly interact with cue  $F(1,38) = .27$ ,  $p = .61$ , and there was also no significant interaction between the three factors of test session, cue, and genotype  $F(1,38) = .03$ ,  $p = .87$ .

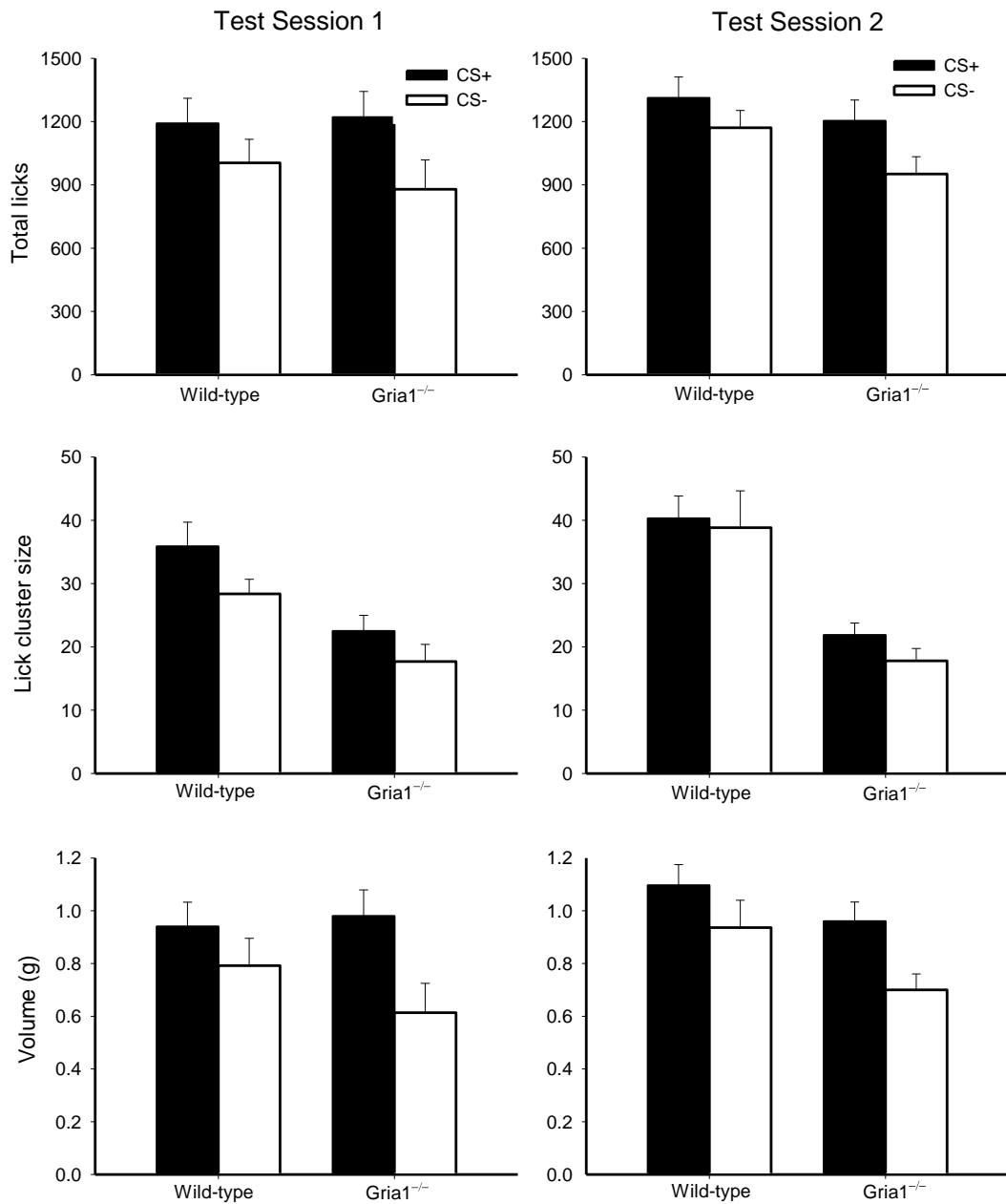
### Lick cluster size:

The mean lick cluster sizes made during consumption of the two flavour cues, by wild-type and *Gria1*<sup>-/-</sup> mice in both test sessions are shown in Figure 3.2 (middle panel). Lick cluster sizes were greater in the wild-type than the *Gria1*<sup>-/-</sup> mice during consumption of both the CS+ and the CS- flavour cues. Lick cluster sizes were however greater during consumption of the CS+ than the CS- for both genotypes, although this effect was seen more in the first than second test session. The ANOVA showed that there was a significant effect of cue on lick cluster size,  $F(1,38) = 6.04$ ,  $p = .019$ , and a significant effect of genotype,  $F(1,38) = 12.4$ ,  $p = .001$ . There was however no significant interaction between cue and genotype,  $F(1,38) < .01$ ,  $p = .99$ . There was a significant effect of test session,  $F(1,38) = 4.5$ ,  $p = .04$ , and a significant interaction between test session and genotype,  $F(1,38) = 5.2$ ,  $p = .028$ . There was no significant interaction between test session and cue,  $F(1,38) = .75$ ,  $p = .39$ , or between the three factors of test session, cue, and genotype,  $F(1,38) = .46$ ,  $p = .50$ .

Simple main effects analysis of the interaction between genotype and test session ,further showed that in both test sessions the wild-type mice made larger lick cluster sizes than the *Gria1*<sup>-/-</sup> mice: first session,  $F(1,38) = 10.3$ ,  $p = .003$ , second test session  $F(1,38) = 12.0$ ,  $p = .001$ . The wild-type mice however showed greater lick cluster sizes in the second session than the first,  $F(1,38) = 12.96$ ,  $p = .001$ , but that there was no significant difference across test sessions for the *Gria1*<sup>-/-</sup> mice,  $F(1,38) = .010$ ,  $p = .92$ .

Volume:

The mean volumes consumed of the two flavour cues, by the wild-type and *Gria1*<sup>-/-</sup> mice during the two test sessions are shown in Figure 3.2 (lower panel). In both test sessions and for both genotypes, a greater volume was consumed of the CS+ than the CS-, with a slight increase in consumption of both cues during the second testing session. The ANOVA showed that there was a significant effect of cue on the volume consumed,  $F(1,38) = 21.8$ ,  $p < .001$ , no significant effect of genotype,  $F(1,38) = 1.4$ ,  $p = .24$ , and no significant interaction between cue and genotype,  $F(1,38) = 2.5$ ,  $p = .12$ . There was a significant effect of test session on consumption, consuming more in the second session than the first,  $F(1,38) = 5.3$ ,  $p = .026$ , an effect that did not significantly interact with genotype,  $F(1,38) = 2.2$ ,  $p = .15$ . There was also no significant interaction between test session and cue,  $F(1,38) = .21$ ,  $p = .65$ , or between the three factors of session, cue, and genotype  $F(1,38) = .33$ ,  $p = .57$ .



*Figure 3.2.* Experiment 9, test stage. The mean numbers of total licks (upper panel), lick cluster sizes (middle panel) and volumes consumed (lower panel), during the first (left) and second (right) testing sessions, by wild-type and Gria1<sup>-/-</sup> mice. Error bars show  $\pm$  SEM.

### 3.1.3 Discussion

The results demonstrate that both genotypes showed a flavour preference for the CS+ flavour compared to the CS- flavour. The wild-type and *Gria1*<sup>-/-</sup> are therefore both able to learn a flavour preference based on contrast effects and differences in perceived sucrose concentration, resulting from pre-exposure to high or low sucrose concentrations. This preference was seen in a higher number of total licks as well as larger amounts being consumed of the CS+ than the CS-. It was also seen in the form of larger mean lick cluster sizes during consumption of the CS+ than the CS-. This suggests that the pre-exposure to the lower sucrose concentration increased the perceived sweetness of the 8% sucrose, in comparison to when the same 8% was presented following exposure to 16% sucrose. The mean lick cluster sizes were also impaired in the *Gria1*<sup>-/-</sup> mice compared to the wild-type mice. However, they did still show the flavour preference in the form of an increase in mean lick cluster size as well as intake.

These results therefore correspond to the previous findings by Dwyer et al. (2018), showing that flavour preference learning can occur as a result of perceived changes as well as actual changes in the sensory properties of the solutions. They also demonstrate that *Gria1*<sup>-/-</sup> mice are able to learn this flavour preference effect to the same degree as the wild-type mice. This result does however go against the prediction that the *Gria1*<sup>-/-</sup> mice would be impaired at showing flavour preference based on contrast effects. This was predicted as a result of the impairment in short-term memory shown by *Gria1*<sup>-/-</sup> mice and the account of GluA1 deletion of slowed short-term memory decay (Sanderson et al., 2009). This slowed decay means that the representation of sucrose would remain in the primary active state for longer, preventing short-term memory (when active in the secondary state) from reducing the associability of stimuli. However in this case, GluA1 deletion, despite impairing short-term habituation, did not seem to impair learning proposed to be dependent on short-term memory.

One explanation for these results is that the flavour preference based on contrast effects is not the result of short-term memory processes as described by Dwyer et al. (2018). It may instead be dependent on a different process that is not affected by

deletion of the GluA1 subunit. One possibility is that the flavour preference may have occurred as a result of the CS+ being more familiar during the test session. As both the wild-type and *Gria1*<sup>-/-</sup> mice consumed more of the CS+ flavour cue during the training sessions, therefore being more familiar than the CS- during consumption in the test sessions. This increased consumption of the CS+ was likely due to mice having already consumed high concentrations of sucrose, becoming more satiated, before presentation of the CS- flavour cue than the CS+. It may therefore be the case that the enhanced flavour preference to the CS+ compared to the CS- shown at test, was a result of mice having a preference towards the relatively more familiar cue.

There is however some evidence against such an account, from Austen, Strickland, & Sanderson (2016) testing the effect of familiarity on licking behaviour. In this experiment, mice were presented with a flavour cue paired with 16% sucrose during training, before being tested with this same flavour and a novel one, also with 16% sucrose. It was found that there were no differences in the total numbers of licks and volume consumed between the familiar and novel flavour cues. Novelty did also not significantly affect lick cluster size, although they were slightly greater to the familiar cue early in the test session. These results suggest that familiarity of a cue does not result in a clear preference for that cue when tested compared to a novel one. This is seen even when the novel flavour has never been previously experienced. In the current experiment the difference in familiarity was not this extreme, being a relative one, as both flavours were experienced in training. It therefore seems even less likely that differences in familiarity caused the flavour preference effect seen in this experiment. However, to fully rule out an explanation based on increased familiarity, a follow up study was carried out. In this experiment, licking behaviour during consumption of a familiar flavour compared to a novel one was assessed.

## 3.2 Experiment 10

### The effect of familiarity on licking behaviour

In the previous experiment mice consumed more of the CS+ flavour cue than the CS- during training, therefore showing a flavour preference for the CS+ compared to the CS-. To rule out the explanation that the flavour preference was due to the CS+ being relatively more familiar than the CS-, mice were run through a secondary study. In this, mice were trained for multiple sessions with one flavour cue that was paired with 4% sucrose. Licking behaviour towards this flavour cue, compared to a novel one, was then assessed. Any preference for the familiar cue compared to the novel one, would be seen in the test sessions as a preference for the flavour previously experienced during the training sessions. In this case, it would suggest that the previous flavour preference effect could have been the result of the CS+ flavour being more familiar, rather than the preference having occurred as a result of short-term memory effects in the training stage.

#### 3.2.1 Methods

##### Subjects and Apparatus

The mice used were the same as in the previous experiment, 3.1, and were all housed and maintained in the same way. The apparatus used were also the same as in experiment 3.1.

##### Procedure

All mice were given access to a new flavour cue, X, paired with 4% sucrose, for four daily 10-minute training sessions, with access to the sipper tube throughout the session. During a single test session, mice were subsequently presented with this familiar flavour cue (X) and also a novel flavour (Y). Each was presented for ten-minutes, with a ten-minute interval between presentations. The flavours were apple and orange Kool-Aid, counterbalanced so that for half of the mice apple was X and orange was Y, for the other half apple was Y and orange was X. The order of presentations in the test session was also counterbalanced with respect to genotype

and sex as far as possible given the numbers. Half of the mice received the familiar flavour first, X, followed by the novel Y, with the remaining mice given flavour Y first followed by flavour X.

### **Statistical analysis**

Mixed model ANOVA were carried out in a similar way to experiment 2.1. For the training data, genotype and session were included as factors, for the test data the factors were genotype and cue.

### **3.2.2 Results and Discussion**

#### Training

##### Total Licks:

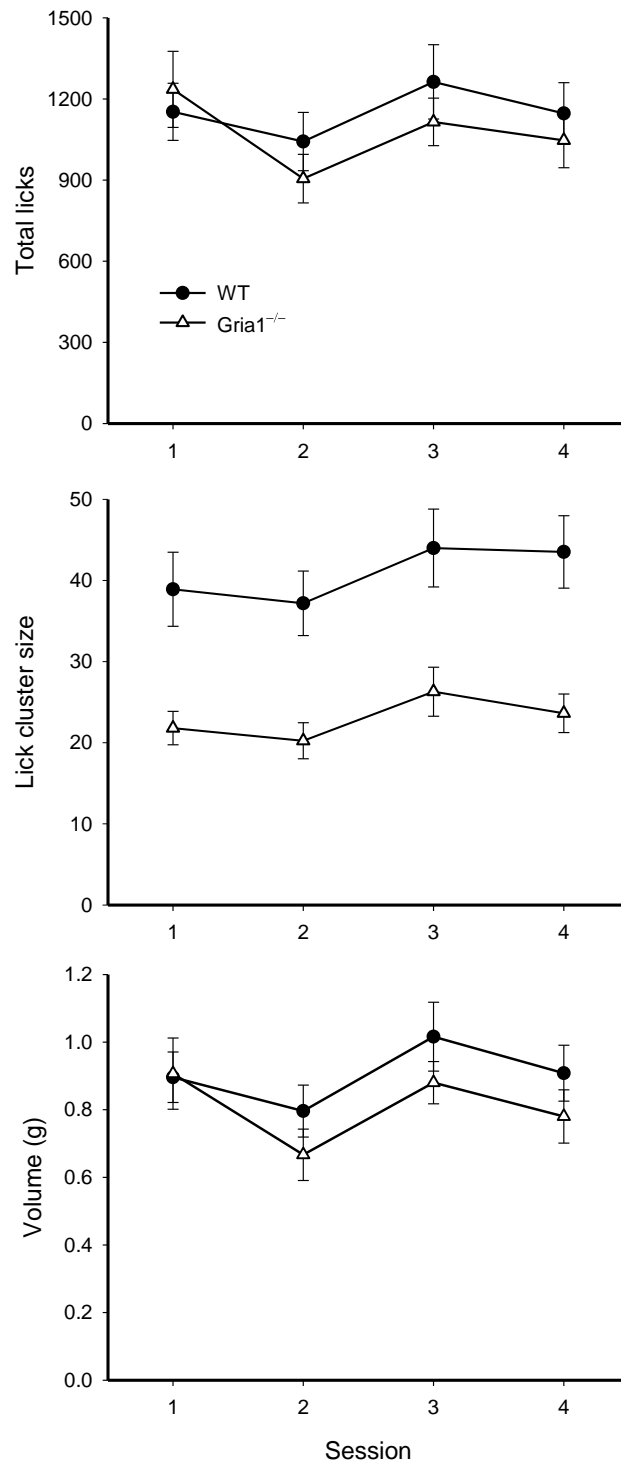
The mean total numbers of licks made during consumption of flavour cue X, by wild-type and *Gria1*<sup>-/-</sup> mice over the four training sessions, are shown in Figure 3.3 (top panel). Both the wild-type and the *Gria1*<sup>-/-</sup> mice made similar numbers of licks during consumption of the sucrose solution over sessions. The ANOVA showed that there was a significant effect of session,  $F(3,114) = 5.97$ ,  $p = .001$ , and no significant effect of genotype,  $F(1,38) = .23$ ,  $p = .63$ . There was also no significant interaction between session and genotype,  $F(3,114) = 1.6$ ,  $p = .19$ .

##### Lick cluster size:

The mean lick cluster sizes made during consumption of flavour X, by both genotypes across the four sessions, are shown in Figure 3.3 (middle panel). The *Gria1*<sup>-/-</sup> mice made smaller lick cluster sizes than the wild-type mice across all four sessions. The mean lick cluster sizes also showed a slight increase over sessions in both the wild-type and *Gria1*<sup>-/-</sup> mice. The ANOVA showed that there was a significant effect of genotype,  $F(1,38) = 10.6$ ,  $p = .002$ , as well as a significant effect of session,  $F(3,114) = 4.4$ ,  $p = .005$ , and there was no significant interaction between session and genotype,  $F(3,114) = .24$ ,  $p = .87$ .

### Volume:

The mean volumes consumed of the flavour cue, by both genotypes across the four sessions, are shown in Figure 3.3 (lower panel). Both the wild-type and *Gria1*<sup>-/-</sup> mice made similar levels of consumption of the cue over sessions. The ANOVA showed that there was a significant effect of session,  $F(3,114) = 6.7$ ,  $p < .001$ , and no significant effect of genotype,  $F(1,38) = .75$ ,  $p = .39$ . There was also no significant interaction between session and genotype,  $F(3,114) = .97$ ,  $p = .39$ .



*Figure 3.3.* Experiment 10, training stage. The mean total numbers of licks (upper), lick cluster sizes (middle) and volumes consumed (lower), by the wild-type (WT) and *Gria1*<sup>-/-</sup> mice. Error bars show ± SEM

## Test

### Total licks:

The mean total numbers of licks made during consumption of the novel and familiar flavour cues, by the wild-type and *Gria1*<sup>-/-</sup> mice, are shown in Figure 3.4 (top panel). The wild-type and *Gria1*<sup>-/-</sup> mice made similar numbers of licks during consumption of the two flavour cues. The ANOVA showed that there was no significant effect of flavour cue,  $F(1,38) = .03$ ,  $p = .86$ , and no significant effect of genotype,  $F(1,38) = .08$ ,  $p = .78$ , or significant interaction between flavour cue and genotype,  $F(1,38) = 2.02$ ,  $p = .16$ .

### Lick cluster size:

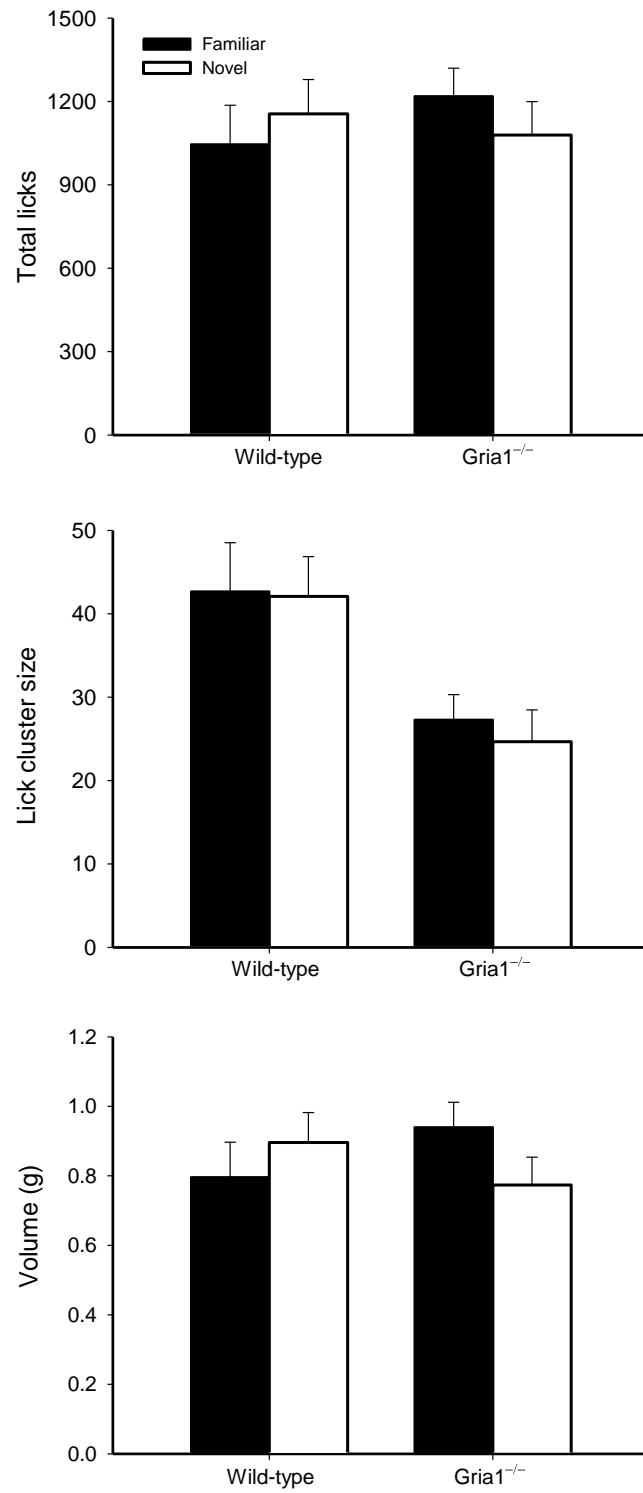
The mean lick cluster sizes made during consumption of the novel and familiar flavour cues, by both genotypes, are shown in Figure 3.4 (middle panel). The mean lick cluster sizes were similar during consumption of the novel and familiar cues in both genotypes. The mean lick cluster sizes were also reduced in the *Gria1*<sup>-/-</sup> mice compared to the control wild-type mice. The ANOVA showed that there was no significant effect of flavour cue,  $F(1,38) = .34$ ,  $p = .56$ , and a significant effect of genotype,  $F(1,38) = 5.9$ ,  $p = .02$ . The interaction between cue and genotype was not significant,  $F(1,38) = .14$ ,  $p = .72$ .

### Volume:

The mean volumes consumed of the novel and familiar flavour cues, by the wild-type and *Gria1*<sup>-/-</sup> mice, are shown in Figure 3.4 (lower panel), with levels of consumption similar in both genotypes. The wild-type mice consumed slightly more of the novel cue compared to the familiar, and the *Gria1*<sup>-/-</sup> mice consumed slightly more of the familiar flavour cue than the novel one. The ANOVA showed that there was no significant effect of cue,  $F(1,38) = .31$ ,  $p = .58$ , or genotype,  $F(1,38) = .008$ ,  $p = .93$ , but the interaction between flavour cue and genotype was significant,  $F(1,38) = 4.9$ ,  $p = .033$ .

Simple main effects analysis of the interaction between flavour cue and genotype, showed that for both the familiar flavour ( $F(1,38) = 1.03$ ,  $p = .32$ ) and novel flavour

cues ( $F(1,38) = .92$ ,  $p = .34$ ), there were no significant differences in volume consumed between the wild-type and *Gria1*<sup>-/-</sup> mice. There were also no significant differences in consumption between the two flavour cues for either the wild-type ( $F(1,38) = 1.8$ ,  $p = .18$ ) or the *Gria1*<sup>-/-</sup> mice ( $F(1,38) = 3.1$ ,  $p = .089$ ). Therefore, although there was a significant interaction between flavour cue and genotype, simple main effects analysis further showed none of these differences reached significance.



*Figure 3.4.* Experiment 10, test stage. The mean total numbers of licks (upper), lick cluster sizes (middle) and volumes consumed (lower), of the familiar and novel flavour cues, by the wild-type and Gria1<sup>-/-</sup> mice. Error bars show ± SEM

The results show that a cue being more familiar, as a result of prior consumption, does not result in a flavour preference for that cue compared to a novel one. This was seen in there being no differences in either the amount consumed, or the mean lick cluster sizes made during consumption, between the novel and familiar flavours during the test sessions. There was therefore no evidence that a flavour cue being familiar results in increased consumption, or palatability, as measured by the mean lick cluster size. Furthermore, in the previous study the difference in familiarity was relative, with both having been previously consumed. In this experiment the difference was even more extreme, comparing a novel cue with a familiar one. Any familiarity effect would therefore be expected to be greater in this experiment compared to the previous one. It therefore seems unlikely that the flavour preference seen in experiment 3.1 occurred as a result of increased familiarity of the CS+ compared to the CS- during the test sessions.

### **3.3 General discussion**

The two experiments in this chapter showed that wild-type and *Gria1*<sup>-/-</sup> mice were able to learn a flavour preference based on contrast effects, despite the prediction that the *Gria1*<sup>-/-</sup> mice would be impaired. Experiment 1 showed that both wild-type and *Gria1*<sup>-/-</sup> mice were able to learn a flavour preference based on contrast effects. Experiment 2 then tested if this flavour preference was a result of greater relative familiarity of the CS+ during the test sessions compared to the CS- flavour cue. The results from this second experiment, although show that there is no difference in licking behaviour between a familiar and novel flavour cue, are a null result. Using a null result to support a lack of effect should be interpreted with some caution. However, the result does correspond to the previous findings of Austen et al. (2016), providing a replication of this study and supporting a lack of effect of familiarity on licking behaviour.

The prediction that the *Gria1*<sup>-/-</sup> would be impaired was due to the proposed role of the GluA1 subunit in short-term memory decay (Sanderson et al., 2009). In particular, Dwyer et al. (2018) explained the contrast based flavour preference in terms of

Wagner's SOP model (Wagner, 1981) and short-term memory decay. The higher concentration of preceding sucrose was proposed to result in more elements being initially processed in a primary active state, compared to the lower concentration. There is then also greater decay of these elements into the secondary active state from this primary one following consumption of the higher sucrose concentration. When the CS- flavour cue is subsequently presented, paired with 8% sucrose, there are relatively few sucrose elements available to enter a primary active state and into an association with the flavour. When the CS+ flavour is presented however, there are fewer elements representing sucrose in the secondary active state, meaning more can enter the primary active state and into an association with the flavour. This results in a flavour preference for the CS+ compared to the CS-, despite both being paired with the same concentration of sucrose. If GluA1 deletion slows this decay rate (Sanderson et al., 2009) then there should be less activation of sucrose elements in the secondary active state. Short-term memory would therefore be less able to prevent subsequent learning about sucrose and generate a flavour preference for the CS+ over the CS-.

One account of these results is that GluA1 deletion does not impair short-term habituation. However, as impairments in short-term habituation have been previously observed in these mice using various procedures (e.g. Sanderson et al., 2009), this explanation seems unlikely. Another potential explanation is that this task does not reflect short-term habituation mechanisms as described by Dwyer et al., (2018), but instead occurred as a result of a different mechanism, such as sensory adaptation. In this case, rather than a cognitive process of decay into short-term memory acting to reduce responding to sucrose, a decline in the physical response to sucrose may instead be the cause of the flavour preference for the CS+. It may be the case that being exposed to a greater concentration of sucrose increases sensory adaptation to sweet tastes such as sucrose. This would mean that the perceived sweetness of the 8% sucrose would be reduced during consumption of the CS- compared to the CS+, causing the flavour preference for the CS+ compared to the CS- flavour. This could also explain the reduced mean lick cluster sizes seen during

consumption of the CS- flavour cue compared to the CS+, as it would be perceived as being less sweet during consumption.

There is some evidence against such an explanation however, in that short-term reductions in responding to sucrose have been found to recover quickly. For example, it has previously found that a period of ten-minutes is sufficient for lick cluster sizes to recover after a short period of access to sucrose in which cluster sizes had quickly declined (Strickland, Austen, & Sanderson, 2018). These results suggest that any effects of sensory adaptation as a result of exposure to sucrose, are likely to be transient and recover over short time periods. As mice received a ten-minute interval between presentations of the sucrose solutions and flavour solutions in the current flavour experiment, it is likely that sensory adaptation effects would be minimal on the processing of the flavour solutions (themselves paired with 8% sucrose) following pre-exposure to sucrose. In these experiments however, mice received far shorter periods of access to sucrose than in the current experiment, meaning the levels of sensory adaptation may not necessarily be comparable to the current experiment.

If the results are however due to learning based on cognitive short-term memory decay, this would suggest that although GluA1 deletion impairs the expression of short-term memory, it does not necessarily affect association formation as may be expected. In this case, it would also suggest that the role of GluA1 may be specific to the expression of short-term memory. This idea corresponds to some of the previous findings in the *Gria1<sup>-/-</sup>* mice, of altered expression but not formation of short-term memory. As although the *Gria1<sup>-/-</sup>* mice showed enhanced responding to a recently experienced stimulus compared to controls, they were still able to respond more to this than a non-recently presented stimulus (Sanderson et al, 2011b). The findings from the experiments in this chapter may support this idea, that GluA1 deletion does not necessarily alter short-term memory encoding but does change the way in which this memory is expressed. This could explain the *Gria1<sup>-/-</sup>* mice still being able learn a flavour preference based on short-term memory of sucrose, while also showing impaired short-term habituation and memory.

The results should however be interpreted with some caution, as the potential effects of sensory adaptation cannot be ruled out from the present results. In this

case, the flavour preference effect may not have been due to cognitive short-term memory decay of the representation of sucrose, but rather memory in the form of differential levels of sensory adaptation and physical responding to sweet sucrose.

## Chapter 4

### **The role of hippocampal NMDA receptors in palatability and flavour preference learning**

The hippocampus, and glutamatergic dysfunction, have been linked to psychiatric disorders including schizophrenia. A meta-analysis of MRI studies of patients with schizophrenia, showed significant bilateral reductions in hippocampal volume (Wright et al., 2000). The NMDA receptor for glutamate has also been linked to the disorder. NMDA receptor antagonists for example result in some of the positive and negative symptoms associated with schizophrenia (Javitt & Zukin, 1991). Furthermore, dysregulation of the GluN1 subunit of the NMDA receptor in the hippocampus was found in a post-mortem study of patients diagnosed with schizophrenia (Vrajová et al., 2010). Genetic association studies have further linked genes encoding NMDA receptor subunits to the disorder (Allen et al., 2008; Begni et al., 2003; Qin et al., 2005). This evidence has led to the glutamate hypofunction model of schizophrenia, with dysregulation of the NMDA receptor suggested to be a potentially important pathology of the disorder (Kantrowitz & Javitt, 2010).

