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Kin recognition in the rat

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KIN RECOGNITION IN THE RAT

by

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I declare that the work described in this thesis is all my own and has not been submitted for any other degree. Part of the work described in chapter 3 has been published in Animal Behaviour, 31, 1177-1191, 1983.

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ABSTRACT

P.G. Hepper: "Kin Recognition in the Rat".

Inherent in Hamilton's (1964 a,b) proposal of the evolution of social behaviour by kin selection is the ability of individuals to recognise their kin. This thesis investigates kin recognition in the rat with respect to its ultimate and proximate causation.

Chapter 1 discusses kin selection theory to examine the possible ultimate causation of kin recognition, and this is investigated in the second half of this thesis. The proximate basis of kin recognition is also considered and a number of possible mechanisms for the acquisition of kin recognition proposed; these are examined in the first half of this thesis.

Chapter 2 investigates individual recognition in the rat by use of a 2 choice discrimination learning task. Results demonstrate that rats can recognise their conspecifics individually.

The next three chapters investigate sibling (Chp. 3), mother-offspring (Chp. 4) and offspring-mother (Chp.5) recognition in the rat and examine the role of socially and genetically mediated mechanisms in the acquisition of kin recognition. The results indicate rats can recognise these kin and evidence was found to support the existence of socially and genetically mediated mechanisms for the acquisition of sibling recognition.

Chapter 6 discusses the proximate causation of kin recognition using results obtained in chapters 2-5, and chapter 7 provides a brief review of 'nepotism' in the rat.

Chapters 8 and 9 investigate huddling and play partner preferences respectively in the rat and examine the effects of acquiring kin recognition through either a social or a genetic mechanism on these preferences. The results indicate that rats prefer their sibs as huddling and play partners and this is linked to the method of acquiring kin recognition.

Chapter 10 considers inbreeding avoidance in the rat. Evidence was found for 2 mechanisms of inbreeding avoidance: delayed reproduction between siblings and behavioural avoidance by sibs in the mating situation.

Chapter 11 summarises the previous chapters and examines the link between the ultimate and proximate causation of kin recognition.

CHAPTER 1

KIN RECOGNITION

1.1
INTRODUCTION

The present research was undertaken to investigate the ability of the laboratory rat, Rattus norvegicus, to recognise its kin. Reports of nepotism amongst kin in many species have become increasingly prevalent in the literature and are suggestive of an important role for kinship in the social behaviour of animals. In recent years the factor of kinship has been argued to be one of the major driving forces in the evolution of social behaviour (see Wilson, 1975). Inherent in these ideas of kinship and the accompanying ideas of nepotism is a mechanism enabling individuals to discriminate between kin and non-kin allowing them to respond appropriately: kin recognition. The aim of this research is to investigate the mechanism of kin recognition in the rat.

1.2
KIN RECOGNITION

Kin recognition is the ability of an individual to distinguish kin from non-kin, or between different classes of kin, and can be operationally defined as the differential responsiveness of an individual to another based upon its degree of kinship to that individual. Such a definition of kin recognition implies nothing about the

intentions/cognitions of the individual and nothing is intended. No conscious ability to calculate different degrees of kinship is implied in the individual's differential treatment of kin and non-kin, just that they behave as if they had calculated the degree of kinship.

Investigation of any biological trait, including behaviour, may take two different ,but not necessarily separate, paths. It may be considered with respect to its ultimate causation, or, with respect to its proximate causation (Baker, 1938). Ultimate causal analysis approaches the problem with a view to elucidating why such a behaviour should have evolved, that is, why that particular trait is adaptive to the individual. The ultimate causation of any trait has been determined by the environmental contingencies to which that species has responded, natural selection shaping the outcome, such a process having taken place over long periods of time (generations). Proximate causal analysis approaches the problem with a view to studying the immediate factors that operate within an individual to produce a particular behaviour. It analyses neurophysiological, developmental, endocrinological factors etc, to comprehend why such a behaviour was emitted. By necessity proximate causal factors operate within the life time of the individual concerned, and usually much more quickly.

Analysis of kin recognition with respect to its proximate causation asks questions concerning how kin are

recognised, for example, what enables kin to be distinguished from non-kin, what senses are used to identify kin, and how individuals acquire the ability to distinguish kin from non-kin. Analysis of kin recognition with a view to its ultimate causation asks questions about why kin are recognised and considers the evolutionary adaptiveness of kin recognition to the individual, to allow explanation of its existence through natural selection.

1.3
THE EVOLUTIONARY ADAPTIVENESS OF KIN RECOGNITION

For any trait to be adaptive it must enhance the individual's fitness, and it may then become a contender for preservation through natural selection. Kinship theory proposes that kin recognition may increase an individual's fitness by allowing differential responsiveness to kin and non-kin.

1.3.1
A Problem For Natural Selection

When Darwin (1859) proposed the theory of natural selection to explain the evolution of different species, he also applied this principle to behaviour. In doing so however he came across a stumbling block in the existence of sterile castes of insects, a problem Darwin thought "actually fatal" to his theory of natural selection (Darwin, 1859, p. 257). The principle of natural selection proposes that individuals will compete with each

other for resources, etc, and, because of the differences between individuals, some will be more successful than others and these will tend to reproduce. Through this differential reproduction species will change and evolve. The problem with sterile insects was that even if being sterile gave the individual some associated advantage in its individual survival, it was difficult to see how this advantage could be passed on to future generations.

Just as the evolution of sterile castes posed problems for Darwin, later day researchers, hoping to apply the principles of natural selection to the evolution of behaviour, ran into problems concerning social behaviour. The frequent observation of favouritism to kin posed some problems, but by far the greatest problem was presented by the occurence of altruistic acts. The problem with altruistic behaviour, defined as "self-destructive behaviour performed for the benefit of others" (Wilson, 1975, p. 578), was that in such behaviour one individual, the altruist, seemingly suffers a loss in fitness whilst increasing the fitness of another individual, the recipient. The problem is to see how such behaviour could evolve, since it apparently reduces the fitness of the altruist.

1.3.2
The Inheritance Of Behaviour

In explaining the evolution of behaviour, there is a tacit assumption that the behaviour in question is

heritable, and I shall briefly consider this here. What is passed from one generation to the next is not the individual, its behaviour, its bodytype (phenotype), but its genes. These are the units of inheritance and any structure/behaviour passed from one generation to the next is done so via the individual's genes (Burns, 1976). In considering altruistic behaviour, individuals do not pass on altruistic behaviour to the next generation, but pass on genes for this behaviour. These genes code for proteins which in turn influence the development of the nervous system, muscles, etc, and eventually produce the particular behaviour (Burns, 1976). Although the route from a particular gene to a particular piece of behaviour is not clearly understood (Kurland, 1977), it is assumed that there are genes which code for particular behaviours (e.g., see Dawkins, 1976), and there is evidence for the inheritance of certain behavioral traits in insects, rodents, domestic animals and humans (e.g., see Ehrman and Parsons, 1977; Fuller and Thompson, 1960).

When considering the evolution of behaviour, single genes are often talked about as coding for a behaviour, for example, a gene for altruism. Most models concerned with the evolution of behaviour postulate single genes for a particular behaviour (e.g., Hamilton, 1964a,b). In reality however, it is more likely that genes work in unison with other genes to produce a particular behaviour (King, 1980). Although individuals may have many genes for a particular behaviour, individuals differing on one

of the genes in a group may behave differently, for example, altruistic behaviour may be controlled by gene complex Y with genes at loci a,b,c,d,e,f. Two individuals may possess the same genes at all loci in complex Y except f, and the difference at this single locus may cause a difference in the altruistic behaviour of the two individuals. Thus one may talk about a single gene causing a difference in behaviour between two individuals (see also Dawkins, 1979, misunderstanding No. 4). In examining the relative merits of single locus modelling for behaviour, Oster et al. (1980) conclude "that although there is certainly some scope for more theoretical and experimental work on genes with interacting effects on fitness, it is reasonable to ask whether a behavioural trait is stable against deviant strategies by examining the conditions for spread of a single gene affecting the trait" (p. 170).

In conclusion, it will be assumed for convenience that behavioural traits can be inherited through genes, and differences in behaviour between two individuals may be due to differences at a single gene locus.

1.3.3
Solutions To The Problem Of The Evolution Of Altruism

To explain the evolution of sterile castes of insects Darwin proposed that selection could act not only at the level of the individual but also at the level of the family. From Darwin's original proposal two different

types of theory have been proposed to explain the evolution of eusociality in insects and altruistic behaviour: Group selection and Kin selection. Although some authors consider the two as part of the same continuum (e.g., Wilson, 1973), in this thesis they will be considered as separate (see Dawkins, 1979, misunderstanding No. 2).

1.3.3.1
    Group Selection

The idea of group selection was proposed in different forms by Haldane (1932), Wright (1945, also see Wright, 1969) _and_ Kalela (1954), but it remained for Wynne-Edwards (1962) to bring these different approaches together and present a comprehensive treatise on group selection. Group selection postulates that "differential extinction of groups can account for the direction of evolutionary change in a trait only when groups differ in this trait and when the difference accounts for the difference in extinction rates" (Alexander and Borgia, 1978, p. 450). The main problem with group selection is that selection would operate more strongly upon individuals within a group to eliminate altruistic acts than would selection between groups to promote such acts (Barash, 1977). For group selection to overcome this the extinction rate of groups must exceed that of individuals (Wilson, 1973). Models have been proposed to find parameters where group extinction would exceed individual extinction and allow group selection to operate, for example, Boorman and Levitt (1972,1973), Levins (1970). Such models however,

require parameters which are unlikely to occur naturally (Wilson, 1973), and lead to the conclusion that, under natural conditions group selection is unlikely to exert any effect (Rothstein, 1980). D.S. Wilson (1980) proposes a new model of group selection which does allow the spread of altruistic genes in a population using naturally occuring parameters; however the model may be viewed as one of individual selection rather than group selection (Krebs and Davies, 1982).

In conclusion, although group selection can provide an explanation of the evolution of altruism under certain conditions, the required conditions are unlikely to occur in nature, and a group selectionist explanation of the evolution of altruism is therefore unlikely.

1.3.3.2
    Kin Selection

This was first proposed by Hamilton in (1963) and later fully expanded (1964a,b) and developed (1970,1971,1972,1975). Kinship theory argues that behavioural interactions between relatives can influence the spread of genes controlling behaviour because relatives have greater similarity than unrelated individuals.

Hamilton (1964 a,b) proposed that an altruistic gene would be spread in a population whenever,

$$\frac{B}{C} > \frac{1}{r_{AR}},$$

where B is the benefit in fitness gained by the recipient (R) of the altruistic act, C is the cost incurred by the altruist (A) from his actions and r_{AR} is the coefficient of relatedness between the altruist and recipient. From this formulation it can be seen that natural selection will favour altruistic acts between relatives when the benefit in fitness gained by the recipient multiplied by the degree of relatedness between the recipient and altruist is greater than the cost to the altruist,

$$\text{i.e., } B.r_{AR} > C,$$

where B is the benefit in fitness gained by the recipient (R) of the altruistic act, C is the cost incurred by the altruist (A) from his actions and r_{AR} is the coefficient of relatedness between the altruist and the recipient. Likewise selfish behaviour would be expected when the benefit to the actor is greater than the cost to the recipient multiplied by the coefficient of relatedness,

$$\text{i.e, } B > C.r_{AR}$$

where B is the benefit in fitness gained by the actor, C is the cost of the act to the recipient and r_{AR} is the coefficient of relatedness between the actor and recipient.

This kinship theory, termed Kin Selection (Maynard-Smith, 1964), relies on two concepts, inclusive fitness and the coefficient of relatedness (r):-

Inclusive Fitness: In the traditional Darwinian approach, fitness refers to a particular individual and is measured ultimately in terms of the individual's reproductive success. Any behaviour may be considered in terms of its effect on the individual's fitness, its reproductive success. As previously discussed, an individual passes on its genes to the next generation and an individual's reproductive success, its fitness, can be determined by the number of its genes in the next generation. Individuals who are genetically related may possess a number of genes in common which are identical by descent (Hamilton, 1964a,b). Thus an individual's reproductive success is not only determined by the success of personal genes but also by the success of these genes present in relatives; this is termed an individual's inclusive fitness (Hamilton, 1964a,b). An individual may suffer a loss in its personal fitness by acting altruistically, but may show a gain in its inclusive fitness if the benefit to its genes in relations is greater than the cost to his personal genes. Natural selection selects behaviours that are adaptive and increase an individual's fitness. Hamilton's kinship theory shows how genes for social behaviour, including altruism, that increase the inclusive fitness of the individual will increase in frequency in the population.

Although of equal importance in kinship theory as the concept of coefficients of relatedness, many authors have tended to neglect the role of inclusive fitness in the evolution of social behaviour and concentrated upon coefficients of relatedness (West-Eberhard, 1975). This seems to be due to the difficulty in establishing a suitable measure of the costs and benefits of a particular behavioural act. Although fitness ultimately refers to the individual's reproductive success, the survival of its genes in the next generation, it may be difficult to interpret quantitatively how a particular behaviour affects this.

Coefficients of Relatedness: This is defined as the number of genes in common between two individuals which are identical by descent. In sexual reproduction, gametes are formed by cellular division, meiosis (Burns, 1976). In diploid species where each gene locus is represented twice, the probability of either of these genes being present in a particular gamete is 0.5. When gametes fuse in sexual reproduction to form a zygote, the zygote has received half of its genes from its father and half from its mother, thus a particular individual has 50% of its genes in common with its mother and likewise 50% in common with its father. The coefficient of relatedness (r) between parents and offspring is 0.5, which represents the probability that at a particular locus a parent and offspring will share a gene that is identical by descent. Now consider the coefficient between siblings: each

sibling receives half of its genes from each parent. The meiosis process is random (Burns, 1976), so each sibling stands a 50% chance of receiving the same gene from their father. Siblings receive 50% of their genes from their father, with a probability of 0.5 of receiving the same gene, thus the probability that two siblings will possess the same gene inherited from their father is 0.25. Similarily sibs will have a probability of 0.25 of receiving the same gene from their mother. Thus for full siblings the probability of having the same gene that is identical by descent at a particular locus is 0.25+0.25=0.5. By similar reasoning coefficients of relatedness of 0.25 for grandchildren and half-siblings, 0.125 for cousins and so on may be obtained.

Although a seemingly simple calculation, the above reasoning assumes no inbreeding, and to take inbreeding into account the formula must be altered (see Crow and Kimura, 1970; Wilson, 1975). As yet however, there has been no comprehensive formula to take into account all possible factors affecting inbreeding (Kurland, 1980).

There have been a number of different ideas on how to measure the coefficient of relatedness (see Kurland and Gaulin, 1979, for a brief review) although a number of these independently derived formula produce identical results (Michod and Hamilton, 1980).

In summary, kinship theory proposes that individuals will maximise their inclusive fitness, and the social behaviour between individuals would be expected to be shaped by the costs and benefits to the actors genotype and the degree of relatedness between the actor and recipient. Many authors have extended the basic principles laid down by Hamilton (1964a,b), for example, Abugov and Michod (1981), Breden and Wade (1981), Charlesworth (1978), Charlesworth and Charnov (1981), Charnov (1977), Craig (1982), Maynard-Smith (1980), Uyenoyama and Feldman (1982), Wade (1980), and there is a considerable amount of work both theoretical (see review by Michod, 1982) and empirical (see review by Kurland, 1980) supporting kin selection.

1.3.4

The Ultimate Causation Of Kin Recognition

The previous discussion has demonstrated that by responding differentially to kin and non-kin individuals may gain advantages in their inclusive fitness. Natural selection acts to maximise an individual's fitness, thus the ability to respond differentially to kin and non-kin would be favoured and kin recognition may be expected to evolve as it increases the fitness of individuals possessing this ability. Behavioural indications of differential responsiveness to kin and non-kin are well documented (see Kurland, 1980), and much nepotistic behaviour exhibited by individuals may be accounted for by kin selection theory.

If kin recognition has arisen through individuals gaining advantages over those individuals unable to recognise kin, one would predict that individuals would exhibit signs of differential responsiveness to kin and non-kin. The second half of this thesis (Vol. 2) investigates this problem and observes the behaviour of the rat, to see if it exhibits signs of differential responsiveness to kin and non-kin, and considers the possible advantages of such behaviour.

1.4
THE PROXIMATE BASIS OF
KIN RECOGNITION

Research attempting to uncover the proximate basis of kin recognition has concentrated on three main questions (Holmes and Sherman, 1983). What is the perceptual sense used in kin recognition?, what is the kin identifier and how is it formed?, and finally how do the individuals acquire the ability to recognise kin?.

1.4.1
The Perceptual Sense Of Kin Recognition

No specific answer to the perceptual sense used to identify kin can be given for all animal species save for the generalisation that the sense used will be that best suited for the ecology of that particular species. One important consideration is that the sense must be able to carry the required information with enough variability to be used by all members of the species. It would, for

example, be no use attempting to designate kinship by the colour of a certain patch if only two colours, black and white, are available, as this stimulus on its own is not variable enough to carry the information required. The ecology of the species is also important in determining what sense would be used, for example, it would be of little use for nocturnal species to rely on vision.

In this thesis all experiments were performed using the rat, and it is probable that the olfactory sense is used to carry information concerning kinship. There is much evidence to suggest that olfaction is the most important sense in rodents (e.g., Bronson, 1971; Brown, 1979; Cheal, 1975). Studies using other rodents have demonstrated the importance of olfactory cues in kin recognition (spiny mouse, Acomys cahirinus, Porter et al., 1979; mouse, Mus musculus, Gilder and Slater, 1978, Kareem and Barnard, 1982; white-footed mouse, Peromyscus leucopus, Grau, 1982). In rats olfactory cues carry information concerning sex (Brown, 1977), status (Krames et al., 1969), and individuality (Carr et al., 1976), and it is likely that information concerning kinship will also be transmitted via the olfactory sense. The rat spends 4% of its daily activity in active sniffing (Bolles and Woods, 1964). Whilst experimental studies have demonstrated the importance of olfaction for the rat, consideration of its habitat suggests why this should be so. Rats, although diurnal, are most active during the night (Barnett, 1975) and visual cues would be of little

value when dark. Rats inhabit burrows (Barnett, 1975;
Lore and Flanelly, 1977) again visual cues would be of
little use in this environment as carriers of information.
Audition could be used, but its effectiveness in burrows
may be reduced. Rats do use auditory signals during
mating (Barfield and Geyer, 1975; McIntosh and Barfield,
1980), and in aggressive encounters (Corrigan and
Flannelly, 1979; Sales, 1972). However there seems to be
little consistency in the calls produced by individuals
(pers. obs.) and they are an unlikely source of kinship
information. In the rat, the primary source of
information concerning kinship is likely to be olfactory,
and the rest of this discussion takes into
consideration this point.

1.4.2
The Kin Identifier

The kin identifier (synonymous with kin recogniser)
is defined as the perceptual signature of the individual
that allows it to be designated as kin, a particular class
of kin, or non-kin. I have previously suggested that the
identifier will be olfactory, here I consider the nature
of this cue. I would like to argue that for a cue to
serve as kin recogniser it must possess two qualities:
Commonality, such that all members of a kin group must
possess it or be delimited by it; and Consistency, in
that it must remain stable over time.

1.4.2.1
    Consistency

For a cue to be used by other individuals as a source of kinship it must remain stable over time. If the cue changes, then the ability of an individual to use it successfully as a source of kinship information decreases.

Odours are a result of both environmental and genetic factors (Stoddart, 1980). The need for consistency in the cue may reduce the role of environmental factors in the production of the kin identifier. Dietary factors are the most important environmental factor affecting odour production (Stoddart, 1980), exerting an effect on the quality of odour (e.g., Leon, 1975). Reliance upon dietary factors may decrease the consistency of the kin identifier. Rats are omnivores (Barnett, 1975) and different members of the same kin group may eat different foods. There is evidence that very small differences in dietary intake will affect odour quality (Galef, 1981) and by eating different foods members of the same kin group may produce different odours. Also seasonal variation in the availability of diet may cause increased variability in the odour produced. Both variability of diet and seasonal variation may cause the cue to vary such that it cannot be used for kin recognition. A further problem with environmental influence upon odour quality through dietary intake is that different kin groups may eat the same food and come to possess a similar odour (kin identifier).

The consistency of the cue may be better maintained by genetic factors which are unlikely to be altered and are unaffected by seasonal variation or dietary intake. Also the problem of different kin groups possessing the same identifier is overcome. Related individuals as they possess genes in common will produce a similar odour, and because non-kin do not have genes in common, the odour of non-kin will be different. There are a number of studies which argue for a major role for genetic factors in the production of the kin identifier, for example, Blaustein and O'Hara (1981), Getz and Smith (1983), Holmes and Sherman (1983), Waldman (1982).

In summary, in order to maintain the consistency required for the kin identifier to be of use it is likely that environmental factors which introduce variability into the production of odours will play a minor role compared to genetic factors in the production the kin identifier.

1.4.2.2 Commonality

For a cue to be used by individuals to delimit kin it must be exclusive to kin and possessed by all. As previously discussed the genetic factors of odour production in the kin identifier may be more important than environmental ones and this would allow for a commonality of identifier amongst kin.

As kin are genetically related they would possess genes of the identifier in common and would produce a similar odour, the more closely related the more similar the odour/identifier. Evidence for a genetic basis for odour is provided by Kalmus (1955) who found that dogs could easily discriminate between unrelated individuals, less easily between relatives and found it very difficult to discriminate between twins, the increasing difficulty of discrimination corresponding to increasing degree of relatedness (see also Greenberg, 1979; Stoddart, 1980).

Other empirical studies also argue for a genetically determined commonally possessed cue. In studies where individuals are separated from their kin, and then given a preference test for unfamiliar kin and unfamiliar non-kin, no preference would be expected if the kin did not have a common identifier. Studies using such a procedure with a variety of animals, for example, Cascades frog, Rana cascadae, Blaustein and O'Hara (1982a); American toads, Bufo americanus, Waldman (1982); sweat bees, Lassioglossum zephyrum, Buckle and Greenberg (1981); mice, Mus musculus, Kareem and Barnard (1982); white-footed mice, Peromyscus leucopus, Grau (1982); pigtail macaques, Macaca nemestrina, Wu et al. (1980), demonstrate that individuals can recognise their unfamiliar kin, indicating the existence of a common identifier.

It may not be necessary to have a commonly possessed

cue: individuals could discriminate kin on the basis of individual cues. Individuals possess an individual identifier (see Halpin, 1980; also lit. rev. Chp. 2, section 2.4), and kin recognition may be accomplished by using this cue combined with an indicator of kinship. Bekoff (1981) proposes individuals could use familiarity, the more familiar an individual is the more closely related that individual is likely to be. To operate such a mechanism requires a good correlation between kinship and familiarity.

Although possible, use of individual cues has disadvantages compared to use of common genetic cues. With use of a genetic cue only one cue has to be used as this is possessed by all kin, however using individual identifiers requires the using of many cues. Individual cues are required to be paired with an indicator of kinship, for example, familiarity, for each individual, the degree of kinship based upon the degree of familiarity. With a common genetic cue, individuals are compared to a single identifier, the degree of matching determining the degree of relatedness (phenotype matching, see Holmes and Sherman, 1983; Lacy and Sherman, 1983). The use of individual cues as delimiters of kinship may be restricted to close kin, for example, siblings, parents-offspring, where numbers may be small. Telle (1966) reports that rats are able to recognise up to twenty rats individually, after this recognition breaks down.

In summary, commonality of the cue may best be achieved by possessing a genetically based kin identifier which kin will share. However close kin may be recognised by individual cues coupled with a common factor of familiarity.

In the preceeding discussion I have attempted to argue that for a kin identifier to be of value it must be both consistent and common, which may be best achieved by a major role for genetic factors in production of the identifier and a minimal role for environmental factors which could introduce variability in the identifier making recognition of kin difficult. In the case of the rat which lives in large groups made up of smaller family units (Telle, 1966), the large group feed and occupy the same home range and so the group is likely to possess the same environmentally determined odour. This may allow for the differentiation of individuals belonging to different groups (Barnett, 1975) but may not provide enough consistent information to allow discrimination between kin groups, and genetically determined cues may be required for this.

A final consideration concerning the identifier is what happens when it is produced. Crozier and Dix (1979) propose two models, see also Holldobler and Michener (1980) who expanded their proposals to four models. An individualistic model in which individual identifiers remain separate, and a 'Gestalt' model in which the

identifiers from different individuals become mixed and produce a single identifier. With odourous stimuli it is likely that they will mix to form one 'Gestalt' odour, however this will be greatly affected by the number of individuals present. Rats in a litter would perceive an odour contributed to by all littermates. However a rat meeting another individually will perceive an odour contributed to mainly by that particular individual.