Animal models provide a way to further investigate the role of NMDA receptors in disorders such as schizophrenia. Mice with impaired NMDA receptor functioning have been shown to be a potential model for some of the negative symptoms of schizophrenia, including impaired behavioural inhibition and social interactions (Halene et al., 2009). One negative symptom associated with schizophrenia, as well as glutamatergic dysfunction, is anhedonia (Der-Avakian & Markou, 2012). The mean lick cluster sizes made during consumption of palatable solutions by rodents have been suggested to provide a measure of palatability and hedonic value. For example, whereas the mean lick cluster sizes have been found to increase with increasing sucrose concentration, the amount consumed shows an inverted-U shape function (Austen et al., 2016; Dwyer, 2012). A microstructural analysis of licking behaviour in the *Grin1*<sup>ΔDGCA1</sup> mice, using mean lick cluster size as well as measures of intake,

therefore provides a way to investigate the role of NMDA receptors in perceived palatability and hedonic value.

The hippocampus has also been linked to the control of eating behaviour and energy intake. For example, it has dense populations of receptors for hormones linked to energy regulation, such as the adiposity hormones of leptin and insulin (Lathé, 2001). It has also been shown to have connections with other areas of the brain linked to energy regulation, such as the hypothalamic nuclei (Cenquizca & Swanson, 2006). Rats with hippocampal lesions also show an inability to use internal energy signals and regulate eating behaviour, resulting in weight gain (Davidson et al., 2009). They also show altered control of eating behaviour, eating more regularly but consuming less in each meal (Clifton et al., 1998). The role of the hippocampus in eating behaviour may also link to its role in learning and memory processes. On a neural level, the satiety hormone Leptin modulates synaptic plasticity through enhancing NMDA receptor functioning and LTP. Rodents that are insensitive to leptin have also been found to show impairments in memory (Harvey, Solovyova, & Irving, 2006), demonstrating that satiety signals in the hippocampus may also act to facilitate learning and memory. Hippocampal functioning has also been linked to an impaired ability to inhibit eating behaviour. Patients with amnesia for example will eat second meals despite having already eaten a full meal (Rozin, Dow, Moscovitch, & Rajaram, 1954). Rats with selective hippocampal lesions also show an increased in appetitive food behaviours, despite already being sated (Clifton et al., 1998). The hippocampus therefore seems to be important in regulating adaptive behaviour linked to internal energy state signals. The precise role of hippocampal NMDA receptors in eating behaviour is, however, unknown.

The first experiment in this chapter investigated the role of hippocampal NMDA receptors in palatability and eating behaviour. A microstructural analysis of licking behaviour was used during consumption of various concentration sucrose solutions by  $\text{Grin1}^{\Delta\text{DGCA1}}$  and control type mice. The mean lick cluster size provides a measure of palatability during consumption of the palatable sucrose solutions. If mean lick cluster sizes are impaired in the  $\text{Grin1}^{\Delta\text{DGCA1}}$  mice, this would correspond with the previous findings of impaired lick cluster sizes in the  $\text{Gria1}^{-/-}$  mice (Austen et al.,

2017). It would also provide further support for a role of hippocampal NMDAR functioning in perceived palatability and hedonic value, suggesting they may provide a potential animal model of anhedonia. Intake was also measured, using the amount consumed and the total number of licks made during consumption. The second experiment in this chapter looked at flavour preference learning in the Grin1<sup>ΔDGCA1</sup> mice. The final experiment in this chapter looks at the roles of flavour-flavour and flavour-nutrient associations, both of which have been suggested to support flavour preference learning, as well as the role hippocampal NMDA receptors may play in these.

## 4.1 Experiment 11

### The role of hippocampal NMDA receptors in licking behaviour

Glutamatergic dysfunction linked to NMDA receptor signalling, including within the hippocampus, has been identified as a potential pathology related to schizophrenia (Kantrowitz & Javitt, 2010). The NMDA receptor has also been linked to symptoms associated with the disorder (Halene et al., 2009). Anhedonia is one negative symptom linked to schizophrenia (Der-Avakian & Markou, 2012). However, the role hippocampal NMDA receptors may play in anhedonia and the perception of hedonic value is not currently known. To investigate the role of hippocampal NMDA receptors in the hedonic response to sucrose, Grin1<sup>ΔDGCA1</sup> mice were given access to four different sucrose concentrations. The mean lick cluster size was used as a measure of the perceived palatability of the sucrose solutions during consumption. Previous studies have found that the mean lick cluster size shows a linear increase with sucrose concentration, whereas the amount consumed follows an inverted-U shape function with increasing concentration. The mean lick cluster size is therefore dissociable from the amount consumed and seems to provide a measure of palatability (Austen et al., 2016; Dwyer, 2012).

Initial lick rates during consumption have also been suggested to provide a potential measure of palatability (Davis & Levine, 1977). Additional analysis, on the total numbers of licks and the mean lick cluster size data from the first 5-minutes of each session, were therefore also carried out. If the Grin1<sup>ΔGCA1</sup> mice show impaired mean lick cluster sizes during consumption of palatable sucrose, this would provide some evidence that hippocampal NMDA receptors may play a role in palatability and hedonic value. It would also suggest that they may provide an animal model for anhedonia and glutamatergic dysfunction linked to schizophrenia.

#### **4.1.1 Methods**

##### **Subjects**

Grin1<sup>ΔGCA1</sup> mice (Bannerman et al, 2012) are NR1 floxed (GluN1<sup>2floxed</sup>) and carry two other transgenes, LC1 and CN12. This allows for doxycycline controlled cre-mediated deletion of the Grin1 gene and expression of the GluN1 subunit that is selective to excitatory hippocampal neurons. Doxycycline is administered to the mothers then removed post-natally, allowing selective hippocampal NMDA deletion upon removal. Heterozygous pairs of Tg<sup>LC1</sup> and Tg<sup>CN12</sup> are bred to give Grin1<sup>ΔGCA1</sup> mice that carry one copy of each transgene, with the remaining mice providing littermate controls that carry neither (just the NR1 gene), or only one of the two transgenes required for the deletion to occur (LC1 or CN12). 29 Grin1<sup>ΔGCA1</sup> mice (14 females, 15 males) and 57 control mice (26 females, 31 male; 18 CN12, 20 LC1, 19 NR1), bred in the life sciences support unit at Durham university and caged in groups of 1-8, were used. They were approximately 7.5-12 months old at the start of testing. During testing they were maintained at 85% of their free feeding body weights, the 85% body weights of the control mice were between 18.4g – 41.1g, the Grin1<sup>ΔGCA1</sup> mice between 23.5g – 42.2g and had ad libitum access to water in their home cages. All mice were naïve and had no previous experience within the apparatus.

##### **Apparatus**

The apparatus used were the same as in previous licking analysis experiments, e.g. 2.1, with four different sucrose concentrations used: 2.5%, 5%, 10%, and 20%. As with previous experiments, sucrose solutions were made up weight/volume with

commercially available sucrose in distilled water. The amount consumed (g) was measured by weighing the pipettes before and after each session of access to one of the sucrose solutions.

### **Procedure**

In each daily testing session mice had 30-minutes of access to one of the sucrose solutions, with the pipette made available after a 5-minute period in the chamber. Each of the four sucrose concentrations were presented for a total of three sessions. The order of presentations was such that there was one presentation of each concentration in every four sessions (a block of trials). The order of presentations of the concentrations were counterbalanced so that half of the mice received the two lower concentrations (2.5% and 5%) in the first two sessions of the first block, with the two higher concentrations (10% and 20%) then given in the final two sessions of the first block. The remaining half of mice received the two higher concentrations in the first two sessions and the lower two concentrations in the final two sessions. The order of presentations within these lower/higher concentration pairings were also counterbalanced. For example, for mice given access to the two lower concentrations in the first two sessions, approximately half received 2.5% first, and the remaining mice received 5% first. For each of these counterbalances, approximately half the mice then received 10% followed by 20% in the two sessions with the higher concentrations, with the other half receiving 20% followed by 10%. This meant there were a total of eight different orders of presentations possible within a block of trials. Genotype and sex were also counterbalanced with respect to presentation order as far as possible given the numbers. After each block of presentations (consisting of one presentation of each concentration) the order of presentations was reversed for each mouse, for a total of three blocks of sessions.

### **Statistical analysis**

For all three measures (total licks, lick cluster size, and volume), and for each of the four sucrose concentrations, the data were averaged over the three blocks of sessions. Statistical analyses were done in a similar way to previous experiments, e.g. 2.1. Mixed model ANOVA, with sucrose concentration and genotype, were carried

out. As initial lick rates have also been suggested to provide a measure of palatability (Davis & Levine, 1977), the total numbers of licks and mean lick cluster sizes were additionally analysed during the first 5 minutes of access to the sucrose solutions. For each minute within the first 5 minutes of access, the data were averaged across the three sessions of access to each concentration. For five mice (all control type), mean lick cluster sizes were unable to be calculated for all concentrations and time bins and were therefore not included in the analysis. Where sphericity of within-subjects variables could not be assumed, a Greenhouse-Geisser correction was applied to produce more conservative p-values. Post-hoc analysis of significant main effects, where relevant, was carried out using the Bonferroni correction for multiple comparisons.

#### **4.1.2 Results**

##### Total Licks:

The mean numbers of total licks made during consumption of the four sucrose concentrations, by control and Grin1<sup>ΔDGCA1</sup> mice, are shown in Figure 4.1 (upper panel). For both control and Grin1<sup>ΔDGCA1</sup> mice the total number of licks showed an inverted-U shape function, with 5% and 10% showing greater numbers of licks than 2.5% and 20%. The ANOVA showed that there was a significant effect of sucrose concentration,  $F(3,252) = 25.5$ ,  $p < .001$ , and that the effect of genotype was not significant,  $F(1,84) = 1.3$ ,  $p = .25$ . There was also no significant interaction between these two factors,  $F(3,252) = 2.1$ ,  $p = .13$ . Post-hoc analysis of the effect of sucrose concentration further showed that there was no significant difference between the lowest concentration (2.5%) and the highest (20%) ( $p = 1.0$ ), or between 5% and 10% ( $p = .60$ ), but that all other comparisons were significant ( $p$ 's  $< .001$ ).

##### Lick Cluster Size:

The mean lick cluster sizes made during consumption of the four sucrose concentration solutions, by control and Grin1<sup>ΔDGCA1</sup> mice, are shown in Figure 4.1 (middle panel). For both genotypes this showed that as sucrose concentration increased the mean lick cluster size also increased, however the Grin1<sup>ΔDGCA1</sup> mice made smaller mean lick cluster sizes than the control mice. The ANOVA showed that

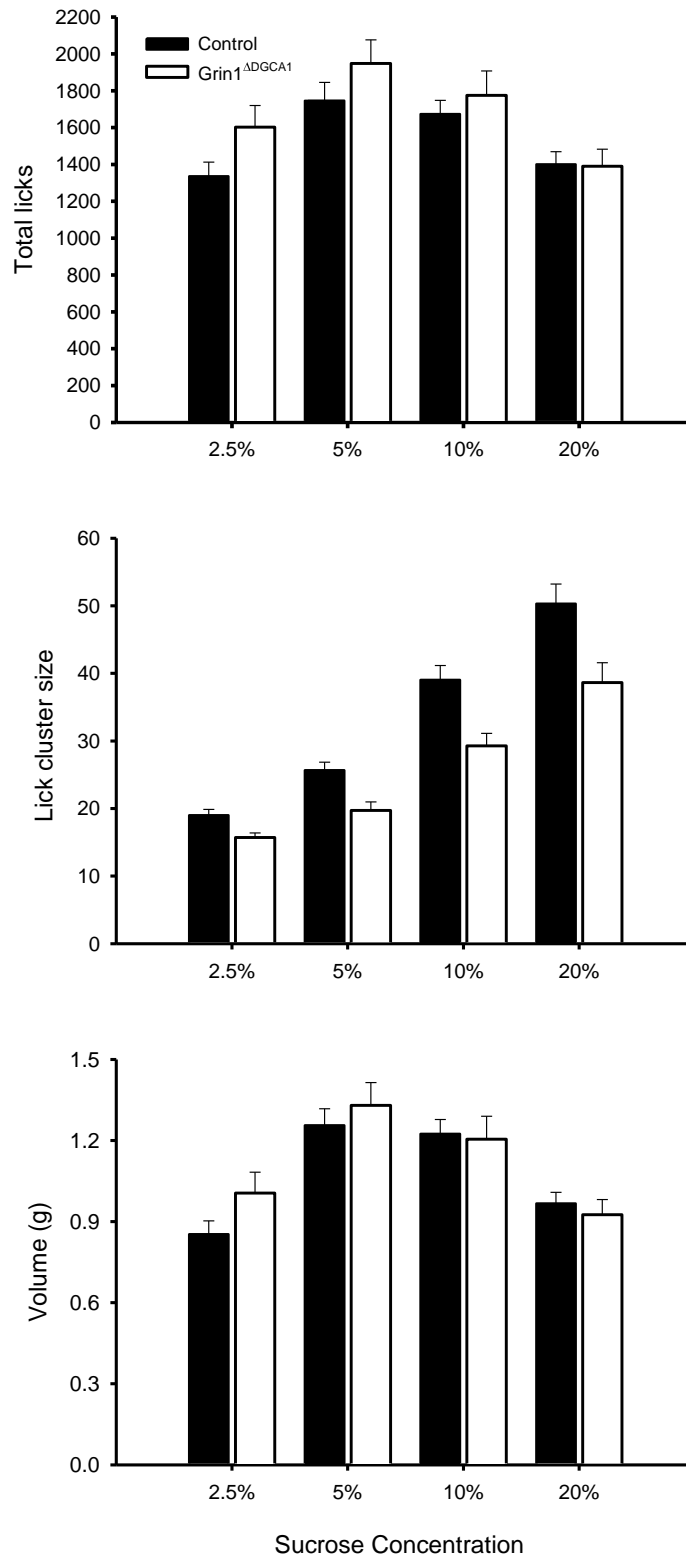
sucrose concentration significantly affected the mean lick cluster sizes made during consumption,  $F(3,252) = 124.5$ ,  $p < .001$ , and that there was also a significant effect of genotype,  $F(1,84) = 10.2$ ,  $p = .002$ . The interaction between these two factors was not significant,  $F(3,252) = 3.03$ ,  $p = .062$ . Post-hoc analysis of the effect of sucrose concentration further showed that the cluster sizes made to the four cues all significantly differed, ( $p$  values  $< .001$ ).

To see if the lick cluster size effect was present from the very first session, additional between subjects analysis using a one-way ANOVA were carried out looking only at the lick cluster sizes from the first session of the experiment. This analysis allows to test if mean lick cluster sizes were reduced in the  $Grin1^{ADGCA1}$  mice from the first exposure to one of the sucrose solutions, or instead if the reduction may have only been apparent after multiple sessions and experience with the various sucrose solution concentrations. The ANOVA showed that there was a significant effect of sucrose concentration on mean lick cluster sizes in the first session alone,  $F(3,78) = 8.7$ ,  $p = .004$ , and a significant effect of genotype  $F(1,78) = 8.7$ ,  $p = .004$ , with no significant interaction between genotype and sucrose concentration,  $F(3,78) = 1.1$ ,  $p = .34$ . Post-hoc analysis of the effect of sucrose concentration further showed that the mean lick cluster sizes made during consumption of the 20% sucrose were significantly larger than during consumption of 2% ( $p = .003$ ), but that no other comparisons reached significance ( $p$  values  $> .39$ ).

#### Volume:

The mean volumes consumed of the four sucrose concentrations, by control and  $Grin1^{ADGCA1}$  mice, are shown in Figure 4.1 (lower panel). As with the total numbers of licks, this shows that for both genotypes there was an inverted-U shape function between concentration and consumption. The ANOVA showed that there was a significant effect of sucrose concentration on the volume consumed,  $F(3,252) = 45.2$ ,  $p < .001$ , with no significant effect of genotype,  $F(1,84) = .28$ ,  $p = .60$ . The interaction between these two factors was not significant,  $F(3,252) = 2.6$ ,  $p = .080$ . Post-hoc analysis further showed that levels of consumption of the lowest (2.5%) and highest sucrose concentrations did not significantly differ ( $p = 1.0$ ), along with no significant

difference between 5% and 10% sucrose, ( $p = .35$ ), but that all other comparisons were significant ( $p$ 's < .001).



*Figure 4.1.* Experiment 11. The mean numbers of total licks (upper), lick cluster sizes (middle) and volumes (lower) consumed, of the four sucrose concentrations, by control and Grin1<sup>ΔDGCA1</sup> mice. Error bars show  $\pm$  SEM.

## Licking behaviour during the first 5-minutes of access

### Total Licks:

The mean numbers of total licks made by the control (upper left panel) and *Grin1<sup>ΔDGCA1</sup>* mice (upper right panel), across the first five minutes of access to the different sucrose concentrations, are shown in Figure 4.2. For both genotypes the mean numbers of total licks made over the first five minutes varied with concentration. The greatest numbers of licks was made during consumption of the 20% sucrose, the lowest to the 2.5%. For each sucrose concentration the number of licks decreased over the five minutes. The ANOVA showed that there was a significant effect of concentration,  $F(3,252) = 80.4$ ,  $p < .001$ , and a significant effect of time bin,  $F(4,336) = 534.5$ ,  $p < .001$ , with no significant effect of genotype,  $F(1,84) = .027$ ,  $p = .87$ , showing that both genotypes made similar patterns of consumption during the first five minutes of access. There was a significant interaction between concentration and time bin  $F(12,1008) = 27.9$ ,  $p < .001$ . All other interactions were not significant,  $F$  values  $< 1.5$ ,  $p$  values  $> .22$ .

### Lick cluster size:

The mean lick cluster sizes made by the control (lower left panel) and *Grin1<sup>ΔDGCA1</sup>* mice (lower right panel), during the first five minutes of consumption, of the four different sucrose concentrations, are shown in Figure 4.2. The control mice made larger mean lick cluster sizes than the *Grin1<sup>ΔDGCA1</sup>* mice, but showed a similar pattern of larger cluster sizes to the higher concentrations of sucrose. The ANOVA showed that the effect of sucrose concentration was significant,  $F(3,237) = 96.7$ ,  $p < .001$ , as was the effect of time bin,  $F(4,316) = 29.0$ ,  $p < .001$ , and the effect of genotype,  $F(1,79) = 9.1$ ,  $p = .003$ . Unlike the total number of licks, the mean lick cluster sizes in the first five minutes of access were reduced in the *Grin1<sup>ΔDGCA1</sup>* mice compared to the control mice. The interaction between concentration and time bin was also significant,  $F(12,948) = 9.7$ ,  $p < .001$ . All other interactions were not significant,  $F$  values  $< 2.4$ ,  $p$  values  $> .088$ .

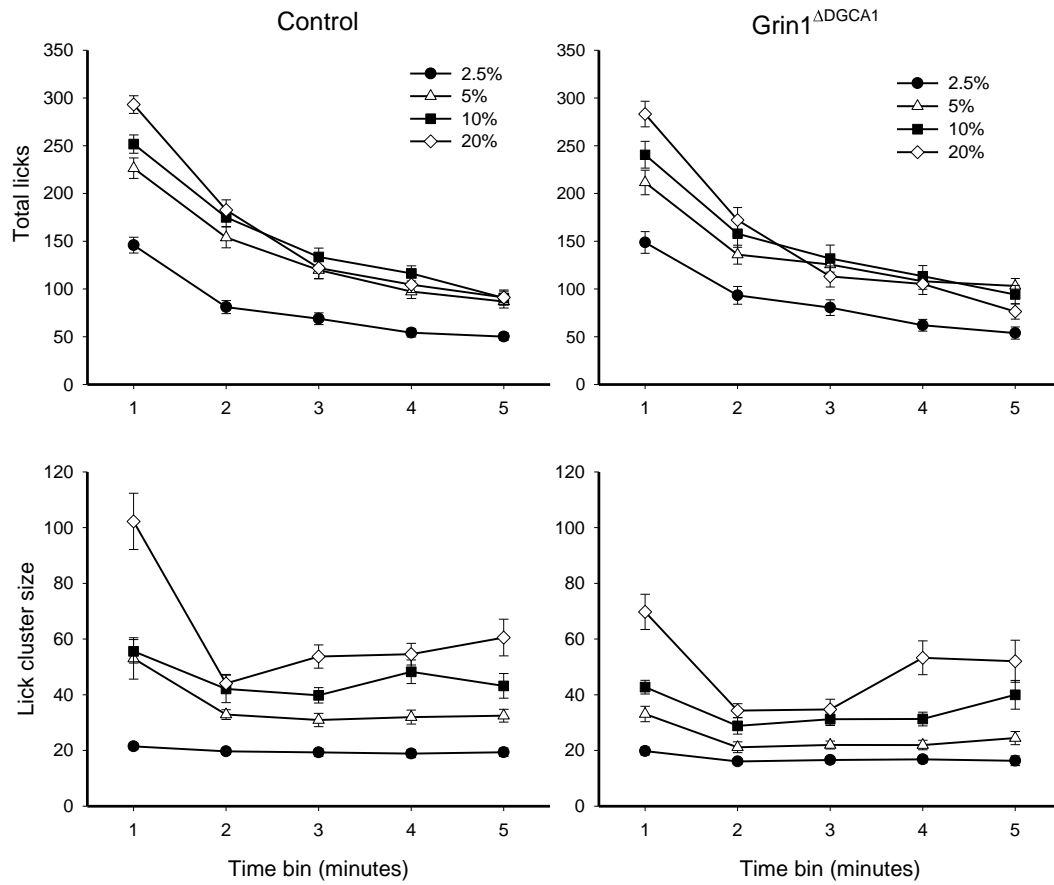


Figure 4.2. Experiment 11, licking behaviour during the first 5-minutes. The mean numbers of total licks (upper) and lick cluster sizes (lower) made during consumption of the four sucrose concentrations, during the first five minutes of access. Control mice are shown in the left panel, Grin1<sup>ΔDGCA1</sup> mice in the right panel. Error bars show ± SEM.

### 4.1.3 Discussion

The results demonstrate that as sucrose concentration increased, both control and Grin1<sup>ΔDGCA1</sup> mice showed an inverted-u shaped function in the total number of licks and the volume consumed, with consumption maximal for the two intermediate sucrose concentrations (5% and 10%). Furthermore, the mean lick cluster sizes made during consumption showed a linear increase with increasing concentration. The mean lick cluster sizes were also reduced in the Grin1<sup>ΔDGCA1</sup> mice compared to the control mice, across concentrations of sucrose. This reduction was also seen from early in training, with an effect of concentration on the mean lick cluster size evident in the first session. This demonstrates that the reduction was evident even with only one session of access to one of the sucrose solutions, showing that lick cluster sizes are reduced, and remain reduced, in the Grin1<sup>ΔDGCA1</sup> mice, rather than occurring after multiple sessions of access and experience with the various sucrose solution concentrations.

Therefore, although the Grin1<sup>ΔDGCA1</sup> mice did show increasing lick cluster sizes with increasing sucrose concentration, deletion of NMDA receptors in the Grin1<sup>ΔDGCA1</sup> mice does seem to reduce mean lick cluster size, a measure of palatability. These results are in line with the previous findings of an inverted-U shape function of consumption, but a linear increase in mean lick cluster size (Austen et al, 2016; Dwyer, 2012). These results also suggest that hippocampal NMDA deletion may reduce the perceived palatability of sweet sucrose solutions, in the form of lower mean lick cluster sizes. In this case, the results would support a role for hippocampal NMDA receptors in hedonic value. Although, it is also possible that this reduction could relate to an impairment in the expression of palatability in the form of mean lick cluster size, rather than altered hedonic value.

The analysis of the first 5-minutes of access also showed that initially the total number of licks was greater as sucrose concentration increased. However, in this measure, there was no effect of genotype. The initial lick rate therefore seems dissociable from the mean lick cluster size. Whereas the mean lick cluster sizes were reduced in the Grin1<sup>ΔDGCA1</sup>, the initial lick rates showed the same pattern as the

control type mice in the first five minutes of consumption. This demonstrates that mean lick cluster sizes are impaired right from the start of consumption in the Grin1<sup>ΔDGCA1</sup> mice. The reduced mean lick cluster sizes seen over the full 30-minute sessions are therefore not due to a different pattern of lick cluster sizes over time and consumption. For example, the lick cluster sizes may have initially been normal but reduced more quickly in the Grin1<sup>ΔDGCA1</sup> mice than the control mice. In this case, this would have resulted in mean cluster sizes being reduced, but the ability to perceive palatability in the form of larger lick cluster sizes, initially being normal. An effect that might be indicative of a satiety or fatigue difference between the genotypes, rather than an impairment in perception of palatability. However, this did not seem to be the case, as mean lick cluster sizes were reduced from early in consumption. Initial lick rates have been suggested to provide a measure of palatability (Davis & Levine, 1977). In this case, the Grin1<sup>ΔDGCA1</sup> mice show reduced palatability only in mean lick cluster size and not in the initial lick rate. This dissociation between the two measures is however indicative that they are likely to measure different aspects of licking behaviour.

However, the impairment in lick cluster sizes could be the result of an inability to express larger lick cluster sizes, rather than in perceived palatability itself. In this case, the Grin1<sup>ΔDGCA1</sup> mice may be just as able to perceive the differences in palatability and hedonic value as the control mice, but not able to make the larger lick clusters to demonstrate it. Although lick cluster sizes did increase in the Grin1<sup>ΔDGCA1</sup> mice as sucrose concentration increased, suggesting they are to some extent able to show larger lick cluster sizes, there may still be a more general impairment in the expression of cluster size. An explanation that is difficult to rule from the currently results.

In the following experiment, the effect of reduced lick cluster sizes on flavour preference learning was tested. This provides a way to assess if an impairment in mean lick cluster sizes, a measure of palatability, also impairs the ability to learn a flavour preference. Normal flavour preference learning would also demonstrate the effect of hippocampal NMDA receptor deletion to be specific to the measure of

palatability (lick cluster sizes), not affecting the ability to discriminate between and learn about, different flavoured sucrose solutions.

## 4.2 Experiment 12

### Flavour preference learning

In the previous experiment, mean lick cluster sizes were reduced in the Grin1<sup>ΔDGCA1</sup> mice during consumption of palatable sucrose solutions. This may suggest that deletion of hippocampal NMDA receptors impairs perceived palatability of sucrose. The amount consumed however, in terms of volume and the total numbers of licks, did not differ between the genotypes. This further demonstrates that consumption is normal in the Grin1<sup>ΔDGCA1</sup> mice, meaning the impairment seems to be specific to mean lick cluster size, a measure of palatability.

In this second experiment, the role of hippocampal NMDA receptors in being able to learn a flavour preference was tested. In flavour preference learning, an association is formed between the higher concentration of the reinforcer (e.g. sucrose) and the flavour paired with it. This results in greater consumption of the CS+ compared to the CS- during test sessions, when both cues are paired with the same concentration of sucrose. Flavour preference learning can be supported by flavour-flavour associations, i.e. between the flavour cue (e.g. apple) and that of the reinforcer (e.g. the sweet taste sucrose). It can however also be supported by an association between the flavour cue and the caloric content of the reinforcer (Ackroff, Dym, Yiin, & Sclafani, 2009). Sucrose, that has both a sweet palatable taste and a high calorie content, is likely to support flavour preference learning based on both flavour-flavour and flavour-nutrient associations. As flavour preference to sucrose may relate to the ability to perceive the palatable taste of it, the impaired lick cluster sizes in the Grin1<sup>ΔDGCA1</sup> mice may also alter the ability to learn this flavour preference. If impaired lick cluster sizes do alter flavour preference learning, this may reduce the preference for the CS+ compared to the CS- cue, in the Grin1<sup>ΔDGCA1</sup> mice compared to the control mice. If however flavour preference learning is normal in the Grin1<sup>ΔDGCA1</sup> mice, this

would suggest that the impairment is specific to the mean lick cluster size, a measure of palatability, not affecting the ability to differentiate between and learn about the sensory and/or nutritional properties of the flavoured sucrose solutions. It would also provide further evidence that associative learning and memory are intact in the Grin1<sup>ΔDGCA1</sup> mice. To test this, Grin1<sup>ΔDGCA1</sup> mice were run through a flavour preference procedure in a similar way to the flavour preference experiments in chapter 2 of this thesis (e.g. 2.1).

#### **4.2.1 Methods**

##### **Subjects**

13 Grin1<sup>ΔDGCA1</sup> mice (6 females, 7 males) and 34 control mice (14 females, 20 males; 11 CN12, 12 LC1, 11 NR1), bred, housed, and caged, in the same way as experiment 4.1, were used. They were approximately 10 - 12 months old at the start of testing and had previously been used in experiment (4.1). During testing they were maintained at 85% of their free feeding body weights, the control mice weighed between 18.4g – 33.6g and the Grin1<sup>ΔDGCA1</sup> mice between 23.5g-35.4g and all mice had ad libitum access to water in their home cages. Mice had previous experience of appetitive magazine conditioning in similar operant chambers and also had experience of consuming sucrose solutions within the same chambers used in this experiment.

##### **Apparatus**

The apparatus used were the same as in previous licking experiments (e.g. 2.1) and the amount consumed was measured by weighing the pipettes before and after each session.

##### **Procedure**

For four daily training sessions, mice were presented with two flavoured sucrose solutions in succession. The CS- paired with 4% sucrose, and the CS+ paired with 32% sucrose. Each daily session consisted of two 10-minute periods of access, one 10-minute session with the CS- and the other with the CS+. Between each period of access, mice were returned to the home cages with an interval of approximately 10-

minutes between sucrose solution presentations. In this time the sipper tubes were weighed and changed to contain the second flavoured sucrose solution. The flavours were counterbalanced across mice, with respect to genotype and sex as far as possible given the numbers. For approximately half the mice the CS- was grape, and the CS+ cherry. For the remaining mice the CS- was cherry and the CS+ was grape. During training all animals received the cherry flavoured solution first followed by the grape, which were then presented in double alternation across training. This meant that half the mice received the CS- first in training and the other half the CS+ first. Flavour preference was then tested in a single test session, with both the CS- and the CS+ presented in the same manner as in training, now both paired with 4% sucrose. The presentation order of the two flavours during the test session was counterbalanced with respect to training order, and so that half received the CS- first and the other half the CS+.

### **Statistical analysis**

Statistical analyses were carried out in a similar way to experiment 2.1. For the training data mixed model ANOVA with genotype, flavour cue, and session as factors, were carried out. In at least one of the training sessions however, lick cluster sizes were unable to be calculated (due to insufficient licks or bouts) for two mice (1 control and 1 Grin1<sup>ΔDGCA1</sup>), meaning the lick cluster size analysis contains 33 controls and 12 Grin1<sup>ΔDGCA1</sup> mice. For the test data one mouse (a control female) was presented with incorrect flavours and was therefore removed from the analysis, meaning the analysis on the test data included 33 control mice and 13 Grin1<sup>ΔDGCA1</sup> mice.

### **4.2.2 Results**

#### Training

#### Total Licks:

The mean numbers of total licks made during consumption of the CS- and CS+ flavour cues, by control and Grin1<sup>ΔDGCA1</sup> mice across the four training sessions, are shown in Figure 4.3 (upper panel). For both control and Grin1<sup>ΔDGCA1</sup> mice the total numbers of

licks were greatest during consumption of the CS+ flavour than the CS-. The ANOVA showed that mice did make a significantly greater number of licks during consumption of the CS+ than the CS-,  $F(1,45) = 220.7$ ,  $p < .001$ , and there was no significant effect of genotype,  $F(1,45) = .028$ ,  $p = .87$ , as well as no significant interaction between flavour cue and genotype,  $F(1,45) = .22$ ,  $p = .64$ . The effect of session was significant,  $F(3,135) = 4.3$ ,  $p = .023$ , and there was no significant interaction between session and genotype,  $F(3,135) = .28$ ,  $p = .71$ . The interaction between flavour cue and session was also not significant,  $F(3,135) = 2.7$ ,  $p = .081$ , along with the interaction between the three factors of flavour cue, session, and genotype,  $F(3,135) = .48$ ,  $p = .59$ .

#### Lick Cluster Size:

The mean lick cluster sizes made during consumption of the CS- and CS+ flavour cues during training, by the control and Grin1<sup>ADGCA1</sup> mice, are shown in Figure 4.3 (middle panel). Both genotypes showed greater mean lick cluster sizes during consumption of the CS+ than CS-. The ANOVA showed that mean lick cluster sizes were greater during consumption of the CS+ than the CS-,  $F(1,43) = 103.6$ ,  $p < .001$ , with no significant effect of genotype,  $F(1,43) = .77$ ,  $p = .38$ , and no significant interaction between these two factors,  $F(1,43) = .001$ ,  $p = .97$ . The effect of session was also not significant,  $F(3,129) = 2.5$ ,  $p = .068$ , and there was no significant interaction between session and genotype,  $F(3,129) = .58$ ,  $p = .61$ . The interaction between flavour cue and session was significant,  $F(3,129) = 3.01$ ,  $p = .047$ , and there was no significant interaction between the three factors of cue, session, and genotype,  $F(3,129) = .78$ ,  $p = .48$ .

#### Volume:

The mean volumes consumed of the CS- and CS+ flavour cues by the control and Grin1<sup>ADGCA1</sup> mice during training, are shown in Figure 4.3 (lower panel). This shows that both control and Grin1<sup>ADGCA1</sup> mice consumed more of the CS+ than the CS- during training. The ANOVA showed that the volumes consumed were significantly greater during consumption of the CS+ than the CS-,  $F(1,45) = 122.2$ ,  $p < .001$ , and there was no significant effect of genotype,  $F(1,45) = .44$ ,  $p = .51$ , along with no significant

interaction between these two factors,  $F(1,45) = .058$ ,  $p = .81$ . The effect of session was significant,  $F(3,135) = 5.9$ ,  $p = .002$ , and there were no significant interactions between session and genotype,  $F(3,135) = .52$ ,  $p = .63$ , flavour cue and session,  $F(3,135) = 2.6$ ,  $p = .078$ , or between the three factors of flavour cue, session, and genotype,  $F(3,135) = .48$ ,  $p = .62$ .

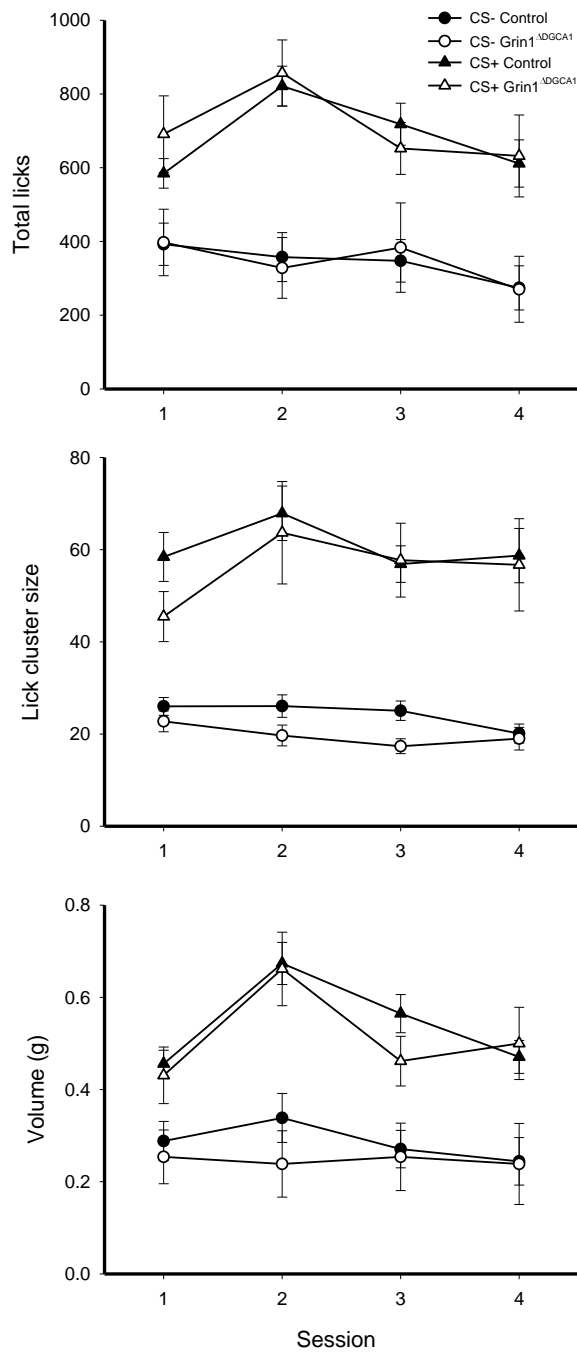


Figure 4.3. Experiment 12, training stage. The mean numbers of total licks (upper), lick cluster sizes (middle) and volumes consumed (lower), of the CS- and CS+ flavour cues, by the control and Grin1<sup>ΔDGCA1</sup> mice during training. Error bars show ± SEM.

## Test

### Total Licks:

The mean numbers of total licks made during consumption of the CS- and CS+, during the test session by the control and Grin1<sup>ΔDGCA1</sup> mice, are shown in Figure 4.4 (upper panel). Both genotypes made greater numbers of licks during consumption of the CS+ than the CS-, demonstrating a flavour preference effect. The ANOVA showed that there was a significant effect of cue,  $F(1,44) = 26.1$ ,  $p < .001$ , and no significant effect of genotype,  $F(1,44) = .19$ ,  $p = .67$ . The interaction between flavour cue and genotype was also not significant,  $F(1,44) = .19$ ,  $p = .67$ . To further investigate the flavour preference effect in each genotype, repeated measures ANOVA were carried out for each the Grin1<sup>ΔDGCA1</sup> mice and the control mice. This showed that for the control mice there was a significant flavour preference,  $F(1,33) = 20.6$ ,  $p < .001$ , and this was also the case for the Grin1<sup>ΔDGCA1</sup> mice,  $F(1,11) = 17.6$ ,  $p = .002$ .

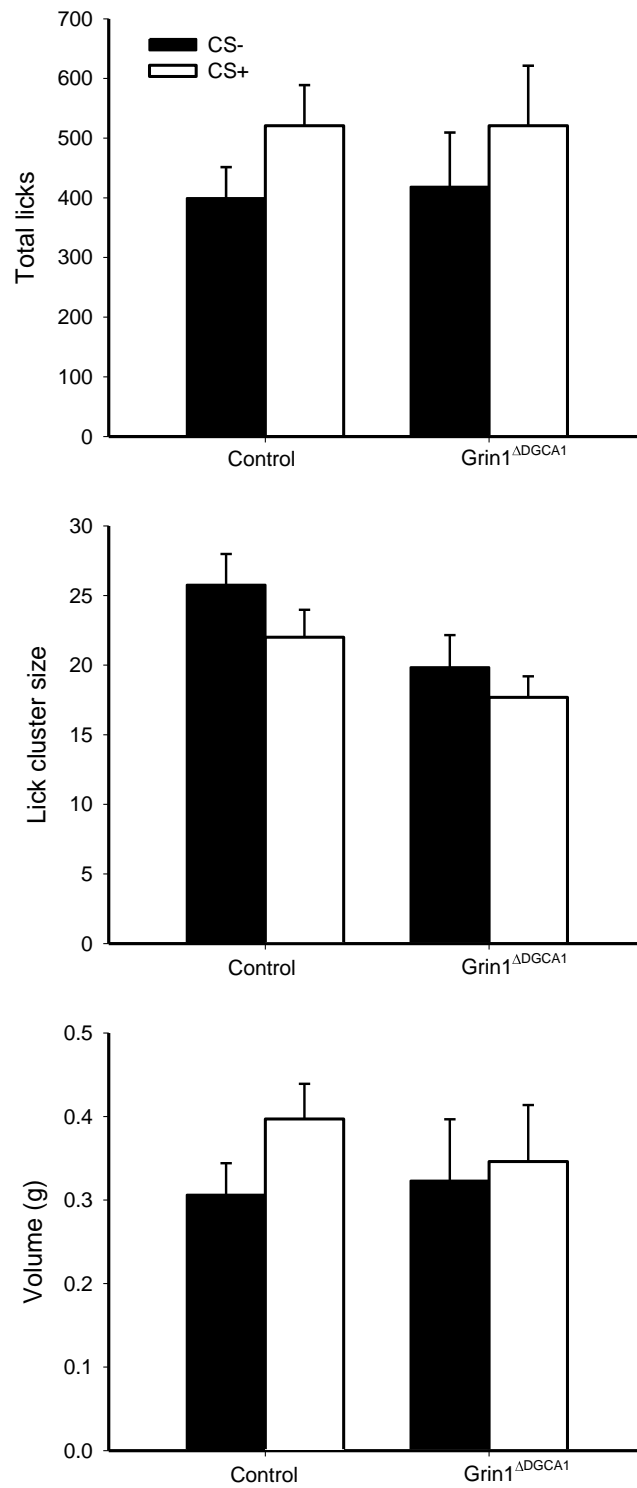
### Lick Cluster Size:

The mean lick cluster sizes made during consumption of the two flavour cues during the test session, by the control and Grin1<sup>ΔDGCA1</sup> mice, are shown in Figure 4.4 (middle panel). Both genotypes showed slightly greater mean lick cluster sizes during consumption of the CS-, rather than the CS+. The ANOVA showed that there was a significant effect of cue,  $F(1,44) = 8.6$ ,  $p = .005$ , meaning mice did consume significantly more of the CS- than the CS+, the opposite of a flavour preference effect. There was also no significant effect of genotype,  $F(1,43) = 2.3$ ,  $p = .14$ , as well as no significant interaction between these two factors,  $F(1,43) = .63$ ,  $p = .43$ .

### Volume:

The mean volumes consumed of the two flavour cues during the test session, by control and Grin1<sup>ΔDGCA1</sup> mice, are shown in Figure 4.4 (middle panel), with both genotypes consuming more of the CS+ than the CS-. The ANOVA showed that there was a significant effect of flavour cue,  $F(1,44) = 6.6$ ,  $p = .014$ , demonstrating a flavour preference effect, and no significant effect of genotype,  $F(1,44) = .052$ ,  $p = .82$ . There was also no significant interaction between the two factors of cue and genotype,

$F(1,44) = 2.3$ ,  $p = .13$ . To further investigate the flavour preference effect in each genotype, repeated measures ANOVA were carried out for each the  $\text{Grin1}^{\Delta\text{DGCA1}}$  mice and the control mice. This showed that for the control mice there was a significant flavour preference effect,  $F(1,33) = 5.4$ ,  $p = .026$ , as was also the case in the  $\text{Grin1}^{\Delta\text{DGCA1}}$  mice,  $F(1,11) = 10.2$ ,  $p = .009$ .



*Figure 4.4.* Experiment 12, test stage. The mean numbers of total licks (upper), lick cluster sizes (middle) and volumes consumed (lower), of the CS- and CS+, by the control and Grin1<sup>ΔDGCA1</sup> mice. Error bars show  $\pm$  SEM.

### 4.2.3 Discussion

The results showed that flavour preference learning was intact in the *Grin1*<sup>ΔDGCA1</sup> mice. This was demonstrated by a significantly greater intake of the CS+ compared to the CS- during the test sessions, with no effect of genotype in either the volume consumed, or the total numbers of licks made during consumption. The effect in terms of the mean lick cluster went significantly in the opposite direction than would be expected for a flavour preference, as both genotypes consumed slightly more of the CS- than the CS+. Previous flavour preference experiments using a similar procedure, found only limited evidence for a lick cluster size effect being seen in terms of larger clusters to the CS+ (Austen et al., 2016). When the results from this experiment are also considered, of larger mean lick cluster sizes in the opposite direction, it seems that flavour preference learning does not result in a clear lick cluster size effect. The results do however show that deletion of NMDA receptors in *Grin1*<sup>ΔDGCA1</sup> mice does not seem to impair the ability to learn a flavour preference, despite previous findings that mean lick cluster sizes are reducing during consumption of sweet sucrose solutions (experiment 4.1). This demonstrates that *Grin1*<sup>ΔDGCA1</sup> mice can differentiate between, and learn about, flavoured sucrose solutions in the form of flavour preference learning.

It is not however known from these results the extent to which flavour-flavour and/or flavour-nutrient associations may have supported the flavour preference with sucrose. The ability to form these two types of associations may also be differentially affected by the lick cluster size impairment seen in the *Grin1*<sup>ΔDGCA1</sup> mice. Flavour-flavour associations, formed between the flavour of the stimulus and that of the reinforcer, are dependent on the ability to perceive the flavour and the palatability of the reinforcer. In flavour-nutrient learning however, an association between the flavour of the stimulus and the post-ingestive consequences of the reinforcer is formed. It is therefore not reliant on the ability to perceive the palatable taste of the reinforcer, only the subsequent caloric benefits. The impaired palatability in the *Grin1*<sup>ΔDGCA1</sup> mice, with the intact ability to perceive and learn about flavoured solutions, provides a way to further investigate these associations during flavour preference learning. It also allows to test the effect of impaired palatability on the

ability to form these different types of associations. As the measure of palatability is impaired in the Grin1<sup>ΔDGCA1</sup> mice, it follows that the ability to form flavour-flavour associations may also be impaired, as they may be less able to form an association between the palatable flavours of the cue and the reinforcer. Flavour-nutrient learning however would be predicted to be less affected, as the Grin1<sup>ΔDGCA1</sup> mice should still be able to learn about the post-ingestive consequences of the reinforcer to the same extent as the control mice. The final experiment in this chapter investigated the role of flavour-flavour and flavour-nutrient associations in flavour preference learning, as well as the effect that impaired lick cluster sizes may have on the ability to learn these associations.

### 4.3 Experiment 13

#### Flavour-flavour and flavour-nutrient associations

Flavour preference learning has been found to be supported by two dissociable types of associations, flavour-flavour and flavour-nutrient associations. In the first case the subject learns to associate the flavour (e.g. cherry) with the flavour of the reinforcer (i.e. sweet taste of saccharin). Whereas with flavour-nutrient learning, the association is between the flavour (e.g. cherry) and the nutritional qualities of the reinforcer (e.g. the high calorie content of sucrose). Evidence for these two types of associations comes largely from the use of different reinforcers, with varying flavour and nutritional properties. Sweet but low-caloric reinforcers like fructose, support flavour preference learning only when the flavour is perceived and not when given directly via intragastric infusion (Sclafani & Ackroff, 1994). This demonstrates it is dependent on perceiving the sweet flavour of fructose, but not any post-ingestive consequences it may have. In contrast, when nutrient rich reinforcers such as polycose, a hydrolzed starch, or glucose, are infused a flavour preference effect is found (Ackroff et al., 2009; Sclafani & Nissenbaum, 1988). These results show that the post-ingestive consequences of these reinforcers alone are sufficient to support flavour preference learning. These two types of associations are also dissociable in

the fact that they show different characteristics. Flavour-flavour associations for example are less able to form when there is a delay between exposure to the flavour and the reinforcer. Whereas flavour-nutrient associations can form even with a relatively long delay between the two (Sclafani & Ackroff, 1994).

Importantly, these two types of associations seem to differentially rely on perception of the flavours of the stimuli. The Grin1<sup>ΔDGCA1</sup> mice provide a way to further investigate the role of palatability on the ability to learn these associations. If perceived palatability is impaired in Grin1<sup>ΔDGCA1</sup> mice, then flavour-flavour associations may be more likely to be affected, but not those based on flavour-nutrient associations. This experiment therefore investigated learning of flavour-flavour and flavour-nutrient associations in Grin1<sup>ΔDGCA1</sup> and control type mice. Two different reinforcers were used to support flavour preference learning, with the procedure similar to that of the previous experiment (4.2). Fructose was used as one of the reinforcers to test flavour-flavour associations. This was used due to fructose having previously been shown to be unable to support flavour preference based on post-ingestive consequences alone, demonstrating the flavour preference to be dependent on perceiving its palatable flavour (Sclafani, Fanizza, & Azzara, 1999). In contrast, maltodextrin was used to test for flavour-nutrient associations, which has been shown to support flavour preference learning based on its post-ingestive consequences (Sclafani & Nissenbaum, 1988). If Grin1<sup>ΔDGCA1</sup> mice are selectively impaired in terms of palatability perception, then learning about fructose (flavour-flavour associations) may be affected. Learning a flavour preference using maltodextrin as the reinforcer however (flavour-nutrient learning), may not be affected by impaired mean lick cluster sizes.

### **4.3.1 Methods**

#### **Subjects**

16 Grin1<sup>ΔDGCA1</sup> mice (8 females, 8 males) and 23 control mice (12 females, 11 males; 7 CN12, 8 LC1, 8 NR1), bred and housed in the same way as experiments 4.1 & 4.2, were used. They were approximately 7.5 - 12 months old at the start of testing were used. They had previously been used in experiment 4.1. During testing they were

maintained at 85% of their free feeding body weights, the control mice had 85% body weights of between 22.2g – 41.1g and the Grin1<sup>ΔDGCA1</sup> mice between 25.0g – 42.2g, with ad libitum access to water in their home cages. Mice had previously been used in appetitive magazine conditioning experiments in similar operant chambers and also had experience consuming sucrose solutions in the same apparatus used in this experiment. They did not however have any previous experience with flavour cues or fructose and maltodextrin.

### **Apparatus**

The apparatus was the same as in the previous flavour preference experiment, (4.2) however maltodextrin (Special Ingredients Ltd, Chesterfield) and fructose (Special Ingredients Ltd) were used as the reinforcers. The flavour solutions were also made in the same way as with sucrose: weight/volume in distilled water.

### **Procedure**

Mice were split into two groups, for one fructose was the reinforcer and for the other maltodextrin was the reinforcer. The groups were counterbalanced with respect to genotype and sex as far as possible given the numbers. The fructose group included 20 mice, 8 Grin1<sup>ΔDGCA1</sup> mice (4 female, 4 male) and 12 were controls (6 females, 6 males, 4 CN12, 4 LC1, 4 NR1). The maltodextrin group included 19 mice, 8 Grin1<sup>ΔDGCA1</sup> mice and 11 controls (6 female, 5 male, 3 CN12, 4 LC1, 4 NR1). For both groups the concentrations of reinforcers were the same. The CS- flavour was paired with 2% and the CS+ flavour was paired with 16%.

The training stage consisted of four daily training sessions, run in the same way as during experiment 4.2. With two 10-minute periods of access, one with the CS- and the other with the CS+, and an interval of approximately ten-minutes between the two periods of access. The flavours used were cherry and grape Kool-aid. These were counterbalanced across animals with respect to genotype, group and sex, as far as possible given the numbers. For half of the mice, the CS- was cherry and the CS+ was grape, for the remaining mice the CS- was grape and the CS+ was cherry. The flavours of cherry and grape were presented in double alternation across training, starting with the cherry flavour for all mice followed by the grape flavour. This meant that

half the mice had the CS- flavour first, followed by the CS+, with the other half the CS+ first and then the CS-. After training there was a single test session. This was run in the same way as the training sessions, with the CS- and the CS+ both presented for 10-minute periods of access. Both were paired with 2% of the reinforcer (either fructose or maltodextrin). The test order was counterbalanced across mice, with respect to group, genotype and flavour allocation, as far as possible given the numbers. Half the mice received the CS- first, the other half the CS+, and vice versa for the remaining mice.

After this test stage the two groups were switched. Mice previously in the fructose group were now in the maltodextrin group. Those in the maltodextrin group were now in the fructose group. They were then run through the flavour preference procedure again, with the same procedure and reinforcer concentrations as used before, but with the new reinforcer. The kool-aid flavours were apple and orange, which were not experienced in the previous flavour preference procedure. As in the first stage, these were presented in double alternation across training sessions. With four daily sessions, each of which had one two periods of access, one with the CS- and one with the CS+. The flavour was counterbalanced so that mice that previously received the CS- first (as a result of being the cherry flavour) now received the CS- second (i.e. the CS- was allocated to the orange flavour). Mice that previously received the CS+ first (as a result of being the cherry flavour) now received the CS- first (i.e., the CS- was allocated to the apple flavour). The test order was similarly reversed, so that those tested with the CS- first in the previous run now received the CS+ flavour first during the test session. Those mice tested with the CS+ first in the previous run now received the CS- first in the second run.

### **Statistical analysis**

Statistical analyses were carried out in a similar way to experiment 4.2. Mixed model ANOVA, with genotype, cue, and session as factors, were carried out for the fructose and maltodextrin groups. This was done due to the interest in the two reinforcers separately, assessing if each can support flavour preference learning in the two genotypes. For the test session mixed model ANOVA, with genotype and cue as factors, were similarly carried out.

### 4.3.2 Results

#### Training

##### Total Licks: fructose

The mean numbers of total licks made during consumption of the CS- and CS+, when paired with fructose by control and Grin1<sup>ΔDGCA1</sup> mice across training sessions, are shown in Figure 4.5 (upper panel, left). Both genotypes made a greater number of licks during consumption of the CS+ than the CS-. There was also a slight downward trend in the numbers of licks made over training sessions by both control and Grin1<sup>ΔDGCA1</sup> mice. The ANOVA showed that there was a significant effect of cue (CS- or CS+)  $F(1,37) = 99.7, p < .001$ , and no significant difference between the genotypes,  $F(1,37) = .31, p = .58$ . There was also no significant interaction between the two factors of cue and genotype,  $F(1,37) = .09, p = .76$ . The effect of session was significant,  $F(3,111) = 7.0, p = .004$ , and there was no significant interaction between session and genotype,  $F(3,111) = 1.2, p = .32$ . There was also no significant interaction between cue and session,  $F(3,111) = .904, p = .40$ , or between the three factors of cue, session, and genotype,  $F(3,111) = .22, p = .76$ .

##### Total Licks: maltodextrin

The mean numbers of total licks made during consumption of the CS- and CS+ flavour cues, when paired with maltodextrin, by control and Grin1<sup>ΔDGCA1</sup> mice across training sessions, are shown in Figure 4.5 (upper panel, right). For both genotypes the numbers of licks were greater to the CS+ than the CS-. The Grin1<sup>ΔDGCA1</sup> mice however made a smaller number of licks to the CS+ than the control mice. The numbers of licks to the CS+ also showed an increase over sessions for both genotypes, with the CS- remained more stable over training sessions. The ANOVA showed that there was a significant effect of cue on the number of licks made,  $F(1,37) = 226.1, p < .001$ , no significant effect of genotype,  $F(1,37) = 2.5, p = .12$ , and a significant interaction between cue and genotype,  $F(1,37) = 10.0, p = .003$ . The effect of session was significant,  $F(3,111) = 10.2, p < .001$ , and the interaction between session and genotype was not significant,  $F(3,111) = .48, p = .61$ . There was a significant interaction between cue and session,  $F(3,111) = 5.7, p = .007$ . The interaction

between the three factors of cue, session, and genotype, was not significant,  $F(3,111) = .41$ ,  $p = .64$ .

Simple main effects analysis of the interaction between cue and genotype, further showed that both genotypes made a significantly greater number of licks during consumption of the CS+ than the CS- (Controls,  $F(1,37) = 201.7$ ,  $p < .001$ ,  $Grin1^{\Delta DGCA1}$  mice  $F(1,37) = 59.9$ ,  $p < .001$ ). They also showed that for the CS+, control mice made a significantly greater number of licks than the  $Grin1^{\Delta DGCA1}$  mice  $F(1,37) = 5.8$ ,  $p = .021$ , but that there was no significant difference between the genotypes during consumption of the CS-,  $F(1,37) = .058$ ,  $p = .81$ .

Lick cluster size: fructose

The mean lick cluster sizes made during consumption of the CS- and CS+ flavour cues when paired with fructose, by control and  $Grin1^{\Delta DGCA1}$  mice, are shown in Figure 4.5 (middle panel, left). For both the CS- and the CS+, the  $Grin1^{\Delta DGCA1}$  mice made smaller lick cluster sizes than the control mice. Both genotypes did however make greater lick cluster sizes during consumption to the CS+ than the CS-. The ANOVA showed that there was a significant effect of cue,  $F(1,37) = 140.4$ ,  $p < .001$  as well as a significant effect of genotype,  $F(1,37) = 8.8$ ,  $p = .005$ , on the mean lick cluster size made during consumption. The interaction between cue and genotype was not significant,  $F(1,37) = 3.5$ ,  $p = .071$ . There was a significant effect of session,  $F(3,111) = 3.2$ ,  $p = .034$ , and no significant interaction between session and genotype,  $F(3,111) = .079$ ,  $p = .96$ . The interaction between cue and session was significant,  $F(3,111) = 7.7$ ,  $p < .001$ , and there was no significant interaction between cue, session, and genotype,  $F(3,111) = 1.5$ ,  $p = .22$ .

Lick cluster size: maltodextrin

The mean lick cluster sizes made during consumption of the CS- and CS+ flavour cues, when paired with maltodextrin by control and  $Grin1^{\Delta DGCA1}$  mice, are shown in Figure 4.5 (middle panel, right). Both genotypes made greater lick cluster sizes during consumption of the CS+ than the CS-, and the control mice made greater lick cluster sizes to both cues than the  $Grin1^{\Delta DGCA1}$  mice. The mean lick cluster sizes also increased over sessions during consumption of the CS+, for both genotypes, but not

during consumption of the CS- flavour cue. The ANOVA showed that there was a significant effect of cue,  $F(1,37) = 96.9$ ,  $p < .001$ , and a significant effect of genotype,  $F(1,37) = 14.2$ ,  $p < .001$ , as well as a significant interaction between cue and genotype,  $F(1,37) = 6.8$ ,  $p = .013$ . The effect of session was also significant,  $F(3,111) = 23.6$ ,  $p < .001$ , with no significant interactions between session and genotype,  $F(3,111) = .67$ ,  $p = .55$ , cue and session,  $F(3,111) = 11.7$ ,  $p < .001$  or between cue, session and genotype,  $F(3,111) = .36$ ,  $p = .69$ .

Simple main effects analysis of the interaction between cue and genotype further showed that both genotypes made significantly greater lick cluster sizes during consumption of the CS+ than the CS- (controls  $F(1,37) = 94.5$ ,  $p < .001$ ,  $Grin1^{\Delta DGCA1}$  mice  $F(1,37) = 22.2$ ,  $p < .001$ ). They also showed that for both the CS- and the CS+, the  $Grin1^{\Delta DGCA1}$  made significantly smaller lick cluster sizes than the control mice (CS-,  $F(1,37) = 8.1$ ,  $p = .007$ , CS+,  $F(1,37) = 12.6$ ,  $p = .001$ ).

Volume: fructose

The mean volumes consumed of the CS+ and CS- flavour cues when paired with fructose, by the control and  $Grin1^{\Delta DGCA1}$  mice across training sessions, are shown in Figure 4.5 (lower panel, left). Both genotypes consumed more of the CS+ than the CS-, and the  $Grin1^{\Delta DGCA1}$  mice consumed slightly less of the CS+ than the control mice. The ANOVA showed that there was a significant effect of cue,  $F(1,37) = 213.8$ ,  $p < .001$ , no significant effect of genotype,  $F(1,37) = .12$ ,  $p = .73$ , but there was a significant interaction between cue and genotype,  $F(1,37) = 4.7$ ,  $p = .037$ . The effect of session was not significant,  $F(3,111) = 2.7$ ,  $p = .076$ , and the interaction between session and genotype was not significant,  $F(3,111) = 1.05$ ,  $p = .38$ . The interaction between cue and session was also not significant,  $F(3,111) = 1.4$ ,  $p = .23$ , and there was no significant interaction between the three factors of cue, session, and genotype,  $F(3,111) = .42$ ,  $p = .62$ .

Simple main effects analysis of the interaction between cue and genotype further showed that both genotypes consumed significantly more of the CS+ than the CS- (controls,  $F(1,37) = 171.6$ ,  $p < .001$ ,  $Grin1^{\Delta DGCA1}$  mice,  $F(1,37) = 65.8$ ,  $p < .001$ ). They also showed that for both the CS- and the CS+ there were no significant differences

in consumption between the control and Grin1<sup>ΔDGCA1</sup> mice (CS-,  $F(1,37) = 2.2$ ,  $p = .15$ , CS+  $F(1,37) = 2.0$ ,  $p = .17$ ).

Volume: maltodextrin

The mean volumes consumed of the CS+ and CS- flavour cues when paired with maltodextrin, by the control and Grin1<sup>ΔDGCA1</sup> mice across training sessions, are shown in Figure 4.5 (lower panel, right). Both genotypes showed greater consumption of the CS+ than the CS-, with the Grin1<sup>ΔDGCA1</sup> mice also showing slightly lower consumption of the CS+ than the control mice. The ANOVA showed that there was a significant effect of cue,  $F(1,37) = 286.7$ ,  $p < .001$ , and no significant effect of genotype,  $F(1,37) = 1.9$ ,  $p = .18$ , although there was a significant interaction between cue and genotype,  $F(1,37) = 5.5$ ,  $p = .024$ . There was also a significant effect of session,  $F(3,111) = 6.9$ ,  $p = .001$ , and no significant interaction between session and genotype,  $F(3,111) = .37$ ,  $p = .711$ . The interaction between cue and session was also not significant,  $F(3,111) = 2.1$ ,  $p = .14$ , and there was also no significant interaction between the three factors of cue, session, and genotype,  $F(3,111) = .702$ ,  $p = .48$ .