1.4.3
The Acquisition Of Kin Recognition

The final question is concerned with how individuals come to respond differentially to kin and non-kin, i.e., recognise their kin. Different authors have proposed different mechanisms (see review Holmes and Sherman, 1983), to account for kin recognition. It is important to remember that whatever the mechanism, the outcome must be to ensure differential responsiveness to kin and non-kin. Here I define two broad classes of mechanisms by which individuals may acquire the ability to recognise kin.

1.4.3.1
Recognition not based upon conspecific cues

In this catagory individuals do not respond on the basis of cues emitted by conspecifics but to other cues. Individuals may show no differential responsiveness to other individuals but act altruistically to all individuals encountered. Such a mechanism would give the appearance of kin recognition if the only individuals met

tended to be kin. As a more advanced stage of this mechanism, individuals may respond to each other on the basis of environmental cues. Individuals who inhabit a home range may respond to individuals encountered within this as kin, but those encountered outside as non-kin. To operate successfully this mechanism requires there to be a high probability of only finding kin in the home range. Such a mechanism of kin recognition has been proposed for certain social insects (see Holldobler and Michener, 1980).

The major problem with this mechanism is that it is open to infiltration by non-kin, and any individual met may be responded to as kin, even though it may not be related. Although such a mechanism is unlikely to function throughout an individual's life, it may function at certain stages. Soon after birth, in altricial species, where young remain in their litter with siblings for a period of development such a mechanism may operate as their is little chance of infiltration by non-kin.

1.4.3.2
Recognition based upon conspecific cues

Two catagories are considered in this section, socially mediated kin recognition and genetically mediated kin recognition. I use these terms not in reference to the mechanisms involved in the acquisition of kin recognition but with reference to its outcome. Socially mediated kin recognition refers to the fact that

individuals are born with a _tabula rasa_ concerning kin, and have to acquire knowledge about kin from their social partners; I include in this the special instance of acquiring knowledge about kin from self. The important fact concerning this catagory is that acquisition is non-selective in that any social partners will be learnt about, and thus to function as a mechanism of kin recognition requires that the individual's social partners are in fact kin. Genetically mediated kin recognition implies that what knowledge the individual acquires about kin is only about kin, and that there is no chance of acquiring the characteristics of non-kin.

Socially Mediated Mechanisms Of Kin Recognition

Here individuals are born with no knowledge of kin and have to acquire this during development. Individuals will learn about the kin identifier from their social partners or possibly from themselves (Dawkins, "armpit effect", 1976,1982). Two different mechanisms for kin recognition may be formulated dependent . upon what the individual acquires.

1) Kin Recognition Based Upon A Common Genetic Identifier: Termed "phenotype matching" by Holmes and Sherman (1982,1983). Individuals possess a common genetically determined kin identifier and individuals learn about this, either from their social partners, or from themselves, to form a template. When encountering

other individuals they are compared to the template, the degree of matching determining the degree of relatedness, and responded to appropriately.

2) Kin Recognition Based Upon Individual Identifiers: Here individuals learn about the individual identifier of their social partners and associate these with some other factor which enables kinship to be assessed. Bekoff (1981) proposes that the more familiar an individual becomes the more closely related that individual is assumed to be.

These mechanisms are non-selective: the individual has no control over who it acquires kinship information from, it simply uses the closest social partners. Thus for kin recognition to be acquired requires good correlation between physical proximity of social partners and kinship. As with recognition based upon non-conspecific cues, individuals acquiring kin recognition through socially mediated mechanisms face the possibility of acquiring the characteristics of non-kin.

Although there have been studies demonstrating the existence of kin recognition (e.g., Holmes and Sherman (1983), see also literature review chapters 3, 4, 5, sections 3.3,4.3,5.2 respectively) demonstration of the existence of kin recognition through social relatedness requires the rearing of unrelated individuals. Unrelated individuals reared together, if they acquire kin

recognition through social experience, should respond to their social partners as kin. Although there have been few studies using this procedure, the ability to acquire kin recognition via socially mediated mechanisms has been demonstrated in spiny mice, Acomys cahirinus, Porter et al. (1981); mice, Mus musculus, Kareem and Barnard (1982); white footed mice, Peromyscus leucopus, Grau (1982); Arctic ground squirrels, Spermophilus parryii, and Belding's ground squirrels, Spermophilus beldingi, Holmes and Sherman (1982).

Genetically Mediated Mechanisms Of Kin Recognition

In contrast to those previously described, this category assures that what is achieved is the ability to recognise only kin, that is, genetically related individuals and there is no possibility of infiltration by non-kin. There are two possible mechanisms to achieve this end.

1) Recognition Genes: This hypothesis, first considered by Hamilton (1964b), proposes that genes can lead to recognition of themselves in other individuals, and has been dubbed the "Green Beard" effect (Dawkins, 1976,1982). To accomplish this, genes are proposed with pleiotrophic effects, first to produce a conspicuous label, the green beard, and second to respond differentially to this label. Although such a gene would be favoured by natural selection and easily spread (Metcalf, 1980), two factors

have cast doubt on its existence and many authors now consider this idea unlikely (Blaustein, 1983).

The existence of recognition genes has been questioned because of their possible "outlaw" status (Alexander and Borgia, 1978). It has been argued that such genes would tend to favour themselves at the expense of all other alleles in the genome (Alexander and Borgia, 1978; Dawkins, 1982). Recent work however has cast doubt on this (Ridley and Grafen, 1981), and their status as outlaws is now in question.

The second criticism concerning the improbability of recognition genes has centered upon their proposed complexity (Blaustein, 1983), and has been widely accepted, for example, Holmes and Sherman (1982,1983), but see Rothstein (1980). Doubts concerning recognition genes due to their complexity have arisen from Hamilton's original proposal (1964b). He proposed these "supergenes" would "affect a) some perceptible feature of the organism, b) the perception of that feature and c) a social response consequent upon what was perceived." (p. 25). Although some authors have tended to combine stages b) and c), for example, Dawkins (1982), the possession of genes with these pleiotrophic effects is regarded by many as too complex, for example, Alexander and Borgia (1978). This may be argued against on two grounds. First, because a gene is proposed to have a complex function this does not mean that it cannot evolve. The Major Histocompatibility

Complex, responsible for the immune response and self-cellular recognition (Dausset, 1981a,b), is a gene complex with three very similar functions to those proposed for recognition genes; production of an identifying cue, recognition of this cue, and an appropriate response. Clearly complex genes may evolve (see also Dawkins, 1979, misunderstanding number 4, where he argues against those who postulate a gene for altruistic behaviour to be too complex to exist).

Second, recognition genes may not be as complex as first suggested. Restating Hamilton's (1964 a,b) formulation for recognition genes, three effects are required, 1) to produce label X denoting kinship, 2) to perceive label X and classify X as a kin identifier, and 3) to respond appropriately to kin. I would argue that recognition genes need only be able to perform stages 1) and 2), to produce and classify label X as kin, the final stage, responding appropriately need not be associated with the same gene. The ability of an individual to respond differentially to kin and non-kin will have evolved by natural selection if such differential responding will benefit the individual. Kinship theory demonstrates how such behaviour (altruism/nepotism) can increase an individual's fitness and will therefore evolve, the gene for this behaviour being spread. Different behaviours require different responses from kin, so, it is likely that each behaviour will evolve with its own modifier for response to kin. Play behaviour is

beneficial to the participants (Fagen, 1981), and individuals who play with kin may gain advantages in their fitness over those who do not and thus play behaviour may evolve with a preference for kin. Mating, however, is disadvantageous with kin and leads to a decrease in fitness (Falconer, 1960), therefore mating may evolve with a preference for non-kin. The point is that each behaviour will evolve with its own modifier for its response to kin, and thus the differential responsiveness to kin will be present in the genotype of the organism. What is required for this to work is a definition of kin.

In instances where identification of kin is acquired socially the same assumption is made that individuals will respond differentially to kin, and what is required is the learning of kinship. Likewise for genetically determined kin recognition what is required is not a gene to recognise and respond to kin but a gene to recognise kin, which will denote what kin are and which can be used by the gene for differential behaviour to kin and non-kin, already selected for by natural selection. Thus when talking about recognition genes what is required is the production of the kin identifier X and the recognition of X as kin, the evolution of a genetic perceptual ability, rather than a more complex system involving both a perceptual ability and a behavioural response on the same gene.

There already exists evidence for genetically

determined perception of certain odours in the rat. Prior exposure to maternal pheromone is unnecessary for rat pups to respond preferentially to it (Schumacher and Moltz, 1982). Likewise response to stress/frustration odour (Mackay-Sim and Laing, 1980; Valenta and Rigby, 1968) requires no experience of this odour. There is also evidence suggestive of an innate ability to recognise predators (Cattarelli, 1982a,b).

2) Intra-Uterine Learning: A second possible mechanism for ensuring recognition of kin is intra-uterine learning. In mammals, individuals are bathed in the uterus by amniotic fluid and individuals may learn from this. As this fluid is produced by the mother, individuals acquiring information (olfactory or gustatory) from this will acquire a genetically similar identifier to that of their kin, and intra-uterine learning may allow for the learning of only the genetically determined identifier. Unlike previous mechanisms of learning (socially mediated kin recognition), invasion of the uterine environment by non-kin is impossible in nature (not with present day surgical techniques), and so individuals are assured of only learning about kin. Studies have demonstrated the ability of rats to learn whilst in utero (Smotherman, 1982a,b; Stickrod et al., 1982) making possible kin recognition by this mechanism.

None of the above mechanisms are mutually exclusive and the role each plays in the acquisition of kin

recognition will be determined by the ecology of the species in question. Individuals who are strictly territorial and defend their home range from all unrelated conspecifics, could rely successfully on recognition based on non-conspecific cues. In most species however total exclusion from non-kin is unlikely and kin recognition must be based upon some mechanism using conspecific cues. Altricial species, in which there is a long period of care after birth, may best acquire kin recognition through social mechanisms, whereas precocial species which may not spend much time together before dispersal may best use genetically mediated kin recognition.

1.4.4
The Proximate Causation Of Kin Recognition

At the beginning of this section three questions were asked pertaining to the proximate causation of kin recognition. I have considered the perceptual sense used in kin recognition and conclude that in the rat this will be primarily olfactory. The nature of the kin identifier was then considered and it was concluded that to be of use it must possess two important factors, commonality and consistency, both best accomplished by genetic factors. Finally the acquisition of kin recognition was considered and two broad classes of mechanisms were considered. It is the aim of the first part of this thesis to investigate more fully the proximate basis of kin recognition and examine the role played in the acquisition of kin recognition by mechanisms using conspecific cues. The

role of both socially and genetically mediated kin
recognition being examined.

1.5
RESEARCH GOALS

The aim of this research is to investigate kin
recognition in the rat with respect to both its proximate
and ultimate causation, and accordingly this thesis is
divided into two sections.

The first section (Vol. 1) is concerned with the
proximate basis of kin recognition, and attempts to
elucidate the mechanisms by which kin are recognised. For
this purpose two assumptions have been made. The first is
that the rat will use olfactory cues to recognise kin, and
the apparatus and experiments were designed to promote the
use of olfactory cues. The second is that the kin
identifier, if it exists, will be mainly genetically
determined. The role of environmental factors in the
odour production were kept to minimum by feeding all rats
the same diet.

Rats were tested for their ability to recognise three
types of kin. The first, natural kin, were genetically
related and were reared together, receiving the same
amount of social experience with each other as expected in
their natural environment. This condition was used to see
if rats could recognise their kin. Second, socially
related kin were genetically unrelated to each other, but

were reared together as kin, receiving identical social experience as natural kin. These kin were used to examine the possibility of acquiring kin recognition through socially mediated mechanisms. The final type of kin used were genetic kin. These individuals were genetically related but received no social experience of one another after birth. These were used to examine the possibility of acquiring kin recognition via a genetically mediated mechanism.

The ecology of the rat makes the existence of both mechanisms of kin recognition (social and genetic) possible. Rats live in large groups made up of smaller family units (Telle, 1966). The family unit may provide a good setting for the social acquisition of kin recognition, whereas the close proximity of other unrelated individuals of the group may favour the genetic acquisition of kin recognition. Chapter 2 uses a discrimination learning task, to examine whether it is possible for rats to learn about individuality and make possible the acquisition of kin recognition by this mechanism. Chapter 3 considers the ability of individuals to recognise their siblings and investigates whether siblings can be recognised and the role of social and genetic mechanisms in this process. Chapter 4 examines the ability of the mother to recognise her offspring, investigating whether individuals can do this and the role played by both social and genetic mechanisms. In chapter 5 the roles are reversed and the ability of offspring to

recognise their mother is investigated, *and* again the role of social and genetic mechanisms examined. Chapter 6 summarises the results of the previous chapters and attempts to explain the mechanisms of kin recognition in the rat from the results of the preceeding experiments.

The second section of this thesis (Vol. 2) investigates the ultimate basis of kin recognition, and considers the possible adaptiveness of this behaviour. From theories of kin selection, it was hypothesised that individuals should act differentially to kin and non-kin in order to maximise their inclusive fitness. In Chapters 8 and 9 the huddling and play behaviour of rats was observed to examine whether individuals prefer to engage in these activities with kin rather than non-kin. Chapter 10 investigates the mating behaviour of rats to observe their preferences for either kin or non-kin. Chapter 11 provides an overview of the preceeding chapters and considers kin recognition in the rat.

1.6

THE SUBJECT

The subjects for this research were laboratory rats. This animal has been subjected to a multitude of laboratory experiments and observation, consequently much is known about its behaviour (Barnett, 1975). I shall make no attempt to review the literature concerning the rat here but will discuss the literature where it is relevant.

The particular subject used in this study was the outbred Sprague-Dawley rat. An important consideration in the evaluation of the degree of relatedness between individuals is the coefficient of inbreeding (f), defined as "the probability that both alleles at one locus in a given individual are identical by virtue of identical descent" (Wilson, 1975, p. 73). In our discussion of kinship it was assumed that the inbreeding coefficient equalled zero, and this produced coefficients of relatedness between parents and offspring of 0.5, and between siblings of 0.5. Although the rats used in this study were outbred, they had an inbreeding coefficient of 4% (personal communication from J.Bantin of B & K (Animal Suppliers) Ltd). Taking this into account, the coefficient of relatedness between parents and offspring, and between siblings now becomes 0.52 and the degree of relatedness between unrelated individuals 0.04. The effect of this is to reduce the difference in relatedness between parents-offspring, siblings and unrelated individuals by 0.02 from 0.5 to 0.48. This is unlikely to exert any effect on the differential responsiveness of individuals to kin (parents-offspring / siblings) and non-kin, and for the rest of this discussion, for literary ease, I shall refer to the coefficients of relatedness of parents-offspring and siblings as r=0.5, and for unrelated individuals as r=0.0.

CHAPTER 2

INDIVIDUAL RECOGNITION

IN THE RAT

2.1

INTRODUCTION

The ability of individuals to perceive differences between members of the same species has been widely reported not only in mammals, but also in fishes, crustacea, and insects, for example, see Halpin (1980). In terms of social behaviour and social organisation the ability to recognise individuals may be important in group cohesion, sexual behaviour, parental behaviour and agonistic encounters. Individual recognition may also play a role in kin selection, providing a mechanism whereby individuals can discriminate kin from non-kin. The aim of this chapter is to examine the ability of rats to discriminate and recognise conspecifics using a discrimination learning task. I shall deal exclusively with individual recognition and not mother-infant recognition or kin recognition. The ability of an individual to discriminate between and recognise individuals must be based on the premise that each individual has a different set of characteristics which separate it from all other individuals. In the case of kin recognition this may not be the case. The ability to discriminate and recognise kin may be based on genetically

determined cues possessed by all kin which allow the discrimination of kin from non-kin but do not necessarily lead to individual recognition. Individuals may be able to discriminate kin from non-kin but not between individuals who are both kin.

2.2 INDIVIDUAL RECOGNITION

Individual recognition is defined as the ability to perceive an individual as distinct from other individuals and as having been previously seen or known. Many authors have tended to use individual recognition and individual discrimination interchangeably (e.g., see Halpin, 1980) and this may lead to a certain amount of confusion. Individual discrimination is defined as the ability of an individual to distinguish between two individuals, it differs from individual recognition in that no reference is made to the individual as having been previously seen or known.

This distinction is important when considering individual recognition as a possible mechanism used in kin recognition. To be of use this mechanism must be individual recognition. When two individuals are encountered, it is of little use only being able to discriminate between them, one must also be able to perceive one as previously known and respond to it on the basis of this previous knowledge. Accordingly kin recognition may only be achieved through individual

recognition and not individual discrimination.

To establish recognition operationally it is important to observe an individual over time, to see whether it responds to an individual in the same way. Confusion has arisen because of the use of discrimination tasks in the investigation of individual recognition. Here individuals are trained to see if they can distinguish between two stimuli, by giving a particular response to a particular stimulus. Although termed a discrimination task, it is really a recognition task, as the individual is required to respond on the basis of previous knowledge.

In this thesis I shall use 'individual recognition' as defined above, and use 'individual identification' as the ability of an individual to distinguish between two conspecifics, and shall use discrimination with reference to learning tasks or in accordance with previous authors usage when discussing their work.

2.3

THE ADAPTIVENESS OF INDIVIDUAL RECOGNITION

In the introductory chapter I discussed how one model, that of kin selection, explains the phenomenon of altruistic behaviour by proposing differential responsiveness between kin and non-kin. Implicated in this was the ability of individuals to recognise kin. One means of achieving this is by use of individual

recognition (see chp. 1, section 1.4.3.2), functioning on the principle that the more familiar an individual is, then the more closely related that individual is assumed to be (Bekoff, 1981). An individual may therefore use the ability to recognise individuals combined with an index of familiarity to recognise its kin. This is not to say that the individual does come to recognise its kin like this, but that if an animal can recognise individuals then it can use this to recognise its kin.

Individual recognition may also play a role in reciprocal altruism. Reciprocal altruism involves the giving of aid to non relatives such that the costs and benefits are dispersed over time (e.g., see Barash, 1977; Wilson, 1975). There are two possible mechanisms by which potential reciprocal altruists may be recognised and such behaviour may evolve. The original formulation by Trivers (1971) argues for individual recognition to be important. Individuals recognise those dispensing reciprocal altruism as individuals and respond altruistically only to these. Later arguments (e.g., see Connor and Norris, 1982) postulate a genetic recognition system, such that the ability to respond altruistically is combined with a recognisable feature indicating the tendency to reciprocate.

Thus the ability of an individual to recognise other individuals, as well as most probably allowing less intense social interactions may provide a mechanism for

allowing individuals to recognise kin, and allow individuals to perform altruistic behaviour to those whom would be most likely to reciprocate. It is for these reasons that the ability of rats to recognise individuals is being investigated.

2.4

INDIVIDUAL RECOGNITION:

LITERATURE REVIEW AND COMMENTARY

There have been a large number of studies claiming individual recognition in a wide variety of species. I shall review the data in taxonomic catagories commencing with insects and finishing with man. Those studies performed on the rat have been omitted from this general review and will be considered separately later. As different experiments are reviewed, methodological problems and flaws will be discussed. Before commencing this review I shall consider briefly the history of individual recognition studies.

One of the first comprehensive works to consider the ability of animals to recognise individuals was "Mental Evolution In Animals" by G.J.Romanes (1883a). In this he proposed a hierachy of the products of intellectual development. On level 23 of this hierachy one finds "recognition of persons", which the taxonomic groups of Fish, Higher Crustacea, Reptiles, Cephlapods, Hymenoptera, Birds and Mammals are said to possess. Here is possibly the first written suggestion of the ability of animals to

recognise individuals. Romanes was plagued by the pre-Darwinian view of a dichotomy between man and animal, and when one studies "Animal Intelligence" (Romanes, 1883b) one finds a number of examples of animals possessing the capability of recognising persons (humans), individually, but no mention of the ability to recognise conspecifics individually. For example, bees have the power to distinguish between persons, and according to a Mr Bingley "the bees not only learn to distinguish persons but lend themselves to tuition by those whom they know" (pp. 188-189). Spiders are not only capable of "sexual passions" (p. 204), but able to distinguish between people and approach those whom are found to be friendly, whilst shunning strangers. Tortoises and snakes are reported to distinguish people, tortoises coming to the calls of favoured people, snakes remember people after an absence of six weeks. Birds are capable of discriminating humans, and elephants are reported to have good recognition for their drivers and remember them well enough to revenge themselves for injuries done to them by particular humans.

Although it is possible to criticise these studies as being anecdotal, which they undoubtedly were, they do represent the first consideration of an animal's ability to recognise individuals from an evolutionary point of view. At the beginning of the 20th century there were a number of pointers that animals other than humans were capable of recognising conspecifics. However, the field remained dormant with only a few and far between studies

on individual recognition until the late nineteen fifties when studies on individual recognition started to appear regularily. These studies will now be described.

2.4.1
Invertebrates

The ability of invertebrates to distinguish among conspecifics has been demonstrated in few species. Studies indicating the ability of males to habituate to a female and then show increased activity to a novel female have been carried out using the halicitid bee, Augochlora pura, and the sweat bee Lasioglossum zephyrum, (Barrows, 1975; Barrows et al., 1975). These studies indicate the ability of males to perceive individual differences amongst females, but according to our definition they cannot be claimed to demonstrate individual recognition.

Other studies have used monogamous pairs to demonstrate individual recognition. Two species of crustacea, the desert woodlouse, Hemilepistus reamuri, (Linsenmair and Linsenmair, 1971), and the banded shrimp, Stenopus hispidus OLIVIER, (Johnson, 1977), have been shown to discriminate their mates from all other conspecifics; in both cases the recognition is based upon chemical cues.

Evidence suggestive of an ability to recognise individuals comes from studies on dominance hierarchies in bees and wasps. Brian (1980) says of societies of bees

and wasps that "these associations are small enough for all females to learn each others individuality...." (p. 380). Similarily, females of the social wasp, Polistes, when forming colonies organise themselves as a dominance hierarchy which appears to be based on individual recognition (Eberhard, 1969).

Behavioural observations indicate that both the clown shrimp, Hymenocera picta, (Wickler and Seibt, 1970) and the hermit crab, Pagurus bernhardus, (Hazlett, 1969) are capable of individual recognition. However in the case of the hermit crab, individuals discriminated a stranger from other members of a familiar group and it is not clear whether this involves individual recognition or recognition of the group as a whole and definite individual recognition cannot be claimed.

Caldwell (1979) reports that the mantis shrimp, Gonodactylus festai, is capable of recognising animals which it had previously fought and defeated. Caldwell (reported in Halpin, 1980) presents evidence indicating these shrimps can distinguish between two different individuals both of which they had encountered previously. This ability based upon chemical cues.

A more generalised form of individual recognition is that of self-recognition and this has been reported in the colonial anemone, Anthopleura elegantissima BRANDT, (Ertman and Davenport, 1981), where nematocyst discharge

is inhibited when the tentacle of a clonemate touches but not when one of a non-clonemate touches.

2.4.2

Fish

Behavioural observations of certain fish have led authors to postulate the existence of individual recognition in these animals. Baerends and Baerends-van Roon (1950) in their study of a number of cichlid fish report that "personal acquaintance of partners prevents severe fighting" (p. 135). The Jewel fish, Hemichromis bimaculatus GILL, is capable of discriminating its mate, and the visual characteristics of the head are important in this discrimination (Noble and Curtis, 1939). It is not clear from these studies whether true individual recognition is being demonstrated or just the ability to discriminate familiar fish (the mate) from unfamiliar, strange fish.

More positive proof of individual recognition can be obtained from studies using operant techniques. In these studies fish are trained to swim to the surface for food when presented with one individual but to escape to a safe retreat to prevent punishment when presented with another individual. Using this method minnows, Phoxinus, (Goz, 1941); yellow bullheads, Ictalurus natalis, (Todd et al., 1967) and brown bullheads, Ictalurus nebulosus, (Richards, 1974) have been shown to be capable of individual recognition.

A further possible case of individual recognition may be found in the electric fish, <u>Gymnotus</u> <u>carapo</u>. Westby (1974) report differences in the electric organ discharge of this fish which represents the social status of that fish. Further investigation may reveal individual differences in this discharge which could be used for individual recognition.

2.4.3
Amphibians

Two studies of amphibians, both using the red-backed salamander, <u>Plethodon</u> <u>jordani</u>, may indicate individual recognition in this species. Madison (1975) demonstrated that the red-backed salamander can discriminate between conspecific neighbours and non-neighbours. This could indicate an ability to discriminate between individuals or just between two classes of conspecifics, familiar/neighbours and unfamiliar/non-neighbours. For conclusive proof of individual recognition the ability to discriminate between two neighbouring or two non-neighbouring individuals should be shown. Jaeger and Gergits (1979) using the same species found that they showed a preference for their own chemical markings as opposed to those of conspecific males. Again this could indicate the ability to discriminate individuals or just the ability to discriminate self from all others.