Simple main effects analysis of the interaction between cue and genotype, further showed that both genotypes consumed significantly more of the CS+ than the CS- (controls,  $F(1,37) = 226.5$ ,  $p < .001$ , Grin1<sup>ΔDGCA1</sup> mice,  $F(1,37) = 90.2$ ,  $p < .001$ ). They also showed that for the CS+ the controls consumed significantly more than the Grin1<sup>ΔDGCA1</sup> mice,  $F(1,37) = 4.4$ ,  $p = .044$ , but there was no significant difference between the genotypes during consumption of the CS-,  $F(1,37) = .009$ ,  $p = .93$ .

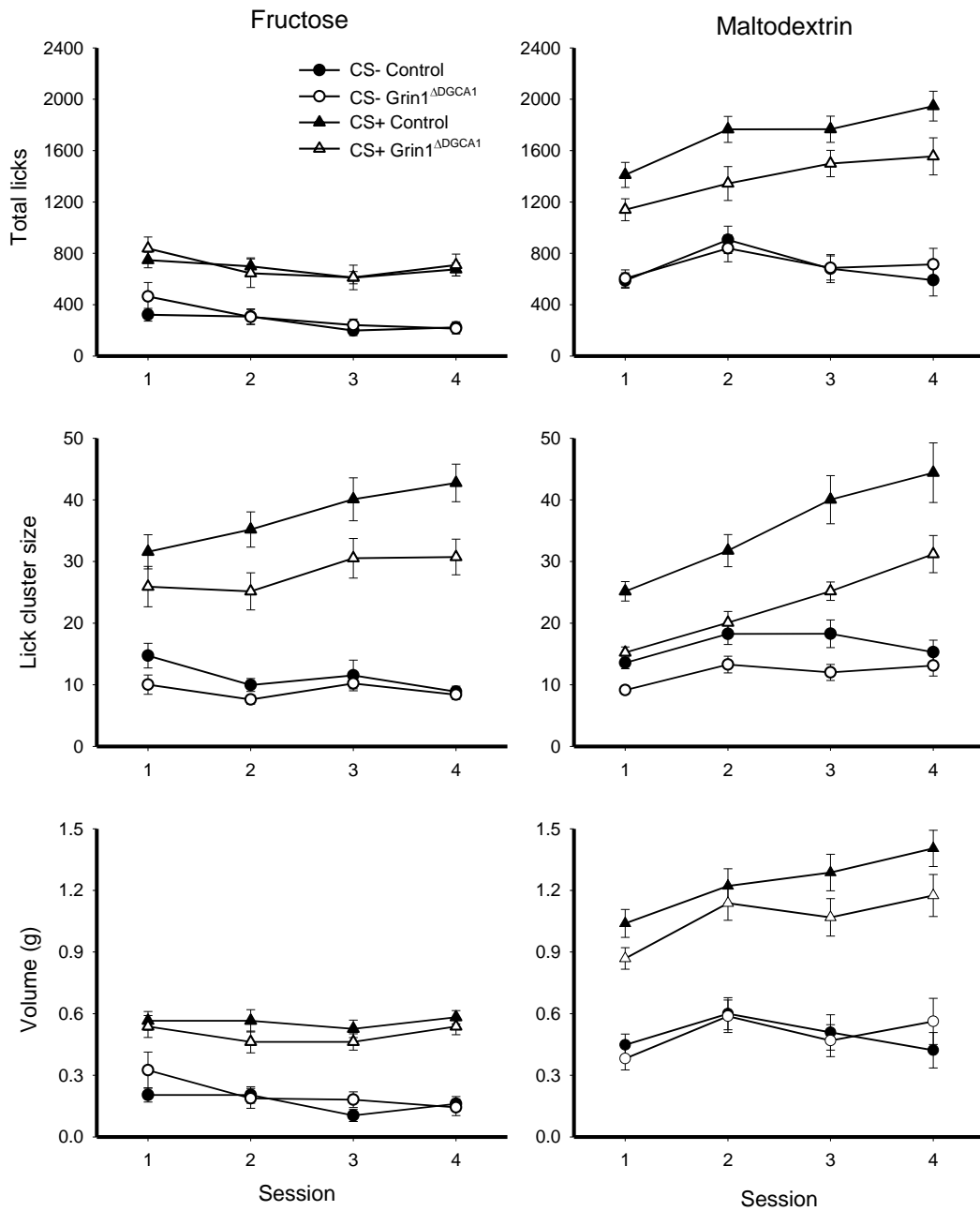


Figure 4.5. Experiment 13, training stage. The mean numbers of total licks (upper), lick cluster sizes (middle) and volumes consumed (lower), of the CS- and CS+, by control and Grin1<sup>ΔDGCA1</sup> mice. Sessions with fructose are shown on the left and maltodextrin on the right. Error bars show ± SEM.

## Test

### Total Licks: fructose

The mean numbers of total licks made during consumption of the CS- and the CS+ flavour cues, when paired with fructose, by the control and Grin1<sup>ΔDGCA1</sup> mice during test, are shown in Figure 4.6 (upper panel, left). Both genotypes made a slightly greater number of licks during consumption of the CS+ than the CS-. The ANOVA showed that the effect of cue (CS- or CS+) was significant,  $F(1,37) = 15.06$ ,  $p < .001$ , and there was no significant effect of genotype,  $F(1,37) = .114$ ,  $p = .74$ . There was also no significant interaction between the two factors of cue and genotype,  $F(1,37) = .029$ ,  $p = .87$ .

### Total licks: maltodextrin

The mean numbers of total licks made during consumption of the CS- and the CS+ when paired with maltodextrin, by the control and Grin1<sup>ΔDGCA1</sup> mice during test, are shown in Figure 4.6 (upper panel, left). Both genotypes made a greater number of licks during consumption of the CS+ than the CS-. The Grin1<sup>ΔDGCA1</sup> mice do however show a slightly smaller difference between the two flavour cues, making a smaller number of licks to the CS+, and a greater number to the CS-, than the control type mice. The ANOVA showed that there was a significant effect of cue,  $F(1,37) = 77$ ,  $p < .001$ , and that there was no significant effect of genotype,  $F(1,37) = .007$ ,  $p = .94$ . There was however a significant interaction between cue and genotype,  $F(1,37) = 4.8$ ,  $p = .034$ .

Simple main effects analysis of the interaction between cue and genotype further showed that there were no significant differences between the genotypes during consumption of either the CS- ( $F(1,37) = 2.007$ ,  $p = .165$ ) or the CS+ ( $F(1,37) = 2.06$ ,  $p = .16$ ). Furthermore, both genotypes showed significantly greater consumption of the CS+ than the CS- flavour (controls,  $F(1,37) = 73.4$ ,  $p < .001$ ; Grin1 s,  $F(1,37) = 18.3$ ,  $p < .001$ ), demonstrating that both genotypes showed a significant flavour preference effect.

#### Lick Cluster Size: fructose

The mean lick cluster sizes made during consumption of the CS- and CS+, by the control and Grin1<sup>ΔDGCA1</sup> mice, when paired with fructose during test, are shown in Figure 4.6 (middle panel, left). Both genotypes made similar lick cluster sizes to the CS- and the CS+, with no differences between the control and Grin1<sup>ΔDGCA1</sup> mice. The ANOVA showed that there was no significant effect of cue on the mean lick cluster size made during consumption,  $F(1,37) = .3.3$ ,  $p = .076$ , and there was no significant effect of genotype,  $F(1,37) = 2.5$ ,  $p = .12$ . There was also no significant interaction between cue and genotype,  $F(1,37) = .54$ ,  $p = .47$ .

#### Lick Cluster Size: maltodextrin

The mean lick cluster sizes made during consumption of the CS- and CS+, by the control and Grin1<sup>ΔDGCA1</sup> mice when paired with maltodextrin during test, are shown in Figure 4.6 (middle panel, right). For both control and Grin1 mice lick cluster sizes were greater during consumption of the CS+ than the CS- cue. The ANOVA showed that there was a significant effect of cue on mean lick cluster size,  $F(1,37) = 32.3$ ,  $p < .001$ , and no significant effect of genotype,  $F(1,37) = 3.2$ ,  $p = .084$ . There was also no significant interaction between cue and genotype,  $F(1,37) = 1.8$ ,  $p = .18$ .

#### Volume: fructose

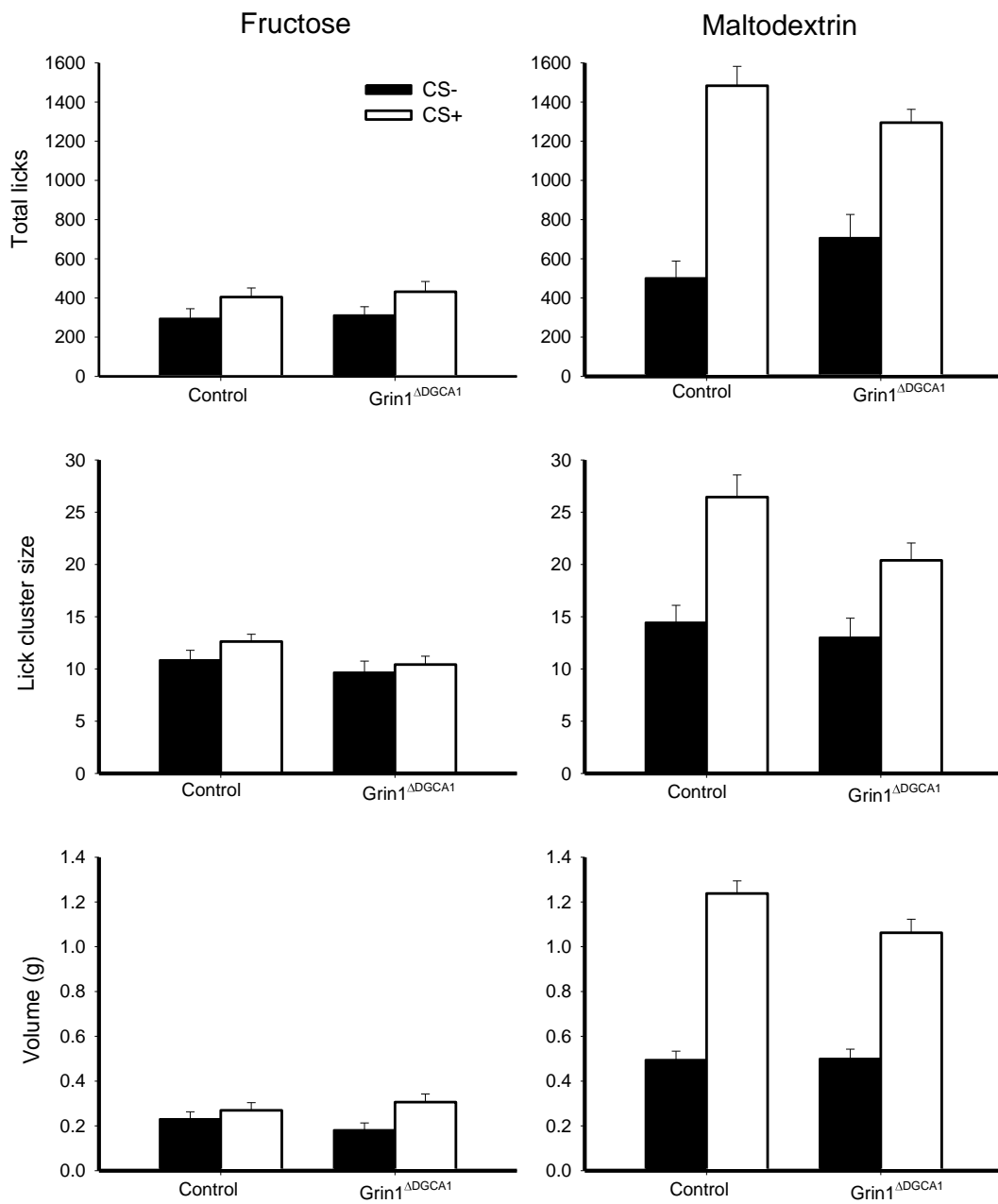
The mean volumes consumed of the CS- and CS+ when paired with fructose, by both control and Grin1<sup>ΔDGCA1</sup> mice during test, are shown in Figure 4.6 (lower panel, left). Both genotypes consumed slightly more of the CS+ than the CS- cue. The ANOVA showed that there was a significant effect of cue,  $F(1,37) = 7.8$ ,  $p = .008$ , and no significant effect of genotype,  $F(1,37) = .026$ ,  $p = .87$ . There was also no significant interaction between cue and genotype,  $F(1,37) = 2.1$ ,  $p = .15$ .

#### Volume: maltodextrin

The mean volumes consumed of the CS- and CS+ during test, when paired with maltodextrin, by the control and Grin1<sup>ΔDGCA1</sup> mice, are shown in Figure 4.6 (lower panel, right). Both genotypes showed greater consumption of the CS+ than the CS-, although the control mice showed slightly greater consumption of the CS+ than the

Grin1<sup>ΔDGCA1</sup> mice. The ANOVA showed that the effect of cue was significant,  $F(1,37) = 286.8$ ,  $p < .001$ , and there was no significant effect of genotype,  $F(1,37) = 1.9$ ,  $p = .18$ . There was however a significant interaction between cue and genotype,  $F(1,37) = 5.5$ ,  $p = .024$ .

Simple main effects analysis of the interaction between cue and genotype, further showed that both control and Grin1<sup>ΔDGCA1</sup> mice consumed significantly more of the CS+ than the CS- (control:  $F(1,37) = 226.5$ ,  $p < .001$ ; Grin1<sup>ΔDGCA1</sup> mice  $F(1,37) = 90.2$ ,  $p < .001$ ). The control mice also consumed significantly more of the CS+ than the Grin1<sup>ΔDGCA1</sup> mice,  $F(1,37) = 4.4$ ,  $p = .044$ , with no significant difference between the genotypes in consumption of the CS-,  $F(1,37) = .009$ ,  $p = .93$ .



**Figure 4.6.** Experiment 13, test stage. The mean numbers of total licks (upper), lick cluster sizes (middle) and volumes consumed (lower), of the CS- and CS+, by control and Grin1<sup>ΔDGCA1</sup> mice. Sessions with fructose are shown on the left and maltodextrin on the right. Error bars show  $\pm$  SEM.

#### 4.3.4 Discussion

The results showed that both fructose and maltodextrin are both capable of supporting flavour preference learning, demonstrated in a greater intake of the CS+ compared to the CS- flavour. Furthermore, this effect was seen in the Grin1<sup>ΔDGCA1</sup> mice and the control type mice. There was also some evidence for flavour preference effects being seen in the mean lick cluster size, as these were greater during consumption of the CS+ than the CS-. However, this was only seen when maltodextrin was used as the reinforcer and not when fructose was used. This effect was however seen in both the Grin1<sup>ΔDGCA1</sup> and control mice. This suggests that flavour preference with maltodextrin, but not fructose, may increase the palatability of the CS+ compared to the CS- flavour during test sessions. This result was seen in the Grin1<sup>ΔDGCA1</sup> mice, demonstrating that the generally reduced lick cluster sizes does not impair flavour preference altering palatability when maltodextrin is used as the reinforcer. Maltodextrin also seemed to differ from fructose in terms of the greater amount consumed and generally larger lick cluster sizes during consumption of it. This was seen in the training stage as well as in the test stage.

The results from this experiment further demonstrate that both fructose and maltodextrin are capable of supporting flavour preference learning. These difference reinforcers have however been shown to be supported by different associations, with fructose linked to flavour-flavour, and maltodextrin flavour-nutrient associations. These results therefore suggest that flavour preference learning is able to be supported either by flavour-flavour and flavour-nutrient associations.

However, the results do not necessarily support the dissociation between fructose and maltodextrin in terms of these types of associations. In the case of fructose, the link to flavour-flavour associations (Sclafani & Ackroff, 1994) means that if either of the two reinforcers should show an effect in terms of altered palatability, it would be more likely to occur with fructose rather than maltodextrin. This is due to the flavour becoming associated with the increased sweet taste and palatability of the higher concentration of fructose. Whereas with maltodextrin this association is instead between the greater post-ingestive consequences of the higher concentration paired with the CS+ (Sclafani & Nissenbaum, 1988). However, the results from this study

showed that flavour preference with maltodextrin, but not fructose, increased the perceived palatability of the CS+. This would suggest that learning a flavour preference based on the flavour-flavour associations supported by fructose, does not alter the perceived palatability of the CS+ during test sessions. The results also suggest that maltodextrin may support the formation of flavour-flavour associations as well as flavour-nutrient associations, as this could explain the increased lick cluster size and palatability effect seen when maltodextrin was used as the reinforcer. This does however support previous results of Dwyer & Quirk (2008), who suggested that maltodextrin may have a taste and be able to support some level of flavour-flavour learning in rodents. Fructose has also been found to generate a small amount of post-ingestive reinforcing signals, and be able to support flavour preference learning based on flavour-nutrient associations (Ackroff, Touzani, Peets, & Sclafani, 2001). It may therefore be the case that both fructose and maltodextrin are able to support, to a more or lesser extent, both flavour-flavour and flavour-nutrient associations.

Something else to consider in relation to the results from this experiment, is that the mice were food deprived throughout. It could be the case that the use of food-deprivation enhanced flavour preference learning based on flavour-nutrient associations compared to flavour-flavour associations (Capaldi, Owens, & Palmer, 1994; Drucker, Ackroff, & Sclafani, 1994; Yiin, Ackroff, & Sclafani, 2005). Although without directly testing this in free-feeding mice the effect of deprivation state in this study is unclear.

The results also showed that although Grin1<sup>ADGCA1</sup> mice have generally reduced mean lick-cluster sizes during consumption, they were still able to learn flavour preferences when either fructose or maltodextrin was used as the reinforcer. Furthermore, these mice also showed the flavour preference to maltodextrin in terms of larger lick-cluster size during consumption of the CS+ than the CS-, although the cluster size to the CS+ was numerically lower compared to the controls. Therefore, although lick cluster sizes are generally reduced in the Grin1<sup>ADGCA1</sup> mice, they are still able to express a flavour preference in the form of enhanced palatability to the preferred flavour cue.

Overall, the results from this experiment demonstrate that the reduced mean lick cluster sizes in the *Grin1<sup>ADGCA1</sup>* mice do not impair the ability to learn a flavour preference based on flavour-flavour and/or flavour-nutrient associations. However, the results do suggest that the dissociation between fructose and maltodextrin, in terms of the different associations supporting flavour preference learning, is not as clear as previous studies would suggest. As the results showed that despite fructose being based on flavour-flavour associations, palatability was not enhanced for the CS+ flavour compared to the CS-. Rather, maltodextrin, based on flavour-nutrient associations, did enhance the palatability of the CS+ compared to the CS-. Suggesting that learning about post-ingestive consequences did enhance the subsequent palatability of the CS+, whereas learning about the flavour of the reinforcer did not alter the palatability of it. It may therefore be that both types of associations can be associated with maltodextrin and/or fructose, but that flavour-flavour are more dominant with fructose and flavour-nutrient with maltodextrin.

One way in which these two types of associations, during flavour preference learning in mice could be further investigated, is to use a delay procedure. As if fructose supports flavour preference based more on flavour-flavour associations, it may be expected that would result in little to no preference effect. Whereas flavour preference learning should still be present when maltodextrin is used as the reinforcer and supported by post-ingestive consequences with a longer time course. If fructose is however still able to support flavour preference learning, this would suggest that fructose is able to support some degree of flavour-nutrient association formation.

## 4.4 General discussion

This chapter aimed to investigate the role of hippocampal NMDA receptors in palatability and flavour preference learning. In the first study it was found that the mean lick cluster sizes, a measure of palatability, were reduced in the *Grin1<sup>ΔDGCA1</sup>* mice compared to the control type mice. This suggests that deletion of hippocampal NMDA receptors may impair palatability during consumption of palatable sucrose solutions, although it could also be due to impaired expression of palatability through mean lick cluster sizes. If the impairment reflects reduced palatability, this also suggests that the *Grin1<sup>ΔDGCA1</sup>* mice may provide an animal model for anhedonia. It would also support the proposed role of hippocampal NMDA receptors in disorders such as schizophrenia (Kantrowitz & Javitt, 2010), as well as previous studies in which NMDA receptor deletion in mice is associated with some of the negative symptoms of disorder (Halene et al., 2009).

The results also add to the evidence for a role of the hippocampus in consumption and eating behaviour. It has previously been found that lesions of the hippocampus alter consummatory patterns, consuming smaller meals more frequently, while total intake remained normal (Clifton, Vickers & Somerville, 1998). The results in this chapter showed that hippocampal NMDA receptor deletion also alters eating patterns, with mean lick cluster sizes impaired, but the amount consumed being normal.

However, it is also possible that the impaired lick cluster sizes seen in the *Grin1<sup>ΔDGCA1</sup>* mice could also be linked to problems in the expression of lick-cluster sizes and palatability, rather than reductions in hedonic value. There was some evidence against this, in the *Grin1<sup>ΔDGCA1</sup>* mice showing normal mean lick cluster sizes during consumption of maltodextrin. This suggests that the impairment may not be related to a wider motor deficit and a complete inability to generate larger lick cluster sizes. However, an impairment in expression is difficult to rule out, as the *Grin1<sup>ΔDGCA1</sup>* mice may be still be unable to generate appropriately sized lick cluster during consumption of palatable solutions, and there may not always have been sufficient sensitivity to see this impairment during the experiments in this thesis.

The final experiment in the chapter looked at flavour-flavour and flavour-nutrient learning, using fructose to investigate flavour-flavour and maltodextrin flavour-nutrient learning. The results showed that the reduced mean lick cluster sizes in the *Grin1<sup>ΔDGCA1</sup>* mice, does not seem to impair the ability to learn a flavour preference based on flavour-flavour associations. However, it was also found that flavour preference using fructose as the reinforcer, did not result in any flavour preference effects on the mean lick cluster sizes, in either the control or the *Grin1<sup>ΔDGCA1</sup>* mice. Whereas flavour preference with maltodextrin did result in a lick cluster size effect, despite having been suggested to be based on flavour-nutrient associations. Further work is therefore required to look at the role of flavour-flavour and flavour-nutrient learning in mice, as well as if impaired lick cluster sizes may differentially affect these associations. In particular if flavour preference using fructose was supported by some flavour-nutrient learning, the role of impaired cluster sizes and palatability on flavour-flavour learning would not have been directly tested in this experiment.

# Chapter 5

## The role of glutamate in matching behaviour

Animals are sensitive to the reinforcement rates of stimuli, with conditioned responding found to match the rate of reinforcement (Harris & Carpenter, 2011). For example, when the cumulative reinforcement rates of two cues are matched (by reinforcing one longer cue 100% of the time, with another shorter cue that is only partially reinforced but presented more often), the rates of responding to the two cues are equal (Harris, Patterson, & Gharraei, 2015). Assessing sensitivity to reinforcement rate in the *Gria1*<sup>-/-</sup> and the *Grin1*<sup>ΔDGCA1</sup> mice, provides another way to look at the role of the GluA1 subunit, and NMDA receptors in the hippocampus, in learning and memory. One way to assess sensitivity to reinforcement rate is to look at matching behaviour during instrumental conditioning. The previous experiments in this thesis focused on the role of glutamate in Pavlovian conditioning, in which the mice learned about presented cues and their reward contingencies. Learning can however also occur instrumentally, with actions rather than cues linked to reward expectancy. In this chapter an instrumental procedure was instead used to assess learning about reinforcement rate, in the *Gria1*<sup>-/-</sup> and the *Grin1*<sup>ΔDGCA1</sup> mice.

The matching law was first outlined by Herrnstein (1961), after observing responding of pigeons towards two concurrently presented cues. In these experiments pigeons were presented with two lights that were each reinforced, as a result of pecking the light, at different rates. This meant that one light resulted in a higher number of reinforcements than the other. The rates of responding (the numbers of pecks) on the two lights, as a proportion of the total responses, were found to be proportional to the numbers of reinforcements earned from each light. This means that if one light were to account for 75% of the numbers of rewards, the number of pecks on that light would account for 75% of the total number of pecks made. The matching law, as shown in equation 1, accounts for this behaviour. It states that the proportion of responding to one cue (behaviour 1, B1) will always equal the proportion of reinforcers earned from that cue (rate of reinforcement for cue 1, R1).

$$B_1 / (B_1 + B_2) = R_1 / (R_1 + R_2) \quad (2)$$

This matching law has since been used to describe the linear relationship shown by animals between levels of responding and reinforcement rate, in animals (Herrnstein, 1961; 1970) as well as humans (Conger, & Killeen, 1974; Vollmer & Bourret, 2000). However, it has also been found that matching is not always as lawful as described by the original matching law. Examples of undermatching, overmatching, and bias towards a response, have all also been observed (McDowell, 2005). During undermatching, animals show a smaller difference in the rates of responding across the two cues than they should, i.e., more equal than would be predicted by the actual reinforcement rate. During overmatching the opposite occurs, with rates of responding more different over the two cues than would be expected by the actual reinforcement rate. Bias occurs when animals show a preference for one behavioural response over the other, even before experiencing the differential reinforcement rates, or when the rates do not actually differ. The most common occurrence of deviation is undermatching, (Fantino, Squires, Delbruck, & Peterson, 1972; Wearden & Burgess, 1982). As a result of these deviations from the original matching law, the 'generalised matching law, shown in equation 2, was later proposed (Baum, 1974; McDowell, 2005).

$$B_1 / (B_1 + B_2) = K (R_1 / (R_1 + R_2))^a \quad (3)$$

This equation accounts for deviations from strict matching by adding two coefficients, with  $K$  representing any bias the animal may have towards a response, and  $a$  representing sensitivity to reinforcement. In the original matching law, these two coefficients would both be set at one. Increasing the value of  $K$  to more or less than 1 alters responding towards one or the other response option. Decreasing the value of  $a$  results in undermatching, increasing its value accounts for overmatching behaviour. The value of  $a$  has been proposed to be around .8 for many animals, including humans (McDowell, 2005; Wearden & Burgess, 1982), to account for the common occurrence of undermatching behaviour. This generalised matching law has proven successful in explaining behaviour under concurrent schedules of

reinforcement (McDowell, 2005, 2013). However, although the generalised matching law provides a good description of how animals can be sensitive to reinforcement rate under concurrent schedules of reinforcement, it does not provide a mechanism for how learning about these rates occurs.

Traditional learning theories, such as the Rescorla and Wagner (1972) model, explain rate sensitivity as a result of prediction error mechanisms and updating of associative strength. When the reinforcer occurs but is not fully predicted, positive prediction error is generated, increasing the associative strength of the predictive cue. When the reinforcer does not occur however, but was at least partly expected, negative prediction error is generated, reducing the associative strength of the cue. Continued summation of these positive and negative prediction errors results in a constantly updating degree of associative strength. Overall, associative strength will be therefore be greater for cues reinforced at a higher rate than cues with a lower rate, resulting in corresponding higher or lower levels of conditioned responding. In this case, assessing rate sensitivity provides a way to test prediction error learning mechanisms in the *Gria1*<sup>-/-</sup> and the *Grin1*<sup>ΔDGCA1</sup> mice.

There is however an alternative explanation to animals sensitivity to reinforcement rate. It has been suggested that animals may more explicitly encode the temporal properties of cues after experiencing them. This means that rather than responding being the result of associative strength, it is instead the result of learning more explicitly about the temporal relationships between cues and outcomes (Balsam & Gallistel, 2009). In this case, assessing rate sensitivity in the *GluA1* and *Grin1*<sup>ΔDGCA1</sup> mice does not provide a measure of prediction error learning. Rather, it would reflect the ability to encode and learn about temporal information. Either way however, learning about the relative rates is still required in order to show accurate matching behaviour. The transgenic mice also provide a way to assess the role of the *GluA1* subunit, and hippocampal NMDA receptors, in learning about reinforcement rate.

In order to assess the ability to learn about the relative reinforcement rates of the two levers, matching behaviour across two levers was tested. In the experiments in this chapter mice were concurrently presented with two levers, each reinforced on different and independent, variable interval schedules of reinforcement. The ability

to learn about the relative reinforcement rates should, according to the matching law (Herrnstein, 1961), result in mice showing matching behaviour across the two levers. This would mean that the relative rates of responding to each of the two levers (the numbers of lever presses) should match the relative rates of reinforcement rates of each of the levers.

Both the *Gria1*<sup>-/-</sup> and the *Grin1*<sup>ΔDGCA1</sup> mice show intact long-term associative memory, demonstrating that learning and prediction error mechanisms are also intact in these mice. If rate sensitivity is due to prediction error mechanisms, as outlined by traditional learning theories, then matching behaviour may also be expected to be normal in these mice. The first experiment in this chapter tested *Gria1*<sup>-/-</sup> mice for rate sensitivity and matching behaviour. However, despite intact prediction error and associative learning, there is evidence that rate calculation is impaired in the *Gria1*<sup>-/-</sup> mice. Austen et al. (in prep), tested *Gria1*<sup>-/-</sup> mice for sensitivity to reinforcement rate. It was found that rather than being sensitive to reinforcement rate, as was shown by the control mice, they were instead sensitive to the number of reinforcements. This suggests that GluA1 deletion does in fact affect rate calculation mechanisms, despite prediction error seeming to be intact in these mice. However, if rate sensitivity may be affected by GluA1 deletion during an instrumental, rather than a Pavlovian conditioning procedure, is not known.

In the second experiment, the *Grin1*<sup>ΔDGCA1</sup> mice were run through a similar matching behaviour procedure. The role hippocampal NMDA receptors may play in rate sensitivity is of interest not only as a result of the importance hippocampal synaptic plasticity in learning, but also due to the hippocampus being implicated in the encoding of temporal information (Howard & Eichenbaum, 2013; Tam & Bonardi, 2012). Time cells, which have temporally specific receptive fields for short intervals between events, have also been identified in the hippocampus (MacDonald, Lepage, Eden, & Eichenbaum, 2011). The encoding of recent events and temporal durations in particular has been suggested to be linked to hippocampal functioning (MacDonald, Fortin, Sakata, & Meck, 2014). Deletion of hippocampal NMDAR's may therefore affect the ability to encode temporal information, impairing rate sensitivity. This may be of importance given the suggestion that rate sensitivity may

be the result of direct encoding of the temporal properties of cues (Balsam & Gallistel, 2009).

As well as looking at rate learning in the form of matching behaviour across the two levers, in both experiments the reward contingencies of the two levers were reversed after the first stage. Reversal learning is often used as a measure of cognitive flexibility, testing the ability for the animal to alter their behaviour after the previously learned reward contingencies are reversed. One particular aspect of learning that reversal procedures have been suggested to test, is the ability to learn about the new reward contingencies received after choosing to make different behavioural responses (Izquierdo, Brigman, Radke, Rudebeck, & Holmes, 2017). The role of glutamate in reversal learning however is currently unclear. Blockade of NMDAR's, using MK-801, has been found to impair spatial reversal learning (Lobellova et al., 2013). Impaired reversal has also been observed after blockade of the GluN2 subunit of the NMDA receptor (Dalton, Ma, Phillips, & Floresco, 2011), further supporting a role for glutamate and NMDA receptors in particular, in reversal learning.

There are however other findings in which altering NMDAR functioning does not seem to affect reversal learning (Svoboda, Stankova, Entlerova, & Stuchlik, 2015). Furthermore, reversal learning in the *Gria1*<sup>-/-</sup> mice during a visual discrimination task has also been found to be intact if not slightly enhanced compared to wild-type controls (Barkus et al., 2012). Although reversal learning in a spatial discrimination task, has been found to be slightly impaired in the *Gria1*<sup>-/-</sup> mice compared to the controls (Bannerman, Deacon, Seeburg, & Rawlins, 2003). The *Grin1*<sup>ΔGCA1</sup> mice have been previously found to show impaired reversal learning in the water-maze (Bannerman et al., 2012). However this impairment was suggested to be linked to altered inhibitory control, rather than necessarily being impaired at learning the new location of the platform. Furthermore, they were not impaired at reversal learning during a non-spatial procedure (Bannerman et al., 2012).

## 5.1 Experiment 14

### GluA1 deletion and matching behaviour

In this experiment, learning about reinforcement rate and the ability to show matching behaviour was tested in the *Gria1*<sup>-/-</sup> mice. Previous experiments have found that rate calculation seems to be impaired in the *Gria1*<sup>-/-</sup> mice (Austen et al., in prep). However, rate learning and sensitivity during an instrumental learning procedure has not previously been assessed in these mice. During this experiment, *Gria1*<sup>-/-</sup> and wild-type mice were able to learn about two concurrently presented levers, each on different and independent variable interval schedules of reinforcement. One lever therefore had a higher rate of reinforcement than the other, with the pellets earned on one not affecting the pellets that could be earned on the other. To further look at rate sensitivity, mice were also split into two groups. For one group of mice the difference between the relative rates of reinforcements of the two levers was larger (the large difference group) than in the other group (the small difference group). For the large difference group the high rate lever accounted for 75% of the total reinforcements that could be earned over a session. For the small difference group this lever accounted for only 60% of the total reinforcements that could be gained.

The matching law predicts that if *Gria1*<sup>-/-</sup> mice are sensitive to the relative rates of reinforcement, they should also show matching behaviour towards the levers. In this case, for the large difference group presses on the high lever should account for 75% of the total responses, but for the small difference group this should be 60%. As well as assessing the acquisition of the relative rates of reinforcement and matching behaviour, the lever contingencies were reversed after 12 training sessions, for mice in both the large and small difference groups. The previously high rate lever was now reinforced on the low rate schedule and the low rate lever now reinforced on the higher rate schedule. This provided a way to further assess the role of the GluA1 subunit in reversal learning, in this case a reversal of the schedules of reinforcements.

### **5.1.1 Methods**

#### **Subjects**

15 *Gria1<sup>-/-</sup>* mice (2 male, 13 female) and 25 wild-type mice (12 male, 13 female), bred and housed in the same way as experiment 2.1, were used. Mice were aged between 8-13 months old at the start of testing and caged in groups of 2-5, with free-feeding weights ranging between 17.6g – 33.1g. Mice had previous experience in appetitive magazine conditioning experiments using auditory and visual cues in the same operant chambers used in this experiment, as well as experience of flavour preference learning in similar apparatus.

#### **Apparatus**

The apparatus used was the same as in experiment 2.5 (blocking using auditory and visual cues) with the addition of two retractable levers (ENV-312-2M, Med Associates) that protruded 2.2cm above the grid floor. Both levers were located on the same wall as the magazine, one to either side of the magazine.

#### **Procedure**

Throughout the experiment both levers were concurrently inserted into the chamber at the start of each daily session. For the first five sessions, each lasting a maximum of 30-minutes, mice were pre-trained to lever press for sucrose pellet rewards. In the first two of these sessions, each lever press, to either of the two levers, resulted in a pellet being delivered into the magazine. Mice could receive a maximum number of 15 pellets per session. If this limit was reached the program ended and the levers retracted from the chamber, with mice removed from the chambers once all mice being tested in that given group of animals had completed the program (either as a result of reaching the reward limit or the time limit was instead reached). In the third session of pre-training, lever presses were reinforced on a 30s variable interval (VI) schedule, with both levers on this same VI schedule of reinforcement. This meant that a lever press, across the two levers, resulted in a sucrose pellet reward on average every 30s, with 30-minutes to receive a maximum of 16 pellets. On the fourth session this schedule was increased to a VI 60s schedule and then again

increased to a 90s schedule on the fifth day. This matched the overall reinforcement that would be given in the main experiment. Throughout the experiment all VI schedules were determined using the Fleshler & Hoffman (1962) distribution.

After pre-training, mice were split into two groups, the large difference group (13 wild-type mice, 7 females, 6 males; 8 *Gria1*<sup>-/-</sup> mice, 7 female, 1 male) and the small difference group (12 wild-type mice, 6 females, 6 males; 7 *Gria1*<sup>-/-</sup> mice, 6 females, 1 male). Both of these groups had the same overall reinforcement rate across the two levers, of 1 pellet every 90s, but the relative rates at which the two levers were reinforced differed. For the large difference group the high lever was reinforced on a VI 120s schedule, and the low lever a VI 360s schedule. A press on the high lever therefore resulted in a sucrose pellet reward on average every 120s. For the low lever this was on average every 360s. For the small difference group the high lever was reinforced on a VI 150s schedule, the low lever on a VI schedule of 225s. In this group a press on the high lever therefore resulted in a pellet on average every 150s, the low lever every 225s. Group allocation (large or small difference) was counterbalanced as far as possible given the numbers with respect to genotype. The lever allocation (high or low) was also counterbalanced so that for approximately half of the mice the left lever was reinforced on the high rate and the right lever the low rate, and vice versa for the other half. Lever allocation was also counterbalanced with respect to group (large or small difference) as far as possible given the numbers.

The first stage of the experiment consisted of 12 daily testing sessions, with each daily session lasting for 24 minutes. This meant that the maximum number of pellets that could be gained, if mice showed optimal behaviour, was 16. For the large difference group, the high lever accounted for 75% of the total reinforcements that could be gained in each session. For the small difference group the high lever accounted for 60% of the total reinforcements that could be gained. After this stage the lever contingencies were reversed for both the large and small difference groups. The lever with a previously high reinforcement rate was now reinforced on the lower VI schedule, and vice versa for the low rate lever. The groups were kept the same, so the large difference group still had a greater difference between the levers and the

small group a smaller difference. These reversed contingencies were presented for 12 sessions and run in the same manner as the previous stage.

### **Statistical analysis**

For each stage of the experiment the total numbers of presses made on each lever (high and low) were recorded and the ratios of presses on the high lever were calculated (high lever presses/ (high lever presses + low lever presses). The numbers of pellets earned on each lever were also recorded. To analyse the number of lever presses made and the number of pellets earned, mixed model ANOVA were carried out, with group, genotype, lever, and session as factors. For the ratios similar analysis were carried out, with group, genotype, and session as factors. This analysis was carried out for the acquisition stage and the subsequent reversal stage. Where appropriate, interactions were analysed with simple main effects analysis using the pooled error term from the original error term and where sphericity of within-subjects variables could not be assumed, a Greenhouse-Geisser correction was applied to produce more conservative p-values.

### **5.1.2 Results and Discussion**

#### Acquisition stage

Numbers of lever presses:

The numbers of lever presses made on the high and low rate levers in the large difference and small difference groups, by the wild-type and *Gria1<sup>-/-</sup>* mice, are shown in Figure 5.1. In both groups and genotypes, the numbers of presses were greater on the high than the low rate of reinforcement lever. The *Gria1<sup>-/-</sup>* mice also made greater numbers of presses than the wild-type mice on both levers. The ANOVA showed that there was a significant effect of lever (high or low),  $F(1,36) = 121.8$ ,  $p < .001$ , and genotype,  $F(1,36) = 12.7$ ,  $p = .001$ , but no significant effect of group, (large or small difference)  $F(1,36) = .55$ ,  $p = .46$ . There was no significant interaction between lever and genotype,  $F(1,36) = .71$ ,  $p = .41$ , but the interaction between lever and group was significant,  $F(1,36) = 9.6$ ,  $p = .004$ . Simple main effects analysis of this interaction further showed that for the low lever the small difference group made a

significantly greater number of presses than the large difference group,  $F(1,36) = 7.0$ ,  $p = .012$ , however there was no significant difference between the groups for the high lever,  $F(1,36) = .32$ ,  $p = .58$ . Furthermore, both large and small difference groups also showed a significantly greater number of presses on the high than the low lever (large difference group:  $F(1,36) = 195.9$ ,  $p < .001$ ; small difference group,  $F(1,36) = 29.8$ ,  $p < .001$ ).

The ANOVA also showed that there was a significant effect of session,  $F(11,396) = 4.6$ ,  $p = .001$ , and no significant interaction between session and genotype,  $F(11,396) = .64$ ,  $p = .64$ , or between session and group,  $F(11,396) = .89$ ,  $p = .47$ . There was a significant interaction between lever and session,  $F(11,396) = 5.0$ ,  $p = .001$ , as well as a significant interaction between the three factors of lever, session and genotype,  $F(11,396) = 5.1$ ,  $p = .001$ . The interaction between the four factors of group, genotype, lever and session, was however also significant,  $F(11,396) = 6.0$ ,  $p < .001$ . All other interactions were not significant,  $F$  values  $< 2.03$ ,  $p$  values  $> .093$ .

To further analyse the interaction between the four factors of group, genotype, lever, and session, mixed model ANOVA were carried out for each group, large and small difference, with genotype, lever, and session as factors. For the large difference group, this showed that there was no significant interaction between the three factors of lever, session, and genotype,  $F(11,209) = .76$ ,  $p = .54$ . For the small difference group however the interaction between genotype, lever, and session was significant,  $F(11,187) = 10.7$ ,  $p < .001$ .

To further analyse this interaction, mixed model ANOVA were carried out for each genotype, with lever and session with factors. For the wild-type mice this showed that there was a significant interaction between lever and session,  $F(11,121) = 8.4$ ,  $p < .001$ . Simple main effects analysis of this interaction further showed that in each session the wild-type mice responded significantly more on the high than the low lever,  $F$  values  $> 12.3$ ,  $p$  values  $< .005$ . For the  $Gria1^{-/-}$  mice there was also a significant interaction between lever and session,  $F(11,66) = 4.3$ ,  $p = .015$ . Simple main effects analysis further showed that they did not always respond significantly more on the high than the low rate lever, although this was the case in the last four sessions, as well as in the third, fourth and seventh sessions prior ( $F$  values  $> 6.6$ ,  $p$  values  $< .042$ ).

In the remaining sessions there were no significant differences in responding between the two levers ( $F$  values  $< 2.7$ ,  $p$  values  $> .095$ ).

#### Ratios:

The ratios of lever pressing during the acquisition stage, for both the wild-type and *Gria1*<sup>-/-</sup> mice, are shown in Figure 5.1. From the second session onwards, the proportion of presses on the high lever was greater in the large difference group than the difference small group, for both genotypes. The ratios of responding were however slightly smaller in the *Gria1*<sup>-/-</sup> mice compared to the wild-type mice. The ANOVA showed that there was a significant effect of session,  $F(11,396) = 2.9$ ,  $p = .001$ , and genotype,  $F(1,36) = 4.4$ ,  $p = .043$ , as well as a significant interaction between session and genotype,  $F(11,396) = 3.4$ ,  $p = .014$ . The effect of group (large difference or small difference) was also significant,  $F(1,36) = 13.2$ ,  $p = .001$ , and there was no significant interaction between session and group,  $F(11,396) = 1.9$ ,  $p = .1$ , or between genotype and group,  $F(1,36) = 2.03$ ,  $p = .16$ . The interaction between the three factors of session, genotype, and group was also significant,  $F(11,396) = 4.6$ ,  $p = .003$ .

To further analyse this three way interaction, mixed model ANOVA, with genotype and session as factors, were carried out for the large and small difference groups. For the large difference group this showed that there was no significant interaction between session and genotype,  $F(11,209) = .27$ ,  $p = .83$ . For the small difference group there was however a significant interaction between these two factors,  $F(11,209) = 9.3$ ,  $p < .001$ . Simple main effects analysis of this interaction further showed that in the first two sessions, the *Gria1*<sup>-/-</sup> mice made significantly lower ratios of responding than the wild-type mice (1<sup>st</sup> session,  $F(1,17) = 21.7$ ,  $p < .001$ ; 2<sup>nd</sup> session,  $F(1,17) = 9.0$ ,  $p = .008$ ), but that in all other sessions there were no significant differences between the genotypes,  $F$  values  $< 3.3$ ,  $P$  values  $> .085$ .

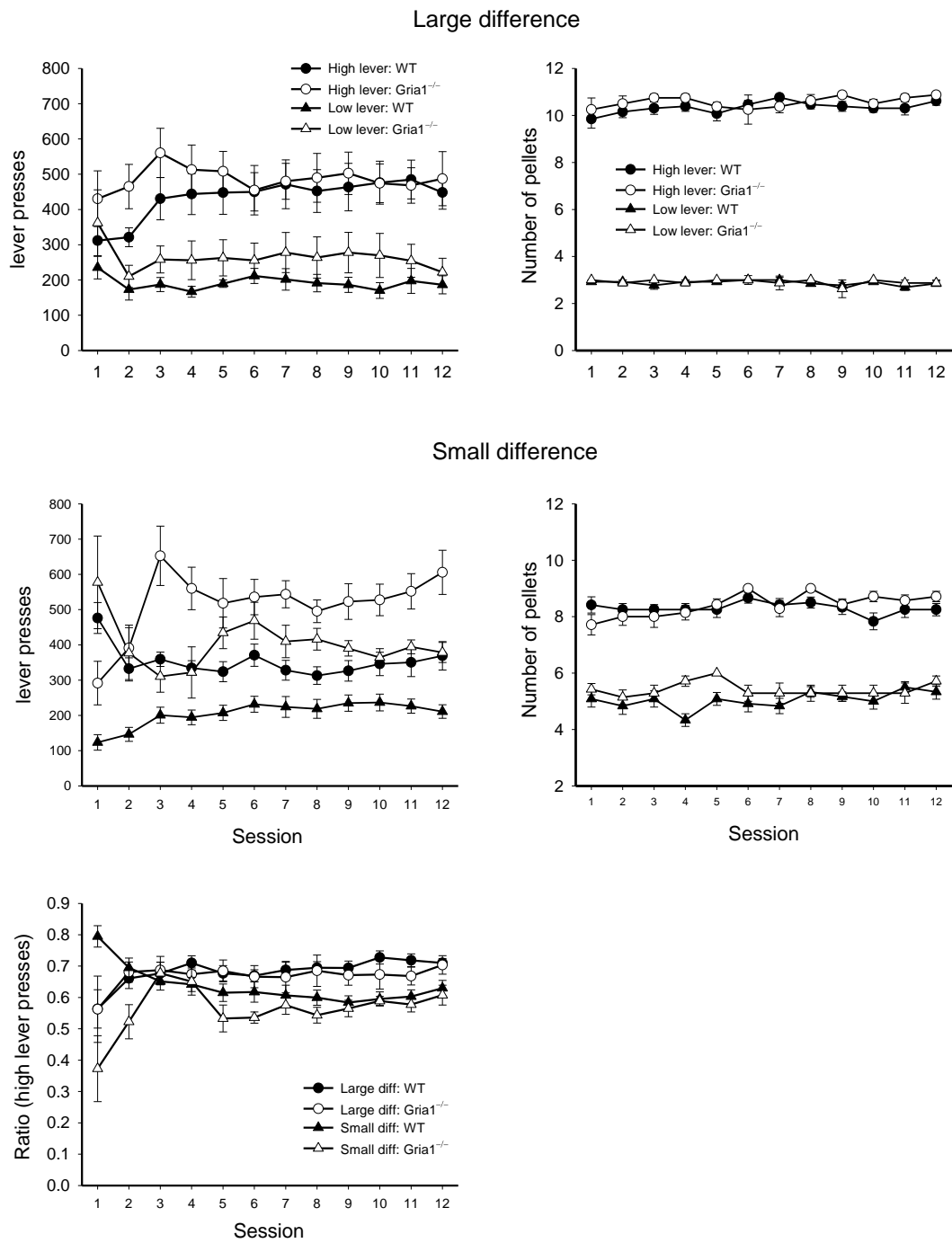
To further test matching behaviour by the end of training, separate one-sample  $t$ -tests were carried out for the large and small difference groups on the ratios from the last training session (as there was no effect of genotype after the second session, this was not added as a factor). The large difference group were compared against the reinforcement rate of .75, the small difference group .6. This allowed to test if

ratios of responding were significantly different from the relative rates of reinforcement in each group. These showed that the large difference group did significantly differ from .75,  $t(20) = -3.1$ ,  $p = .006$ , but that the small difference group did not significantly differ from .60,  $t(18) = 1.1$ ,  $p = .28$ .

Numbers of pellets:

The numbers of pellets earned on the high and low levers during the acquisition stage, by both genotypes in the large and small difference groups, are shown in Figure 5.1. In both groups a greater number of pellets were earned on the high than the low lever, with little difference between the genotypes. The numbers of pellets earned across the two levers also neared the maximum amount (16 in total) from the first training session. The ANOVA showed that there was a significant effect of lever (high or low),  $F(1,36) = 9716.0$ ,  $p < .001$ , and no significant effect of group (small or large difference),  $F(1,36) = 2.2$ ,  $p = .14$ , or session,  $F(1,36) = 1.7$ ,  $p = .076$ . There was however a significant effect of genotype, with the *Gria1*<sup>-/-</sup> mice earning a statistically greater number of rewards than the wild-type mice,  $F(1,36) = 5.8$ ,  $p = .022$ . There was no significant interaction between genotype and group,  $F(1,36) = .46$ ,  $p = .50$ , or between lever and genotype,  $F(1,36) = .10$ ,  $p = .75$ . There was a significant interaction between lever and group,  $F(1,36) = 1666.0$ ,  $p < .001$ , as well as between the three factors of lever, genotype, and group,  $F(1,36) = 4.5$ ,  $p = .041$ . All other interactions were not significant,  $F$  values  $< 1.3$ ,  $p$  values  $> .22$ .

To further analyse the interaction between lever, genotype, and group, mixed model ANOVA were carried out for each group (large and small difference), with lever and genotype as factors. For the large difference group this showed that there was no significant effect of genotype,  $F(1,19) = 1.2$ ,  $p = .29$ , a significant effect of lever,  $F(1,19) = 14880.7$ ,  $p < .001$ , and no significant interaction between lever and genotype,  $F(1,19) = 2.5$ ,  $p = .13$ . All other effects and interactions were not significant,  $F$  values  $< 1.6$ ,  $p$  values  $> .10$ . For the small difference group there was a significant effect of genotype,  $F(1,17) = 7.1$ ,  $p = .017$ , and lever,  $F(1,17) = 1174.1$ ,  $p < .001$ , with no significant interaction between these two factors,  $F(1,17) = 2.1$ ,  $p = .17$ . All other effects and interactions were not significant,  $F$  values  $< 1.7$ ,  $p$  values  $> .065$ .



**Figure 5.1.** Experiment 14, acquisition stage. The mean numbers of lever presses (left side) and the numbers of pellets earned on each lever (right side), for the large difference group (upper) and small difference group (middle), by wild-type (WT) and *Gria1*<sup>-/-</sup> mice. The lower panel shows the ratio of lever press responding (high lever presses/ (high lever presses + low lever presses)). Error bars show  $\pm$  SEM.

The results from the acquisition stage demonstrate that both the wild-type and *Gria1*<sup>-/-</sup> mice were sensitive to the relative rates of reinforcement of the two levers. This was seen in both genotypes making a greater number of presses on the lever with the higher rate of reinforcement than the low, with no significant difference between the small and large difference groups. This shows that both wild-type and *Gria1*<sup>-/-</sup> mice were able to detect the relative differences in reinforcement rates and respond more to the lever with the higher reinforcement rate.

It was also the case however that the *Gria1*<sup>-/-</sup> mice made higher numbers of presses, to both levers, than the wild-type mice. When looking at the ratios of responding, the large difference group also showed higher ratios than the small difference group, for wild-type and *Gria1*<sup>-/-</sup> mice. This demonstrates that greater differences in relative reinforcement rates, result in greater differences in the relative rates of responding. There was a significant effect of genotype on the ratios, with the *Gria1*<sup>-/-</sup> mice making significantly smaller ratios of responding than the wild-type mice. However, when looking at the results, it can be seen that in the first couple of sessions the ratios in the *Gria1*<sup>-/-</sup> mice are particularly low. The wild-type mice, in the first session, also show particularly high ratios of responding. There therefore seemed to be a large amount of variability in the first couple of sessions that was not seen after a couple of sessions of training.

It may have been the case that going into the acquisition stage of the experiment the mice were not responding equally across the two levers, resulting in the initial variability in the ratios of responding. This suggests that mice, both *Gria1*<sup>-/-</sup> and the wild-types, may have had particular bias towards one of the levers which in pre-training did not differ in terms of reinforcement rates. When the reinforcement rates did then differ in the experiment, the learning about these relative rates then limited the effect of the previous bias, resulting in the more stable matching behaviour subsequently seen. This effect was evident in the significant interaction between genotype and session, with the ratios only significantly lower earlier in training. This shows that although there was a significant effect of genotype, this occurred as a result of the high level of variability in the first two sessions. In the subsequent sessions when ratios of responding were more stable, there was no longer an effect

of genotype, demonstrating there was no clear difference between the wild-type and *Gria1*<sup>-/-</sup> mice in learning about the relative reinforcement rates.

There was however a slight difference in the number of pellets earned between the genotypes. This effect was only seen in the small difference group, in which the *Gria1*<sup>-/-</sup> mice earned slightly more than the wild-type mice. In general however, this did not seem to alter learning about the relative reinforcement rates, as the effect of genotype was attributable to the high level of variability early in training. The slight increase in the number of pellets in the small difference group did therefore not seem to affect learning about the relative reinforcement rates. An additional finding from the results of the acquisition stage was that although both genotypes were sensitive to the relative rates of reinforcement across the two levers, there was some evidence of undermatching behaviour. This was seen in the large difference group, with the ratios of responding significantly lower than the actual rate of reinforcement. The small difference group did not however significantly differ, although there is the potential that the smaller relative difference meant it was not possible to see any undermatching behaviour.

#### Reversal stage

##### Numbers of lever presses:

The numbers of presses made on the high and low levers by both groups and genotypes during the reversal stage, are shown in Figure 5.2. In the large and the small difference groups, responding was greater on the high than the low lever, by both the wild-type and *Gria1*<sup>-/-</sup> mice. This effect was also smaller in the small difference group compared to the large difference group. The *Gria1*<sup>-/-</sup> mice also made higher numbers of presses on the two levers than the wild-type mice in both groups. The ANOVA showed that there was a significant effect of lever (high or low),  $F(1,36) = 9.7$ ,  $p = .004$ , and genotype,  $F(1,36) = 20.9$ ,  $p < .001$ , as well as group (large difference or small difference),  $F(1,36) = 4.2$ ,  $p = .047$ , and a significant interaction between genotype and group,  $F(1,36) = 5.04$ ,  $p = .031$ . The interaction between lever and genotype was also significant,  $F(1,36) = 7.8$ ,  $p = .008$ , as was the interaction between lever and group,  $F(1,36) = 9.8$ ,  $p = .004$ . The effect of session was significant,

$F(11,396) = 3.5, p = .003$ , and there was no significant interaction between session and genotype,  $F(11,396) = 1.7, p = .12$ , but the interaction between session and group was significant,  $F(11,396) = 2.4, p = .032$ . There was also a significant interaction between lever and session,  $F(11,396) = 28.2, p < .001$ , and also between the three factors of lever, session, and genotype,  $F(11,396) = 4.3, p < .001$ , as well as between the three factors of lever, session, and group,  $F(11,396) = 3.5, p = .002$ . All other interactions were not significant,  $F$  values  $< 1.96$ ,  $p$  values  $> .078$ .

To further investigate the interaction between the three factors of lever, session, and genotype, mixed model ANOVA were carried out for each genotype, with lever and session as factors. This showed that for the wild-type mice there was a significant interaction between lever and session,  $F(11,264) = 11.9, p < .001$ . Simple main effects analysis of this interaction further showed that in the first ( $F(1,24) = 19.2, p < .001$ ) and second session ( $F(1,24) = 5.5, p = .027$ ) responding was significantly lower on the high than the low rate lever. In all other sessions responding was not significantly different to the two levers,  $F$  values  $< 3.6$ ,  $p$  values  $> .068$ , apart from the final session, in which responding was significantly greater on the high rate lever,  $F(1,24) = 4.8, p = .038$ . For the *Gria1*<sup>-/-</sup> mice there was also a significant interaction between lever and session,  $F(11,154) = 13.0, p < .001$ . Simple main effects analysis of this interaction further showed that in the first session responding was significantly lower on the high than the low rate lever,  $F(1,14) = 23.0, p < .001$ , but in the following 4 sessions there was no significant difference between the levers,  $F$  values  $< 3.2$ ,  $p$  values  $> .095$ . For the remaining 7 sessions however responding was significantly greater on the high than the low rate lever,  $F$  values  $> 5.2$ ,  $p$  values  $< .039$ .

To further investigate the interaction between the three factors of lever, session, and group, mixed model ANOVA were carried out for each group, with lever and session as factors. This showed that for the large difference group there was a significant interaction between lever and session,  $F(11,220) = 26.0, p < .001$ . Simple main effects analysis of this interaction further showed that in the first session responding was significantly lower on the high than the low rate lever,  $F(1,20) = 27.0, p < .001$ . Responding did not significantly differ between the levers for following two sessions (second session  $F(1,20) = .026, p = .87$ ; second session,  $F(1,20) = 3.1, p = .093$ ). In all

subsequent sessions however responding was significantly greater on the high than the low rate lever,  $F$  values  $> 5.6$ ,  $p$  values  $< .028$ . For the small difference group there was also a significant interaction between lever and session,  $F(11,198) = 5.3$ ,  $p < .001$ . Simple main effects analysis of this interaction further showed that in the first session responding was significantly lower on the high than the low rate lever,  $F(1,18) = 14.7$ ,  $p = .001$ , with no significant differences between the levers in any of the following sessions ( $F$  values  $< 2.7$ ,  $p$  values  $> .88$ ).

#### Ratios:

The ratios of responding for the wild-type and  $Gria1^{-/-}$  mice in the large and small difference groups, during the reversal stage, are shown in Figure 5.2. The  $Gria1^{-/-}$  mice made higher ratios of responding than the wild-type mice in both groups, a pattern seen across training sessions. The ANOVA showed that there was a significant effect of session,  $F(11,396) = 27.8$ ,  $p < .001$ , and genotype,  $F(1,36) = 6.1$ ,  $p = .019$ , with no significant interaction between these two factors,  $F(11,396) = 1.5$ ,  $p = .18$ . There was also a significant effect of group,  $F(1,36) = 12.02$ ,  $p = .001$ , and no significant interaction between genotype and group,  $F(1,36) = .36$ ,  $p = .55$ . The interaction between session and group was significant,  $F(11,396) = 5.3$ ,  $p < .001$ , and there was no significant interaction between the three factors of session, genotype, and group,  $F(11,396) = 1.1$ ,  $p = .38$ . Simple main effects analysis of the interaction between session and group further showed that in the first, second and fifth sessions, there were no significant differences in ratios of responding between the groups,  $F$  values  $< 3.6$ ,  $p$  values  $> .067$ . In all remaining sessions responding was significantly greater in the large than the small difference group,  $F$  values  $> 4.3$ ,  $p$  values  $< .046$ .