2.4.4

Reptiles

There have been very few studies investigating the ability of reptiles to recognise individuals. Although Noble and Clausen (1936) report that the cloacal gland of snakes may produce individually different odours, there has been little research to follow this up. I found only one study, using the water snake, Nerodia sipedon sipedon, which indicated these snakes can discriminate the odours of conspecifics using lingual air sampling (Scudder et al., 1980).

2.4.5

Birds

The ability of birds to produce individual songs has long been recognised by keen ornithologists who often report being able to distinguish the song of particular birds. Thorpe and North (1965) add their support to this idea, saying that "in many species the song is capable of sufficient individual variation within the overall specific fixity of pattern to differentiate one individual bird from another" (p. 219). Such field observations have no doubt stimulated the large body of research which has been carried out in the field of individual recognition in birds.

Most studies have concentrated on the acoustic signals of birds in attempts to show that these animals possess individual recognition capabilities. Two broad

experimental techniques have been used: Playback experiments in which songs of different individuals are recorded then played back to another individual and its response noted and sonographic analysis of bird song to show consistent individual differences exist between the songs of two individuals.

Playback experiments have been performed to demonstrate the ability of territorial birds to discriminate between the song of a neighbouring individual and that of a stranger. The rationale behind these experiments is that if an individual can discriminate between the two, as it is more familiar with the neighbour there should be a less intense reaction to the neighbour's song than the strange bird's song. Studies demonstrating this have been carried out on the indigo bunting, Passerina cyanea, (Emlen, 1971); ovenbirds, Sciurus aurocapillus, (Weeden and Falls, 1959); blue grouse, Dendragapus obscurus, (Falls and McNicholl, 1979); field sparrows, Spizella pusilla, (Goldman, 1973); song sparrows, Melospiza melodia, (Harris and Lemon, 1976; Kroodsma, 1976); great tits, Parus major, (Krebs, 1971); rufous - sided towhees, Pipilo erythrophthalamus, (Richards, 1979); stripe-backed wrens, Campylorhyncus nuchalis, (Wiley and Wiley, 1977) and yellowthroats, Geothlypis trichas, (Wunderle, 1978).

Although these studies claim individual recognition they cannot be treated as such because, as Beer (1970)

points out, the results demonstrate the ability to discriminate familiar and unfamiliar sounds and not the ability to discriminate individuals. For conclusive proof, the ability to discriminate between two neighbouring or strange birds should be demonstrated. Studies by Falls and Brooks (1975) on the white-throated sparrow, Zonotrichia albicollis, demonstrated a different response to a neighbour's song depending on where the song was played back from, indicating that location may play a role in this discrimination. They do report a stronger response to the song of a strange bird compared to that of a neighbour when both songs were played back in the centre of the subject's territory, but, again this only demonstrates the ability to discriminate between familiar and unfamiliar, and not between individuals. Kroodsma (1971) points out that there are similarities amongst the songs of neighbouring birds but this similarity declines the further apart the birds are (see also Lemon, 1967). Thus although each bird's song is individually different it is more similar to its neighbour's song than to a stranger's song, and this may allow discrimination without involving individuality.

The second type of playback experiments have made use of mating pairs of birds. Here monogamous pairs are suggested to have powers of individual recognition which enable them to recognise their mate from other birds. Kunkel (1974) gives a table of monogamous tropical birds, all of which as they remain pair-bonded may be able to

recognise individuals. Studies playing back the call of a bird's mate and of a strange conspecific have been carried out on the manx shearwater, _Puffinus puffinus_ (Brooke, 1978); white-throated rails, _Dryolimus cuvieri aldabranus_, (Huxley and Wilkinson, 1979); zebra finches, _Taeniopygia guttata_, (Miller, 1979a); least terns, _Sterna albifrons_, (Moseley, 1979); American goldfinches, _Carduelis tristis_, (Mundinger, 1970); bou-bou shrikes, _Laniarius aethiopicus major_, (Thorpe and North, 1966); kittiwake gulls, _Russa tridactyla_, (Wooler, 1978) and have demonstrated the ability of these individuals to recognise their mate's calls. It need not only be auditory cues that are used to recognise the mate; Ficken (1963) in his investigation of courtship in the common grackle, _Quiscalis quiscula_, found that the females recognise the males by auditory cues, but that the males probably recognise their mates by a combination of visual cues and location. Although the authors claim individual recognition has been demonstrated, this may not necessarily be the case. Paired birds are known to learn each others song and produce a common song between them (duetting). This may allow the mate to be discriminated from all other birds on the basis of familiarity, but it does not signify the ability to discriminate individuals, the birds may be incapable of discriminating between two unfamiliar conspecifics.

Playback experiments rely on their proof of individual recognition on a different response being given

to one of two individuals. These experiments are often confounded by having one individual familiar and the other not, and because of this, only the ability to discriminate between familiar and unfamiliar individuals can be claimed, and not the ability to recognise individuals.

The other major technique used with birds has concentrated on establishing that individuals possess individual characteristics in their song. Borror and Gunn (1965) report of the white-throated sparrow, Zonotrichia albicollis, "since each bird usually sings songs of a single pattern, it is often possible to recognise individual birds" (pp. 46-47). Other studies have used detailed sonographic analysis of bird song and individual differences capable of allowing individual recognition have been found in the savannah sparrow, Passerculus sandwichensis, (Chew, 1981); pinon jays, Gymnorhinus cyanocephalus, (Berger and Lignon, 1977); great-tailed grackles, Quiscalus mexicanus prosopidicola, (Kok, 1971); black capped chickadees, Parus atricapillus, (Mammen and Nowicki, 1981); common crows, Corvus brachyrhynchos, (Thompson, 1969) and sandwich terns, Sterna sandvicensis, (Hutchinson et al., 1968). These studies, whilst demonstrating individual differences capable of permitting individual recognition, do not actually demonstrate individual recognition. For this some behavioural evidence must be provided, for example, Brooks and Falls (1975a,b) demonstrate that certain song features are important in the recognition of neighbours and that

individuals can actually use these to recognise them as such. Similarly , White and White (1970) and White et al. (1970), demonstrate the existence of individual calls in the gannet, Sula bassana, and also that these differences can be used to recognise their mate. Although both studies cannot be said to prove the existence of individual recognition, they do provide behavioural evidence for the possible use of individual cues.

Not all studies have concentrated on bird song as the mechanism for individual recognition. Bertram (1977) demonstrated that there is variation in the wing song of the flappet lark, Mirafa rufocinnamomea, which may allow individual recognition. Ferns (1978) showed there are individual differences in the head and neck plummage of ruddy turnstones, Arenaria interpres, which may allow individual recognition. Chickens, Gallus sp., are reported to use visual cues to discriminate individuals. Guhl (1962) points out that the features of the chicken's neck and head enable them to be discriminated as individuals and thus maintain their peck order. However, it may not be as simple as this, as Maier (1964) found the social recognition of hens was destroyed when the physical context and performing of the dominance determining ritual was not formed. Thus the recognition of individual chickens may be situationally determined and not true individual recognition.

Two studies have attempted to investigate individual

recognition by using a discrimination training paradigm. Trillmich (1976) trained budgerigars, Melopsittacusundulatus, to discriminate between conspecifics, which they achieved, even managing to discriminate when slides were given rather than the actual birds. The features of the head apparently carried sufficient information for individual recognition. Ryan (1982) found that chickens, Gallus gallus, could be trained to discriminate between two pictures of individual chickens and that some birds could transfer this learning to a different slide of the same bird never before seen. Little information is given concerning the familiarity or unfamiliarity of the stimulus animals to the subjects in either of these experiments so it is difficult to establish whether they were discriminating on the basis of individual cues. However as the experimental technique required that the birds respond in a particular way to the same individual, recognition has been shown as the subjects respond to the stimuli in the same way over a period of time.

2.4.6

Mammals

A wide variety of techniques have been used to demonstrate the ability of a large number of mammalian species to recognise individuals. The technique used is largely determined by the experimenter's thoughts on the source of the identifier. Most authors agree that the source of the identifier is likely to be linked to the

most important perceptual sense for that animal. Thus, for example, most studies on rodents' ability to recognise individuals have used techniques to promote olfactory cues. Likewise in birds, most studies have promoted auditory cues. It is likely that as one advances through the mammalian order more than one sense comes into play and individual recognition may be attained by using more than one cue. For example, Wilson (1975) reports "the faces of gorillas, chimpanzees and red-tailed monkeys (Ceropithecus nictitans) are so variable that human observers can tell individuals apart at a glance. It is plausible that the equally visual non-human primates can do so as well (Marler 1965, van Lawick-Goodall 1971)" (p. 205). Fox (1982) reports that chimpanzee, Pan troglodytes, females may be distinguished by the volatile fatty acid content of their vaginal secretion and that chimpanzees may use this to distinguish between individuals. Thus it is possible that in chimpanzees there are at least two cues which allow individual recognition. However few studies consider two possible cues for recognition of individuality and most concentrate on one.

Some studies investigating the ability of mammals to recognise individuals have used discrimination learning techniques. Sheep (Baldwin and Meese, 1977) were able to discriminate between pairs of odours from urine, wool, faeces, saliva, interdigital pouch secretions, inguinal pouch secretions and infraorbital pouch secretions from

different sheep. Similarily studies of mice, Mus musculus, (Bowers and Alexander, 1967; Hahn and Simmel, 1968; Hahn and Tumulo, 1971) have demonstrated the ability of mice to discriminate between odours from two different mice of the same inbred strain. Pigs (Large white, Meese et al., 1975) have been shown to discriminate between two urine samples from other pigs in order to obtain food. Pettyjohn (1981) using a different technique demonstrates individual recognition in the mongolian gerbil, Meriones unguiculatus, by using a conditioned aversion technique. Similarily guinea-pigs, Cavia porcellus, using an "aversively motivated learning task" were shown to discriminate between anogenital odours of individuals from the same colony (Ruddy, 1980). The use of conditioned discrimination techniques has its advantages in that it allows a particular independent reponse to be perfomed over time to the same individual and thus indicates individual recognition rather than individual identification. However, this technique has its disadvantages in that although it demonstrates that the animals are capable of individual recognition, it does not establish whether they can actually use it. Many of the experiments used cues, for example, urine, separated from the individual, which when placed back in the context of the individual as a whole may not enable individual recognition.

A second experimental technique, similar to playback experiments in birds, has been to present stimuli from two

different individuals and observe the behavioural reaction
to these. If there is a different response to the stimuli
one can assume individual recognition. Red foxes, Vulpes
vulpes show a higher frequency of marking behavior when
presented with urine and anal sac odours from unfamiliar
foxes than familiar conspecifics (Blizard and Perry,
1979). The desert woodrat, Neotoma lepida lepida, showed
a higher level of rubbing behaviour to conspecific odour
than their own (Fleming and Tambosso, 1980). The collared
lemming, Dicronstonyx groenlandicus, showed a preference
for the odour of their former partner over that of a
stranger (Huck and Banks, 1979), and genets, Genetta
genetta, sniffed the scent marks of unfamiliar individuals
more than familiar individuals (Roeder, 1980). Not all
studies have used olfactory cues: Snowdon and Cleveland
(1980) report that individual pygmy marmosets, Cebuella
pygmaea, respond differentially to the vocalisations of
different individuals when played back, and Waser (1977)
demonstrates that playback of the "whoopgobble" call of
the grey-cheeked mangabey, Cercocebus albigena, transmits
information of individuality. These studies, like those
performed in birds using the playback technique, suffer
from the criticism that they demonstrate only the ability
to discriminate between familiar and unfamiliar cues and
not necessarily between individuals. One study by Rasa
(1973) overcomes this problem by using two unfamiliar
individuals and demonstrates that the African dwarf
mongoose, Helogale undulata rufula, is able to
discriminate between two unfamiliar individuals on the

basis of odour from the anal glands.

Studies using an habituation paradigm have demonstrated the ability of the mongolian gerbil, Meriones unguiculatus, (Halpin, 1974,1976); the brown lemur, Lemur fulvus, (Harrington, 1976) and the ring-tailed lemur, Lemur catta, (Mertl, 1975) to recognise individuals. As with the conditioning studies these studies demonstrate the ability of individuals to perceive differences between two individuals but not whether they use them.

Other studies have attempted to identify the individual identifiers to see whether and where the individual differences occur. Buss et al. (1976) examined the temporal gland of the African elephant, Loxodonta africana BLUMENBACH, and found individual differences in the cholestrol levels of these glands which may serve a function as an individual identifier. Similar differences in cues which would allow individual recognition have been found in the aliphatic acid of chimpanzee, Pan troglodytes, vaginal lavage (Fox, 1982); the adult type ɪv vocalisations of the grasshopper mouse, Onychomys torridus (Hafner and Hafner, 1978); inguinal gland secretions in rabbits, Oryctolagus cuniculus, (Goodrich and Mykytowycz, 1972); the relative concentrations of 6-carboxylic acids from the pocket gland secretion of the mongoose, Herpestes auropunctatus, (Gorman, 1976); the vocalisations of stumptail macaques, Macaca arctoides, (Lillehei and Snowdon, 1978); the

pant-hooting vocalisations of chimpanzees, P. troglodytes, (Marler and Hobbett, 1975); the anal sac secretion of the hyena, Hyaena hyaena, (Wheeler et al., 1975); the tarsal gland secretion of the black tailed deer, Odocoileus hemoines, (Brownlee et al., 1969) and the tarsal gland secretions of the reindeer, Rangifer tarandus, (Andersson et al., 1975; Muller-Schwarze et al., 1977). Although these studies demonstrate differences in certain cues which would enable individuals to be recognised they do not demonstrate that the animals do actually use these to recognise individuals (for an exception to this see Muller-Schwarze, 1971).

Evidence of individual recognition also comes from studies of self-recognition in primates. Gallup (1970,1977), and Suarez and Gallup (1981) have demonstrated the ability of chimpanzees Pan troglodytes, and orangutans, Pongo pygmaeus, to recognise themselves. Whether this ability can be taken to represent an ability to recognise individuals is debatable, however it does indicate that these animals are capable of recognising themselves.

A further source of evidence for the ability to recognise individuals comes from studies of the Bruce effect, in which pregnancy is blocked in the female by exposure to a strange male. First demonstrated in the laboratory mouse, Mus musculus, (Bruce, 1959,1960) it has now been found in the prairie vole, Microtus ochrogaster,

(Stehn and Richmond, 1975); the meadow vole, Microtus pennsylvanicus, (Clulow and Landford, 1971); the bank vole, Clethrionomys glareolus, (Clarke et al., 1970) the meadow vole, Microtus agrestis, (Clulow and Clark, 1968); the wild house mouse, mus musculus, (Chipman and Fox, 1966) and the deer mouse, Peromyscus maniculatus bairdii, (Bronson and Eleftheriou, 1963). As with many of the previous studies discussed these results may indicate the ability to identify or recognise individuals but all that can be said with certainty is that they show the ability of animals to discriminate between familiar and unfamiliar conspecifics.

Studies have also been performed to examine individual recognition in marine mammals. Caldwell et al. (1972a) demonstrated the ability of Atlantic bottle-nosed dolphins, Tursiops truncatus, to discriminate between the whistles of conspecifics and have also demonstrated the existence of individual differences in the calls of these animals (Caldwell et al., 1970). Futhermore these dolphins have been shown to be capable of discriminating between the whistles of individuals of another species, the common dolphin, Delphinus delphinus, (Caldwell et al., 1972b). Studies have been carried out examining the signature whistles of other marine species, and individual differences have has been found in the calls of Pacific white-sided dolphin, Lagenorhynchus obliguidens, (Caldwell and Caldwell, 1971); the north Atlantic pilot whale, Globicephala melaena, (Taruski, 1979); the spotted

dolphin, <u>Stenella</u> <u>plagiodon</u>, (Caldwell et al., 1973); the minke whale, <u>Balaenoptera</u> <u>acutorostrata</u>, (Thompson et al., 1979); the sperm whale, <u>Physeter</u> <u>catodon</u>, (Bachus and Schevill, 1966); the fin whale, <u>Balaenoptera</u> <u>physalus</u>, (Thompson et al., 1979) and the humpbacked whale, <u>Megaptera</u> <u>novaeangliae</u>, (Hafner et al., reported in Winn and Olla, 1979). Again these studies, whilst demonstrating the existence of individual differences in the whistles of certain marine mammals, which would allow recognition of individuals, do not actually demonstrate they can recognise individuals.

The ability of humans to recognise individuals is well known to most members of this species. Humans are visually distinct from one another, with the possible exception of identical twins, but even these can be told apart by people 'close' to them. Similarly , although slightly more difficult to perform, humans can be identified and recognised by their voice. Experimental studies have also been carried out to examine the ability of humans to recognise individuals by smell. Ellis (1934) first reported that "the infant, the adult, and the aged person each has his own smell" (p. 45). Wallace (1977) demonstrated the ability of humans to discriminate between individuals on the basis of hand odour alone, and Hold and Schleidt (1977) report the ability of humans to correctly identify their own odour and that of their partner, however in their test sample only approximately 33% of the subjects were able to do this. The existence of

individual odours in humans has been demonstrated by Rappolt et al. (1979) and Kalmus (1955) who demonstrated that dogs had the ability to discriminate between human odours. One interesting form of self-recognition in humans has been reported by Beardsworth and Buchner (1981), who report humans are able to recognise themselves from a recorded point light display of their movements, and they are better at recognising themselves, whom they have never seen walking from an external point of view than their friends whom they see walking every day.

2.4.7

Critique

There have been many studies covering a wide range of animals claiming the ability of individuals to recognise conspecifics. However there are a large number of limitations in these experiments which mean one cannot claim individual recognition from them. I shall now briefly review these.

Experiments showing differential responsivity of one individual to another when presented with two conspecifics, cannot be used to claim individual recognition when one of the stimulus animals is familiar and the other unfamiliar to the test individual. It may indicate individual recognition but all that has been shown is the ability to recognise familiar and unfamiliar stimuli.

Studies demonstrating the existence of cues which allow individuals to be recognised, although providing a means whereby individual recognition could be accomplished, do not say anything about the ability of animals to recognise individuals, only that the mechanism is present for this ability.

Experiments relying on a single test for example, habituation tests, do not demonstrate individual recognition, just individual identification. As has been previously said for recognition to be demonstrated individuals must respond to a particular individual in the same way over time. Operant techniques where the individual is required to repeat a particular operant response in the presence of a particular individual do demonstrate recognition as the response is repeated over time.

Experiments using operant techniques can demonstrate the ability of animals to recognise individuals, but whilst showing that they can recognise conspecifics they do not show whether the animals use this ability naturally.

Finally, experiments which use as stimuli particular cues can only be said to show the ability of an individual to recognise that particular cue. When that cue is 'reunited' with the animal as a whole its significance and salience may be altered to such an extent that individual

recognition is no longer possible.

2.4.8
Summary

If one adopts such strict criteria very few of the studies described could be used to claim individual identification and even fewer to claim individual recognition. Many of the studies indicate that individual differences do exist between individuals but lack the behavioural data to indicate whether individuals can actually use this ability. One notable exception to this is the study by Beer (1970) on individual recognition in the laughing gull, Larus atricilla, where behavioural observations of individual recognition are combined with evidence obtained from controlled experiments on the individual's ability to discriminate between individuals plus sonographic analysis of the birds' song to provide evidence of individual characteristics in the birds' song.

In summary, the review of the literature produces a large number of studies giving an indication of individual recognition, with very few providing concrete evidence of individual recognition. One suprising fact to emerge from this review is that Romanes back in 1883 may have got it right. He suggested that all vertebrates and the invertebrate groups of hymenoptera and crustacea would possess the ability to "recognise persons". The data available at the moment suggests that individual recognition is present in these groups.

2.5

INDIVIDUAL RECOGNITION IN THE RAT

The laboratory rat is one of the most extensively
studied animals and has been used in a countless number of
widely varying experiments. Accordingly the social
behaviour of this animal is well documented. An
indication of the ability of rats to recognise individuals
comes from behavioural observations. Barnett (1975)
reports that rats have the power to distinguish group
members from strange animals and Telle (1966) argues that
rats in small groups (up to twenty individuals) possess
the ability to recognise each other as "acquaintances".
Thus observation of rats' social behaviour gives an
indication of the ability of the Norway rat to recognise
individuals.

Studies examining the sexual choice of rats can also
be used to give an indication of the rats' ability to
recognise individuals. Carr et al. (1980) demonstrated
that males with monogamous mating experience, if given a
choice of the same female and a novel female prefer the
novel female. Females however show the reversed
preference and prefer the same male over an unfamiliar one
(Carr at al., 1979a). This may involve the ability to
recognise individuals but only the ability to recognise
familiar and unfamiliar individuals has been demonstrated.
Sokolov and Lyapunova (1979) demonstrate the rats' ability
to discriminate familiar and unfamiliar conspecifics by
showing male and female rats investigate the odour of an

unfamiliar conspecific longer than a familiar one. Tolman (1961) similarily demonstrated the ability of juvenile rats to discriminate between familiar and unfamiliar rats.

The above mentioned studies, whilst not directly investigating the rats ability to recognise individuals, can be used to give some indication that rats may possess this ability. Studies directly examining the question of individual recognition have used operant techniques. Carr et al. (1976) found that both immature and mature males sniffed a cardboard tube which had housed a novel immature male longer than one which had housed an immature familiar male; however they showed no preference for mature strangers over mature cagemates. This study demonstrated the ability to recognise familiar and unfamiliar conspecifics. In a second experiment they used an habituation technique in which rats were presented for ten minutes with two tubes containing the odour of the same cagemate, then the subject was presented with two tubes, one containing the odour of the same cagemate as phase 1, the other, odour from another cagemate. The subject rat investigated the tube containing the novel cagemate longer than the familiar one. The results show that both mature and immature male rats can discriminate between their cagemates. However this may not be the case if the tube presented in phase 2 was also used in phase 1 to habituate the subject rat. For then, this tube would contain not only the odour of the cagemate, but also the odour of the subject rat who had previously investigated this tube in

phase 1. When presented a second time with this tube, it would possess more cues than the tube containing the odour of the novel cagemate, and the subject could respond on the basis of this, by recognising its own odour on one tube and recognising this as having been previously investigated. Indeed the third experiment in this paper does present evidence that rats can identify their own odour.

Krames (1970), also using an habituation task, demonstrated that female Long-Evans rats could discriminate between the odours of two males. The odorous stimuli were presented in cardboard tubes, first two tubes containing the odour of the same animal, then two tubes one of which contained the same odour, the other the odour of a novel male. In order to ensure that the odour of the new rat was the only novel odour introduced, each of the two tubes was housed in a cardboard sleeve in the habituation phase, and in the test phase both the habituated and the novel odour tubes were placed in the two sleeves to equate for the odour deposited by the subject.

Sharpe and Cooper (1966) used a simultaneous discrimination task in which one cagemate was designated the S+, the subject receiving food if it entered the compartment containing this animal. The other animal was designated the S-, and if the subject entered the compartment containing this animal it received footshock.

Both males and females were used to discriminate same sex cagemates. The results suggest that both males and females were able to discriminate between their two cagemates. However, this may not be due to individual recognition. The response emitted by the subject was to enter the compartment containing one of the stimulus animals; if it entered the S- side then both the subject and S- animal were shocked. Experiments investigating the effects of stress have demonstrated that stressed rats emit an odour which other rats avoid (Mackay-Sim and Laing, 1980; Valenta and Rigby, 1968). Administering of electric shocks to animals causes stress and the emission of stress odour. Thus once the S- had been stressed the subject animal only has to discriminate between stressed and non-stressed rats. Support for this comes from a comparison of the two subject arrangements used in this study. In arrangement 1 the same stimulus rat was used as the S- (i.e., received shock) for two subjects and never served as a subject or the S+, in arrangement 2, each rat served as a subject, S+ and S-. If stress odour played a role in the discrimination, then the animals in arrangement 1 should be easier to discriminate than those in arrangement 2 as one subject is the S- for both subjects and thus receives a 'double dose' of shock and would be expected to be more stressed and thus produce more stress odour. This prediction is supported by Sharpe and Cooper's results, where subjects in arrangement 2 "made over three and a half times more errors in reaching criterion than arrangement 1" (p. 156). Further support

for the notion that the animals were not being discriminated on the basis of their individuality comes from Sharpe and Cooper's observation that the procedure of shocking and reward produced different behavioural reactions in the discriminative stimuli when approached by the subject. The S- rats froze when approached by the subjects whereas the rewarded rats became active. Thus as well as the stress odour, the discriminative stimuli exhibited gross behavioural differences which would allow the discrimination to be made. It is likely that although the subjects used in this experiment gave the appearance of being able to discriminate between their cagemates, this discrimination was not based on individuality but on cues associated with stress.

A final study using operant techniques was performed by Husted and McKenna (1966) who trained Wistar rats to discriminate between conspecifics by using a successive discrimination task. Four rats were trained to press a bar in the presence of one rat but not in the presence of a second rat. All rats learned to make this discrimination although there were wide individual differences in the rate of learning. Thus the rats were able to recognise their conspecifics as they performed the same response (a bar press) in the presence of a particular rat over time. However no idea of the housing arrangements of the subject animals is given and thus it is difficult to say whether the task was performed using cues of individuality or of familiarity.

The ability of male Charles River C.D. rats to discriminate between the urine odours of males has been demonstrated by Fass et al. (1978). Males were exposed to the urine of one male for eleven hours and then presented with the odour of the same urine or that from a novel male, the subject preferred the familiar odour. This study demonstrates the ability of rats to discriminate between urinary odours of males but not whether they actually use this in recognition of individuals.

2.6
AN INVESTIGATION OF THE ABILITY OF RATS TO
RECOGNISE CONSPECIFIC INDIVIDUALS

As I argued above, the previous studies on individual recognition in the rat do not demonstrate the existence of this ability conclusively. The aim of this experiment is to demonstrate that both male and female rats can recognise individual conspecifics. To do this a conditioned simultaneous discrimination task is used in which subjects are trained to respond to one individual to get food. If the animals learned this task then recognition can be said to have been demonstrated, as the individual repeats a particular reponse to a particular individual over time. Indeed, if the individual did not recognise the animal associated with the food it could not learn the task. To overcome problems of discrimination based upon familiarity, the discriminative stimuli used were unfamiliar to the subject.

2.6.1

Experiment 2.1:

The Ability Of Male And Female Rats To Discriminate

Between Unfamiliar Same Sex Conspecifics

In this experiment food deprived male and female rats were trained to discriminate between two same sexed unfamiliar conspecifics in a simultaneous discrimination task to obtain food.

2.6.1.1

Subjects

Rats (8 male and 8 female), born in the Department of Psychology, University of Durham, from adult Sprague-Dawley rats supplied by B & K (Animal Suppliers) Ltd. were used. The rats were 91 days old (+/- 1 day) at the start of the experiment. Two male and two female rats were used as the discriminative stimuli and were housed in same sex pairs in plastic Bowman's cages (16"x12"x7") for the duration of the experiment. These stimulus rats were genetically unrelated (r=0.0) to each other and to all other rats in this experiment and had been housed together since birth. The remaining 12 rats (6 male and 6 female), were designated the subjects and were housed in same sex groups of three in plastic Bowman's cages. All rats were kept on a 12 hour light/dark cycle (darkness starting 1200 hours) with food and water freely available until 91 days old. All rats had previously been used in observational tests of huddling and play partner preference, and were ear punched for individual identification.

2.6.1.2
Apparatus

A modified 'T-maze' was used (see plate 2.1, fig. 2.1). The maze was constructed of aluminium and mounted on wooden blocks, with a smoked perspex top. The start box (S.B.) was separated from the rest of the maze by means of a door, which when raised allowed the rat access to the maze. The maze was 3.5" wide by 6" high and raised 3" off the ground. The rats serving as disciminative stimuli were housed in cages, which were 6"x6"x6" and made of 0.5" wire mesh. Both cages stood in a tray of sawdust and protruded 3" into the maze, leaving a gap of 3" between the top of the cage and the maze roof. Doors were present either side of the choice point which allowed each arm of the maze, including the discriminative stimuli, to be isolated from the rest of the maze. The arms of the maze were dog-legged and the reward placed in the second half such that the rat could not see the reward from the choice point. The perspex roof was hinged after the S.B. to allow the subject to be placed in the S.B. and the whole roof could be lifted off the maze to remove the rat after each trial. The reward used was 2 Precision food pellets supplied by Campden Instruements Ltd., and weighed 45 mg per pellet.

2.6.1.3
Procedure

The experimental subjects had to discriminate between two conspecific agemates of the same sex, in order to get
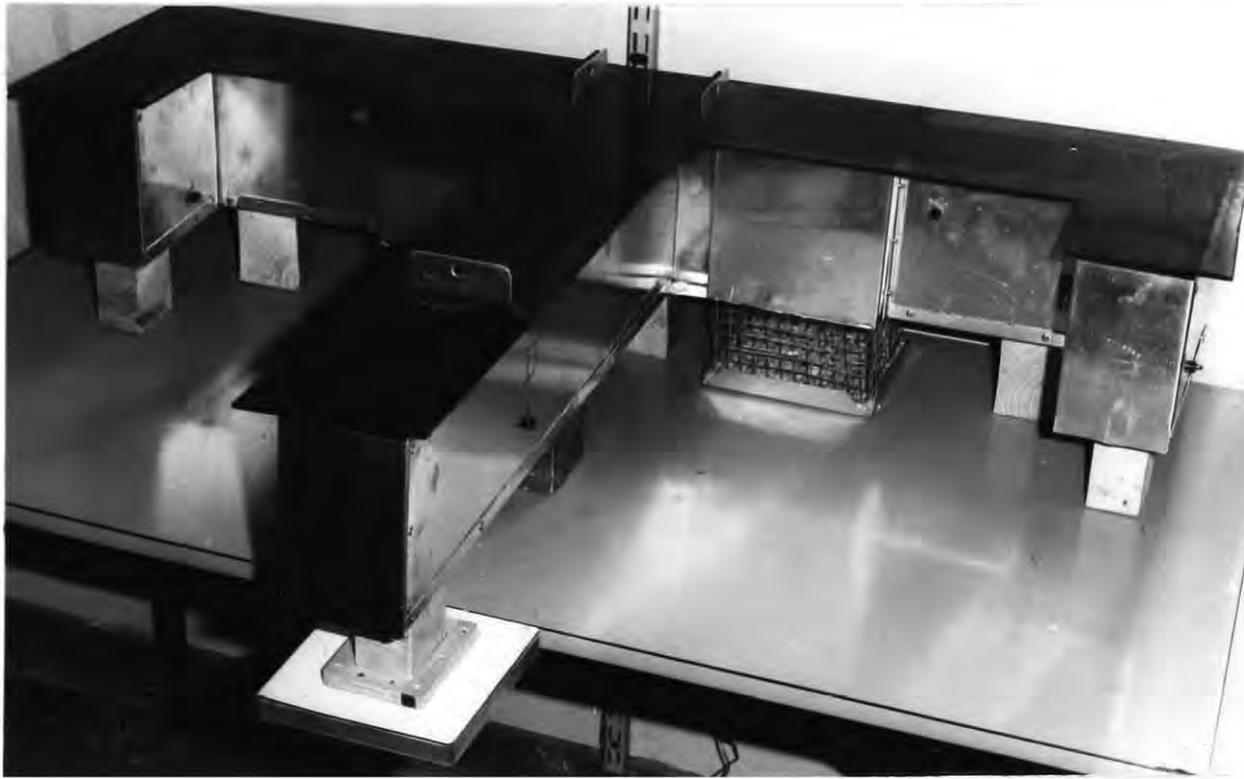
PLATE 2.1
T-maze used in Experiments 2.1 and 2.2.
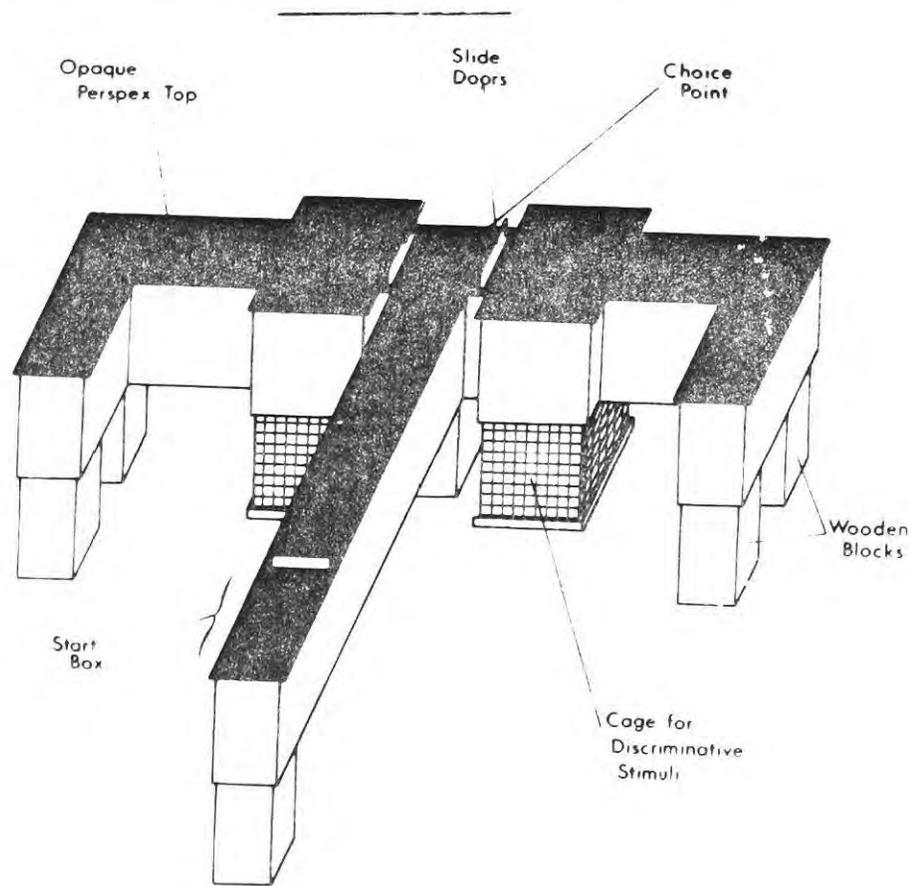
FIGURE 2.1
Diagrammatic representation of T-maze apparatus used in experiments 2.1 and 2.2.

food. The experiment was divided into two periods, a pretraining phase, followed by a discrimination training phase.

Pretraining: All experimental subjects were deprived of food for 22 hours daily from 91 days old and this was maintained through out the experiment. The rats were fed at the same time each day (1200-1400 hours). After three days of food deprivation the subject rats were placed in the maze in their cage groups of three, for twenty minutes with reward pellets abundantly available in each goal box, in order to habituate them to the maze and get them used to feeding in the maze. After five days of exposure in threes, the rats were placed in the maze individually, first in the S.B. and then admitted to the maze by raising the door. They were left until all the food had been eaten plus three minutes and then removed from the maze. This training was continued for four days after which all individuals showed no hesitation in entering and eating in the maze.

The discriminative stimuli were placed in the cages in the maze for half an hour per day to habituate them to the maze. All the discriminatve stimuli were placed in the maze for seven days prior to the start of discrimination training.

Discrimination training: The male and female rats were run separately in two squads, each rat receiving ten

trials per day. Within each squad all six rats had their nth trial before any had their n+1th trial. A gap of three minutes was left between the end of one trial and the start of the next. For each trial the order of running of the six rats was randomised. Ten different running orders of six rats were constucted (see appendix 2.1) and a different order chosen for each trial. This was necessary to prevent any rat obtaining cues from the rat which had immediately preceeded it in the maze.

The two discriminative stimuli (Sd) were labelled S1 and S2 and the position of these was randomly chosen for each trial between left and right according to a prearranged Gellerman series (after Gellerman, 1933). Ten sequences of left and right were constructed with the following constraints: 1) no more than three consecutive trials to the same side, and 2) in the ten trials there were to be an equal number of left and right (see appendix 2.2). A different sequence was used each day and after ten days the cycle was restarted. Both male and female experienced the same positioning of the Sd each day. For half of the subjects in each squad the S1 was the positive stimulus and food was obtained if it responded positively to this, for the other half the S2 was the positive stimulus.

All trials were carried out under red light between 0900 and 1200 hours each day. The experimental subjects were taken to the test room and placed in individual

holding cages (N.K.P. Cages Ltd., M3, 19"x6"x5") for fifteen minutes prior to the first trial. Similarly the Sd were placed in their cages in the maze. The reward (2 pellets, 90mg of food) was placed on the side of the Sd which was positive for that particular subject. An experimental subject was then placed in the S.B. and the door opened admitting the rat to the maze. Once the subject had reached the choice point (C.P.) and chosen one side, that is, crossed over the cage containing one of the Sd, the door was shut preventing it from returning to the C.P. The rat was now left until it entered the G.B. On entering the G.B. the rat was left until it had eaten the food plus twenty seconds, and then removed from the maze. The same procedure was followed for the next rat. After all rats had had trial one a three minute period was left before the start of trial two. If the discriminative stimuli were to be changed over they were removed from their cages and put into the appropriate cage. Thus the cages remained on the same side through out the experiment with only the Sd changing sides. After three minutes the second trial was started with the rats running in a different order from that of the previous trial. The procedure was continued until all rats had ten trials. The squad of females was run first and then the squad of males.

If a rat did not run and remained at the C.P. for 120 seconds then the rat was gently ushered into the correct arm of the maze to get food. The response was not

scored. On each trial it was recorded whether the rat made a correct response and a rat was said to have learned the discrimination if it made a minimum of eighteen correct responses on two consecutive days.

2.6.1.4
Results

The day each rat completed the criterion of eighteen correct in two successive days was recorded for both male and female rats (see table 2.1, figure 2.2). The experiment was terminated on day 76 except for one female subject (No. 2) who was run for an extra day to see if criterion could be attained. All subjects, except two females learned the discrimination and it is probable that if the experiment had continued these females would have eventually learned the discrimination. These results indicate that both male and female rats are able to discriminate between two conspecific agemates of the same sex, housed together since birth. Thus although rats may come to possess a common group odour (Barnett, 1975), they can also recognise individuals within this.

Observation of the time taken by females to learn the task suggests that they took longer to acquire the discrimination than males. A comparison was then carried out between the time taken to reach criterion by the male and female rats by means of a t-test (see Robson, 1975). For the purposes of this test the two females which did not learn were given values of 78 days to reach crierion

TABLE 2.1

The time taken (in days) for subjects in experiment 2.1 to achieve the required criterion (18/20).

SUBJECT	MALES	FEMALES
RAT 1	48	XX
RAT 2	45	77
RAT 3	27	30
RAT 4	32	XX
RAT 5	21	72
RAT 6	67	47

XX=Did not achieve criterion.
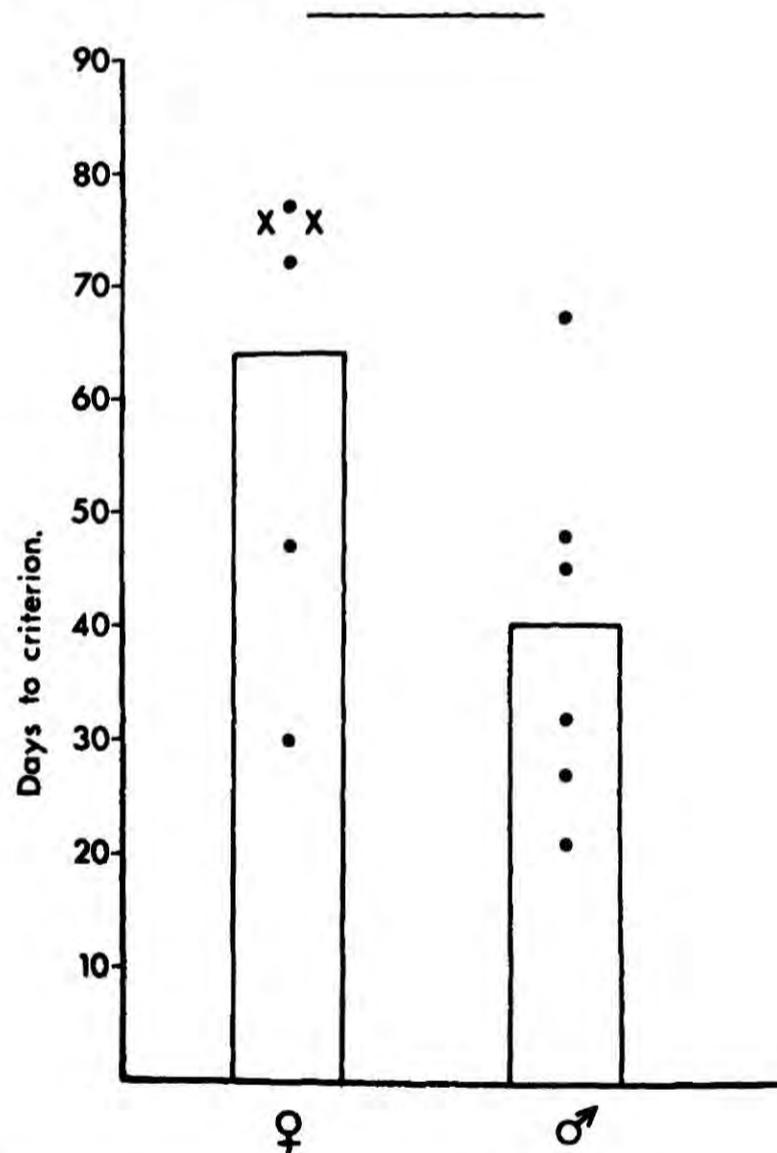
FIGURE 2.2
Mean time for males and females (bar) and time each individual (dot) took to achieve criterion in experiment 2.1 in days.
X = Did not achieve criterion.

which is the earliest they could have achieved this after the experiment was stopped on day 76. The results indicate that the difference in the time taken to achieve criterion by the males (mean 40 days) and the females (mean 63.6667 days) is not significant (t=2.1970, df=10, 0.1>p>0.05). However, the results do approach significance and if one assumes, as is likely, that both females which did not learn took longer than the extra two days to learn the discrimination then the result would become significant. Also one of the males (No. 6) was bitten by one of the Sd during the course of the experiment and stopped running for a few days which may have caused him to take longer to learn the discrimination. Thus there does seem to be an indication that there may be a difference in the ability of males and females to learn to discriminate between individuals of the same sex, the females being poorer learners than the males.

2.6.1.5
Discussion

The results of these experiments indicate that rats can discriminate between unfamiliar individuals of the same sex. All rats except two females learned this discrimination and it is likely that if the experiment had been continued both of these would have learned. Individuals showed wide differences in the time taken to learn the task. A similar variation in individuals performance was reported by Husted and McKenna (1966).

The results indicate that the males may be able to learn the discrimination more quickly than the females. This could be due to one or both of two factors. First, it may be task dependent: the females might be able to discriminate between the individuals, but something about the discrimination task causes poorer learning. Second, the females may for some reason be poorer discriminators than the males and are poorer learners because of this reduced ability to discriminate. Sharpe and Cooper (1966) found that males made fewer errors to criterion than did females and, although this difference was non-significant, it could be indicative of a poorer discriminative ability of females.

From a theoretical point of view there should be no difference or, if any, the females should be better at discriminating than males. Both males and females can gain equal advantages in their inclusive fitness by responding differentially to kin and non-kin. However the ability to discriminate kin from non-kin may be more important for the females. If the aim of an individual is to reproduce and so preserve its genes in the next generation (Dawkins, 1976), then the mating situation becomes crucial. One possible danger in this is inbreeding which can cause decreases in fitness (Falconer, 1960). The female invests more in the mating situation than the male (Barash, 1977) and as such stands to loose more if mistakes are made. Accordingly the female should have at least the same powers of discrimination as the

male and possibly better.

Pietras and Moulton (1974) investigated the olfactory sensitivity of both male and female rats for various chemicals. They found that the female's olfactory sensitivity fluctuates with her hormonal cycle, being most sensitive at the time of oestr us. More interestingly, in comparison with male olfactory sensitivity which showed no cyclicity, the female always had poorer sensitivity except on the day of oestr us where sensitivity was equivalent. Such fluctuations may affect the ability of females to discriminate between the stimuli. Pietras and Moulton also found that if one artificially raised the levels of oestrogens in females one achieved higher performance in odour detection plus increasing the excitability of the olfactory system. Thus by using ovariectomized females with oestrogen replacement one can induce a state of permenant oestrous and thereby produce a permanently higher olfactory sensitivity with no fluctuations.

Another factor which may contribute to the poor performance of females is that they had to discriminate females whereas for the males, males were the discriminative stimuli. There may be some difference in the odour produced by the males and females which would affect the performance of males and females. One such factor which may be involved is the odours produced by the female during the oestrous cycle. Male rats have been trained to discriminate between odours of receptive and

non-receptive females (Carr and Caul, 1962) indicating their are odorous changes during the oestrous cycle. More evidence of this comes from studies demonstrating the existence in males of a preference for odours of receptive females over non-receptive females (Carr et al., 1965,1966). Sexually experienced or non-receptive females show no preference to the odours of receptive and non-receptive females, whereas naive females prefer the non-receptive female odour (Carr et al., 1970). This experiment indicates that both receptiveness and sexual experience play a role in the females' preferences for these odours. There can be little doubt that females do produce different odours during the oestrous cycle and this may affect the females' perception of the discriminative stimuli in such a way that their individuality is masked. If ovariectomized stimuli were used then there would be no fluctuating hormones and thus the odour of these females should remain the same as there are no oestrous changes.

The results of experiment 2.1 indicate that both male and female rats can discriminate between two unfamiliar cagemates. However this may not be due to individual recognition. If individual characteristics are under genetic control, which to a certain extent they must be, the previous discrimination could be based on the ability to discriminate between two genetically unrelated individuals, and if so the individuals would not be expected to discriminate between genetically related

individuals.

To examine this, and why females are poorer
discriminators than males, the experiment was repeated
using sibling cagemates as the Sd. Two further conditions
were added. First, ovariectomized females were used as
discriminative stimuli to overcome the problems of
differential odour production during the oestrous cycle.
Second, ovariectomized oestrogen primed females were used
as subjects to increase the olfactory sensitivity.

2.6.2
Experiment 2.2:
The Ability Of Male And Female Rats To Discriminate Between Same Sexed Sibling Cagemates With Controls For The Effect Of Female Hormones On Olfactory Sensitivity And Odour Production

The procedure and apparatus used in these experiments
were identical to those used in experiment 2.1 and will
not be discussed again. I shall here limit the discussion
to that of the subjects only.

2.6.2.1
EXPERIMENT 2.2a: The Ability of Males to Discriminate between Unfamiliar Sibling Cagemates

Eight Sprague-Dawley males born in the Department of
Psychology, University of Durham, were used in this
experiment. The rats were 100 (+/-1) days old at the

start of the experiment. Six rats were designated the experimental subjects and were housed in two groups of three in plastic Bowman's cages (16"x12"x7"). Two rats (the discriminative stimuli - Sd) were housed similarly together in a Bowman's cage. These rats were siblings (r=0.5), and unrelated (r=0.0) and unfamiliar to all other rats in this experiment and had been housed together since birth. All rats were kept on a 12 hour light/dark cycle (darkness starting at 1200 hours) with food and water freely available until the start of the pretraining phase. The six experimental subjects were placed on 22 hour food deprivation on day 100 and then placed on pretraining followed by the discrimination phase, the procedure in these phases identical to that described earlier (Experiment 2.1, section 2.6.1.3).

2.6.2.2

EXPERIMENT 2.2b: The Ability of Females to Discriminate between Unfamiliar Sibling Agemates

Eight Sprague-Dawley females born in the Department of Psychology, University of Durham, were used. The rearing conditions, procedure, etc., were identical to those described above for the males, with the single exception that these were female rats.

2.6.2.3

EXPERIMENT 2.2c: The Ability of Oestrogen primed Females
to Discriminate Unfamiliar Sibling Cagemates

Eight female Sprague-Dawley rats, born in the
Department of Psychology, University of Durham, were used
in this experiment. The rearing conditions were identical
to those described for the females in experiment 2.2b with
the exception of the six experimental subjects. These had
been ovariectomized under ether anesthesia (see Waynforth,
1980, for surgical procedure) on day 50 and had been left
in dioestr-us until day 98. On this day the experimental
subjects were injected with 0.1cc oestrogen
(β-estradiol-3-Benzoate, 10 μg/0.1cc). This continued
daily until day 110, when the dosage was altered to 0.2cc
and given every other day. The effect of these injections
was to place the female rats into permenant receptivity by
increasing their circulating levels of oestrogen. By
doing this the olfactory sensitivity and excitability was
increased above that of normal females.

2.6.2.4

EXPERIMENT 2.2d: The Ability of Females to Discriminate
Ovariectomized Unfamiliar Sibling Cagemates

Again the rearing conditions of these subjects were
identical to those described for the females in Experiment
2.2b with the exception of the rats serving as the
discriminative stimuli. These were ovariectomized under
ether anesthesia (see Waynforth, 1980, for surgical

procedure) on day 50 and were thus in permanent non-receptivity. Eight Sprague-Dawley females were used, born in the Department of Psychology, University of Durham, six of these were used as subjects, the remaining two were the discriminative stimuli.