To further test ratios of responding relative to the actual rates of reinforcement, one-sample  $t$ -tests were carried out for both the large and small difference groups on the data from the 12<sup>th</sup> training session. As there was a significant effect of genotype, these were done for the wild-type and the  $Gria1^{-/-}$  mice in each group. For the large difference group, the  $Gria1^{-/-}$  mice did not significantly differ from .75,  $t(7) = -1.9$ ,  $p = .103$ , however the wild-type mice were significantly lower,  $t(12) = -3.8$ ,  $p = .003$ . For the small difference group the  $Gria1^{-/-}$  mice again showed ratios of responding not significantly different from .60,  $t(6) = -.901$ ,  $p = .402$ , with the wild-type mice also

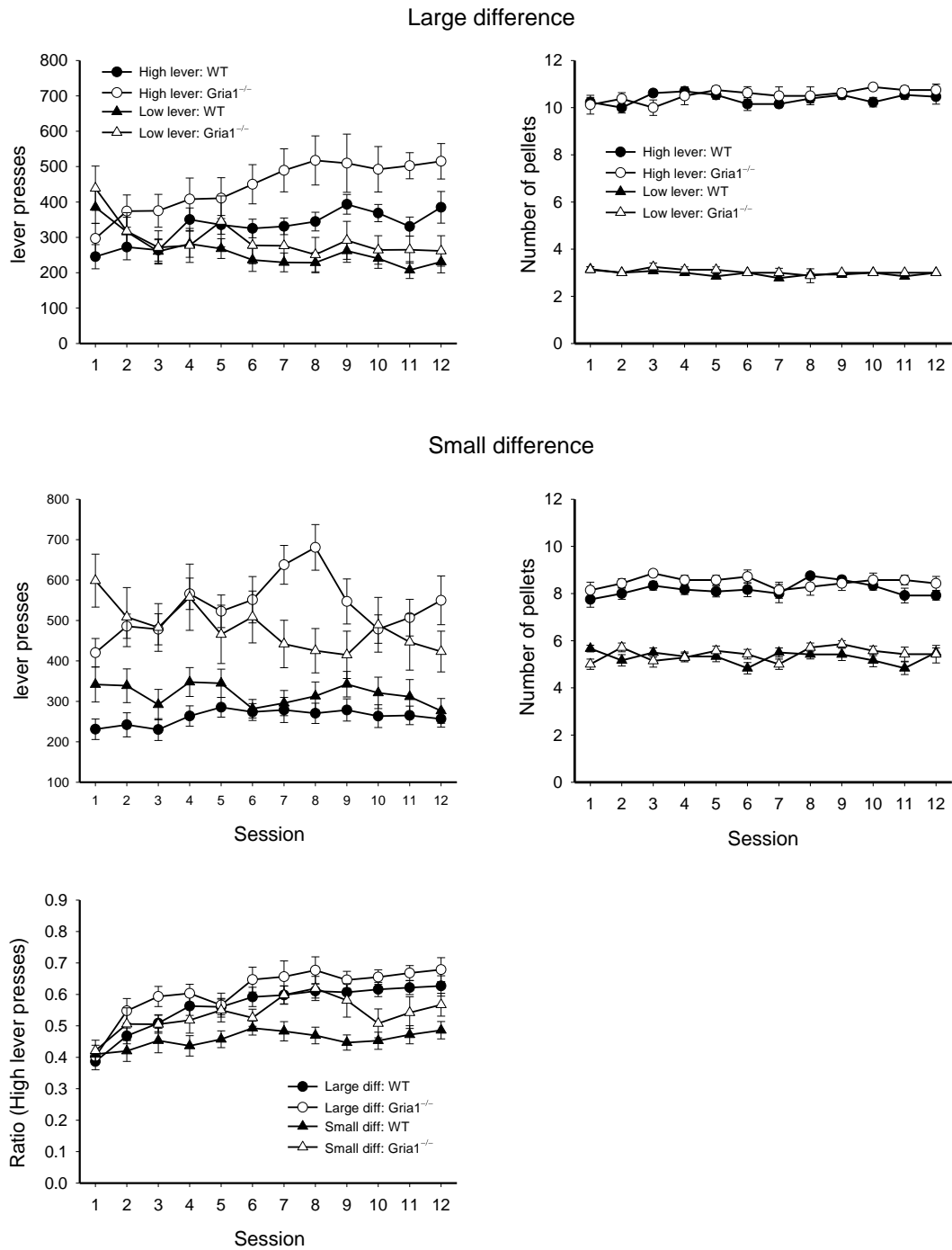
again being significantly lower than the actual rate,  $t(11) = -4.1$ ,  $p = .002$ . Furthermore, these wild-type mice did not significantly differ from .5,  $t(11) = -.50$ ,  $p = .63$ , showing no preference for the lever with a higher reinforcement rate (although wild-type mice in the large group did make ratios of responding significantly greater than .5,  $t(12) = 3.9$ ,  $p = .002$ ).

Numbers of pellets:

The numbers of pellets earned on each lever during the reversal stage, by both genotypes in the large and small difference groups, are shown in Figure 5.2. The numbers of pellets gained from the high lever was greater than on the low lever in both groups. The wild-type and the  $Gria1^{-/-}$  mice also showed near maximum numbers of pellets being earned across the two levers, an effect seen from the first session of training. The ANOVA showed that there was a significant effect of lever,  $F(1,36) = 8903.4$ ,  $p < .001$ , and a significant effect of genotype, with the  $Gria1^{-/-}$  mice earning more pellets than the wild-type mice,  $F(1,36) = .024$ . The total numbers of pellets earned over the 12 sessions on average by the wild-type mice was 134, and for the  $Gria1^{-/-}$  mice this was 137. The effect of group was not significant,  $F(1,36) = 2.7$ ,  $p = .11$ , and there was no significant interaction between genotype and group,  $F(1,36) = .49$ ,  $p = .49$ , and also no significant effect of session,  $F(11,36) = 1.6$ ,  $p = .10$ . The interaction between lever and group was significant,  $F(1,36) = 16663.6$ ,  $p < .001$ .

To further analyse this interaction, simple main effects analysis were carried out. This showed that both the large and small difference groups received a significantly greater number of pellets from the high than the low rate lever, (large difference group,  $F(1,36) = 9680.8$ ,  $p < .001$ ; small difference group,  $F(1,36) = 1357.9$ ,  $p < .001$ ). They also showed that for high rate lever the large difference group received significantly more pellets than the small difference group,  $F(1,36) = 363.4$ ,  $p < .001$ . For the low rate lever, the small difference group received significantly more pellets than the large difference group,  $F(1,36) = 1550.9$ ,  $p < .001$ . The interaction between the four factors of lever, session, genotype, and group neared significance,  $F(11,396) = 1.8$ ,  $p = .059$ . All other interactions were not significant,  $F$  values  $< 1.6$ ,  $p$  values  $> .088$ .

It is possible that the higher ratios of responding in the *Gria1*<sup>-/-</sup> mice could have been the result of the higher numbers of pellets earned by these mice compared to the controls. As the increased exposure to the rewards and therefore the reward contingencies of the levers, could have aided learning about the new relative reinforcement rates of the levers. However, the real difference between the numbers of pellets was very small, equating to 3 pellets more on average in the *Gria1*<sup>-/-</sup> mice compared to the wild-type mice. This means they received only one extra pellet, across both the levers, approximately every 4 training sessions. To further test the effect of pellets on the ratios of responding an additional mixed model ANOVA was carried out on the ratio data. Genotype, group, and session were added as factors and the total number of pellets, earned over the 12 reversal sessions for each mouse, added as a covariate. This showed that even with the numbers of pellets as covariate, the effect of genotype was significant,  $F(1,35) = 5.3$ ,  $p = .027$ , and there was no significant effect of the number of pellets earned,  $F(1,35) = .019$ ,  $p = .89$ .



**Figure 5.2.** Experiment 14, reversal stage. The mean numbers of lever presses (left side) and the numbers of pellets earned on each lever (right side), for the large difference group (upper) and small difference group (middle), by wild-type (WT) and *Gria1*<sup>-/-</sup> mice. The lower panel shows the ratio of lever press responding (high lever presses/ (high lever presses + low lever presses)). Error bars show  $\pm$  SEM.

During the reversal stage, as with the acquisition stage, both wild-type and *Gria1*<sup>-/-</sup> mice made a greater number of presses to the higher rate of reinforcement lever, demonstrating sensitivity to the relative rates of reinforcement of the two levers. The *Gria1*<sup>-/-</sup> mice also made higher numbers of lever presses than the wild-type mice, also an effect that was seen during the acquisition stage. The ratios of responding also showed that the large difference group, again as was also seen during the acquisition stage, made higher ratios of responding than the low, an effect that was seen in both wild-type and *Gria1*<sup>-/-</sup> mice. This further demonstrates sensitivity to the relative reinforcement rates in both genotypes. The wild-type mice did however show lower ratios of responding than the *Gria1*<sup>-/-</sup> mice, an effect that continued across training sessions. The wild-type mice also showed ratios of responding that differed from the actual rate of reinforcement, whereas the *Gria1*<sup>-/-</sup> mice did not significantly differ. The *Gria1*<sup>-/-</sup> mice therefore seem to be enhanced relative to the wild-type mice at responding to the reversed contingencies. As they showed more accurate matching behaviour compared to the wild-type mice, that under-matched in both the large and small difference groups. This effect in the wild-type mice was especially apparent when the relative difference between the levers was small, with the wild-type mice in the small difference group showing ratios of responding that did not significantly differ from .5. (equal levels of responding across the two levers).

One potential explanation for the enhanced reversal learning in the *Gria1*<sup>-/-</sup> mice is that they were simply exposed more to the relative reinforcement rates, something that could have occurred as a result of the greater numbers of presses made on the two levers. The number of pellets earned provided a way to assess any differences in the exposure to the contingencies, with the *Gria1*<sup>-/-</sup> mice earning significantly more than the wild-type mice in this stage. However, although this effect was statistically significant, the real difference in the numbers of pellets earned was small. The total numbers of pellets earned over the 12 sessions on average by the wild-type mice was 134, and for the *Gria1*<sup>-/-</sup> mice this was 137. There was therefore only a real difference of 3 pellets on average, equating to only one extra pellet across both the levers every 4 sessions. It seems unlikely that this would result in the enhancement in reversal learning seen in the *Gria1*<sup>-/-</sup> mice, particularly as this enhancement was also seen

from the second session in this stage. Differences in pellets earned during the acquisition stage also seem unable to account for the enhancement in this stage. As only the *Gria1*<sup>-/-</sup> mice in the small difference group significantly differed in pellets earned from the wild-type mice, yet the *Gria1*<sup>-/-</sup> mice in both groups showed the enhancement during the reversal stage. Furthermore, this difference in the acquisition stage equated to a difference of fewer than 6 pellets, again a small proportion of the overall pellets earned (160.6 in the wild-types compared to 166 in the *Gria1*<sup>-/-</sup> mice). Finally, when the total numbers of pellets earned was added as a covariate into the analysis with the ratios of responding, there was also found to be no significant effect of pellets earned.

### 5.1.3 Discussion

The results showed that both wild-type and *Gria1*<sup>-/-</sup> mice were sensitive to the relative reinforcement rates of the levers, with greater responding on the lever with the higher rate of reinforcement. Furthermore, this effect was also greater when the differences between the levers were larger, as shown by higher ratios in the large difference than the small difference group. Therefore, despite the previous findings by Austen et al. (in prep) of impaired rate calculation in *Gria1*<sup>-/-</sup> mice, they do show sensitivity to reinforcement rate in the form of matching behaviour to these relative rates.

Traditional learning theories, such as Rescorla & Wagner (1972), explain rate sensitivity through moment-by-moment prediction error correction and updating of associative strength. Moments in which a lever is not reinforced generates negative prediction error, decreasing the associative strength of it, whereas moments of reinforcement increase the associative strength. This results in the higher rate of reinforcement lever gaining greater associative strength than the lower rate lever, leading to the differing levels of conditioned responding on each of the two levers. If learning about the relative rates of reinforcement in this study was dependent on associative strength and prediction error learning, then this means that *GluA1* deletion does not impair the ability to learn about rate in this manner. Rate sensitivity has however also been suggested to be due to encoding of the temporal properties of cues and their relationships with reward (Balsam & Gallistel, 2009;

Molet & Miller, 2014). In this case, the intact matching behaviour in the *Gria1*<sup>-/-</sup> suggests that the GluA1 subunit is not required for encoding of the relevant temporal information in this experiment.

It is possible however that the *Gria1*<sup>-/-</sup> mice were not learning about reinforcement rate, but instead about the numbers of reinforcements associated with each lever. In the previous studies by Austen et al. (in prep) although the *Gria1*<sup>-/-</sup> mice were impaired at calculating the rate of reinforcement, they were sensitive to the numbers of reinforcements associated with a cue. This meant that the *Gria1*<sup>-/-</sup> mice responded more to cues leading to higher numbers of reinforcements, rather than the actual reinforcement rate. In the previous experiment, the lever with the higher reinforcement rate led to a higher number of pellets. It may be that the matching behaviour shown by the *Gria1*<sup>-/-</sup> mice in this study was due to learning about the numbers of pellets associated with each lever, rather than necessarily the reinforcement rate. This would explain the previous findings of impaired rate calculation in the *Gria1*<sup>-/-</sup> mice as well as the intact matching behaviour shown in this study. It would also suggest that the *Gria1*<sup>-/-</sup> mice were not necessarily directly learning about the temporal properties of cues, due to learning instead about only the numbers of reinforcements associated with each lever (Balsam & Gallistel, 2009; Molet & Miller, 2014).

The intact matching behaviour in the *Gria1*<sup>-/-</sup> mice was also seen despite showing higher rates of responding to both the levers. The conditioning procedure used here was instrumental, with mice learning to lever press for the reward. The prediction error signals, required for learning in traditional learning theories (Rescorla & Wagner, 1972), may therefore have occurred in response to the outcome of each lever press. Any non-reinforced lever presses therefore result in negative prediction error and the rewarded presses positive prediction error. The *Gria1*<sup>-/-</sup> mice made a greater number of presses than the wild-type mice, with the numbers of rewards showing only a very slight difference. This means that although the positive prediction errors should be similar across the two genotypes, the *Gria1*<sup>-/-</sup> mice would be expected to have lower contingencies between lever pressing and rewards, due to having greater experience with more non-reinforced lever presses than the wild-

type mice. This would mean that the probability of reinforcement and associative strength would be predicted to be lower in the *Gria1*<sup>-/-</sup> mice compare to the wild-type mice, for both levers. This did not however seem to be the case, as both genotypes showed similar ratios of responding and sensitivities to the different reinforcement rates during the acquisition stage. The *Gria1*<sup>-/-</sup> mice did therefore not seem to be affected by the enhanced exposure to the negative contingencies between the levers and the rewards, showing similarly high ratios of responding across the two levers. They also continued to press more than the wild-type mice, whereas greater exposure to non-reinforcement of the levers would be expected to reduce the associative strength and levels of conditioned responding to the levers. This result is however in line with previous work suggesting that the balance between positive and negative prediction error signalling may be altered in the *Gria1*<sup>-/-</sup> mice (Austen et al., in prep). This seems to occur despite *Gria1*<sup>-/-</sup> mice still being sensitive to non-reinforcement and negative prediction error, as shown by extinction learning being intact (Austen et al., in prep).

As well as showing intact matching behaviour in the acquisition stage, the *Gria1*<sup>-/-</sup> mice were also enhanced in the reversal stage. Whereas the wild-type mice under-matched relative to the actual rate of reinforcement during the reversal stage, this was not the case in the *Gria1*<sup>-/-</sup> mice. The finding of enhanced reversal learning is however in line with some of the previous findings in the *Gria1*<sup>-/-</sup> mice, in which reversal learning was found to be intact and even slightly enhanced in the early stages of reversal (Barkus et al., 2012). This enhancement did also not seem to be due to altered learning prior to the reversal stage, as both genotypes showed similar acquisitions in the first stage. This was demonstrated by the similar ratios of responding across the two levers by both wild-type and *Gria1*<sup>-/-</sup> mice in the first stage. Going into the reversal stage, the associative strength of the levers, whether based on learning about the count or rate, would therefore be expected to be similar for both genotypes. The enhancement therefore suggests that the *Gria1*<sup>-/-</sup> mice were more sensitive to the new relative reinforcement rates of the two levers. It has been suggested that reversal learning reflects the ability to learn about reward contingencies after changing the responses made to the given stimuli (Izquierdo et

al., 2017). GluA1 deletion may therefore increase the ability to learn from changes in reward contingencies, in this case from pressing the newly reversed high and low rate levers. How this might occur however is unclear. It is possible that the enhanced reversal links to a more general enhancement in associative learning, previously shown in these mice (Sanderson et al., 2009). However, learning and acquisition of matching in the first stage was not enhanced, something that would be expected if this were the case. Although it could be that the enhancement was only able to be seen in the reversal stage, due perhaps to being sufficiently difficult, without any floor/ceiling effects that would otherwise mask differences between the genotypes. This may be the case during reversal learning, explaining the small enhancement shown in this experiment and also in previous studies with the *Gria1*<sup>-/-</sup> mice (Barkus et al., 2012).

Overall, both the wild-type and *Gria1*<sup>-/-</sup> mice showed matching behaviour to the relative reinforcement rates of the levers during an instrumental conditioning procedure. However, whereas the wild-type mice may have been calculating reinforcement rate, the *Gria1*<sup>-/-</sup> mice likely learned about the relative number of rewards rather than the relative rates. The *Gria1*<sup>-/-</sup> mice also seemed to show enhanced reversal learning when the contingencies of the two levers were reversed. Although this could be related to increased cognitive flexibility, it is possible it could also be related to a more general enhancement in associative learning that is only evident given sufficient sensitivity.

## 5.2 Experiment 15

### Hippocampal NMDAR deletion and matching behaviour

In this experiment, Grin1<sup>ΔDGCA1</sup> mice were tested for the ability to learn about reinforcement rates and show matching behaviour. The hippocampus has been found to play an important role in the encoding of temporal information and timing (Howard & Eichenbaum, 2013). Evidence for this includes findings of hippocampal lesions impairing trace conditioning, in which there is a delay between the CS and US, but not when there is no delay between the two (Bangasser, 2006). Timing of conditioned responding has also been found to be linked to the hippocampus. For example, Tam and Bonardi (2012) observed that timing of conditioned responses during a peak procedure was impaired in rats with lesions to the dorsal hippocampus, with responding peaking earlier than the target duration (of 40s). Further evidence for the hippocampus playing a role in timing, comes from the findings of 'time cells', which have specific temporal fields and seem to encode moments of time in the interval between events (MacDonald et al., 2011). These results suggest that the hippocampus may be required for encoding of recent temporal durations and events, particularly of short-term duration of seconds to minutes (MacDonald et al., 2014).

To further investigate the role of the hippocampal NMDA receptors in rate sensitivity, the Grin1<sup>ΔDGCA1</sup> mice were tested on the same matching behaviour procedure as the previous experiment (experiment 14). Traditional learning theories explain temporal sensitivity as a result of prediction error mechanisms increasing and decreasing associative strength (e.g. Rescorla & Wagner, 1972, Wagner, 1981). It has also however been suggested that temporal sensitivity in animals is a result of direct encoding of the temporal properties of cues, with these associations used to direct conditioned behaviour (Gallistel & Gibbon, 2000).

## 5.2.1 Methods

### Subjects

16 Grin1<sup>ΔDGCA1</sup> mice (8 female, 8 male) and 23 control mice (7 CN12, 8 LC1, 8 NR1; 13 female, 10 male), bred and housed in the same way as experiment 4.1, were used. Mice were caged in groups of 1-6 and were between 12.5 – 17 months old at the start of testing and were maintained at 85% of their free-feedings weights. The control mice had 85% body weights of between 22.2g – 41.1g and the Grin1<sup>ΔDGCA1</sup> mice between 25.0g – 42.2g. Mice had previous experience with appetitive magazine conditioning within the same apparatus used in this experiment, as well as of flavour preference learning in similar apparatus.

### Apparatus

The apparatus used were the same as the previous experiment (5.1).

### Procedure

The procedure used was the same as the previous experiment (5.1), with the addition of a third stage in the experiment. In this stage, the schedules of reinforcement for the small difference group were changed, so that they now received the variable interval schedules of reinforcement from the large difference group. The large difference group however continued to receive the same reinforcement contingencies as the previous training stage. This meant that both groups now received the same VI schedules of reinforcement with the larger difference between the two levers. The high rate lever was reinforced on a VI 120s schedule and the low lever a VI 360s schedule. The lever contingences remained the same for both groups, so that the lever (either left or right) that was reinforced at a higher rate in the previous stage continued to be the higher rate lever in this third stage. These contingencies were presented for 8 sessions in total. Group allocation was counterbalanced in the same way as experiment 5.1. The large difference group included 12 control mice (4 CN12, 4 LC1, 4 NR1; 6 female, 6 male) and 8 Grin1<sup>ΔDGCA1</sup> mice (4 female, 4 male). The small difference group included 11 control type mice (3 CN12, 4 LC1, 4 NR1; 7 female, 4 male) and 8 Grin1<sup>ΔDGCA1</sup> mice (4 female, 4 male).

## Statistical analysis

Statistical analyses were carried out in the same way as the previous experiment (5.1), with the addition of a third training stage, analysed in the same way as the previous two stages. In the lever reversal stage there were two sessions in which one of the levers failed to operate (once in the 10<sup>th</sup> and 12<sup>th</sup> session). In these cases the mouse was given the average numbers of presses from either the preceding and following sessions (for session 10) or the two preceding sessions (session 12), and the ratio calculated as normal. One mouse died between the second and third stages of training (female control, CN12, large difference group), meaning the analysis on the third stage was on a total of 38 mice (22 controls and 16 Grin1<sup>ΔDGCA1</sup> mice).

### 5.2.2 Results

#### Acquisition stage

##### Number of lever presses:

The number of presses made on the high and low levers by both genotypes during the acquisition stage, are shown in Figure 5.3. The Grin1<sup>ΔDGCA1</sup> mice made greater numbers of presses than the control mice, in both the large difference and small difference groups, but both genotypes made greater numbers of presses on the high than the low lever. The ANOVA showed that there was a significant effect of lever (high or low),  $F(1,35) = 119.5$ ,  $p < .001$ , and genotype,  $F(1,35) = 9.9$ ,  $p = .003$ , with no significant interaction between these two factors,  $F(1,35) = 2.05$ ,  $p = .16$ . The effect of group (large or small difference) was not significant,  $F(1,35) = .12$ ,  $p = .73$ , with no significant interaction between genotype and group,  $F(1,35) = 1.0$ ,  $p = .32$ , and the interaction between lever and group was significant,  $F(1,35) = 9.5$ ,  $p = .004$ . There was a significant effect of session,  $F(11,385) = 4.5$ ,  $p < .001$ , but no significant interactions between session and genotype,  $F(11,385) = 1.7$ ,  $p = .12$ , or session and group,  $F(11,385) = .35$ ,  $p = .904$ . All other interactions were also not significant,  $F$  values  $< 1.6$ ,  $p$  values  $> .20$ .

Simple main effects analysis of the interaction between lever and group, further showed that for both levers there were no significant differences in responding

between the large and small difference groups (high lever:  $F(1,35) = 2.0$ ,  $p = .17$ , low lever  $F(1,35) = 2.6$ ,  $p = .12$ ). They also showed that in both groups responding was significantly greater on the high rate lever than the low rate lever (large difference group,  $F(1,35) = 99.9$ ,  $p < .001$ , small difference group  $F(1,35) = 30.3$ ,  $p < .001$ ).

#### Ratios:

The ratios of responding made by the  $Grin1^{\Delta DGCA1}$  mice and control mice, during the acquisition stage of training, are shown in Figure 5.3. The proportions of responding across the two levers remained relatively stable over sessions, with the large difference group showing higher ratios of responding than the small difference group, with little difference between the two genotypes. The ANOVA showed that there was no significant effect of session,  $F(11,385) = .90$ ,  $p = .44$ , or genotype,  $F(1,35) = .59$ ,  $p = .45$ , as well as no significant interaction between these two factors,  $F(11,385) = .39$ ,  $p = .75$ . There was however a significant effect of group (large or small difference),  $F(1,35) = 10.9$ ,  $p = .002$ , with no significant interaction between genotype and group,  $F(1,35) = .11$ ,  $p = .75$ , or between session and group,  $F(11,385) = 1.5$ ,  $p = .22$ . The interaction between the three factors of session, genotype, and group was also not significant,  $F(11,385) = .33$ ,  $p = .79$ .

To further test if the groups were showing accurate matching behaviour, one-sample t-tests were carried out for the large and small difference groups on the data from the 12<sup>th</sup> training session in this stage. These showed that the large difference group made ratios of responding significantly lower than the actual reinforcement rate of .75,  $t(19) = -2.3$ ,  $p = .03$ . The small difference group however did not significantly differ from the actual reinforcement rate of .6,  $t(18) = -.69$ ,  $p = .50$ .

#### Number of pellets:

The mean numbers of pellets earned by both genotypes on each of the levers, in the large and small difference groups during the acquisition stage, are shown in Figure 5.3. In both groups, a higher number of pellets were gained on the high compared to the low lever, with little difference between the wild-type and  $Grin1^{\Delta DGCA1}$  mice. The ANOVA showed that there was a significant effect of lever (high or low),  $F(1,35) = 4800.9$ ,  $p < .001$ , and no significant effect of group (large or small difference),  $F(1,35)$

= 1.8,  $p = .18$ , and also no significant effect of session,  $F(11,385) = .69$ ,  $p = .75$ . There was however a significant effect of genotype, with the  $\text{Grin1}^{\Delta\text{DGCA1}}$  mice earning a higher number of pellets than the control mice,  $F(1,35) = 5.7$ ,  $p = .022$ . The control mice gained an average of 155.3 pellets in total over the 12 sessions, the  $\text{Grin1}^{\Delta\text{DGCA1}}$  mice only earned a very slightly larger number; an average of 160.56 pellets in total across the 12 sessions. The interaction between genotype and group was not significant,  $F(1,35) = 1.7$ ,  $p = .20$ , and there was also no significant interaction between lever and genotype,  $F(1,35) = 3.1$ ,  $p = .089$ . There was a significant interaction between lever and group,  $F(1,35) = 843.0$ ,  $p < .001$ . All other interactions were not significant,  $F$  values  $< 1.2$ ,  $p$  values  $> .27$ .

The results from the acquisition stage showed that both genotypes were able to learn about the relative reinforcement rates of the levers, demonstrating similar levels of matching behaviour. This was seen in there being no difference between the control and  $\text{Grin1}^{\Delta\text{DGCA1}}$  mice in the ratios of responding made across the two levers. The results also showed that the  $\text{Grin1}^{\Delta\text{DGCA1}}$  mice made higher numbers of presses than the control mice, however, as there was no difference in the ratios of responding this did not seem to affect the accuracy of matching behaviour. The ratios of responding were also higher in the large difference group, for both genotypes, further demonstrating sensitivity to the relative reinforcement rates of the levers in both genotypes. The matching behaviour was also seen early in training, with no significant effect of session on the ratios of responding in either genotype.

The  $\text{Grin1}^{\Delta\text{DGCA1}}$  mice did however earn a slightly greater number of pellets than the control mice. This did not seem to affect learning about the relative rates of reinforcement, as the ratios of responding were not affected by genotype. Furthermore, although this difference between the numbers of reinforcements was statistically significant, it equated to a difference of only 5.2 pellets across all 12 sessions (control mice 155.3 pellets total,  $\text{Grin1}^{\Delta\text{DGCA1}}$  mice 160.56 pellets in total). In terms of the accuracy of the matching behaviour, the large difference group did show slight undermatching relative to the actual reinforcement rate. This was not seen in the small difference group, something that could be due to more accurate matching behaviour when the relative difference between the levers is smaller. Although this

result could also be due to an inability to see undermatching when the difference in reinforcement rates of the levers is smaller.

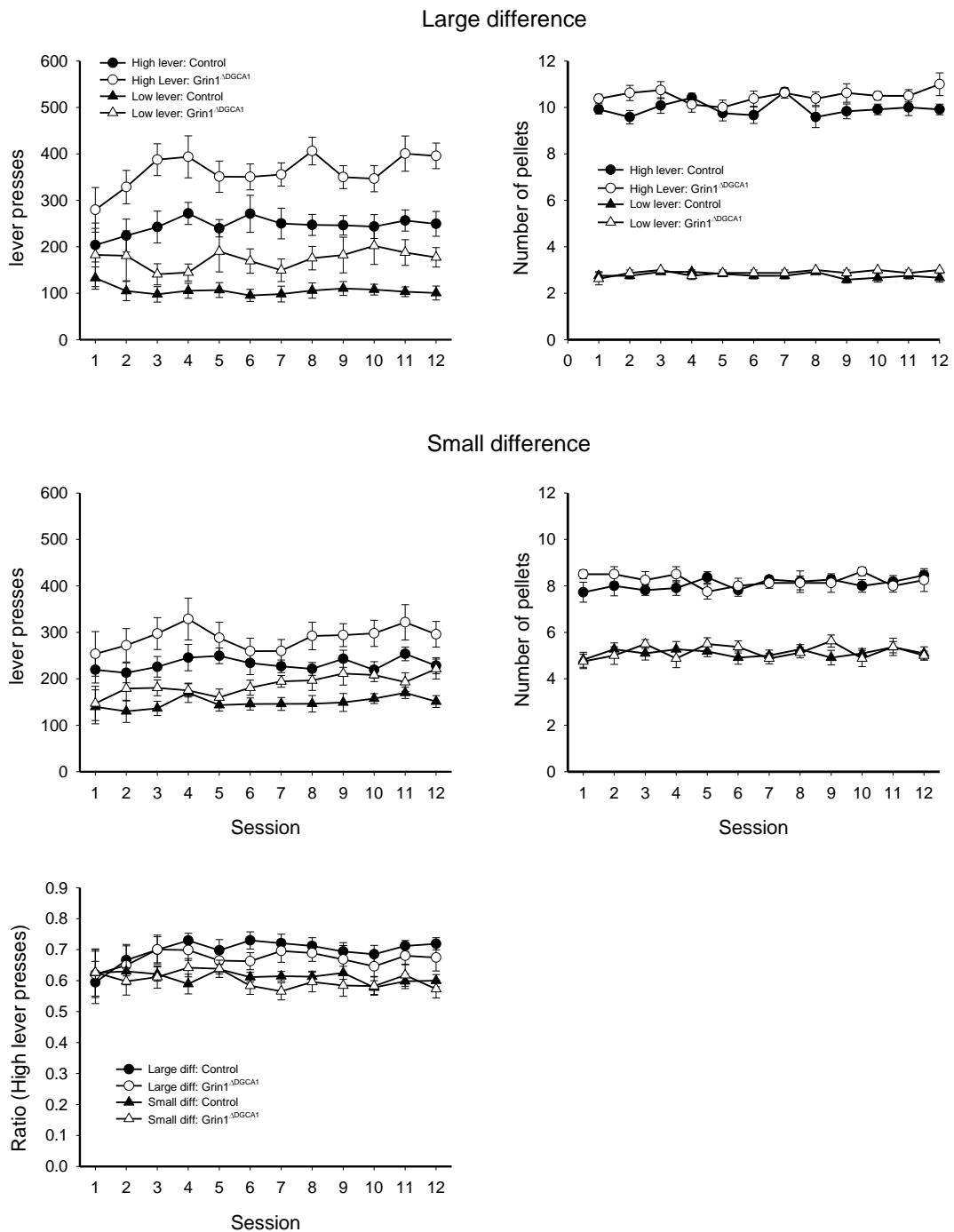


Figure 5.3. Experiment 15, acquisition stage. The mean numbers of lever presses (left side) and the numbers of pellets earned on each lever (right side), for the large difference group (upper) and small difference group (middle), by control and Grin1<sup>ΔD/GCA1</sup> mice. The lower panel shows the ratio of lever press responding (high lever presses/ (high lever presses + low lever presses)). Error bars show ± SEM.

Lever reversal stage

Numbers of lever presses:

The numbers of lever presses made on the high and low rate levers during the reversal stage, by both genotypes in the large and small difference groups, are shown in Figure 5.4. In the large difference group, the *Grin1*<sup>ΔD<sub>GCA1</sub></sup> mice made a greater number of presses than the control mice, particularly on the high lever. Both genotypes did however make a greater number of presses on the high than the low rate lever. In the small difference group, the numbers of presses were also greater on the high than the low lever, with the *Grin1*<sup>ΔD<sub>GCA1</sub></sup> mice making slightly higher numbers of presses than the control mice.