The experiments were carried out at the same time each day and one squad of rats run for ten trials before the next squad started. The pretraining and discrimination training phases were identical to that used in experiment 2.1, see section 2.6.1.3. The criterion of 18 correct trials in two successive days was maintained and the day each rat obtained this recorded.

2.6.2.5
 Results

The experiment was terminated after 87 days, at which point all subjects except one female in experiment 2.2b had learned the discrimination to criterion, (see table 2.2, fig. 2.3). The female in experiment 2.2b which did not learn the discrimination was diagnosed as suffering from Chronic Respiratory Disease (C.R.D.) on day 4 of the experiment. She was immediately separated and given antibiotics (Tetracycline. Trade name Terramycin soluble powder, Pfizer Ltd.) and allowed to complete the experiment. However symptoms persisted and she was later withdrawn from the experiment. None of the other animals gave any indication of having C.R.D. either during the experiment or in the 50 days following the experiment.

TABLE 2.2

The time taken (in days) for the subjects in experiment 2.2 to achieve the required criterion (18/20).

SUBJECT	Expt. 2.2a MALES	Expt. 2.2b FEMALES	Expt. 2.2c OVX FEMALE SUBJECTS	Expt. 2.2d OVX FEMALE STIMULI
RAT 1	32	36	52	49
RAT 2	42	87	47	55
RAT 3	18	57	63	46
RAT 4	40	47	63	30
RAT 5	48	XX	34	45
RAT 6	27	57	37	54

XX=Did not achieve criterion.
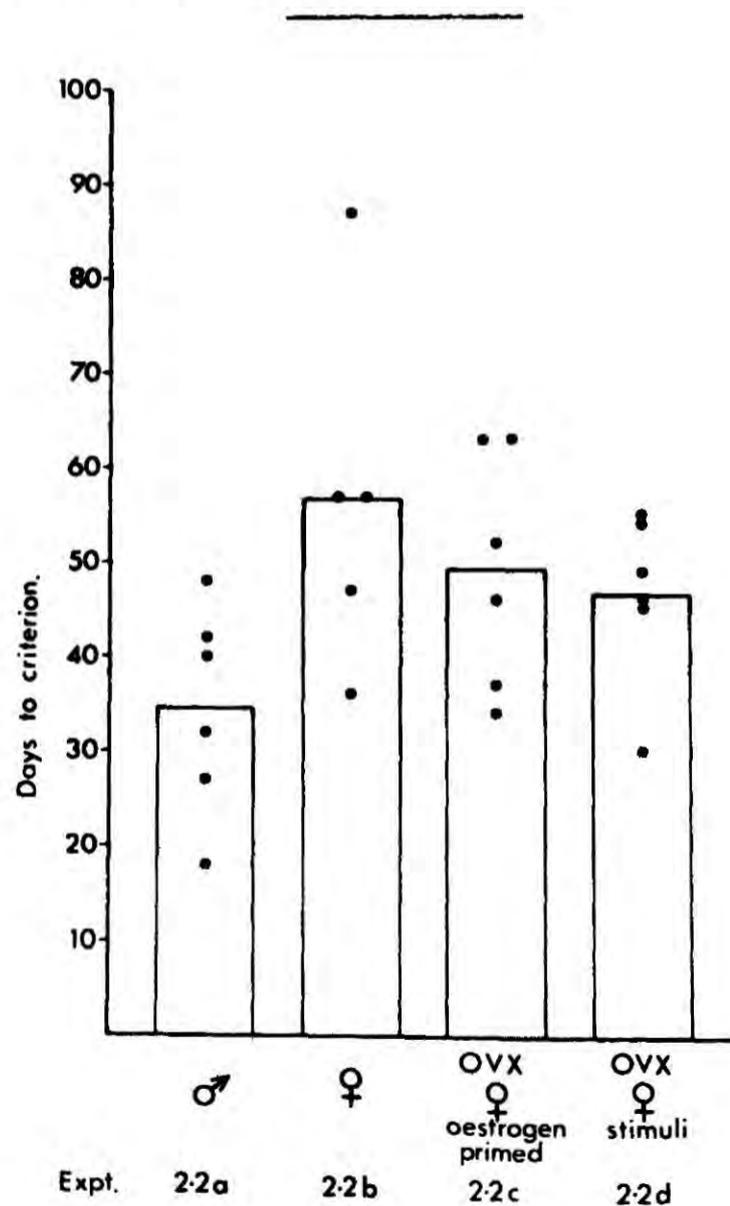
FIGURE 2.3
Mean time (bar) and time each individual (dot) took to achieve criterion in experiments 2.2 (a-d) in days.

The results indicate, as with the previous experiment, that rats are able to recognise their conspecifics.

There was again evidence of a difference in the time taken by males (Expt. 2.2a) and females (Expt. 2.2b) to reach criterion, males achieving criterion more quickly than females (mean number of days to criterion: males, 34.5; females, 56.8). To test the significance of this an independent t-test was performed, (see Robson, 1975) and this indicated that males did learn the discrimination significantly more quickly than females (t=2.4435, df=9, 0.05>p>0.02).

The oestrogen primed ovariectomized females in experiment 2.2c also seemed to learn the discrimination faster than the females in 2.2b, (mean number of days to achieve criterion: oestrogen primed OVX females, 49.3; normal females, 56.8). However a t-test revealed this to be non-significant (t=0.7862, df=9, 0.5>p>0.4). It seems that increasing the olfactory sensitivity of females did not increase their ability to learn the task.

When the females were discriminating between rats which were ovariectomized (Expt. 2.2d) instead of normal animals (Expt. 2.2b), they took less time to reach criterion (mean number of days to achieve criterion: females discriminating OVX females, 46.5; females discriminating normal females, 56.8). This difference however proved to be non-significant when tested with a

t-test (t=1.1863, df=9, 0.3>p>0.2). Thus removing the attractive odour associated with oestrous from the stimulus rats does not increase the females speed of learning the task.

This experiment has demonstrated that both males and females can discriminate between and recognise two unfamiliar conspecifics who are siblings and cagemates. The males are quicker at achieving criterion in this task than females, and neither increasing the olfactory sensitivity of females nor removing oestrous odours from stimulus rats significantly reduced the time taken by females to achieve criterion.

2.6.2.6 Discussion

As in the previous experiment rats were found to be able to discriminate between their conspecifics. Males were quicker to learn this discrimination than females. Husted and McKenna (1966) report that males made fewer errors to reach criterion than females, but this difference was not significant. Trillmich (1976) trained budgerigars to discriminate between individuals and found that males reached the required criterion, taking on average 200-400 trials, quicker than females, taking on average 300-600 trials. It may be that these results are due to the female being a poorer discriminator of individuality than males. As previously discussed there should, theoretically, be little difference in the males

and females' ability as both sexes would benefit by possessing this ability.

In the above experiment two possibilities were considered which could have accounted for the poorer performance of females. Females exhibit cyclicity in their olfactory sensitivity linked to their oestrous cycle, and on three out of four days of this cycle their sensitivity is poorer than the males and on the fourth day equivalent to the males (Pietras and Moulton, 1974). By using oestrogen primed OVX females, the level of circulating oestrogens was raised, increasing the olfactory sensitivity of the females. If the female's olfactory sensitivity was inhibiting its performance in this task it would have be been expected that the oestrogen primed females would learn the task faster than normal females. This turned out not to be the case, and increasing the females' olfactory sensitivity did not lead to females achieving the criterion faster than normal females. Thus the possibility that decreased olfactory sensitivity is the main reason for poorer female learning can be ruled out.

The second factor which may have influenced the females' performance was that of odours produced by the stimulus females during their oestrous cycle. Females produce odours at oestrous which are attractive to other rats (Carr et al., 1965,1966) and these odours may interfere with odours of individuality. Ovariectomized

females, as they have no oestrous cycle, do not produce these odours. If these odours affected the females' performance it would be expected that females discriminating OVX female stimuli would achieve criterion faster than those discriminating normally cycling female stimuli. No difference was found between these learning rates and thus the effect of oestrous odours on the females' performance can be overlooked as a major factor causing poorer learning.

It is therefore unlikely that the females' poorer performance in this task is due to their poorer ability at olfactory discriminations. Measures taken to increase their olfactory sensitivity and make more salient the cues of individuality by removing interfering odours had no effect on the females' performance. The poorer performance is probably due to either a poorer ability of females to associate food with individuality or more likely some task dependent variable which adversly affects the female.

The most important task dependent variable is the motivational state of the male and female. The reward used in this experiment was food and to make animals work for this reward they were food deprived for 22 hours per day. It may be that this deprivation period affected males and females differently. Females are smaller than males (average body weight, males 330 grams, females 225 grams, B and K, (Animal Suppliers) Ltd.) and require less

food. As such they may have been able to achieve more of their daily food requirement than males during the two hour feeding period. At the start of the experiment the males may have been hungrier than the females and were more motivated to learn the task. Observations of the males and females during the task did indicate that males performed each trial quicker than the females and this may be a reflection of their differing motivational states. (A similar phenomenon was found during a first year practical demonstration, where all rats were kept on 21 hour deprivation to learn a simple bar press and males learned the task quicker than females). It may be that the males and females were of different motivational states during the experiment and this caused the difference in performance. A better index of deprivation would have been to maintain the subjects at 80% body weight rather than use 22 hour food deprivation.

Another task dependent variable to be considered is that of stress of the animals serving as discriminative stimuli. Rats produce an odour when stressed which conspecifics will avoid (Mackay-Sim and Laing, 1980; Valenta and Rigby, 1968). It is possible that the female discriminatory stimuli may have been more stressed than their male counterparts. The female subjects would be more affected by this and therefore learn the discrimination more poorly. Both male and female stimuli were given lengthy exposure to their cages prior to the experiment, and at the start of the experiment all

stimulus animals showed no signs of distress at being placed in the cages and most went to sleep. It is therefore unlikely that the females were stressed more than the males, and this factor can be discarded as exerting a major influence on the behaviour of male and female subjects.

One final factor to consider is that the females may be poorer at associative learning. If so, as this experiment requires that subjects associate individuality with food, the females may take longer to reach the criterion than males. There is little evidence to suggest this and it is probable that any such constraint on learning would affect males and females equally.

In conclusion, the difference between males and females in reaching criterion is not due to the female being a poorer discriminator of individuality than males but rather due to some task related variable. Most probably there was a difference in the motivational states of the males and females, the female being less motivated, which could have caused the difference in time taken to reach the required criterion.

The aim of these experiments was to demonstrate that rats can recognise individuals, the discrimination task being the tool used to do this. The time taken to reach criterion is not an indication of the ability to discriminate individuals but of the ability to associate

individuality with food, and as such differences between individuals are due to factors affecting this association rather than the the ability to discriminate individuals. Thus the differences between individuals do not reflect differences in discrimination but rather differences in the learning of the association and as such are of minor importance compared to the original aim of demonstrating recognition of individuals.

2.7
INDIVIDUAL RECOGNITION IN THE RAT

The aim of these experiments has been to demonstrate the ability of rats to recognise individuals. The results from experiment 2.1 indicate that males and females can discriminate between two unfamiliar same-sex conspecifics who are cagemates. Similarly experiment 2.2 demonstrates that males and females can discriminate between two unfamiliar same sex conspecifics who are cagemates and siblings. From these studies can we conclude that rats do possess the ability to recognise individuals?.

As discussed earlier there are two criterion to be met before individual recognition can be demonstrated. First, the ability to perceive differences between two individuals and second, the ability to respond to the individual as previously seen or known. I shall first consider the ability of rats to distinguish individuals. The above experiments demonstrated the ability of rats to discriminate between two conspecifics, but is this based

on individual cues?. Previous studies in other species, for example, Goldman (1973), Weeden and Falls (1959), failed to demonstrate individual recognition because they used cues differing on familiarity, one individual being familiar the other unfamiliar. In our experiment the stimuli were both unfamiliar to the subject. Both stimulus animals came from the same cage overcoming the possibility of subjects discriminating on the basis of different cage odours. In experiment 2.1, the discriminative stimuli were unrelated and thus there was a genetic difference between them. The possibility exists that genetically related individuals produce a particular odour common only to kin. Thus two genetically unrelated individuals may be distinguishable on the basis of this odour, but genetically related individuals indistinguishable. In experiment 2.2 the stimuli were siblings (r=0.5) and so, even if kin produce similar odours there is enough variability between them to allow them to be distinguished individually by others. We can be confident that the subjects do use individual cues in this task and therefore it is the individual that is being identified.

The second criterion for recognition requires that the subject responds to the individual as having been previously seen or known. The nature of the task requires the subject to learn to associate a particular individual with food each day. If the subject learns the task it can be said to have learned to associate the same individual

with food and thus responds in the same way to the same individual. From this it can be said that the individual responds to the stimulus individual as that which it had previously seen or known.

The experiment satisfies the two criterion for individual recognition, that is, demonstrates the ability of rats to distinguish between individuals and to respond to the stimuli as one which they had previously experienced. We can therefore conclude that rats do possess the ability to recognise individuals of the same species.

2.7.1
   The Difficulty Of Individual Recognition

Previously I have mentioned that the time taken to reach the required criterion is not an index of the ability to recognise individuals but rather the ability to form an association between food and a particular individual. The length of time taken to learn this may be due to a number factors. It may be due to a poor ability to discriminate, and a number of exposures are required before the subject can distinguish individuals. This is unlikely. If individual recognition is to be of use to animals, the individuality of conspecifics must be immediately available and not require a number of exposures before it can be identified.

Another possibility is that the association of food

with conspecifics may present some problems. The time taken to learn the association may be affected by some constraint on learning. Rats can easily form taste/odour-toxicosis aversions, (Domjan and Wilson, 1972) even as neonates (Gemberling and Domjan, 1982) but it takes them much longer to form taste/odour-shock aversions (Garcia and Koelling, 1966; Garcia et al., 1968). A similar difficulty may be experienced when trying to associate food with individual odours. In the natural environment food is usually found in a particular place and to get to this spatial cues are used, except when in very close contact when olfactory cues may be used to locate the food. Studies investigating olfactory discriminations have found that spatial cues tend to interfere with the discrimination and prolong the learning whereas if spatial cues are removed, the olfactory discrimination is much more easily learned (Eayrs and Moulton, 1960). In this experiment subjects were required to make a spatial decision at the choice point, to go left or right, and this may have interfered with their perception of olfactory cues and prolonged the learning. Another factor which could have prolonged the learning is the contiguity between the stimulus and reward. Experiments on olfactory discrimination learning using close stimulus-reward contiguity (e.g., Lashley and Sperry, 1943) have been able to demonstrate rapid learning, whereas those using widely separated stimulus and reward (e.g., Swann, 1933) found it very difficult or impossible to achieve any learning. In this experiment

the stimulus animal and food reward were quite widely separated and this may have prolonged the time taken to learn this task.

One final possibility is that by not cleaning the maze the rats were influenced by the performance of the previous rats. Rats are known to produce a different odour depending on whether they were rewarded or not which is detectable by other rats (Morrison and Ludvigson, 1970). The odour of non-reward seemingly exerting more influence than that of reward (Taylor and Ludvigson, 1980). This may have affected the perfomance of the rats in this experiment. However once the rat had chosen a side and crawled over the cage containing the stimulus a door was closed preventing access to the choice point. Therefore it could not deposit any odour associated with reward/non-reward at the choice point and thus this odour is unlikely to exert an effect on the rat at the choice point. This odour if deposited and detected could influence the rat from the stimulus cage to the goal box, causing it to run slower and reduce the contiguity between stimulus and reward. The maze was not cleaned throughout the experiment and the build up of odours may have masked any one particular odour, making the detection of non-reward odour difficult. This build up may also have affected the salience of the stimulus odours making the task more difficult for the subjects.

The time taken to achieve criterion in this task is a

reflection of the ability to associate the individual's odour with the food reward and this is affected by the interference of spatial cues, the stimulus-reward contiguity and possibly interference from odour cues deposited by other rats in the maze.

2.8
CONCLUSION

The experiments in this chapter have demonstrated that rats do possess the ability to recognise individuals and support previous behavioural observations suggestive of the ability of rats to recognise conspecifics (e.g., Telle, 1966).

The ability of rats to recognise and respond to conspecifics on the basis of individual cues may allow rats to respond altruistically to non-kin - reciprocal altruism. If a rat can recognise an individual as one whom it has observed giving aid to others and returning the aid given to it by others it would allow the rat to respond altruistically to this individual and it would enable rats not to respond altruistically to individuals it had not observed dispensing or reciprocating aid.

The ability to recognise individuals opens the door for one possible mechanism by which rats could achieve kin recognition - that based on the acquisition of individual identifiers. Animals would become familiar with particular individuals and respond on the basis of this,

the more familiar an individual, the more closely related that individual is assumed to be (see Bekoff, 1981). These experiments do not say whether rats use this mechanism to recognise kin but that the possibility exists for kin to be recognised via individually distinct characteristics.

CHAPTER 3

SIBLING RECOGNITION IN THE RAT

3.1

INTRODUCTION

In mammals, with the exception of monozygotic twins, siblings along with parents and offspring are the closest genetically related individuals having a coefficient of relatedness (r) of 0.5. This close genetic relatedness may favour interactions between siblings for two reasons. First, for any particular behavioural act an individual can gain a greater increase in its inclusive fitness by performing this with the closest related individuals, for example, siblings. Second, as altruism is favoured when the cost to the altruist is less than the benefit to the recipient, weighted by the degree of relatedness (Hamilton, 1964a,b), siblings will require a smaller gain in fitness to participate in altruistic acts than other less closely related individuals and thus more altruistic acts may be possible between siblings than other less related individuals.

Sibling interactions may also be favoured by environmental factors. Bekoff (1981) lists four conditions which favour sibling interactions: more than one individual per litter; individuals being born immature and requiring a period of development; the litter remaining in contact during development and finally

the litter being relatively isolated from unrelated
individuals. These conditions are well met in the rat
(e.g., see Barnett, 1975) and thus the environment of the
rat may predispose it towards sibling interactions.

Due to the increased likelihood of sibling
interactions and the greater gain in fitness individuals
may achieve by responding to siblings than other less
related individuals, it may be expected that individuals
will possess the ability to recognise siblings. This
chapter investigates the ability of rats to recognise
their siblings and the mechanisms used to achieve this.

3.2
THE ADAPTIVENESS OF SIBLING RECOGNITION

As siblings are likely to interact with one another,
the ability to recognise siblings may confer advantages
upon individuals capable of this. I have previously
discussed (see Chp. 1, section 1.3.3.2) how kin
recognition can increase the inclusive fitness of an
individual and shall not return to this here.

Recognition of siblings may reduce competition for
resources between siblings, possibly commencing in the
uterus (see Seger, 1976). Porter et al. (1980) report
that siblings of the spiny mouse, Acomys cahirinus, show
greater amounts of food sharing than do non-siblings.
Sibling recognition may increase co-operation amongst
sibling individuals. Huddling behaviour, a dynamic

process (Alberts, 1978a), allows a relatively constant temperature to be maintained (Alberts, 1978b). Co-operation by siblings in huddling may lead to better temperature regulation. Play behaviour, important for the development of the rat (e.g., Einon and Morgan, 1977; Einon et al., 1978; Morgan, 1973; see also Chp. 9, section 9.3), is a behaviour in which siblings may gain advantages in their fitness by preferentially playing with sibs. Finally sibling recognition may be important in the avoidance of inbreeding, which can lead to a decrease in fitness (e.g., Wilson, 1975).

3.3
SIBLING RECOGNITION:
LITERATURE REVIEW AND COMMENTARY

Until recently there has been little work concerned with the recognition of siblings (compare literature reviews of individual recognition, Chp. 2, and mother-infant recognition, Chps. 4, 5, sections 2.4, 4.3, 5.2 respectively). Here as with the review of work investigating individual recognition I shall consider the available data in taxonomic groups.

3.3.1
Invertebrates

Behavioural observation of the sphecid wasp, Trigonopsis cameronii, indicates this invertebrate may be capable of sibling recognition. Individuals of this species steal much less prey from their nestmates, who are

likely to be related, than from non-nestmates who are not related (Eberhard, 1972).

Other studies have been performed in the laboratory and have demonstrated sibling recognition in a number of different species. Workers of the honey bee, _Apis mellifera_, are able to discriminate full sisters from half sisters preferring to swarm with their full sisters (Getz et al., 1982). Recognition making use of a genetically determined identifier (Getz and Smith, 1983). The sweat bee, _Lassioglossum zephyrum_, can distinguish between different classes of kin using a genetically determined odorous identifier (Greenberg, 1979) which it learns from its nestmates (Buckle and Greenberg, 1981). Desert woodlice, _Hemilepistus reamuri_, are capable of sibling recognition and use a family specific chemical badge to discriminate siblings from non-siblings (Linsenmair, 1972).

3.3.2

Fish

Fish may be capable of sibling recognition. Kuhme (1963) reports the young of the cichlid fish, _Hemichromis bimaculatus_, orientate to water from their sibs in preference to that from similarily aged young of other species. This may be due to the ability to recognise siblings or simply due to the ability to discriminate between species. For definite evidence of sibling recognition individuals should be tested for their ability

to discriminate between siblings and non-siblings of the same species.

3.3.3
Amphibians

Amphibians have been shown to be capable of sibling recognition. Tadpoles of the Cascades frog, Rana cascadae, are able to recognise their siblings (O'Hara and Blaustein, 1981) by use of olfactory cues (Blaustein and O'Hara, 1982a), even when reared apart from other siblings (Blaustein and O'Hara, 1981), maternal cues rather than paternal cues being more important in the discrimination (Blaustein and O'Hara, 1982b). Similarly tadpoles of the American toad, Bufo americanus, prefer to associate with siblings over non-siblings in laboratory conditions (Waldman and Adler, 1979) and in their own habitat (Waldman, 1982), experience with their siblings not being essential for recognition and maternal cues being more important than paternal ones (Waldman, 1981).

3.3.4
Reptiles

As with individual recognition, the ability of reptiles to recognise siblings has been neglected and I found no studies indicative of the ability of reptiles to recognise siblings.

3.3.5

Birds

There have been many studies of the ability of birds to discriminate between individuals (see previous chapter, section 2.4.5) but there has been much less work on the ability of birds to recognise siblings. Mallard ducklings of the species, Anas platyrhynchos, inhibit their own distress call during that of their siblings, exhibiting almost perfect alternation response (Gaioni and Platter, 1982) and this may indicate the ability to recognise siblings. Japanese quail, Coturnix coturnix japonica, are able to discriminate between siblings, cousins and unrelated individuals showing a preference for their cousins (Bateson, 1978a,1982), this preference being used to achieve an "optimal balance" between inbreeding and outbreeding (Bateson, 1980).

Observational reports of the mating behaviour of certain species may be taken as evidence of the ability to recognise siblings. The Tasmanian hen, Tribonyx mortierii, due to an excess of males often breeds in groups of three, two males and a female, most commonly two brothers and an unrelated female. The tolerance of the male for another male in the group is dependent upon the other male being its brother (Maynard Smith and Ridpath, 1972). Similar co-operative mating has been observed in the Rio Grande turkey, Melagris gallopavo intermedia, (Watts and Stokes, 1971, but see Balph et al., 1980).

3.3.6

Mammals

In this group most research has concentrated upon rodents and primates. The white-footed deermouse, Peromyscus leucopus, shows signs of sibling recognition by investigating related individuals less than unrelated individuals (Grau, 1982). Adult male mice, Mus musculus, prefer unfamiliar females to their littermate sisters, but females show no preference for related or unrelated males (Hayashi and Kimura, 1983; see also Kareem and Barnard, 1982). Spiny mice, Acomys cahirinus, prefer to huddle with siblings (Porter et al., 1978; Porter and Wyrick, 1979), recognising sibs on the basis of olfactory cues (Porter et al., 1978) which appear to be learnt during early development (Porter and Wyrick, 1979). Food sharing also provides evidence for sibling recognition in the spiny mouse, A. cahirinus. Sibling pairs of this mouse were more active in exploring a novel environment containing limited food resources and engaged in a greater degree of co-operative feeding or food sharing than did non-sibs (Porter et al., 1980). Sibling recognition has been demonstrated in three species of ground squirrel, Richardson's ground squirrel, Spermophilus richardsonii, (Sheppard and Yoshida, 1971), the Arctic ground squirrel, S. parryii, and Belding's ground squirrel, S. beldingi, (Holmes and Sherman, 1982). In all these species individuals show less aggression to sibs than to non-sibs.

In non-human primates most evidence for sib

recognition comes from observations of nepotism amongst individuals of this group. Rhesus monkey, Macaca mulatta, siblings form alliances with brothers more often than with non-kin and intervened less in sexual encounters where a sibling brother was involved than expected (Meikle and Vessey, 1981). The grooming behaviour of bonnet macaques, Macaca radiata, exhibits signs of nepotism to kin as females groom relatives more than unrelated individuals. Females are more likely to support relatives than non-relatives incurring greater risks when they do so and direct more severe forms of aggression to non-relatives (Silk, 1982; Silk et al., 1981).The baboon, Papio anubis, prefers to play with its siblings more than would be expected (Owens, 1975). Baboons show a preference for siblings in grooming behaviour and were observed to intervene in an agonistic encounter on the behalf of siblings but never for unrelated individuals (Nash, 1978).