The ANOVA showed that there was a significant effect of lever (high or low),  $F(1,35) = 39.3$ ,  $p < .001$ , and genotype,  $F(1,35) = 14.4$ ,  $p = .001$ , as well as a significant interaction between lever and genotype,  $F(1,35) = 7.5$ ,  $p = .01$ . The effect of group (large or small difference) was not significant,  $F(1,35) = .43$ ,  $p = .52$ , and there was no significant interaction between genotype and group,  $F(1,35) = 1.5$ ,  $p = .23$ . There was a significant interaction between lever and group,  $F(1,35) = 23.4$ ,  $p < .001$ , and also between the three factors of lever, genotype, and group,  $F(1,35) = 10.3$ ,  $p = .003$ . There was a significant effect of session,  $F(11,385) = 7.2$ ,  $p < .001$ , and no significant interaction between session and genotype,  $F(11,385) = .62$ ,  $p = .66$ , or between session and group,  $F(11,385) = 1.6$ ,  $p = .16$ . The three way interaction between session, genotype, and group, was also not significant,  $F(11,385) = .604$ ,  $p = .67$ . There was a significant interaction between lever and session,  $F(11,385) = 37.6$ ,  $p < .001$ , and between the three factors of lever, session, and genotype,  $F(11,385) = 4.1$ ,  $p = .001$ . There was also a significant interaction between the three factors of lever, session, and group,  $F(11,385) = 7.5$ ,  $p < .001$ , as well as between the four factors of lever, session, genotype, and group,  $F(11,385) = 2.7$ ,  $p = .018$ .

To further analyse this four way interaction, mixed model ANOVA for the large and small difference groups were carried out, with genotype, lever, and session, as factors. For the small difference group this showed that the interaction between lever, session, and genotype was not significant,  $F(11,187) = 2.1$ ,  $p = .073$ , with no

significant interaction between lever and genotype,  $F(1,17) = .20$ ,  $p = .66$ , or between session and genotype,  $F(11,187) = .78$ ,  $p = .59$ , but there was a significant interaction between lever and session,  $F(11,187) = 7.1$ ,  $p < .001$ . Simple main effects analysis of the interaction between lever and session showed that in the first session the numbers of presses were significantly greater on the low compared to the high lever,  $F(1,17) = 19.5$ ,  $p < .001$ . In the fifth session, presses were significantly greater on the high lever,  $F(1,17) = 5.02$ ,  $p = .039$ , with this also true in the following 8<sup>th</sup>, 10<sup>th</sup>, 11<sup>th</sup>, and 12<sup>th</sup> sessions,  $F$  values  $> 5.3$ ,  $p$  values  $< .035$ . In all other sessions there was no significant difference between the two levers,  $F$  values  $< 3.3$ ,  $p$  values  $> .087$ . For the large difference group there was a significant interaction between the three factors of lever, session, and genotype,  $F(11,198) = 4.5$ ,  $p = .002$ .

To further analyse this three way interaction, mixed model ANOVA were carried out for each genotype (in the large difference group), with lever and session as factors. For the control type mice there was a significant interaction between lever and session,  $F(11,110) = 4.7$ ,  $p = .003$ . Simple main effects analysis of this interaction further showed that in the first,  $F(1,10) = 6.8$ ,  $p = .027$ , and second session,  $F(1,10) = 5.6$ ,  $p = .039$ , responding was significantly lower on the high rate than the low rate lever. In most subsequent sessions responding did not significantly differ between the two levers,  $F$  values  $< 3.7$ ,  $p$  values  $> .084$ , although responding was significantly greater on the high rate lever in sessions 7 ( $F(1,10) = 5.4$ ,  $p = .042$ ) and 10 ( $F(1,10) = 6.1$ ,  $p = .033$ ). For the  $\text{Grin1}^{\Delta\text{DGCA1}}$  mice there was also a significant interaction between lever and session,  $F(11,77) = 4.2$ ,  $p = .014$ . Simple main effects analysis of the interaction further showed that in the first session responding was significantly greater on the low than the high rate lever,  $F(1,7) = 12.7$ ,  $p = .009$ , but that in most other sessions there were no significant differences between the levers,  $F$  values  $< 5.3$ ,  $p > .054$ , apart from session 8, in which responding was significantly greater on the high rate lever than the low rate lever,  $F(1,7) = 8.6$ ,  $p = .022$ .

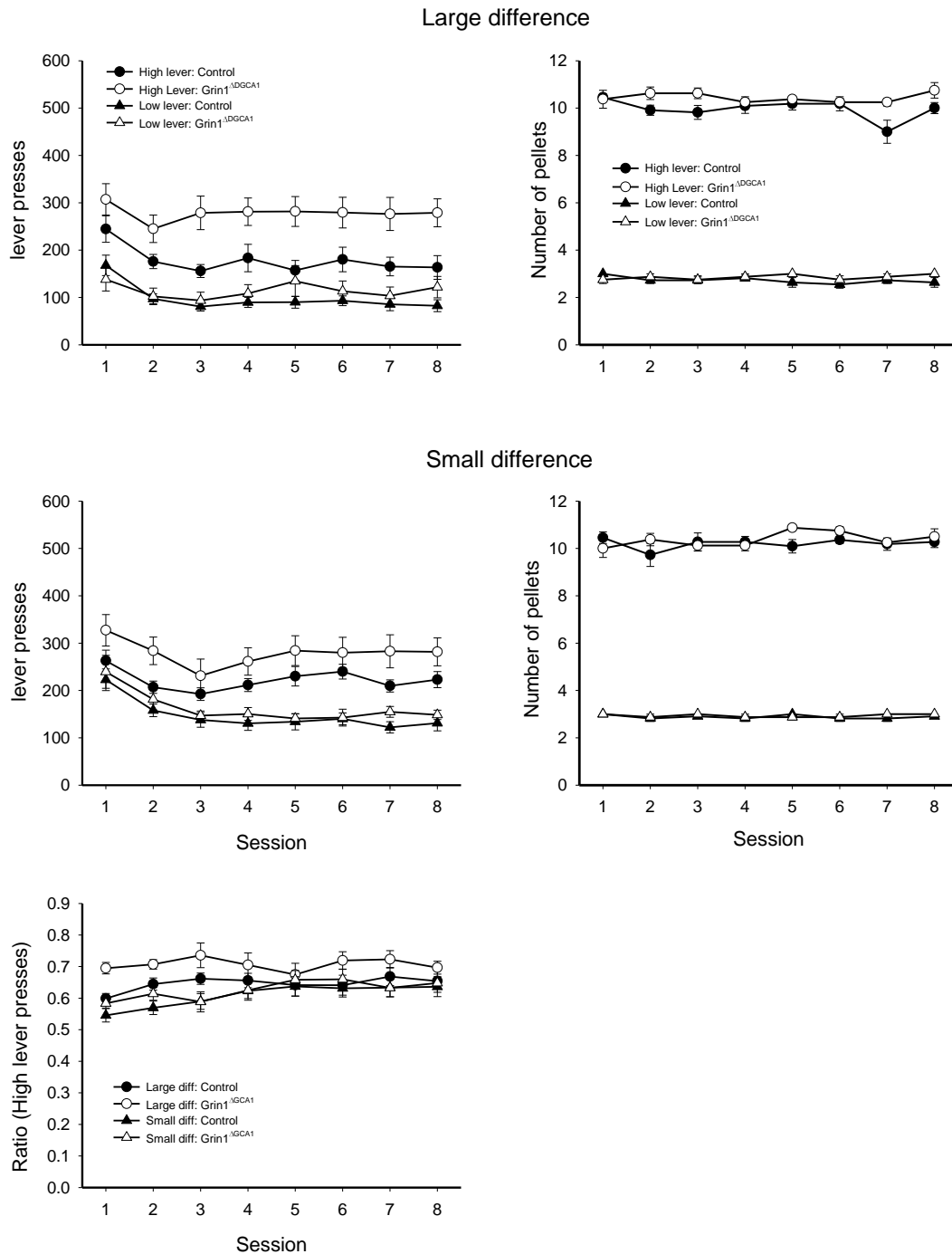
## Ratios:

The ratios of responding during the reversal stage, by Grin1<sup>ΔDGCA1</sup> and control mice in the large and small difference groups, are shown in Figure 5.4. The ratios of responding showed a general increase over training sessions and were also greater in the large than the small difference group. The Grin1<sup>ΔDGCA1</sup> mice showed higher ratios than the control mice, particularly in the large difference group. The ANOVA showed that there was a significant effect of session,  $F(11,385) = 34.04$ ,  $p < .001$ , and no significant effect of genotype,  $F(1,35) = 3.1$ ,  $p = .085$ , or significant interaction between these two factors,  $F(11,385) = 1.7$ ,  $p = .15$ . The effect of group (large or small difference) was significant,  $F(1,35) = 23.9$ ,  $p < .001$ , as was the interaction between genotype and group,  $F(1,35) = 5.2$ ,  $p = .029$ . The interaction between the three factors of session, genotype, and group was not significant,  $F(11,385) = 1.5$ ,  $p = .18$ . Simple main effects analysis of the interaction between genotype and group, further showed that the Grin1<sup>ΔDGCA1</sup> mice in the large difference group made significantly higher ratios than those in the small difference group,  $F(1,35) = 21.8$ ,  $p < .001$ . The control mice also showed near significantly greater ratios in the large difference group,  $F(1,35) = 4.1$ ,  $p = .050$ . In the large difference group the Grin1<sup>ΔDGCA1</sup> mice also made significantly greater ratios of responding than the control mice,  $F(1,35) = 8.3$ ,  $p = .007$ , with no significant difference between the two genotypes in the small difference group,  $F(1,35) = .13$ ,  $p = .73$ .

To further test the accuracy of the matching behaviour, separate one-sample t-tests were carried out for both groups on the data from the final testing session of this stage. These were done separately for each genotype in the large but not the small difference group, due to there being a significant effect of genotype on the ratios of responding in the large difference, but not the small difference, group. These showed that the control mice in the large difference group made significantly lower ratios than the actual reinforcement rate of .75,  $t(19) = -3.8$ ,  $p < .001$ , whereas the ratios of responding made by Grin1<sup>ΔDGCA1</sup> mice did not significantly differ,  $t(7) = -1.1$ ,  $p = .33$ . The small difference group also made significantly lower ratios than the actual reinforcement rate of .60,  $t(18) = -4.4$ ,  $p < .001$ , although they were still significantly greater than .5,  $t(18) = 2.4$ ,  $p = .028$ .

Number of pellets earned:

The mean numbers of pellets earned on the two levers, by both genotypes in the large and small difference groups during the reversal stage, are shown in Figure 5.4. In both the large and small difference groups more pellets were earned on the high than the low rate lever, with little difference between the genotypes. The ANOVA showed that there was a significant effect of lever (high or low),  $F(1,34) = 3007.1$ ,  $p < .001$ , no significant effect of genotype,  $F(1,34) = 1.2$ ,  $p = .28$ , or group (large or small difference),  $F(1,34) = 1.9$ ,  $p = .18$ , as well as no significant effect session,  $F(11,374) = .56$ ,  $p = .78$ . The interaction between genotype and group was also not significant,  $F(1,34) = .804$ ,  $p = .38$ , and there was no significant interaction between lever and genotype,  $F(1,34) = .058$ ,  $p = .81$ , but there was a significant interaction between lever and group,  $F(1,34) = 496.7$ ,  $p < .001$ , and between lever and session,  $F(11,374) = 2.8$ ,  $p = .005$ . All other interactions were not significant,  $F$  values  $< 1.8$ ,  $p$  values  $> .081$ .



*Figure 5.4.* Experiment 15, reversal stage. The mean numbers of lever presses (left side) and the numbers of pellets earned on each lever (right side), for the large difference group (upper) and small difference group (middle), by control and Grin1<sup>ΔDGA1</sup> mice. The lower panel shows the ratio of lever press responding (high lever presses/ (high lever presses + low lever presses)). Error bars  $\pm$  SEM.

The results from the reversal stage showed that the Grin1<sup>ΔDGCA1</sup> and control mice, in both groups were sensitive to some extent, to the reversal of the relative reinforcement rates of the high and low levers. As with the acquisition stage, the Grin1<sup>ΔDGCA1</sup> mice also made higher numbers of presses than the control mice, particularly on the high lever in the large difference group. The ratios of responding were also higher in the Grin1<sup>ΔDGCA1</sup> mice compared to the control mice in the large difference group, but not in the small difference group. There was however no difference in the numbers of pellets earned, either between the genotypes or the groups.

Therefore, despite pressing the levers significantly more, the Grin1<sup>ΔDGCA1</sup> mice did not earn a significantly greater number of rewards. The Grin1<sup>ΔDGCA1</sup> mice in the large difference group also showed more accurate matching behaviour, as they did not significantly differ from the actual rate of reinforcement, whereas the control mice under-matched. In the small difference group both Grin1<sup>ΔDGCA1</sup> and control mice showed undermatching relative to the actual reinforcement rate. The Grin1<sup>ΔDGCA1</sup> mice therefore showed enhanced reversal learning compared to the control mice, although this was only seen in the large and not the small difference group. In the third stage, the VI schedules in the small difference group were shifted to the same VI schedules as the large difference group. This allowed to test if the Grin1<sup>ΔDGCA1</sup> mice in the small difference group may also show enhanced reversal learning, given a sufficiently large difference between the relative rates of reinforcement of the two levers. This meant that in the 3<sup>rd</sup> stage, mice in the large difference group remained on the same reward contingencies as the previous stage, but for mice in the small difference group the relative difference between the rates of levers was slightly larger than previously experienced.

Stage 3: small difference group switched to large difference group VI schedules

Number of lever presses:

The number of presses made on the high and low rate levers, for the large difference group and the small difference group (now with the large difference VI schedules), by control and Grin1<sup>ΔDGCA1</sup> mice, are shown in Figure 5.5. For both groups (large and small difference, with both now on the same VI schedules) the number of lever presses remained greater on the higher rate of reinforcement lever. The Grin1<sup>ΔDGCA1</sup> mice also continued to make greater numbers of presses than the control type mice. The ANOVA showed that there was a significant effect of lever (high or low),  $F(1,34) = 122.2$ ,  $p < .001$ , and genotype,  $F(1,34) = 7.4$ ,  $p = .010$ , as well as a significant interaction between these two factors,  $F(1,34) = 10.1$ ,  $p = .003$ . The effect of group (large difference or small, now on large difference schedules) was near significant,  $F(1,34) = 4.1$ ,  $p = .051$ , and there was no significant interaction between genotype and group,  $F(1,34) = .37$ ,  $p = .55$ , or between lever and group,  $F(1,34) = 1.8$ ,  $p = .19$ . There was a significant effect of session,  $F(7,238) = 16.9$ ,  $p < .001$ , with no significant interaction between session and genotype,  $F(7,238) = 1.0$ ,  $p = .41$ , or between session and group,  $F(7,238) = .79$ ,  $p = .54$ . The interaction between the three factors of lever, session, and group was significant,  $F(7,238) = 2.8$ ,  $p = .016$ , but all other interactions were not significant,  $F$  values  $< 2.1$ ,  $p$  values  $> .061$ .

To further analyse the interaction between lever, session and group, mixed model ANOVA, with session and lever as factors, were carried out for the large and small difference groups. For the large difference group there was no significant interaction between lever and session,  $F(7,126) = .57$ ,  $p = .78$ . For the small difference group however, there was a significant interaction between lever and session,  $F(7,126) = 5.5$ ,  $p < .001$ . Simple main effects analysis of the interaction between lever and session in the small difference group further showed that in all sessions the number of presses were significantly greater on the high than the low rate lever,  $F$  values  $> 19.6$ ,  $p$  values  $< .001$ .

## Ratios:

The ratios of lever presses made by control and Grin1<sup>ΔDGCA1</sup> mice, in the large and small difference groups (with the small difference group during now on the large difference VI schedules), are shown in Figure 5.5. The small difference group did show a slight increase in the ratios of responding over sessions, whereas the large difference group showed more stable ratios of responding over sessions. The ANOVA showed that there was a significant effect of session,  $F(7,238) = 3.9$ ,  $p < .001$ , no significant effect of genotype,  $F(1,34) = 3.4$ ,  $p = .076$ , and no significant interaction between these two factors,  $F(7,238) = .68$ ,  $p = .63$ . The effect of group was significant,  $F(1,34) = 7.5$ ,  $p = .010$ , and there was no significant interaction between genotype and group,  $F(1,34) = 1.0$ ,  $p = .32$ . There was however a significant interaction between session and group,  $F(7,238) = 2.7$ ,  $p = .023$ , but no significant interaction between the three factors of genotype, session, and group,  $F(7,238) = .29$ ,  $p = .91$ .

Simple main effects analysis of the interaction between session and group further showed that the small difference group made significantly lower ratios in the first 3 sessions,  $F$  values  $> 15.3$ ,  $p$  values  $< .001$ , but this was not the case in most of the following sessions,  $F$  values  $< 3.5$ ,  $p$  values  $> .067$ . The exception was session 7, in which ratios were significantly higher in the large difference group compared to the small difference group,  $F(1,34) = 4.4$ ,  $p = .043$ .

To further look at the accuracy of matching behaviour in this stage, separate one-sample  $t$ -tests were carried out for the large and small difference groups on the data from the final session of this stage (both against the actual reinforcement rate of .75). These showed that both groups now significantly under-matched relative to the actual rate (large difference group,  $t(18) = -3.4$ ,  $p = .004$ ; small difference group,  $t(18) = -5.6$ ,  $p < .001$ ).

Number of pellets earned:

The mean total numbers of pellets gained from each lever, by both genotypes in the large and small difference groups (with the small difference group now on the same VI schedules as the large difference group), are shown in Figure 5.5. Both genotypes in the two groups earned a greater number of pellets on the high than the low rate lever, with little difference between the genotypes. The ANOVA showed that the effect of lever was significant,  $F(1,34) = 8464.4$ ,  $p < .001$ , the effect of group was not significant,  $F(1,34) = 1.5$ ,  $p = .23$ , but that the  $Grin1^{\Delta DGCA1}$  mice did earn a significantly greater number of pellets than the control mice,  $F(1,34) = 5.8$ ,  $p = .021$ . The difference in terms of the real numbers of pellets was however small, as the control mice earned an average total of 103 pellets across the 8 sessions, and the  $Grin1^{\Delta DGCA1}$  mice an average of 107.4 pellets. The interaction between genotype and group was not significant,  $F(1,34) = 1.3$ ,  $p = .26$ , and there was also no significant effect of session,  $F(7,238) = 1.2$ ,  $p = .29$ . The interaction between lever and genotype was not significant,  $F(1,34) = 2.1$ ,  $p = .15$ , and there was also no significant interaction between lever and group,  $F(1,34) = .024$ ,  $p = .88$ . All other interactions were also not significant,  $F$  values  $< 1.8$ ,  $p$  values  $> .084$ .

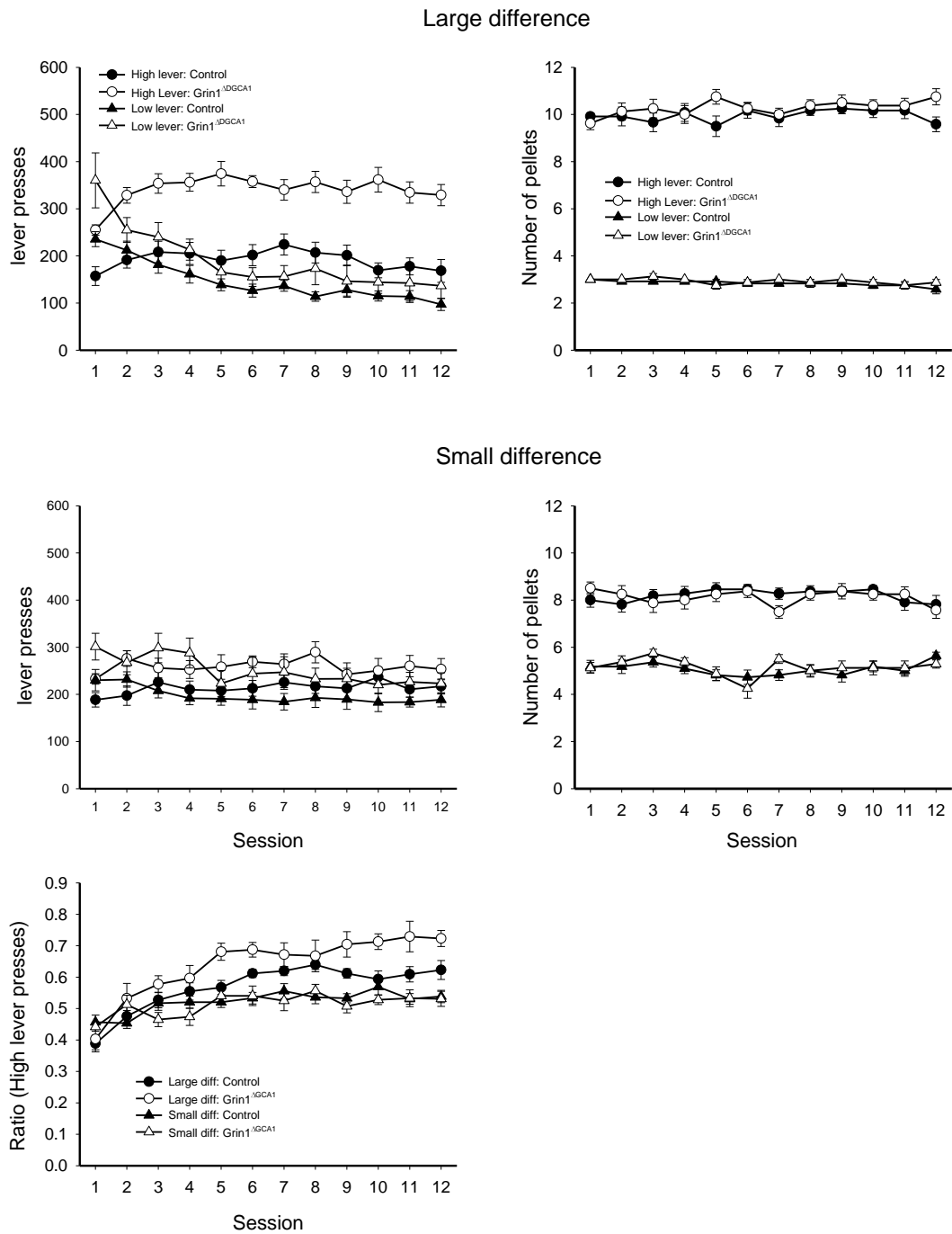


Figure 5.5. Experiment 15, stage 3. The mean numbers of lever presses (left side of the panel) and the numbers of pellets gained on each lever (right side), for the large difference group (upper) and small difference group (middle), by control and Grin1<sup>ΔDGGCA1</sup> mice. The lower panel shows the ratio of lever press responding (high lever presses/(high lever presses + low lever presses)). Error bars show ± SEM.

In the third stage, the mice in the small difference group did show some learning to the new relative reinforcement rates of the two levers. This was seen in the ratios of responding in the small difference group being initially lower than the large group, but not significantly differing by the end of the 8 sessions of training. The Grin1<sup>ΔDGCA1</sup> mice did not however show any enhancement compared to the control mice, despite being shifted to the VI schedules from the large difference group. However, the previous experiences of the lever reversal and small difference VI schedules, may have prevented any enhancement being seen at this point. The results also showed that the Grin1<sup>ΔDGCA1</sup> mice continued to make a greater number of lever presses than the controls, something that again did not seem to affect the ratios of responding. They also earned a significantly greater number of pellets in total across the two levers than the control mice, as was seen in the initial acquisition stage. This did not however affect the ratios of responding across the two levers, as there was no effect of genotype on the ratios of responding. This difference was again also very small relative to the total numbers of pellets earned across the two levers, with a difference of only 4.3 pellets on average over the 8 sessions of training. By the end of this stage, both groups and genotypes showed undermatching relative to the actual reinforcement rate, as was also seen in the previous stages in the large difference group.

### **5.2.3 Discussion**

The results from this experiment showed that both control and Grin1<sup>ΔDGCA1</sup> mice were sensitive to the relative reinforcement rates of the two levers, with a greater proportion of responses made on the high rate than the low rate lever. The ratios were also greater in the large difference group compared to the small difference group, further demonstrating sensitivity to the relative reinforcement rates of the levers. The Grin1<sup>ΔDGCA1</sup> mice also generally made a higher number of lever presses than the control type mice. This did not however affect the ratios of responding, as both the control and Grin1<sup>ΔDGCA1</sup> mice showed similar ratios and therefore accuracy of matching behaviour. The results from the reversal stage also showed that both Grin1<sup>ΔDGCA1</sup> and control mice were sensitive to the reversal and able to adapt responding to be greater to the new higher rate lever. They also showed that the

Grin1<sup>ΔDGCA1</sup> mice were enhanced relative to the control mice, in the large difference group but not the small difference group. One potential explanation of the reversal learning is that the Grin1<sup>ΔDGCA1</sup> mice were more exposed to the reward contingencies of the levers than the control mice. The Grin1<sup>ΔDGCA1</sup> mice did however earn more pellets than the controls in the first stage, but this difference was small, of only around 5 pellets over the 12 sessions. There was also no difference in the number of pellets earned in the reversal stage, meaning differences in exposure to the reward contingencies of the levers seem unlikely to explain the enhancement seen during the reversal stage.

Both genotypes did however show a degree of under-matching when the difference between the levers was larger, suggesting matching behaviour may be more accurate when the difference between the levers is smaller. This effect, rather than being due to more accurate learning about the smaller differences, could be linked to a performance deficit in the large difference group. When the difference between the levers is greater, mice are required to make a greater difference in the relative rates of responding across the two levers. It may be that although they learn the relative rates fine, it is more difficult to express this in the form of even greater responding on one lever. It does however seem that as commonly seen in previous matching experiments (e.g., McDowell, 2005), there was evidence of undermatching relative to the actual reinforcement rate. The results also showed that both control and Grin1<sup>ΔDGCA1</sup> mice were also able to show reversal learning when the high rate and low rate levers were switched. Furthermore, the Grin1<sup>ΔDGCA1</sup> mice in the large difference group actually showed an enhancement relative to the control mice. Not only were their ratios of responding greater than the control mice, but in the reversal stage they no longer significantly undermatched, as was seen in the control type mice.

Overall, the results show that deletion of hippocampal NMDAR's does not seem to impair rate sensitivity in the form of showing matching behaviour, even though hippocampal functioning has been implicated in the coding of temporal information (Howard & Eichenbaum, 2013). Furthermore, reversal learning and sensitivity to the present reinforcement rates seems to be slightly enhanced in the Grin1<sup>ΔDGCA1</sup> mice compared to the control mice. This suggests that hippocampal NMDA deletion may

in some cases actually enhance cognitive flexibility in the form of enhanced reversal learning, in this instance about the relative reinforcement rates of levers.

### 5.3 General discussion

In the two experiments in this chapter, *Gria1*<sup>-/-</sup> and *Grin1*<sup>ΔDGCA1</sup> mice were tested for rate sensitivity and matching behaviour during an instrumental conditioning procedure. In both of these experiments it was found that the knockout mice were able to acquire matching behaviour to the same extent as the control type mice. Both the *GluA1* and *Grin1*<sup>ΔDGCA1</sup> mice also showed higher rates of responding to the levers compared to the controls, making greater numbers of lever presses, but with only a very slight increase in the numbers of pellets earned. This suggests that in both experiments the knockout mice were not affected by the lower reward contingencies that would result from the greater numbers of presses, but similar numbers of pellets being earned. It was also the case that mice showed undermatching compared to the actual rate of reinforcement, particularly in the large difference groups and during the first acquisition stage. This result matches the findings from previous animal studies in which under-matching is often seen (McDowell, 2005). The results also suggest that undermatching is more likely to be observed when the differences between the relative rates of reinforcement are greater. Although rather than being due to more accurate matching in the small difference group, it could also be related to a lack of sensitivity when the difference between the levers is smaller.

In the *Gria1*<sup>-/-</sup> mice, the intact matching behaviour was seen despite the previous findings of impaired rate sensitivity during Pavlovian conditioning, being sensitive to reinforcement number rather than to reinforcement rate (Austen et al., in prep). The intact matching behaviour in the *Gria1*<sup>-/-</sup> mice can be similarly explained using reinforcement number. In this case, rather than learning about the differential rates of the two levers, they may instead have learned about the different numbers of reinforcements associated with the levers. *GluA1* deletion does therefore not impair matching behaviour, even though it does seem to alter the processing of temporal and numerical information.

The intact matching behaviour in the  $Grin1^{\Delta DGCA1}$  mice demonstrates that despite the hippocampus playing an important role in encoding temporal information (Howard & Eichenbaum, 2013; Tam & Bonardi, 2012), deletion of the NMDAR's in the hippocampus does not affect rate sensitivity and matching behaviour. It may be that the matching behaviour in this experiment was not dependent on the encoding of temporal information related to hippocampal functioning. It is possible for example, that as with the  $Gria1^{-/-}$  mice, the  $Grin1^{\Delta DGCA1}$  mice may have learned about something other than reinforcement rate, such as reinforcement number. Something that is not known from the present set of experiments, with further work into timing and rate calculation in the  $Grin1^{\Delta DGCA1}$  mice required to investigate this.

Both the  $Gria1^{-/-}$  and the  $Grin1^{\Delta DGCA1}$  mice were however enhanced during the lever reversal stage, showing more accurate matching behaviour relative to the control mice. Reversal learning has been suggested to reflect the ability to learn from reward contingencies after choosing a response (Izquierdo et al., 2017). Deletion of the GluA1 subunit of the AMPA receptor, or hippocampal NMDAR's, may therefore enhance sensitivity to changes in relative reinforcement rates of the two levers. However, they do not seem more generally sensitive to reinforcement rate, as they did not show an enhancement during the acquisition stage. It may also be that there is a more general enhancement in discrimination learning in the  $Gria1^{-/-}$  and the  $Grin1^{\Delta DGCA1}$  mice, with this only seen under sufficient levels of task difficulty. In the  $Gria1^{-/-}$  mice at least, this would match some previous findings in the  $Gria1^{-/-}$  mice of enhanced learning (Sanderson et al., 2009) and reversal learning (Barkus et al., 2012). However, if it is the case that learning is more generally enhanced, then this enhancement is only seen under limited conditions, as it was not seen in these experiments during the initial acquisition stage, or during the additional third stage with the  $Grin1^{\Delta DGCA1}$  mice.