Whilst there have been many field reports of nepotism amongst siblings in primates (see above; also Bekoff, 1981), there have been fewer laboratory studies of sibling recognition. Wu et al. (1980) investigated the ability of infant pigtail macaques, Macaca nemestrina, to recognise their half-sisters. Individuals were observed to prefer their half-sisters to unrelated individuals.

Sibling recognition may be present in the lion, Panthera leo, where groups of male lions are often related. Such co-operative behaviour leads to a longer

tenure of female prides and enables production of more offspring than single lions (Bygott et al., 1979).

The ability of humans to recognise siblings by visual and probably auditory cues is well known, although little is known of the development of this ability. Recently the ability of individuals to recognise siblings by olfactory cues has been demonstrated (Porter and Moore, 1981).

3.3.7
Summary and Critique

Studies of sibling recognition have used two main strategies. Firstly studies have observed the naturally occuring behaviour of individuals and recorded the distribution of behaviour to siblings and non-siblings, to see if there are any signs of differential responding. Most of these studies have been performed in the wild, for example, Bygott et al., (1979); Maynard Smith and Ridpath, (1972); Nash, (1978). Such studies may be criticised on two grounds.

First, the studies often fail to give the expected value of occurence of a particular behaviour by chance. Individuals who are surrounded by kin have more opportunity to interact with kin, and therefore behaviour between kin would be expected to occur more often due to their spatial distribution alone, and not necessarily through any intentional nepotism or sib recognition (see also Altman and Walters, 1978).

A second problem occurs in the assessment of the genetic relatedness of individuals observed in the wild. It is often difficult to determine accurately the degree of relatedness between two individuals, paternity especially posing a problem (Kurland, 1977). Without knowlege of the genealogies involved assertion of a behaviour as being differentially given to kin and non-kin is speculative (see for example Balph et al.'s, 1980, criticism of Watts and Stokes, 1971).

The second technique to investigate sib recognition has used preference measures and has been mainly used by laboratory studies, for example, Bateson (1978a,b,1982); Breed (1981); Grau (1982); Kuhme (1963). Such techniques allow easy assessment of genetic relatedness through the use of laboratory bred animals and this technique has been used to document the ability of individuals to recognise their siblings in a wide variety of species.

3.4
THE ACQUISITION OF SIBLING RECOGNITION

A number of researchers, as well as examining the ability of animals to recognise siblings have also attempted to elucidate the mechanisms involved in the acquisition of this ability. Studies have attempted to investigate the role of socially and/or genetically mediated sibling recognition. I shall now review these.

The assessment of genetically mediated sibling recognition has used the general technique of testing unfamiliar siblings and comparing these with unfamiliar non-sibs, individuals showing a preference for either siblings or non-siblings or exhibiting signs of differential responsiveness, being judged to recognise siblings through a genetically mediated mechanism. Although many studies have demonstrated the ability of individuals to recognise unfamiliar siblings (see below), the methodology used does not allow a distinction to be made between the use of a genetically determined identifier and that of a genetically mediated recognition ability.

A number of different techniques have been used to establish unfamiliar siblings. Studies on amphibians, the American toad, <u>Bufo</u> <u>americanus</u>, and Cascades frog, <u>Rana</u> <u>cascadae</u>, have used individuals isolated from conspecifics until the time of testing (Waldman, 1981; O'Hara and Blaustein, 1981). Although the technique excludes experience with other kin the opportunity to learn from oneself is still present, and by isolation rearing the salience of one's own odour is increased, and such studies can only be used to argue for a genetically mediated identifier rather than genetically mediated acquisition of sibling recognition. A similar criticism applies to Wu et al. (1980) where pigtail macaques, <u>Macaca</u> <u>nemestrina</u>, were reared in isolation.

Breed (1983) and Buckle and Greenberg (1981), investigating the role of genetic factors in sibling recognition in the honey bee, Apis mellifera, and the sweat bee, Lasioglossum zephyrum, respectively, separated a group of kin and reared these together, apart from other kin. They then test this group individually with unfamiliar kin. Although this technique excludes the possibility of learning about the test individuals, kin are present during rearing and individuals may learn the cues from these individuals and use this to recognise their siblings. Again all that can be said from these studies is that the cue is genetically determined and nothing can be said about the mechanism of acquisition. Similar criticism can be applied to Grau (1982) who tested siblings from successive litters and to Kareem and Barnard (1982) who tested paternal half siblings. Although the test individuals were unfamiliar to each other they had the opportunity to learn about the identifier from other kin present during rearing. In an attempt to reduce the possibility of learning from other kin, individuals should be cross-fostered in non-kin litters. Holmes and Sherman (1982) go some of the way to achieve this and cross-fostered two individuals into a new litter such that each rearing litter consisted of two pairs of siblings. The opportunities for learning from kin are reduced as not all littermates are kin, but as the individuals are reared with a sibling the opportunity to learn from kin is still present.

Although studies have attempted to demonstrate the existence of genetically mediated sibling recognition, they have failed to distinguish between genetically mediated kin recognition and socially mediated kin recognition based upon a common genetic identifier. By rearing sibs with other kin (e.g., Breed, 1983; Buckle and Greenberg, 1982; Grau, 1982; Holmes and Sherman, 1982; Kareem and Barnard, 1982) or in isolation (e.g., Blaustein and O'Hara 1981; Waldman, 1981; Wu et al., 1980), individuals have the opportunity to learn about their siblings through a commonly possessed kin identifier and therefore make possible socially mediated kin recognition based upon a common genetic identifier as well as sib recognition via a genetically mediated mechanism. To overcome these problems individuals should be reared apart from ALL kin to prevent them from learning about kin, and reared with unrelated individuals to reduce the salience of their own identifying cues and thus decrease the possibility of learning about sibs from themselves.

Examination of the acquisition of sibling recognition via a social mechanism requires the exclusion of all possible genetic cues. The technique used to achieve this is to rear unrelated (r=0.0) individuals together and then test for a preference or for differential responsiveness to their littermates when compared to unfamiliar unrelated individuals. Buckle and Greenberg (1981) reared two sibling groups of the sweat bee, Lasioglossum zephyrum, together and found that individuals reared with another

sibling group would accept unfamiliar individuals of this sibling group more often than individuals reared only with their own sibling group, indicating the ability to learn about nestmates. The desert woodlouse, Hemilepistus reamuri, has the ability to learn new "badges" of other kin groups and will respond to these as its own family group (Linsenmair, 1972). Grau (1982) although testing non-sibling cagemates of the white-footed deermouse, Peromyscus leucopus, mentions little about these concentrating on the behaviour of siblings reared apart. Observation of his graphs indicates that non-sibling cagemates perform the behaviours, Ward, Chase, and Avoid less than do non-sibling non-cagemates and show decreased duration of nasal contact between cagemates than non-cagemates. Such results are indicative of an ability to learn about littermates and respond to these as kin. Kareem and Barnard (1982) using a similar rearing procedure to Buckle and Greenberg (1981) reared two different sibling groups of laboratory mice, Mus musculus, together and found that non-siblings reared together showed less sniffing, investigation and total interaction, but more touching and exploring than non-siblings reared apart. Similarily Porter et al. (1981) found by rearing unrelated individuals of the spiny mouse, Acomys cahirinus, together, these individuals when tested at a later date huddled together more than biological siblings reared apart and non-siblings reared apart. Holmes and Sherman (1982) found that in the Arctic ground squirrel, Spermophilus parryii, and Belding's ground squirrel, S.

beldingi, by rearing two pairs of siblings together these unrelated individuals when tested showed less agonism to each other than individuals reared apart. Although these studies demonstrate the ability of individuals to learn about littermates and respond to them as kin, the question of what is learned is unanswered. Do they learn the individual characteristics of the individual, or some commonly possessed group feature.

3.5
AN INVESTIGATION OF SIBLING RECOGNITION
IN THE RAT
EXPERIMENTS 3.1-3.4

Despite the probable important influence of siblings for each others social development, due to their close genetic relatedness and spatial proximity during development which affords good opportunity for sibling interactions, there have been no studies on sibling recognition in the rat.

The experiments here aim to investigate the ability of rats to recognise their siblings and the role played in this ability by both socially and genetically mediated mechanisms of kin recognition. To assess the possible role of socially mediated acquisition of sibling recognition pups were cross-fostered into other litters, such that no other kin were present and then tested for their ability to discriminate between littermates (with whom they were socially related but genetically unrelated,

r=0.0) and unfamiliar individuals. Pups were also tested for their ability to recognise siblings by use of a genetically mediated mechanism. Pups were examined for their ability to discriminate between siblings from whom they had been reared apart (genetically related, r=0.5, but not socially related) and unfamiliar individuals.

The pups were tested in a T-maze for their preference between 'siblings' and unfamiliar litters. Carr et al. (1979b) found that at 12 days of age, rat pups preferred familiar bedding odours, at 16 days showed no preference and at 20 days showed a reversed preference, preferring the unfamiliar bedding. From these results and Tolman (1961), who found pups preferred unfamiliar conspecific agemates at 22 days of age, it was hypothesised that if the pups could discriminate between their 'sibling' and unfamiliar pups, they would show a preference for the 'siblings' at 12 days, at 16 days show no preference and at 20 days show a preference for the unfamiliar pups.

In using a preference technique to assess whether individuals possess the ability to discriminate between two stimuli one runs across a major problem, in that the absence of a preference does not necessarily imply a failure to discriminate. It may be that the individual can discriminate between the two stimuli but shows no preference for either. One reason for this may be that neither stimulus is attractive to the individual. To test the 'attractive quality' of the litters used in this

experiment a further test was added. This involved having a litter on one side of the T-maze and an empty box on the other. It was conjectured that if the litter was an attractive stimulus, then the pups would prefer it to an empty box. Thus if the pups showed a preference for the litter in this test but showed no preference when confronted by 2 conspecific stimuli, this is unlikely to be due to a lack of attractive quality of the stimuli.

Another reason why no preference may be exhibited is because the pups have not the necessary physiological apparatus or development to enable them to detect the discriminative stimulus. In the rat the discriminative stimulus will be of an olfactory nature as this is ecologically the most important sense for the rat (see Chp. 1, section 1.4.1). Thus no preference may be due to the olfactory system being underdeveloped. Studies using pure chemical stimuli have indicated that rat pups can respond to these at birth (Small, 1899). However, to ensure that the pups could detect more complex olfactory stimuli (conspecific odour) they were tested for their preference for their own litter vs no litter at an early age, to see when they could respond to the chemical stimuli.

3.5.1

Subjects

Rat pups born in the Department of Psychology, University of Durham from outbred Sprague-Dawley rats

supplied by B & K (Animal Suppliers) Ltd. were used. Care was taken in breeding to ensure that all adult rats were unrelated (r=0.0). All cages were inspected twice daily and if a litter was found it was noted and that day termed day 0 in the pups life. All litters used in this experiment were born within 3 days of one another, so that on any particular day all pups were the same age +/- 1 day. All litters were culled to 7 pups on day 2 and on day 4 placed in one of 2 rearing conditions.

Rearing Condition 1: One litter was used in this condition consisting of its original members. This ensured that all rats in this condition were both socially, littermates, (S) and genetically, siblings, (G) related.

Rearing Condition 2: Seven litters were used in this condition. Each litter was numbered, such that the first litter contained rats all marked 1, litter 2 marked 2 and so on. These 7 original litters were then rearranged to make 7 new litters each containing 7 members, one from each of the 7 litters. Each new litter contained rats marked 1,2,3,4,5,6,7.

Thus no siblings were now in the same litter and these litters were left for the duration of the experiment. This rearing condition provided 2 experimental conditions: first the $S\widetilde{G}$ condition in which the rats were socially related (S) but were not

genetically (G̃) related, and secondly, the S̃G condition in which rats were socially unrelated (S̃) but were siblings (G).

The S̃G̃ condition consisted of pups unfamiliar, that is, neither genetically nor socially related to the pups in the SG, SG̃, S̃G conditions. All rats were marked daily with a Carioca Black felt-tip pen from day 4 to day 12 (marking took the form of writing the appropriate number on the back of the rat). From day 13 all rats were marked with alcoholic Picric acid every 3 days.

Rat pups were kept in plastic Bowman's cages (16"x12"x7") with food and water freely available on a 12 hour light/dark cycle, (darkness starting at 1200 hours).

3.5.2
Apparatus

Three different sizes of T-maze were used to accommodate the growing pups. All mazes were functionally identical differing only in size; because of this only apparatus 2 will be described in detail.

The apparatus (see fig. 3.1, plate 3.1) was a T-maze constructed of 2" diameter clear perspex tube. The start tube was 2" long and made such that the pup could be placed completely in the tube with its head at the choice point. The arms of the maze were 5" long and ended at the goal box. The goal boxes were made of clear perspex and were

PLATE 3.1
Preference testing apparatus.
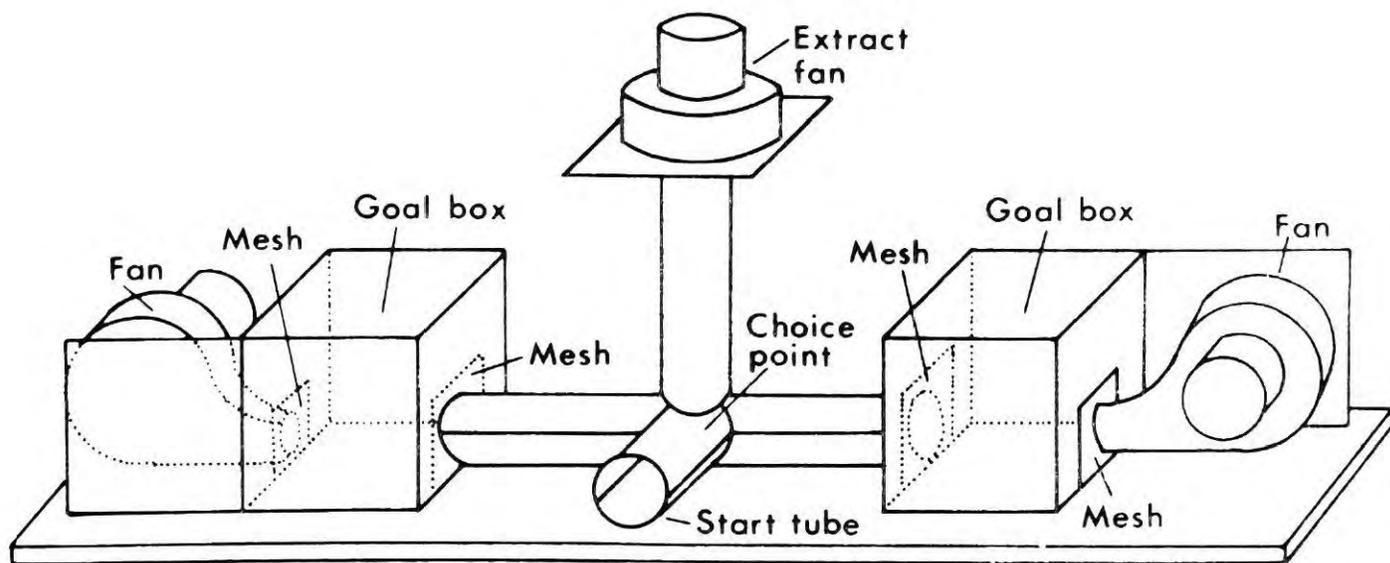
FIGURE 3.1
Diagrammatic   representation   of   preference   testing
apparatus.

5"x5"x5" square. Two 2" diameter holes were formed, both had half inch mesh across. Each arm of the T-maze fitted tightly into one of these holes, pups entrance to, and exit from, the goal box being inhibited by the wire mesh. Into the other hole of each goal box fitted an electric fan (Dayton Model IC 939, Dayton Electric MFG Co, Chicago 48) connected to an electricity supply and controlled by means of variable resistance to equate the running speed of each fan. The fans blew air through the goal box and into the arms of the T-maze. Each goal box had an airtight top with 6 holes to allow air to be drawn into the box. Immediately above the choice point was an exhaust tube, connected to a fan which sucked air out of the maze. The T-maze had been cut in half with the top half left clear but the bottom half painted matt black to make the surface less slippery to enable the rats to crawl in the T-maze.

Apparatus 1 differed only from apparatus 2 by the fact that the arms of the maze were only 2.5" long. Apparatus 3 differed from apparatus 2 in a number of aspects. Firstly, the maze was constructed of 3" diameter perspex, the start tube was 3.5" long and the arms of the maze 10.5" long. The goal box was similarly made of clear perspex but 7"x7"x7" square with a partition which enabled the width of the box to be restricted to 5". The holes in each goal box which accommodated the maze were enlarged to 3.25" but the holes for the fans left the same size. In all other aspects apparatus 1 and 3 were

identical to apparatus 2.

Apparatus 1 was used for all pups until and including day 7. The short arms were necessary because such young pups have weakly developed crawling responses. Apparatus 2 from day 8 to day 14 inclusive, longer arms were used as the rats now crawl readily. Apparatus 3 was used from day 15 onwards, the larger diameter tube giving the pups greater mobility in the maze having outgrown apparatus 2. All pups had been placed in the T-maze individually for 2, 15 minute sessions prior to experimental testing.

3.5.3

Procedure

A number of experiments were carried out to investigate the preferences of infant rats for socially and/or genetically related individuals. The procedure for each experiment was identical with only the stimuli varying; thus the first experiment will be discussed in detail and only points of difference mentioned for the other experiments.

3.5.3.1

EXPERIMENT 3.1: The preference of infant rats for $S\widetilde{G}$ vs $\widetilde{S}\widetilde{G}$ pups

Rats were tested in this experiment for their preference for pups with which they had been reared since day 4 but which were not siblings, that is, the $S\widetilde{G}$ condition of rearing condition 2. A litter of $S\widetilde{G}$ rats

were placed in one goal box and a litter of unfamiliar conspecific agemates ($\widetilde{SG}$) placed in the other. (All pups had previously been exposed to the goal box in litters for 2, 15 minutes sessions prior to the experiment). Both litters were placed on tissue paper in the goal box and left for 10 minutes with all 3 fans on, such that air was drawn over each litter, carried to the choice point and then sucked up the exhaust tube. A test pup was then removed from the goal box containing the $\widetilde{SG}$ pups, held in tissue paper for 30 seconds before being placed completely in the start tube with its head at the choice point. The pup was then left until it crawled to one side or the other its head touching the mesh of the goal box separating it from the maze arm. The rat was said to have preferred that side and its response, which side it had chosen, recorded. The rat was then removed from the maze by removing the top half of the maze, and replaced in the goal box. Both halves of the maze were thoroughly cleaned with Tego (an odourless disinfectant which is used to eliminate the odour trail of the pups) and water, flushed with water and dried. The top was replaced and left for one minute before the next rat was removed from the goal box and placed in the start tube. This procedure was repeated until all the test rats of the $\widetilde{SG}$ litter had been tested. Both litters were removed and both goal boxes cleaned with Tego and water, water, and dried. Two new litters were placed in the goal box one a $\widetilde{SG}$ litter, the other unfamiliar ($\widetilde{SG}$) to the $\widetilde{SG}$ litter and the procedure repeated. Ten rats were tested from 2 litters. Each rat

was given 1 trial per day for 15 days commencing day 10
and ending day 24. All tests were carried out at
approximately the same time each day, each $\widetilde{SG}$ litter
experiencing the same unfamiliar litter and being randomly
placed in one of the goal boxes.

3.5.3.2
EXPERIMENT 3.2: The preference of infant rats
for $\widetilde{SG}$ vs $\widetilde{\widetilde{SG}}$ pups

Rats in this experiment were tested for their
preference for siblings (from whom they had been separated
since day 4) as opposed to unfamiliar pups of equivalent
age. The litters of siblings were obtained from the
litters in rearing condition 2 by reconstructing their
original birth litter and placed in one of the goal boxes.
A litter of similar aged pups but unfamiliar to those in
the $\widetilde{SG}$ condition were placed in the other goal box. The
procedure was identical to that described in experiment 1.
Thirteen animals were tested from 2 different litters,
testing commencing on day 10 and terminating on day 24.

3.5.3.3
EXPERIMENT 3.3: The preference of rat pups for
$S\widetilde{G}$ pups vs a clean goal box

Rats in this experiment were tested for their
preference for littermates (who were socially but not
genetically related, thus $S\widetilde{G}$) against a clean goal box.
Rat pups from rearing condition 2 ($S\widetilde{G}$) were placed in one
goal box on tissue paper. The other goal box was left

clear except for pieces of tissue paper crumpled up to 'represent pups'. Test animals were taken from the $\widetilde{SG}$ litter and placed in the start tube. The procedure was then identical to that described in experiment 3.1. Ten animals were tested from 2 different $\widetilde{SG}$ litters from day 10 to day 24.

3.5.3.4

EXPERIMENT 3.4: The development of pup
attraction to social stimuli

Rats in this experiment were tested for their preference for their own litter (SG) as opposed to a clean goal box. Eight individuals from a single litter were used. These were placed in one goal box with nothing in the other except tissue paper again used to simulate pups. This experiment used rats of a younger age than experiments 3.1-3.3, using rat pups aged 4 days and was an attempt to plot the developing attraction of rat pups to littermates. Rats in this experiment began testing in apparatus 1 and were tested from day 4 to day 12. The procedure for testing was the same as that used in the other experiments.

3.5.4

Analysis

The number of rats crawling to the side of their "siblings", that is, $\widetilde{SG}$ or $\widetilde{SG}$ on each day was expressed as a percentage and plotted graphically. Previous research (Carr et al., 1979b; Gregory and Pfaff, 1971) has shown

that rats at 12 days exhibit a preference for familiar odours but at 20 days this preference is reversed and rats show a preference for unfamiliar odours. To examine this possibility, the individual rats results were divided into two sections, days 10-14 (mean day 12) inclusive and days 18-22 (mean day 20) inclusive. Within each time period the side the rat pup preferred overall (defined as the side with the greatest number of reponses) was obtained. Thus for each rat we had the side preferred in the 2 time periods. The number of rats preferring the 'sibling' and unfamiliar litter in each time period was calculated and a 2x2 contingency table drawn up of time (days 10-14, days 18-22) and litter preference ('sibling'; $\widetilde{S}G$ or $S\widetilde{G}$, unfamiliar; $\widetilde{S}\widetilde{G}$). A Fisher Exact Probability (F.E.P.) test (see Siegel, 1956) was then carried out to determine whether the 2 time periods differed significantly in the proportion of preferences expressed for 'sibling' and unfamiliar.

3.5.5

Results

3.5.5.1

EXPERIMENT 3.1: $S\widetilde{G}$ vs $\widetilde{S}\widetilde{G}$

The number of pups preferring the $S\widetilde{G}$ litter is expressed as a percentage and plotted graphically in figure 3.2. Observation of the graph indicates that more than 50% of rats preferred the side containing socially related pups in the first half of the test and in the second half less than 50% preferred the familiar $S\widetilde{G}$
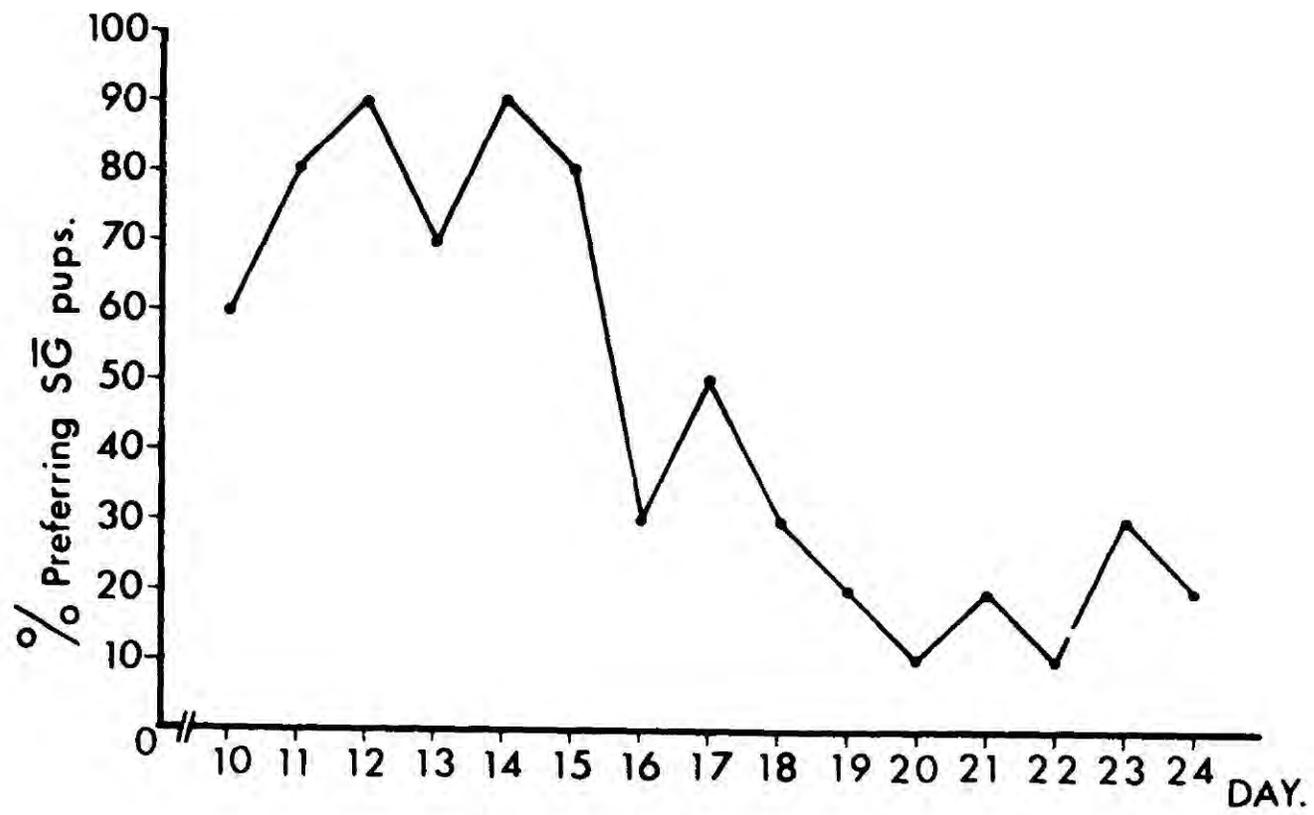
FIGURE 3.2
The percentage of pups preferring the socially related
litter (SG̃) on each day of testing.

litter.

The Fisher Exact Probability test (see table 3.1) indicates there is a significant difference in the proportion of rats preferring the $\widetilde{SG}$ and unfamiliar littermates in the two time periods (p<0.001). Binomial tests (see Siegel, 1956) indicating significantly more pups prefer the $\widetilde{SG}$ littermates on days 10-14 (N=10, x=0, p=0.001, 1-tailed), but more rats preferring the unfamiliar rats at days 18-22 (N=10, x=1, p=0.011, 1-tailed). One tailed tests were used as it had been predicted prior to the experiment which side the pups should prefer in each age group. One tailed probabilities were used as it was predicted which side the pups would prefer in each time period prior to the experiment.

The results indicate that pups do show a preference for littermates and this preference changes so that by day 20 the pups prefer unfamiliar agemates. Thus we can infer that rats do possess the ability to recognise socially related rats.

3.5.5.2
EXPERIMENT 3.2: $\widetilde{SG}$ vs $\widetilde{\widetilde{SG}}$

Observation of figure 3.3 indicates that in the first half of the experiment the pups prefer their siblings, but in the second half more rats prefer their unfamiliar agemates.

TABLE 3.1

Summary of the number of pups showing an overall preference for the 'sibling' and 'unfamiliar' litters at 10-14 and 18-22 days of age for experiments 3.1 - 3.3 with Fisher probability.

	SIDE PREFERRED	TIME PERIOD (DAYS)		FISHER PROBABILITY
		10-14	18-22	
EXPT. 3.1	$\widetilde{SG}$	10	1	p<0.001
	$\widetilde{\widetilde{SG}}$	0	9	
EXPT. 3.2	$\widetilde{SG}$	12	3	p<0.01
	$\widetilde{\widetilde{SG}}$	1	10	
EXPT. 3.3	$\widetilde{SG}$	9	9	p~1
	O	0	0	
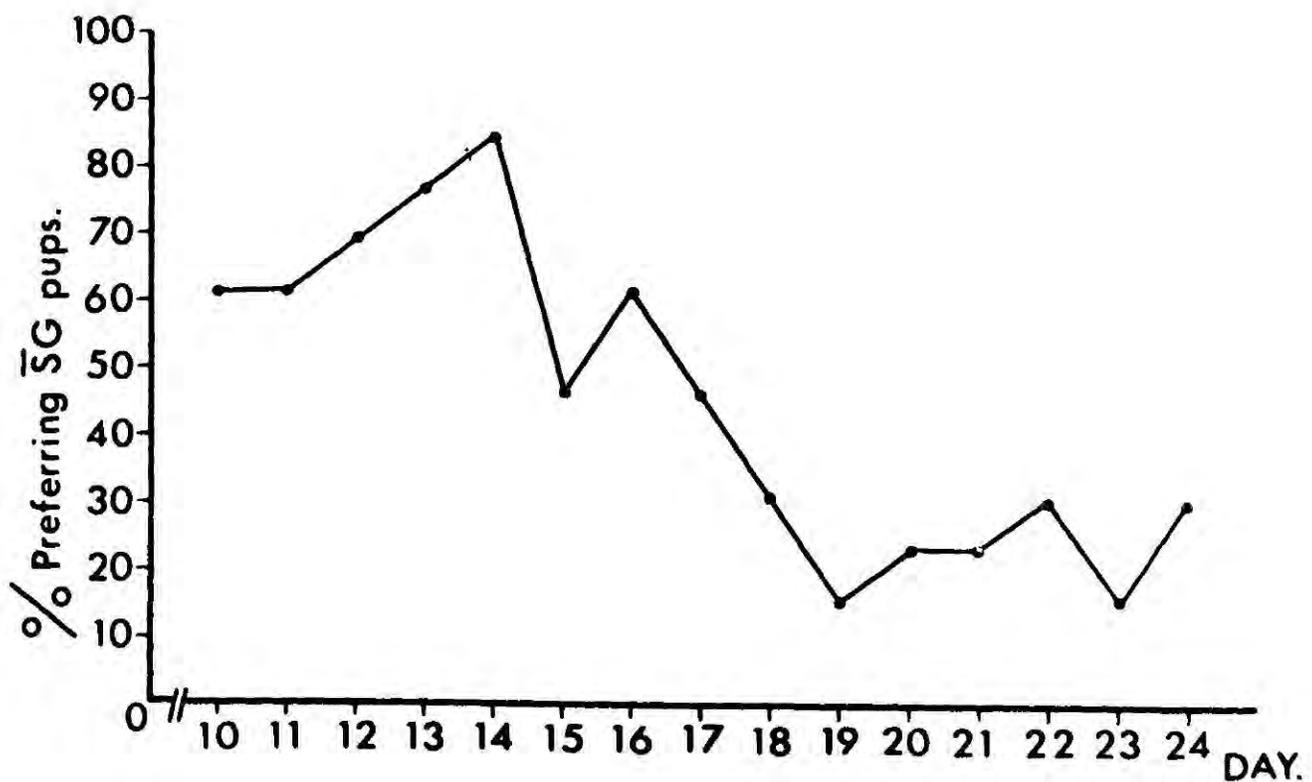
FIGURE 3.3
The percentage of pups preferring the genetically related litter ($\widetilde{SG}$) on each day of testing.

The F.E.P. test (see table 3.1) indicates there is a significant difference in the proportion of rats preferring the familiar ($\widetilde{S}G$) and unfamiliar litters in both time periods (p<0.01). Binomial tests for each time period indicate more pups prefer their siblings on days 10-14 (N=13, x=1, p=0.002, 1-tailed) but at days 18-22 more pups prefer the unfamiliar litter (N=13, x=3, p=0.046, 1-tailed).

The results indicate that the pups do show a preference which, like experiment 3.1, shows a reversal over the course of the experiment. We can infer from the data that rat pups can recognise their siblings even though they had been separated from them at 4 days of age.

3.5.5.3
EXPERIMENT 3.3: S$\widetilde{G}$ pups vs a clean goal box

Analysis of this experiment was confined to 9 pups as one died after the start of the experiment and its results were discarded. The graphical record (see fig. 3.4) of daily preferences indicates that the pups consistently preferred the side containing their S$\widetilde{G}$ related littermates over the side of the empty goal box. There being no significant difference between the distribution of preference on days 10-14 and days 18-22 (see table 3.1).

The results indicate that rats are able to discriminate social stimuli from no stimuli and that a litter of similarly aged rat pups have an attractant
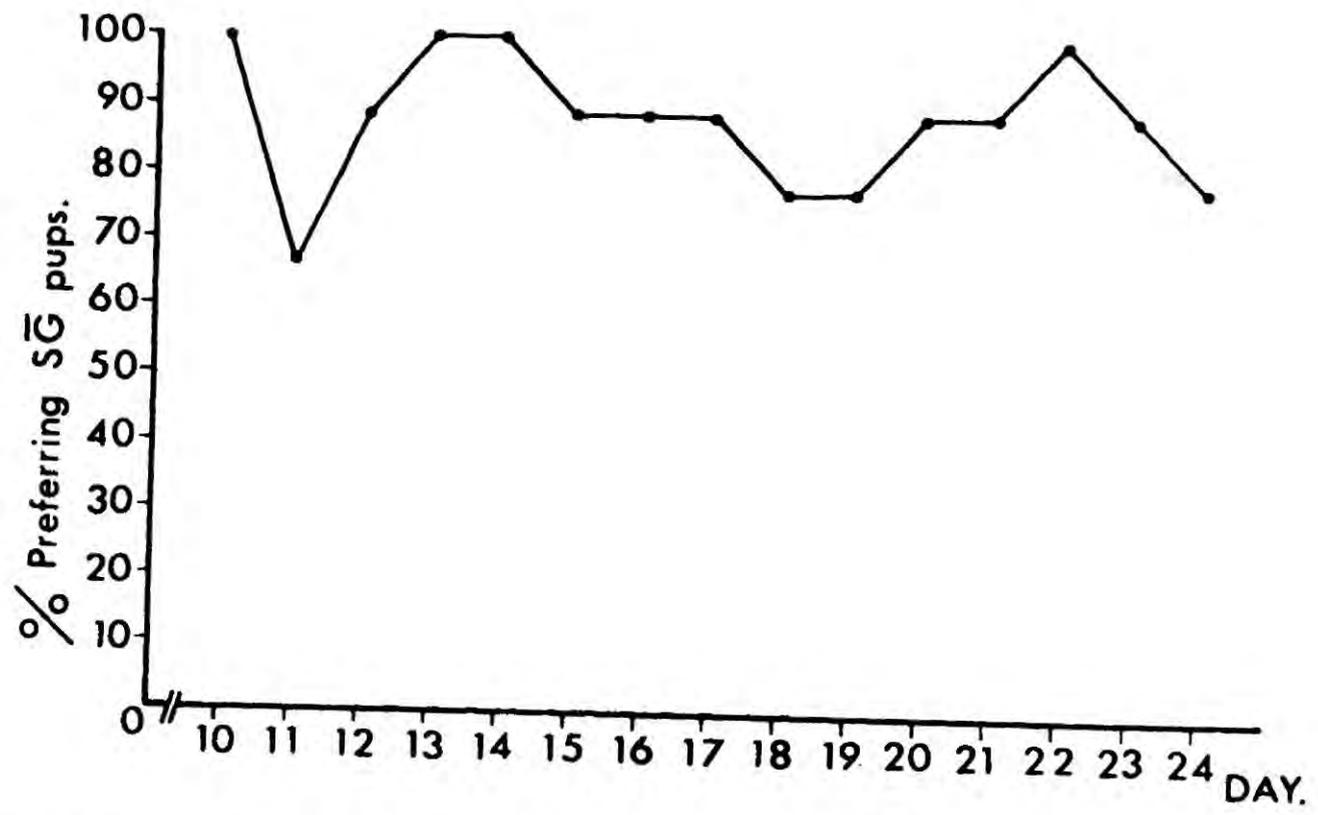
FIGURE 3.4
The percentage of pups preferring the litter of S$\tilde{G}$ pups on each day of testing.

quality.

3.5.5.4
EXPERIMENT 3.4: The development of pup attraction to social stimuli

Due to the young age of the pups a supplementary reponse measure was taken to augment the crawling response. As the very young rats, days 4-5, have an underdeveloped ambulatory response, the crawling response may be an inappropriate measure for preferences so head orientation was used. For this two white lines were painted in each arm of apparatus one, level with the edges of the start tube. If a pup crossed this line and remained for 15 seconds and no crawling response observed, the side the pup was facing was recorded. As for the crawling response the number of rats showing a head orientation to the SG litter was noted each day and plotted graphically (see fig. 3.5).

It was intended to continue the experiment until day 12, but observation of the graphical record indicated the experiment had started too late as by day 4, 70% of the pups showed head orientation to the SG litter and from day 5 - day 8 a minimum of 6 out of 8 rats crawled to the litter. Thus the experiment was terminated on day 8.

The results of this experiment indicate that as early as day 4 infant rats can detect and respond to odours emitted by their littermates.
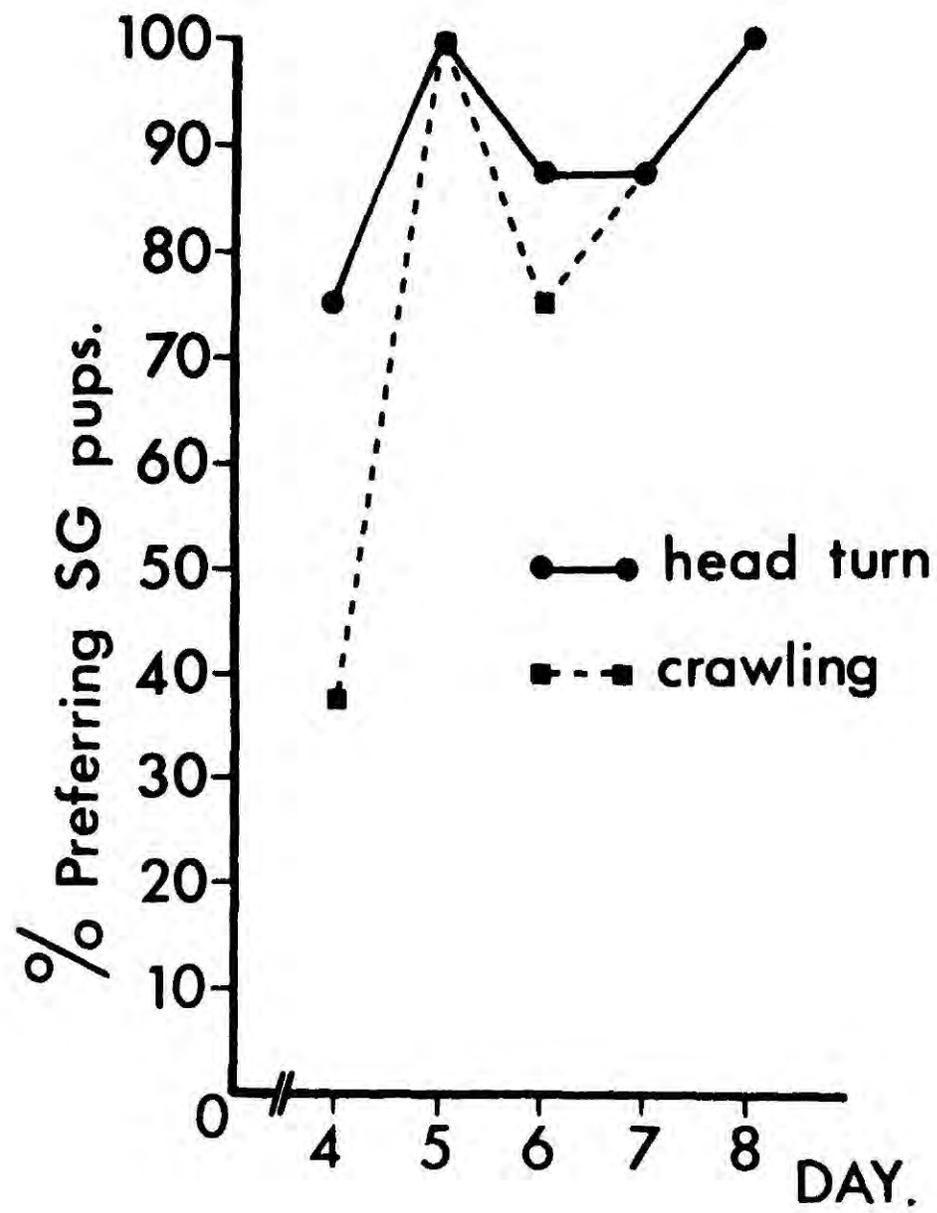
FIGURE 3.5
The percentage of pups preferring the litter of SG pups with the preference being measured by a crawling response and a head turn on each day of testing.

3.5.6
Discussion

The results of experiment 3.1 showed that individuals do have the ability to discriminate between socially related pups and unfamiliar agemates, demonstrating that pups can learn the characteristics of their littermates and do respond differentially to them. Similarly in experiment 3.2, the pups showed an ability to discriminate between siblings from whom they been separated since day 4 and unfamiliar pups. As the pups responded to their siblings as familiar it can be concluded that they did recognise them as siblings. Experiment 3.3 shows that the litter does provide an attractive stimulus to the pups and the result of experiment 3.4 shows that even as early as day 4 and probably earlier the pups can detect and respond to the stimulus provided by other pups.

As the pups were able to recognise the various stimuli we should consider how this is performed. First, what is the cue used?. As has been said previously it is likely that olfaction is the main cue used and the apparatus was designed to promote olfactory information. It is unlikely that other cues are used for the following reasons.

Auditory stimuli may be divided into 2 classes, first audible auditory stimuli (0-20 KHz). Throughout the experiment a white noise generator was used to provide a low level masking stimulus, but occasionally squeaks from

the pups in the goal box could be heard above this. Observation of the test rat indicated no immediate response or orientation to these sounds. The pups external auditory meatus did not open until day 9/10 and the pups showed no reaction to a hand clap until this time, thus the role of audition in experiment 3.4 can be completely excluded. Second, young pups also produce ultrasounds in the 60-80 KHz range when cold (Allin and Banks, 1971; Sales and Smith, 1978). The possibility that these calls could provide cues was examined by monitoring the ultrasound calls, with a specially built detector. Of all the calls detected, 48% of the pups went to the side of the caller, and in no case was an immediate orientation or response to the side of the caller noted. It is unlikely that auditory stimuli played a role in the discrimination.

The pups' eyes did not open until day 14/15 and since the pup is able to discriminate between the stimuli before this, it is unlikely that vision is essential for the discrimination. In experiments 3.1 and 3.2, the visual stimuli presented by each side were controlled by having equal numbers of pups in each goal box. Visual stimuli were controlled in experiment 3.3 by the use of white tissue paper placed in the empty goal box. It is therefore unlikely that visual stimuli play a role in the discrimination, especially considering the generally poor visual ability of albino rats (Birch and Jacobs, 1979; Lashley, 1930).

It may be that the tactile sense could play a role, when the rats were moving around in the goal box vibration may travel down the arm of the maze and provide a cue to the rat in the start tube. In experiments 3.1 and 3.2 any tactile stimuli would be randomly distributed between the two goal boxes and observations indicated no uneven distribution of movement between the boxes. In experiments 3.3 and 3.4, there were no rats on one side and tactile cues came only from one side. Observations of the pups in the goal boxes indicated that there was little movement during the experiment in these boxes, and the test pup showed no immediate orientation or response to one side after prolonged movement. Such evidence suggests that tactile cues were not utilised by the pups.

From this discussion it seems probable that olfaction is the sense used to discriminate between the stimuli. However before accepting that rats can recognise siblings, there are two other possibilities to consider.

The effect may be due to some familiarisation effect, that is, when the pups were placed in the goal box they become familiar with the other rats and may respond solely on the basis of this exposure prior to the experiment.

It is also possible that individuals may be responding by means of spatial cues. When removed from the goal box the pups might remember where they have been removed from and regard this side as familiar. At the

start of the study the pup returns to the 'familiar' side but in the second half of the experiment the pups prefer the unfamiliar side. The changeover in preference from the familiar to the unfamiliar pups could be due to either a centralised process which affects all preferences or it may be due to the onset of spontaneous alternation (Dember and Fowler, 1958; Douglas, 1966; Glanzer, 1953; Montgomery, 1952; Tolman, 1925). Spontaneous alternation usually begins at 28 days (Douglas, 1975), but in stressed rats the onset of spontaneous alternation is accelerated and occurs circa day 16 (Douglas, 1975). The pup may become stressed due to separation from the mother, handling or from the novel environment and this may cause the early onset of spontaneous alternation. Thus when removed from the goal box, from day 16 onwards, it responds to its placement in the goal box as trial one and when in the start tube alternates and crawls to the unfamiliar goal box.

Thus the ability to recognise siblings may be due to a familiarisation effect and/or spatial cues. To assess the importance of these the experiments were repeated with modifications to the procedure and equipment. A further condition was added, that of natural siblings, that is, genetically related (r=0.5) and littermates, the SG condition. Only points of difference will be described below.

3.6

FURTHER STUDIES ON SIBLING RECOGNITION

IN THE RAT

EXPERIMENTS 3.5-3.8

Two major procedural changes were used in these experiments to overcome the possible effects of familiarisation and spatial cues. First, the maze was rotated through 180 degrees for half the animals tested each day, and secondly test rats were never placed in the goal box, and thus never came into contact with the stimulus rats.

3.6.1

Subjects

The subjects were reared as before, except in this experiment pups were culled to 8 pups per litter and placed in two rearing conditions, but on day 2; three litters being placed in rearing condition 1 and 8 litters in rearing condition 2. All other details were as before.

3.6.2

Apparatus

The apparatus was unaltered except for being mounted on a turntable, which allowed it to be rotated through 180 degrees. For half the pups in each experiment each day the turntable was rotated through 180 degrees prior to the pups placement in the start tube. Therefore for these pups the spatially 'familiar' position is in the unfamiliar position, so if the pups were responding by

spatial cues no preference would be shown.

3.6.2.1

EXPERIMENT 3.5: The preference of rat pups for

SG vs S̃G̃ pups

This experiment involves a stimulus condition not previously used, that of natural siblings (individuals both socially related, littermates, and genetically related, siblings, r=0.5), SG from rearing condition 1. The procedure used is identical to that detailed in experiment 3.1 with two exceptions. First, for half the pups each day the apparatus was rotated through 180 degrees after pups removal from the goal box to control for any response to spatial cues. Second, each test pup was left in the maze for 2 minutes and the time the rat spent in each arm of the maze recorded. Once the rat's head had crossed the lines level with the edge of the start tube the timer corresponding to that side was started and stopped whenever the rat's head was not between that line and the goal box. Sixteen pups were tested in this condition from 2 litters from day 7 to day 22.

3.6.2.2

EXPERIMENT 3.6: The preference of rat pups for

S̃G vs S̃G̃ pups

The conditions were those of experiment 3.1 with two adjustments to control for the possible familiarisation and spatial learning effect. The major difference from

that experiment was that the test pups were never placed
in the goal box and thus had no chance to become familiar
with either the individuals in the goal box or its
position. Also experimental pups were left for two
minutes in the maze and the time spent in each arm
recorded. The maze was rotated through 180 degrees for
half the pups each day. All other procedural details were
identical to experiment 3.1. Eighteen pups were tested in
the experiment commencing day 6 and terminating day 22.

3.6.2.3
EXPERIMENT 3.7: The preference of rat pups for $\widetilde{S}G$ vs $\widetilde{S}\widetilde{G}$ pups

As with experiment 3.6 this study provided a
replication, in this case for experiment 3.2. In this
experiment however the siblings had been separated on day
2 and not day 4 as in experiment 3.2. The procedure used
was identical to experiment 3.6, that is, no test pups
used as stimuli, the time the test pups spent in each arm
in the 2 minute trial recorded, and the maze rotated for
half the pups each day. Eighteen pups were tested in this
condition commencing day 6 and terminating day 22.

3.6.2.4
EXPERIMENT 3.8: The development of pup attraction to social stimuli

This study is intended as a repeat of experiments 3.3
and 3.4, but with certain modifications. Experiment 3.4
had demonstrated that by day 5 75% of pups tested show a

response to the litter stimuli. Thus this experiment commenced on day 2 and used 24 pups per day to test for the onset of response to litter stimuli, from day 14 onwards the pups were tested every other day. The procedure was identical to that used in experiment 3.5.

3.6.3
Analysis

The analysis was identical to that used in experiments 3.1-3.4 with the exception of the response measure, which in these experiments was the side on which the pup spent the greatest amount of time in the two minute trial. Again graphs were drawn of the percentage of rats preferring the familiar/sibling litter, and the F.E.P. test was carried out to assess the significance of changeover in pup's preference.