Importantly, the results suggest that neither the GluA1 subunit or hippocampal NMDA receptors, seem to be required for rate sensitivity in the form of matching behaviour. Traditional learning theories (e.g., Rescorla & Wagner, 1972) explain rate sensitivity as a result of prediction error and associative strength. In this case, the results further suggest that deletion of the GluA1 subunit or hippocampal NMDA

receptors, do not impair prediction error learning. Although this learning could have been about the reinforcement rates, it may also have been due to learning about reinforcement number. It has also been suggested however that rate sensitivity is due to more direct encoding of temporal properties of cues (Balsam & Gallistel, 2009; Gallistel & Gibbon, 2000). In this case, the GluA1 and Grin1<sup>ΔDGCA1</sup> mice are able to encode the relevant temporal properties required to show matching behaviour.

# Chapter 6

## Discussion

The main aim of this thesis was to investigate the effects of glutamate dysfunction in learning and memory, either as a result of deletion of the GluA1 subunit, or deletion of hippocampal NMDA receptors. This was done by testing *Gria1<sup>-/-</sup>* and *Grin1<sup>ΔDGCA1</sup>* mice on various learning procedures.

The GluA1 subunit of the AMPA receptor is of particular interest in learning due to the role it plays in LTP (Kessels & Malinow, 2009). In *Gria1<sup>-/-</sup>* mice, short-term habituation is impaired, but long-term associative memory is intact. The role of the GluA1 subunit has therefore been explained in terms of Wagner's SOP model (Wagner, 1981), slowing the decay rate between the primary and secondary active states of memory (Sanderson et al., 2009). This account predicts that associative retrieval and cue-competition effects should be intact or even enhanced in the *Gria1<sup>-/-</sup>* mice. This prediction was directly tested in chapter 2, using blocking and overshadowing procedures. Although it was found that blocking and overshadowing were normal when using auditory and visual cues, blocking of flavour preference learning was impaired in the *Gria1<sup>-/-</sup>* mice. The results suggest that GluA1 deletion may affect cue-competition when cues are normally processed in a more configural, compared to a more elemental manner.

The account of GluA1 deletion, of slowing short-term memory decay (Sanderson et al., 2009), also predicts that short-term stimulus associability may be increased. This is due to the stimulus representation remaining in a primary state of activation for longer, subsequently being more likely to enter into associations with other stimuli concurrently active in this state. Previous studies with the *Gria1<sup>-/-</sup>* mice provided some evidence for this, with trace conditioning resulting in inhibitory learning in the wild-type mice but remaining excitatory in the *Gria1<sup>-/-</sup>* mice (Sanderson et al., 2017). The role of GluA1 deletion in the associativity of short-term memory was investigated in chapter three. This was done by testing *Gria1<sup>-/-</sup>* and wild-type mice on a flavour

preference procedure, in which the flavour preference learning has been suggested to be dependent on short-term memory decay (Dwyer et al., 2018). As this decay process is proposed to be slowed in the *Gria1*<sup>-/-</sup> mice, this flavour preference process should therefore also be impaired compared to the control mice. It was found however, that both the wild-type and *Gria1*<sup>-/-</sup> showed similar flavour preference learning based on having a short-term memory for the recently experienced stimulus. It is however difficult to rule out sensory adaptation, something that could also result in the flavour preference effect that was seen. This could be due to greater levels of adaptation potentially occurring before processing the CS- than before the CS+ flavour cue. However, if the effect was due to short-term memory decay, then this result would suggest that GluA1 deletion impairs the expression, but not the formation of, short-term memory.

The role of GluA1 deletion in learning was also tested in chapter 5, using matching behaviour to assess learning about the relative reinforcement rates of two levers during instrumental conditioning. Despite previous findings of impaired rate calculation in the *Gria1*<sup>-/-</sup> mice (Austen et al., in prep), both wild-type and *Gria1*<sup>-/-</sup> mice were able to show matching behaviour and therefore rate sensitivity to the two levers. The *Gria1*<sup>-/-</sup> mice have however been found to be sensitive to reinforcement number, the times a cue is paired with reinforcement, rather than rate of reinforcement (Austen et al, in prep). It may therefore have been the case that the *Gria1*<sup>-/-</sup> mice were learning about the numbers of reinforcements associated with the two levers, rather than the reinforcement rate. An additional finding was that reversal learning was enhanced in the *Gria1*<sup>-/-</sup> mice, showing more accurate matching when the reinforcement rates of the levers were reversed.

The *Grin1*<sup>ΔDGCA1</sup> mice lack NMDA receptors specifically within the hippocampus, resulting in impaired hippocampal synaptic plasticity (Bannerman et al., 2012). In chapter 4, the role of hippocampal NMDA receptors in palatability, measured using mean lick cluster sizes made during consumption, was tested. It was found that the mean lick cluster sizes were reduced, suggesting a reduction in perceived palatability. Although, it is also possible this reduction may relate to an impairment in the expression of palatability, in the form of mean lick cluster sizes, rather than altered

hedonic value. Deletion of hippocampal NMDA receptors in *Grin1<sup>ΔDGCA1</sup>* mice does not however impair the ability to learn a flavour preference, despite mean lick cluster sizes, a measure of palatability, being reduced in these mice. The results suggest that hippocampal NMDA receptors may play a role in perceived palatability but are not required for flavour preference learning. The ability to learn a flavour preference related to flavour-flavour associations, tested using fructose as the reinforcer, was also not impaired in the *Grin1<sup>ΔDGCA1</sup>* mice, despite mean lick cluster sizes being reduced. Flavour preference learning based on flavour-nutrient associations also seemed to be normal in the *Grin1<sup>ΔDGCA1</sup>* mice. However, the extent to which fructose and maltodextrin may independently support these difference associations was not clear from the results of this experiment.

The role of hippocampal NMDA receptors in learning about reinforcement rate was tested in chapter 5, assessing matching behaviour across two levers with different rates of reinforcement. It was found that despite the hippocampus being important for temporal encoding (Howard & Eichenbaum, 2013) matching behaviour was intact compared to the control mice. Furthermore, reversal learning was found to be enhanced when the relative rates of the levers were switched in the second stage.

## **6.1 The role of the GluA1 subunit in learning and memory**

The results from the *Gria1<sup>-/-</sup>* mice across the experiments in chapters 2, 3 and 5, showed a few key findings, each of which will be discussed in this section. Firstly, associative learning was normal in *Gria1<sup>-/-</sup>* mice. Secondly, when using auditory and visual cues, cue-competition effects of blocking and overshadowing were intact in the *Gria1<sup>-/-</sup>* mice. There was also some evidence that GluA1 deletion, despite impairing the expression of short-term memory, may not necessarily impair the formation of short-term memory. Finally, mean lick cluster sizes were also impaired across the studies that used a microstructural analysis of licking behaviour. There were however some additional findings, suggesting that information may be processed qualitatively differently in *Gria1<sup>-/-</sup>* mice compared to wild-type mice. This includes the failure to observe blocking of flavour preference learning. The *Gria1<sup>-/-</sup>* mice also showed

enhanced reversal learning in chapter five, when the reinforcement schedules of the two levers were switched.

The first main finding, of intact associative learning in the *Gria1<sup>-/-</sup>* mice, was seen in experiments throughout this thesis. These results are consistent with previous findings of intact long-term memory in these mice (e.g., Sanderson et al., 2009). The first evidence for intact learning in this thesis was seen in chapters two and three, in which flavour preference learning was found to be normal. For example, this was seen during the second test stages in the blocking of flavour preference experiments, when learning in the first training phase was tested and the *Gria1<sup>-/-</sup>* mice showed a preference for the CS+, compared to the CS-, flavour cue. This may even have been slightly enhanced, as the wild-type mice failed to show a flavour preference despite showing blocking of flavour preference. This enhancement is consistent with previous findings of slight enhancements in associative spatial memory that have also been seen in the *Gria1<sup>-/-</sup>* mice (Sanderson et al., 2009; Schmitt et al., 2003). However, flavour preference learning was generally not found to be enhanced in the *Gria1<sup>-/-</sup>* mice compared to the wild-type control mice. Any enhancement therefore seems to be slight, seen only in these experiments when tested in an additional test stage, having already extinguished and no longer being seen in the wild-type mice.

Intact associative learning was also seen in chapter 2, in the blocking and overshadowing experiments using auditory and visual cues. This was seen in both the wild-type and *Gria1<sup>-/-</sup>* mice being able to learn to respond to the reinforced cues more than to the non-reinforced cues. As well as during cue-competition procedures, normal associative learning was also seen in chapter 5 during an instrumental conditioning, matching behaviour study. This was seen in there being no effect of genotype on acquisition of matching behaviour across the two levers during the first stage of training. Deletion of the GluA1 subunit therefore did not impair the ability to differentiate between and learn about, the different relative rates of reinforcement. Overall, the *Gria1<sup>-/-</sup>* mice therefore showed normal associative learning and memory in the forms of flavour preference learning, conditioned responding to auditory and visual cues, and in terms of matching behaviour across two levers.

One of the aims of this thesis was to test the prediction that cue-competition effects, such as blocking and overshadowing, would be intact, or perhaps even enhanced, in the *Gria1*<sup>-/-</sup> mice. This prediction was the result of the role of the GluA1 subunit being proposed to be selective to the decay rates between the primary and secondary active states of memory in Wagner's SOP model (1981) (Sanderson et al., 2009). Cue-competition effects should be evident due to association formation and subsequent memory retrieval, also being intact in these mice. This means that the representation of the outcome retrieved by an associated cue, will still act to reduce the degree of prediction error and the ability to further learn about any cues present and in a primary active state of memory. Furthermore, the proposed role of GluA1 means that cue-competition effects may even have been enhanced, due to the increased duration of cues in a primary active state of memory. The finding of intact blocking and overshadowing, when using auditory and visual cues at least, supports GluA1 deletion not impairing cue-competition. There was however no evidence for enhanced cue-competition effects, although it is possible this could be related to a lack of sensitivity in the experiments in this thesis.

However, when using flavour preference learning, blocking was impaired in the *Gria1*<sup>-/-</sup> mice compared to the wild-type control mice. It was also found that neither genotype showed overshadowing of flavour preference learning. It is likely that flavour cues, that are mixed together when presented in compound, may have been processed more configurally rather than elementally (Pearce, 2002; Dwyer et al., 2011). The lack of overshadowing for example can be explained by configural processing of the flavour cues, with high levels of generalisation between these flavours (Dwyer et al., 2011). The GluA1 subunit does therefore seem to play a role in cue-competition, but only when the effect is dependent on configural processing and the level of generalisation between the cues. It could be that deletion of the GluA1 subunit enhances the tendency to process stimuli in a more elemental rather than configural manner. In this case, the failure to see blocking of flavour preference learning could be explained by a greater formation of within-compound associations between the flavour cues in the *Gria1*<sup>-/-</sup> mice. The extent of sensory preconditioning of taste aversion was not however found to differ between the genotypes, suggesting

that the *Gria1*<sup>-/-</sup> mice do not have an enhanced tendency to form within-compound associations. However, as the sensory preconditioning effect was small, this procedure may not have been sensitive enough to see differences in the formation of these associations.

It may therefore still be the case that the *Gria1*<sup>-/-</sup> mice are more likely to process stimuli in an elemental compared to a configural manner. Further work is therefore needed to investigate the degree of elemental and configural processing in *Gria1*<sup>-/-</sup> mice compared to wild-type control mice. Summation for example, when separately pre-trained stimuli are presented together during test sessions, could be used to test for elemental processing, as elemental theories (e.g., Rescorla & Wagner, 1972) predict conditioned responding to summate, but configural theories do not (Pearce, 1994, 2002). Evidence for an enhanced tendency to show summation in the *Gria1*<sup>-/-</sup> mice compared to the wild-type control mice would provide support for more elemental, rather than configural, processing. Overall, the results from chapter two do support a selective role for the GluA1 subunit in short-term memory processes, as proposed by Sanderson et al. (2009). However, under situations in which cues are processed more configurally, cue-competition effects may be impaired. This could be as a result of altered processing of the stimuli, such as the *Gria1*<sup>-/-</sup> continuing to process in a more elemental manner when the wild-type mice are processing the stimuli configurally.

In chapter three, the effect of the impaired short-term memory on learning about recently presented stimuli was tested. In particular, the ability to learn a flavour preference based on recent sucrose presentation and having a short-term memory for this sucrose was tested. The account of GluA1 deletion in short-term memory decay predicts that stimulus associability may be enhanced, due to the slowed decay rate and increased duration of primary state activation (Sanderson et al., 2009). As GluA1 deletion impairs short-term habituation and increases short-term stimulus associability, it was predicted that they may be impaired at learning the flavour preference in this procedure. This was due to the flavour preference being suggested to be dependent on short-term memory decay between the primary and secondary active states of memory. More specifically, the preference towards the CS+

compared to the CS- occurs due to fewer elements representing sucrose having decayed to the secondary active state of memory when the CS+ is presented compared to the CS- (Dwyer et al., 2018). It was found however, that the *Gria1*<sup>-/-</sup> mice learned this flavour preference as well as the wild-type mice. If the account by Dwyer et al. (2018) is true, then this result suggests that GluA1 deletion does not impair the ability to learn a flavour preference dependent on short-term memory decay. This is despite short-term memory decay seeming to be impaired in the *Gria1*<sup>-/-</sup> mice. This would suggest that although the expression of short-term memory is impaired in the *Gria1*<sup>-/-</sup> mice, they are still able to form short-term memory representations that are able to enter into associations. It would also provide some evidence against the slowed decay rate between the primary active states, as the *Gria1*<sup>-/-</sup> mice learned based on this decay process as well as the wild-type controls. However, this result should be taken with some caution, due to an inability to rule out sensory adaptation to sucrose. Rather than the flavour preference being due to differential levels of short-term memory decay, it could be due to differential amounts of sensory adaptation to sucrose. This could have been due to the CS+ being presented following a lower concentration of sucrose, meaning any short-term adaptation effects would be lower during consumption of it compared to the CS-, something that could have resulted in the flavour preference for the CS+. To rule this out would require demonstrating specificity of the flavour preference effect, showing that it is not related to a more general adaptation to sweet tasting sucrose.

The *Gria1*<sup>-/-</sup> mice have also been previously shown to have impaired lick cluster sizes, a measure of palatability. Glutamatergic dysfunction and the GluA1 subunit of the AMPA receptor have also been linked to schizophrenia (Ripke et al., 2013). Anhedonia is one of the negative symptoms associated with the disorder (Der-Avakian & Markou, 2011). The *Gria1*<sup>-/-</sup> mice have therefore been suggested to provide an animal model of altered glutamatergic signalling and anhedonia (Austen et al., 2017). Impaired mean lick cluster sizes during consumption were also seen in this thesis, throughout chapters two and three, with mean cluster sizes reduced in the *Gria1*<sup>-/-</sup> compared to the wild-type control mice across experiments using a microstructural analysis of licking behaviour. The results also replicated the findings

that despite a reduction in mean lick cluster size, *Gria1*<sup>-/-</sup> mice can learn a flavour preference (Austen et al., 2017). This demonstrates that deletion of the GluA1 subunit, that impairs mean lick cluster sizes, does not impair the ability for *Gria1*<sup>-/-</sup> mice to be able to discriminate between and learn about flavour cues. If reduced mean lick cluster sizes are reflective of a reduction in palatability (Dwyer, 2012), then this would also mean that reductions in perceived palatability do not subsequently impair flavour preference learning.

An additional finding was that when maltodextrin was used as the reinforcer, mean lick cluster sizes were not reduced in the *Gria1*<sup>-/-</sup> mice compared to the wild-type mice during the test sessions, although they were reduced during the training stage. Maltodextrin has been found to support preference learning through flavour-nutrient, rather than flavour-flavour associations, as it is able to support flavour preference learning when given via intragastric infusion (Sclafani & Nissenbaum, 1988). The intact mean lick cluster sizes during the flavour preference test sessions with maltodextrin, may suggest that GluA1 deletion does not affect increases in perceived palatability based on post-ingestive consequences. It may however reduce palatability for the perceived sensory properties of solutions, when the actual higher or lower concentrations are directly experienced. This would explain the reduced lick cluster size being specific to the training and not the test sessions. However, it should also be considered that the *Gria1*<sup>-/-</sup> mice may have impaired cluster sizes not as a result of reduced palatability and hedonic value, but as the result of impaired expression of palatability, in the form of mean lick cluster sizes. Therefore, the *Gria1*<sup>-/-</sup> mice may be unable to show an increase in the perceived palatability due to being less able to make the larger lick cluster sizes required. In this case, the *Gria1*<sup>-/-</sup> mice may still be able to perceive the increased sweetness as well as the wild-type mice, but are less able to express this in the form of an increase in mean lick cluster sizes during consumption. Such an explanation cannot be fully ruled out, although the finding that other aspects of licking behaviour (volume consumed per lick and lick duration) are normal, provide some evidence against a wider general motor deficit (Austen et al., 2017).

One further result from the experiments in this thesis to consider is the enhanced reversal learning shown by the *Gria1*<sup>-/-</sup> mice in chapter 5. In this experiment, there were no differences between the genotypes during the acquisition stage. However, when the reward contingencies of the levers were switched, the *Gria1*<sup>-/-</sup> mice showed an enhancement compared to the wild-type mice. The ability to learn about the new reward contingencies during reversal learning has been suggested to provide a test of cognitive flexibility (Izquierdo et al., 2017). In this case, deletion of the GluA1 subunit therefore seems to enhance cognitive flexibility. This enhanced flexibility suggests that the *Gria1*<sup>-/-</sup> mice are more able to learn about the present reinforcement contingencies. The GluA1 subunit may play a role in determining the influence of previous learning, as the *Gria1*<sup>-/-</sup> mice seem more sensitive to the present temporal context and better able to learn about the new reinforcement rates of the levers. However, spatial reversal learning in the *Gria1*<sup>-/-</sup> mice has been found to be impaired, rather than enhanced (Bannerman et al., 2003). Whereas cognitive flexibility in the *Gria1*<sup>-/-</sup> mice seems to be enhanced when learning about the reinforcement rates of the levers, it seems to be impaired during spatial reversal learning.

In conclusion, the results from this thesis further demonstrate that GluA1 deletion does not impair long-term memory based on associative retrieval. This supports the proposed role of the GluA1 subunit being selective to short-term memory processes (Sanderson et al., 2009). However, it does seem that GluA1 deletion may alter the way in which stimulus representations are processed. The impaired blocking of flavour preference could, for example, be linked to enhanced elemental compared to configural processing. In addition, the enhanced reversal learning could be related to increased sensitivity to the reward contingencies in the present temporal context.

## 6.2 The role of hippocampal NMDA receptors in learning and memory

The results from the studies with the Grin1<sup>ΔDGCA1</sup> mice, in chapters four and five, showed a few main findings, each of which will be discussed in this section. Firstly, NMDA receptors in the hippocampus were not required for associative learning. It was also shown that mean lick cluster sizes made during consumption were impaired. It was also found that reversal learning, during the matching behaviour study in chapter 5, was enhanced in the Grin1<sup>ΔDGCA1</sup> mice compared to the control mice. Finally flavour preference learning, based either on flavour-flavour or flavour-nutrient learning, was intact in the Grin1<sup>ΔDGCA1</sup> mice. This is despite mean lick cluster sizes, a measure of palatability, being impaired.

Intact associative learning in the Grin1<sup>ΔDGCA1</sup> mice was evident in both chapters four and five. These results are in line with previous findings of intact spatial learning in these mice (Bannerman et al., 2012; Taylor et al., 2014). In chapter four, the Grin1<sup>ΔDGCA1</sup> mice showed no difference compared to the control mice in flavour preference learning, consuming more of the CS+ compared to the CS-. This was seen not only when sucrose was used as the reinforcer, but also when either fructose or maltodextrin were used to support flavour preference learning. This demonstrates that the Grin1<sup>ΔDGCA1</sup> mice were able to learn an association between the flavour cue and higher reinforcer concentration paired with it during training. In chapter five, intact associative learning was seen in the form of normal matching behaviour during the acquisition stage. As both the control and Grin1<sup>ΔDGCA1</sup> mice showed sensitivity to the relative rates of reinforcement of the two levers, with no difference between the genotypes in the ratios of responding across the two levers. The normal associative learning in these experiments, as well as in previous experiments (Bannerman et al., 2012), demonstrates that hippocampal NMDA receptors are not required for associative learning and memory. This provides evidence against the suggestion that hippocampal LTP provides a potential neural basis of learning and memory (Bliss & Collingridge, 1993).

The second finding, of reduced mean lick cluster sizes in the *Grin1<sup>ΔDGCA1</sup>* mice, was seen during the experiments in chapter 4. In this chapter, consummatory behaviour to a range of sucrose concentrations was assessed in the *Grin1<sup>ΔDGCA1</sup>* and control type mice. It was found that both genotypes consumed similar amounts, with an inverted-U shape function of intake as sucrose concentration increased. This replicates the previous findings of highest intake at intermediate concentrations with an inverted U-shape function of consumption (Austen et al., 2016; Dwyer, 2012). Deletion of hippocampal NMDA receptors does therefore not seem to affect consummatory behaviour in terms of the amount consumed of palatable sucrose solutions. Therefore, despite the hippocampus being implicated in the regulation of eating behaviour (Konoski & Grill, 2017), impaired hippocampal synaptic plasticity does not seem to affect overall intake of sucrose solutions in *Grin1<sup>ΔDGCA1</sup>* mice. Hippocampal NMDA receptors, within the dorsal DG and CA1 subfields do however seem to be important for being able to show appropriate mean lick cluster sizes during consumption of palatable solutions. As mean lick cluster size provides a measure of palatability, this would suggest that these receptors may play a role in hedonic value, with deletion of these reducing the palatability of the solutions in the *Grin1<sup>ΔDGCA1</sup>* mice. If this is the case, then this result provides evidence for a role of the hippocampus, and the dorsal DG and CA1 subfields in particular, in hedonic value, something that theories of hippocampal functioning currently do not directly incorporate. It could for example build on from the proposed role of the hippocampus in appetitive behaviours and incentive motivation, but not consumption behaviour (Tracy et al., 2001; Jarrard, 1973), suggesting that palatability and hedonic value during consumption are also related to hippocampal functioning.

The deletion of NMDA receptors in the *Grin1<sup>ΔDGCA1</sup>* mice does also extend into the olfactory bulb as well as in the hippocampus (Bannerman et al., 2012), something that could potentially contribute to the ability to perceive flavours that might have an olfactory component, as with Kool-Aid solutions. Importantly however this did not seem to impair flavour preference learning in the experiments in this thesis, demonstrating that the *Grin1<sup>ΔDGCA1</sup>* mice are still able to differentiate between the different flavours and concentrations of solutions consumed. It cannot however be

completely ruled out that the effect of NMDA receptor deletion in the Grin1<sup>ΔDGCA1</sup> mice, of reduced mean lick cluster sizes, are a result of effects not exclusive to regions of the hippocampus alone.

The results also replicated the previous findings of consumption following an inverted U-shape function with increasing sucrose concentration, but mean lick cluster sizes showing a linear increase (Austen et al., 2016; Dwyer, 2012). It was however found that the mean lick cluster sizes, a measure of palatability, were reduced in the Grin1<sup>ΔDGCA1</sup> mice compared to the control mice. However, although these results suggest that perceived palatability may be reduced in the Grin1<sup>ΔDGCA1</sup> mice, the result may also be linked to an impairment in the expression of palatability. It could be the case that the Grin1<sup>ΔDGCA1</sup> mice are less able to generate the larger lick cluster sizes required to express palatability, something that cannot be ruled out from the current results.

The third finding from the Grin1<sup>ΔDGCA1</sup> mice was the enhanced reversal of matching behaviour seen in chapter 5. During the acquisition stage, the control and Grin1<sup>ΔDGCA1</sup> mice did not differ in the matching behaviour across the two levers. When the relative reinforcement rates of the two levers were reversed, the Grin1<sup>ΔDGCA1</sup> mice showed enhanced matching behaviour compared to the control mice. Deletion of hippocampal NMDA receptors therefore seemed to enhance the ability to learn about the new reward contingencies of the two levers. This result is however in contrast to the previous findings of impaired spatial reversal learning in the Grin1<sup>ΔDGCA1</sup> mice (Bannerman et al., 2012), suggesting that the role of hippocampal NMDA receptors is not specific to reversal learning.

The final result from the Grin1<sup>ΔDGCA1</sup> mice, was the finding that flavour preference learning was intact, when either fructose or maltodextrin were used as the reinforcers. This was seen despite mean lick cluster sizes, a measure of palatability, being reduced in the Grin1<sup>ΔDGCA1</sup> mice. Fructose has been found to support flavour preference learning through the formation of flavour-flavour associations, not supporting preference learning when given via intragastric infusion (Sclafani et al., 1999). In contrast, maltodextrin seems to support flavour preference learning based on flavour-nutrient associations, as it can support preference learning when given via

intra-gastric infusion (Sclafani & Nissenbaum, 1988). The reduction in palatability, as indicated by a reduction in mean lick cluster sizes in the *Grin1<sup>ΔDGCA1</sup>* mice, did not however seem to affect flavour preference learning with fructose. This is despite fructose being more dependent on flavour-flavour associations, something that would be more likely to be affected by altered palatability. If the reduced mean lick cluster sizes are reflective of impaired palatability, then this suggests that reduced hedonic value/palatability does not impair the ability to learn based on flavour-flavour associations.

However, it was also not clear the extent to which flavour-flavour and flavour-nutrient associations supported flavour preference with fructose and maltodextrin. As maltodextrin, but not fructose, resulted in a flavour preference in the form of increased intake and mean lick cluster size. If fructose does support flavour preference learning based on flavour-flavour associations, then this should be seen in the form of increased palatability. Yet lick cluster size effects were not seen during the test stage with fructose. Equally, maltodextrin, based on flavour-nutrient associations, did result in a lick cluster size effect. This suggests that learning an association between a flavour with a greater concentration of maltodextrin, does result in an increase in palatability. The results therefore question the dissociation between flavour-flavour and flavour nutrient associations in respect to fructose and maltodextrin, as well as the link between flavour-nutrient associations and mean lick cluster size, a measure of palatability. With maltodextrin, that seems to support flavour preference learning based on flavour-nutrient associations, seeming to increase the palatability of the CS+. This supports previous suggestions that maltodextrin may support both flavour-flavour as well as flavour-nutrient associations, although flavour-nutrient associations may be predominant (Dwyer & Quirk, 2008).

The results from this experiment did however show that when using maltodextrin, the mean lick cluster sizes made during consumption were not impaired in the *Grin1<sup>ΔDGCA1</sup>* mice. This could be related to an inability to see lick cluster size effects in the *Grin1<sup>ΔDGCA1</sup>* mice, due perhaps to ceiling effects in the mean cluster size when using maltodextrin. If there is no impairment however, this could suggest that lick

cluster sizes are normal in the *Grin1<sup>ΔDGCA1</sup>* mice when the flavour preference learning may be largely based on the positive post-ingestive consequences of the reinforcer. In this case, the results would suggest that the role of hippocampal NMDA receptors in palatability may be specific to flavour preference learning based on sensory properties. It is not clear from the current results however if the failure to see the lick cluster size impairment when using maltodextrin was due to lack of sensitivity, or an absence of an effect.

Overall, the results from the experiments in this thesis provide further evidence that long-term memory, based on associative retrieval, is intact in *Grin1<sup>ΔDGCA1</sup>* mice. Hippocampal NMDA receptors are therefore not required for associative learning and memory, despite hippocampal synaptic plasticity being proposed to be an important neural basis of learning. The results also show that mean lick cluster sizes, a measure of palatability, are generally reduced in the *Grin1<sup>ΔDGCA1</sup>* mice. It is however possible that this reduction relates to impaired expression of palatability, in the form of mean lick cluster sizes during consumption, rather than reductions in hedonic value. Altered NMDA receptor signalling has also been linked to schizophrenia, one of the negative symptoms of which is anhedonia. The *Grin1<sup>ΔDGCA1</sup>* mice may therefore provide an animal model for aspects of the altered glutamatergic signalling, and anhedonia, associated with disorders such as schizophrenia. Although it is not possible to rule out impaired expression of palatability in the form of mean lick cluster size from the present results. Finally, the results also demonstrate that deletion of hippocampal NMDA receptors enhanced reversal learning of matching behaviour. Therefore, although the role of hippocampal NMDA receptors does not seem to be specific to reversal learning, they may affect it under some situations. Taken together, the role of hippocampal NMDA receptors does not seem to be linked to the neural basis of associative learning, but deletion of them does seem to alter stimulus processing. The precise mechanisms that may relate to this altered processing are however not clear from the results in this thesis.

### 6.3 General conclusions

The *Gria1*<sup>-/-</sup> and *Grin1*<sup>ΔDGCA1</sup> mice provide a way to investigate different types of glutamate dysfunction in learning and memory. However, there were a few main findings that were evident in both transgenic mice. Firstly, impaired synaptic plasticity, either as a result of deletion of the GluA1 subunit, or hippocampal NMDA receptors, does not impair long-term memory based on associative retrieval. Glutamate dependent synaptic plasticity does therefore not seem to be required for associative learning.

Secondly, reversal learning of matching behaviour was enhanced in the knockout mice compared to the control mice. This suggests that glutamate may play a role in determining sensitivity to the reward contingencies in the present temporal context. It may, for example, reduce the degree of interference from previously learned associations, resulting in the enhanced the ability to learn about the relative rates of reinforcement in the present context.

Finally, it was also shown that the mean lick cluster sizes, a measure of palatability, were reduced in both the *Gria1*<sup>-/-</sup> and *Grin1*<sup>ΔDGCA1</sup> mice. A finding that was seen throughout the experiments, to a more or lesser extent, in this thesis using a microstructural analysis of licking behaviour, (chapter 2, experiments 1-4, 7-8; chapter 3, experiments 1 & 2; chapter 4, experiments 1-3). Although it is not clear if this reduction relates to an impairment in perceived palatability, or expression of palatability. However, if palatability is reduced, this would suggest that the *GluA1* and *Grin1*<sup>ΔDGCA1</sup> mice may provide animal models for the glutamatergic dysfunction and the negative of symptom of anhedonia, associated with schizophrenia. Furthermore, if the reduced mean lick cluster sizes in these mice are reflective of a reduction in palatability and hedonic value, then this would suggest that the more localised deletion of NMDA receptors in the dorsal DG and CA1 in the *Grin1*<sup>ΔDGCA1</sup> mice (as well as possibly also in the olfactory bulb) is necessary and sufficient for a reduction in palatability, as measured by mean lick cluster size. This means that theories of hippocampal functioning may need to consider a possible role for dorsal DG and CA1 subfields in particular in palatability and hedonic value.

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