3.6.4
Results

3.6.4.1
EXPERIMENT 3.5: SG vs $\widetilde{SG}$

The graphical record (fig. 3.6) indicates that in the first half of the experiment the pups prefer their siblings (SG), but in the second half exhibit a preference for the unfamiliar animals. The F.E.P. (see table 3.2) indicates there is a significant difference in the distribution of pups preferring the sibling and unfamiliar stimuli in the 2 time periods ($p < 0.0001$). Binomial tests indicating pups prefer their sibling littermates on days

FIGURE 3.6
The percentage of pups preferring the socially and genetically related litter (SG) on each day of testing.

10-14 (N=16, x=2, p=0.002, 1-tailed) and the unfamiliar pups on days 18-22 (N=16, x=0, p<0.002, 1-tailed). Using stimuli of natural siblings, the results indicate that pups were able to discriminate between the two stimuli and recognise their sibs.

3.6.4.2
EXPERIMENT 3.6: $\widetilde{SG}$ vs $\widetilde{\widetilde{SG}}$

Figure 3.7 indicates that more pups prefer the $\widetilde{SG}$ litter in the first half of the experiment and as in experiment 3.1 this preference is reversed and in the second half of the experiment pups prefer the unfamiliar litter. The F.E.P. (see table 3.2) shows there is a significant difference in the number of rats preferring the socially related and unfamiliar stimuli in the two time periods (p<0.0001). Binomial tests indicate that significantly more pups prefer the $\widetilde{SG}$ litter on days 10-14 (N=18, x=4, p=0.015, 1-tailed) and significantly more pups prefer the unfamiliar litter on days 18-22 (N=18, x=0, p<0.001, 1-tailed), the effect seems somewhat smaller on days 10-14 than 18-22 and more will be said of this later. The results of this experiment replicate experiment 3.1 and provide evidence of the pups ability to learn the characteristics of their littermates and to use this to discriminate them from unfamiliar rat pups.

TABLE 3.2

Summary of the number of pups showing an overall preference for the 'sibling' and 'unfamiliar' litter at 10-14 and 18-22 days of age for experiments 3.5 - 3.7 with Fisher probability.

	SIDE PREFERRED	TIME PERIOD (DAYS)		FISHER PROBABILITY
		10-14	18-22	
EXPT. 3.5	SG	14	0	p< 0.0001
	$\widetilde{SG}$	2	16	
EXPT. 3.6	S$\widetilde{G}$	14	0	p< 0.0001
	$\widetilde{S}\widetilde{G}$	4	18	
EXPT. 3.7	$\widetilde{S}$G	13	3	p< 0.005
	$\widetilde{S}\widetilde{G}$	5	15	
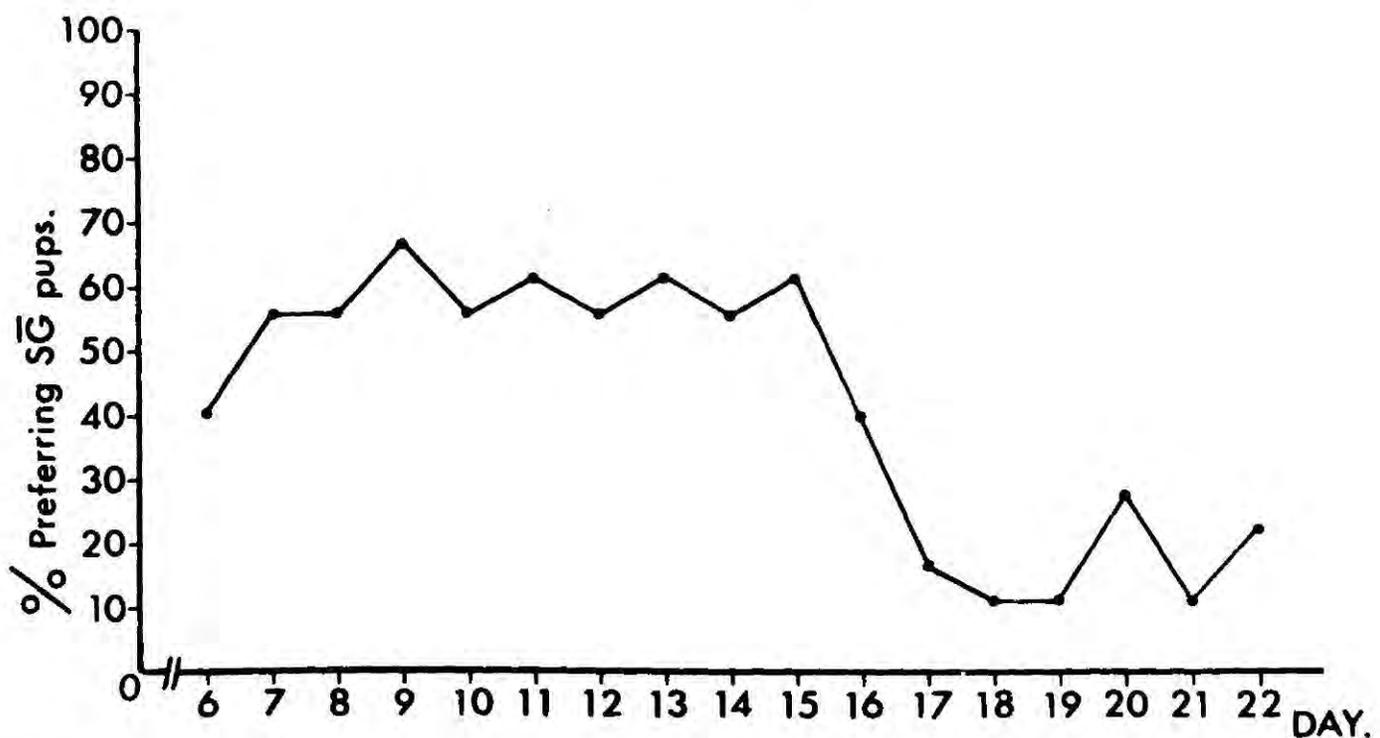
FIGURE 3.7
The percentage of pups preferring the socially related litter (S$\widetilde{G}$) on each day of testing.

3.6.4.3
EXPERIMENT 3.7: $\tilde{S}G$ vs $\tilde{\tilde{S}}\tilde{G}$

The results here provide a replication of the results of experiment 3.2. The graphical record (fig. 3.8) indicates that more pups prefer their siblings in the first half of the experiment but prefer the unfamiliar pups in the second half. The F.E.P. (table 3.2) indicates there is a significant difference in the proportion of pups preferring their siblings in the two time periods ($p < 0.005$). Binomial tests indicating significantly more pups prefer their siblings on days 10-14 (N=18, x=5, p=0.048, 1-tailed) but on days 18-22 significantly more prefer the unfamiliar pups (N=18, x=3, p=0.004, 1-tailed). This experiment again provides evidence of the pups ability to discriminate between the two litter stimuli and to recognise its siblings, even though they had been separated since day 2.

3.6.4.4
EXPERIMENT 3.8: The development of pup attraction to social stimuli

The results of this experiment are plotted graphically in figure 3.9. Observation of this graph indicates that as early as day 2 over 70% of the pups preferentially respond to the litter stimuli. This result supports and extends the observations of experiment 3.4. The results of experiment 3.3 are similarily replicated although there does seem to be a drop in the pup's preference around day 16.

FIGURE 3.8
The percentage of pups preferring the genetically related litter (S̃G) on each day of testing.
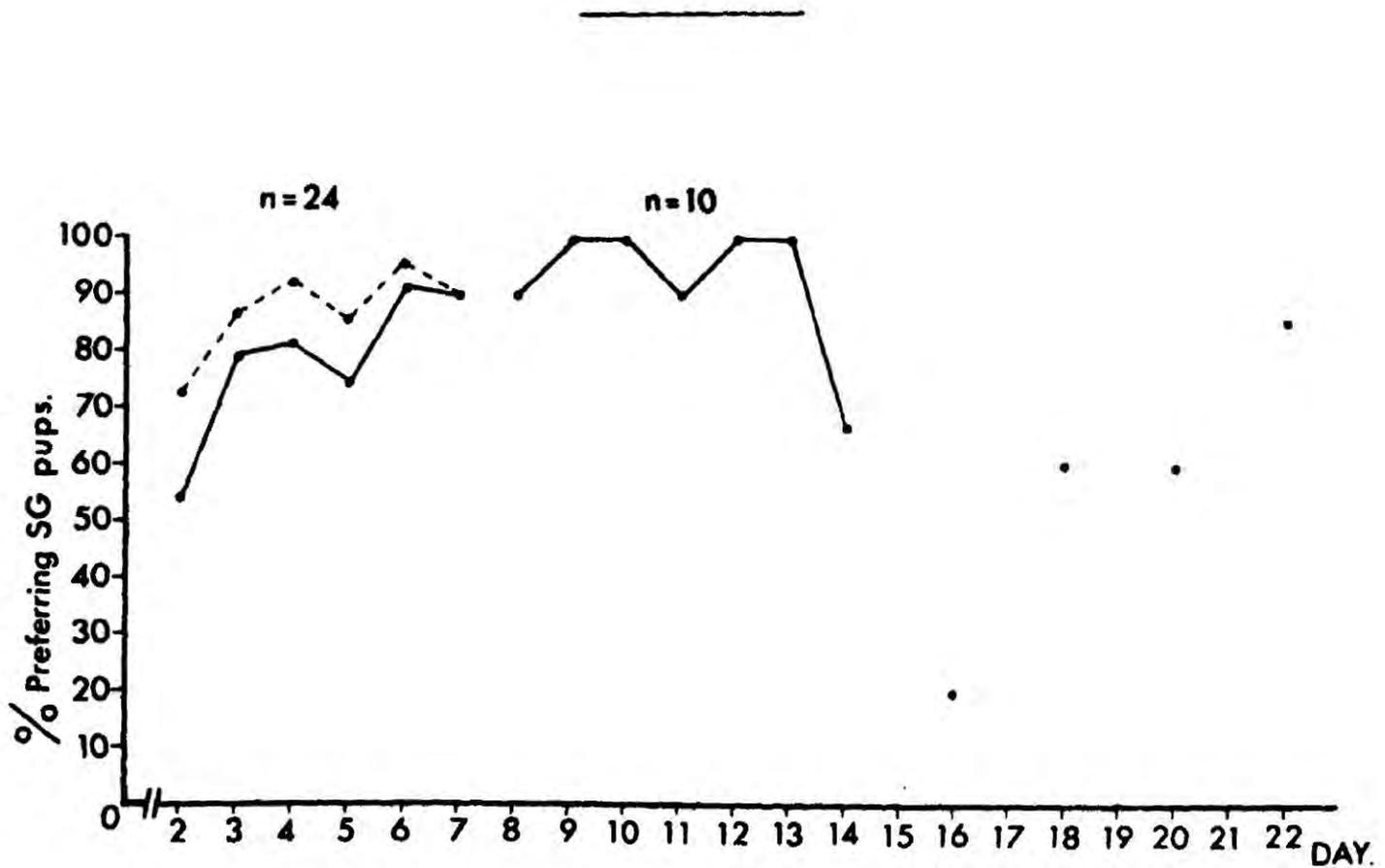
FIGURE 3.9
The percentage of pups preferring the litter of SG pups n=24 on days 2-7, preference excluding pups who showed no response (●----●) and preference using all pups (●——●), n=10 on days 8-22, pups tested daily until day 14 then every other day.

3.6.5

<u>Comparisons Between Experiments 3.1-3.7</u>

The results of these experiments provide evidence that pups can recognise related animals (their 'siblings') and that as early as 2 days old they have a functioning olfactory system. If one compares the graphs of experiment 3.1 and 3.2 with experiments 3.6 and 3.7 respectively certain differences can be seen. Pups in experiment 3.6 at days 10-14 seem to show a smaller preference than the pups in experiment 3.1 at the same age, and the graph of experiment 3.7 seems to show more variability than that of experiment 3.2. It could be that the effect obtained in experiments 3.6 and 3.7, although significant, is less strong than that in experiments 3.1 and 3.2, implying that spatial cues and familiarity did have an effect in the first experiment.

To assess this, comparisons were carried out between experiments 3.1 and 3.6 and between 3.2 and 3.7. The number of times each pup in experiment 3.1 (S$\widetilde{G}$ vs $\widetilde{S}\widetilde{G}$) preferred the 'sibling' side on days 10-14 was compared with the number of times each pup in experiment 3.6 (S$\widetilde{G}$ vs $\widetilde{S}\widetilde{G}$) preferred the 'sibling' side on days 10-14 by means of an independent t-test (see Robson, 1975). The results indicate there is a significant difference between the two experiments (t=2.8772, df=26, 0.01>p>0.001), the pups in experiment 3.1 showing a greater number of preferences for the 'sibling' side (mean preference 3.9 out of 5) than pups in experiment 3.6 (mean preference 2.888 out of 5).

Further comparisons were then carried out between the preference of pups in experiments 3.1 and 3.6 on days 18-22 (t=0.2448, df=26, 0.8>p>0.7, mean preference for 'siblings': experiment 3.1, 0.9; experiment 3.6, 0.8333 out of 5), experiments 3.2 (S̃G vs S̃G̃) and 3.7 (S̃G vs S̃G̃) on days 10-14 (t=0.3716, df=29, 0.8>p>0.7, mean preference for siblings: experiment 3.2, 3.5385; experiment 3.7, 3.3888 out of 5) and days 18-22 (t=1.3761, df=29, 0.2>p>0.1, mean preference for siblings: experiment 3.2, 1.2308; experiment 3.7, 1.7222 out of 5), none reaching significance.

The results of these experiments suggest that familiarisation and/or spatial cues could have had an effect on the choice of socially related rats at 10-14 days enhancing an already significant effect to prefer their socially related littermates. It is probable that this effect is due to familiarisation rather than spatial cues. Both conditions (SG̃, S̃G) having the same potential spatial cues but different familiarisation ones. In the SG̃ case the pups are placed in the goal box with pups with whom they have been with for the past 24 hours, however in the S̃G case the pups were placed in the goal box with pups (their siblings) from whom they had been separated for the past 24 hours. It may be that the time together, that is, when the pups in the S̃G condition are placed in the goal box is not enough to significantly increase the pups' preference or memory for these pups, but he same time spent apart, that is, when the SG̃ pups are separated prior

to testing may decrease the pups memory for their littermates. Thus one may expect no effect in the $\widetilde{S}G$ condition but would expect a decrease in preference in the $S\widetilde{G}$ condition, where littermates are separated prior to testing, which occurs. By the time the pups are tested at days 18-22 the effect has disappeared because the pups have learned their 'siblings' characteristics better and now suffer no loss in retention for their characteristics over the time of separation.

A series of comparisons were then carried out within experiments 3.1 and 3.2 and experiments 3.5, 3.6 and 3.7, to assess whether the different rearing conditions had an effect on the strength of preference. The number of preferences of each pup for the 'sibling litter' was compared between experiment 3.1 ($S\widetilde{G}$ vs $\widetilde{S}\widetilde{G}$) and experiment 3.2 ($\widetilde{S}G$ vs $\widetilde{S}\widetilde{G}$) by use of an independent t-test. At days 10-14 (t=1.2375, df=21, 0.3>p>0.2) and days 18-22 (t=0.6909, df=21, 0.5>p>0.4) no significant differences between the magnitude of preferences shown by the pups at both age ranges were found. Thus the effect of both social relatedness (S/$\widetilde{S}$) and genetic relatedness (G/$\widetilde{G}$) were equivalent in both experiments. Similar comparisons were then carried out between experiments 3.5, 3.6 and 3.7 (see table 3.3). On days 10-14 the only significant difference occurs between pups SG related and pups S$\widetilde{G}$ related, the SG pups showing a stronger preference for their siblings (mean preference, 3.625 out of 5) than S$\widetilde{G}$ pups (mean preference, 2.8889 out of 5).

TABLE 3.3

Summary of t-test results for comparison of the number of rats having a preference for their 'sibling' litter between experiments 3.5 - 3.7 on days 10-14 and days 18-22.

DAYS 10-14.

	EXPT. 3.5	EXPT. 3.6	EXPT. 3.7
EXPT. 3.5	X	t=2.0357 df=32 0.05>p>0.02	t=0.5732 df=32 0.6>p>0.5
EXPT. 3.6	X	X	t=1.2888 df=34 0.3>p>0.2
EXPT. 3.7	X	X	X

DAYS 18-22

	EXPT. 3.5	EXPT. 3.6	EXPT. 3.7
EXPT. 3.5	X	t=2.0172 df=32 0.1>p>0.05	t=4.7949 df=32 p<0.001
EXPT. 3.6	X	X	t=4.2942 df=34 p<0.001
EXPT. 3.7	X	X	X

This could be due to either these pups being innately predisposed to recognise siblings, or having been together for longer (since conception as opposed to day 2 after birth), they have had more opportunity to learn the characteristics of their siblings, for example, in utero, or a combination of these two, (further discussion of the importance of these two factors will be given later).

At the age range 18-22, SG pups (expt. 3.5) exhibited a greater preference for the unfamiliar pups than did S̃G pups (expt. 3.7), mean number of preferences by SG for siblings, 0.5 out of 5 and by S̃G pups, 1.7222 out of 5. Similarily SG̃ pups (expt. 3.6) showed a greater preference for the unfamiliar litter than did the S̃G pups (expt 3.7), mean number of preferences by SG̃ for 'siblings', 0.8333 out of 5 and by S̃G pups, 1.7222 out of 5. No significant differences were found between the preferences exhibited by the pups in experiment 3.5 and experiment 3.6.

The results obtained from these comparisons suggest that pups who are littermates show a stronger attraction to the unfamiliar litter at days 18-22 than do S̃G pups. This may indicate that the SG and SG̃ pups have learned who are their siblings more strongly than the S̃G pups. This might be expected since they are together for 24 hours of the day.

The results of experiments 3.5, 3.6, 3.7 and 3.8

replicate and extend the findings of experiments 3.1-3.4 providing controls for possible effects of familiarisation and spatial cues, although comparisons of the two series of experiments indicate these factors only exert a significant effect in the $S\tilde{G}$ condition at days 10-14 enhancing the pups' preferences for their littermates. Within series comparisons indicate at days 18-22 pups socially related (SG and $S\tilde{G}$) show a greater preference for the unfamilair litter than do socially unrelated ($\tilde{S}G$) pups.

3.7 EXPERIMENT 3.9: SIBLING RECOGNITION AT 40 DAYS

The previous experiments have demonstrated the ability of rat pups to recognise their siblings between 7-22 days of age. Comparisons between the various conditions indicated that the ability of only socially related individuals ($S\tilde{G}$) increased over the duration of the experiment, whereas the ability of only genetically related individuals ($\tilde{S}G$) may have decreased. This may be expected since socially related pups are together for 24 hours of the day, whereas genetically related pups have been separated since day 2. In this experiment pups were tested for their preference for either natural siblings (SG), socially related ($S\tilde{G}$) or genetically related ($\tilde{S}G$) siblings and unfamiliar ($\tilde{S}\tilde{G}$) pups at day 40.

3.7.1

Method

The subjects were those used in experiments 3.5,3.6 and 3.7 and the procedure used identical to that for these experiments. The subjects had been weaned on day 25, and in this experiment rats were tested only once, on day 40. The time each individual spent in each side of the maze was recorded.

3.7.2

Analysis and Results

The results for each condition SG, $S\tilde{G}$ and $\tilde{S}G$ were analysed separately. Two analyses were performed, first the side on which the individual spent the greatest amount of time was obtained and a binomial test (see Siegel, 1956) performed to see if there was any difference in the preferences of the pups. Secondly the time spent by the pups on either side of the maze ('siblings' or unfamiliar) was analysed by means of a matched pairs t-test (see Robson, 1975).

Natural siblings (SG) still retained the ability to recognise their siblings. The binomial test indicated significantly more pups spent a greater amount of time on the side of the unfamiliar pups (N=16, x=2, p=0.004, 2-tailed). Similarily the matched pairs t-test indicated pups spent significantly longer on the side of the unfamiliar litter (mean time, 30.1 secs.) than on the side of their siblings (mean time, 19.6 secs.),

t=4.8361, df=15, p<0.001.

Significantly more socially related pups (S$\widetilde{\text{G}}$) spent a greater amount of time on the side of the unfamiliar pups (binomial test N=18, x=2, p=0.002, 2-tailed). Similarily the matched pairs t-test indicated pups spent significantly longer on the side of the unfamiliar litter (mean time, 28.3 secs.) than on the side of their siblings (mean time, 16.6 secs.), t=4.7628, df=17, p<0.001. These results indicate that socially related pups still possess the ability to recognise their 'siblings'.

Results from pups only genetically related (S̃G) indicated these pups could no longer recognise their siblings. The binomial test indicated pups did not spend a greater amount of time on one side or the other (N=18, x=6, p=0.238, 2-tailed) a result supported by the matched pairs t-test which indicated their was no difference between the time spent on the side of siblings (mean time, 31.9 secs.) and on the side of the unfamiliar pups (mean time, 28.1 secs.), t=1.3035, df=17, 0.3<p<0.2.

3.7.3

Discussion

The results of this experiment indicate that a period of social exposure to siblings after birth is essential for the maintenance of sibling recognition as individuals with no social experience of siblings (S̃G) show no

preference for them, whereas individuals with social experience SG, SG̃ still show a preference.

The reason for the lack of preference to sibs by S̃G individuals could be due to two reasons: loss of the ability to discriminate and/or loss of the preference.

The results of experiment 3.7 indicated these individuals (S̃G) did have a preference , that is, they could discriminate between their siblings and unfamiliar individuals. Their lack of preference now could be due to a loss of discriminatory powers. This is unlikely as other individuals in different rearing conditions, SG and SG̃ can still discriminate. It may be the lack of ability to discriminate is due to the discriminatory stimulus rather than the loss of discriminative ability by the test individual. The litter stimulus S̃G was constructed by reforming the original birth litter. It may be that by rearing with other individuals, siblings may come to possess a group odour and this may mask their genetic odour. Thus the stimulus presented by the litter is not only of the test pups but also of other pups. However the same may apply to experiment 3.7. but pups here were still able to recognise their siblings (S̃G).

It is more likely that this result may be explained by the loss of the preference for these individuals rather than a loss of discriminatory ability. The reason for this may be due to selective pressure on individuals to

have an adaptable mechanism for kin recognition. Individuals that find themselves only able to respond to one set of individuals, kin, may find themselves in difficulty if all other kin die, individuals would be left with no-one to respond to. Thus it may pay individuals to learn to respond preferentially to surrounding littermates if they do not receive any exposure to kin. I shall refer to this point later.

3.8 EXPERIMENT 3.10: THE USE OF OLFACTION IN SIBLING RECOGNITION

In the introduction and the discussion following experiments 3.1-3.4 it was argued that the most likely signal of kinship was olfactory. To examine this, experiment 3.5 was repeated using an identical procedure with the exception that the holes from the goal box into the arms of maze were blocked with a clear perspex bung and the fans were not run. This procedure was used to prevent any olfactory stimuli from the litter entering the maze. The pups in this experiment had access to both visual and auditory cues but were deprived of olfactory cues. Twenty pups were used in this experiment from five litters reared as for rearing condition 1. Pups were tested from day 10 to day 22 for their preference for their own litter (SG) or an unfamiliar litter ($\widetilde{SG}$).

3.8.1
Results

The results demonstrate the importance of olfactory cues in sibling recognition. The graph (fig. 3.10) indicates that pups, when denied access to olfactory cues, show no preference for their sibs in the first half of the experiment nor a preference for the unfamiliar litter in the second half (cf. experiment 3.5, fig. 3.6, where pups were allowed access to olfactory cues). The F.E.P. test (see table 3.4) indicates there to be no significant changeover in preference between days 10-14 and days 18-22 (p=0.3549) and binomial tests indicate no significant preference for siblings on days 10-14 (N=20, x=9, p=0.824, 2-tailed) or days 18-22 (N=20, x=10, p~1, 2-tailed).

The results of this experiment confirm previous discussions that siblings (and possibly other kin) are recognised on the basis of olfactory cues.

3.9
EXPERIMENT 3.11: THE USE OF INDIVIDUAL PUPS AS STIMULI

The previous experiments have all tested the ability of individuals to recognise litters of stimuli rather than particular individuals. Litters were chosen as these represent a valid stimulus for individual pups. Rats under natural conditions at the time of testing remain in their litters and any individual lost and trying to find its way back to its nest is likely to encounter a litter rather than a single individual. In order to examine

TABLE 3.4

Summary of the number of pups showing an overall
preference for the sibling and unfamiliar litter (expt.
3.10) and individual (expt. 3.11) at 10-14 and 18-22 days
of age with Fisher probability.

	SIDE PREFERRED	TIME PERIOD (DAYS) 10-14	18-22	FISHER PROBABILITY
EXPT. 3.10	SG	9	10	n.s.
	S̃G̃	11	10	
EXPT. 3.11	SG	11	5	p<0.05
	S̃G̃	5	11	
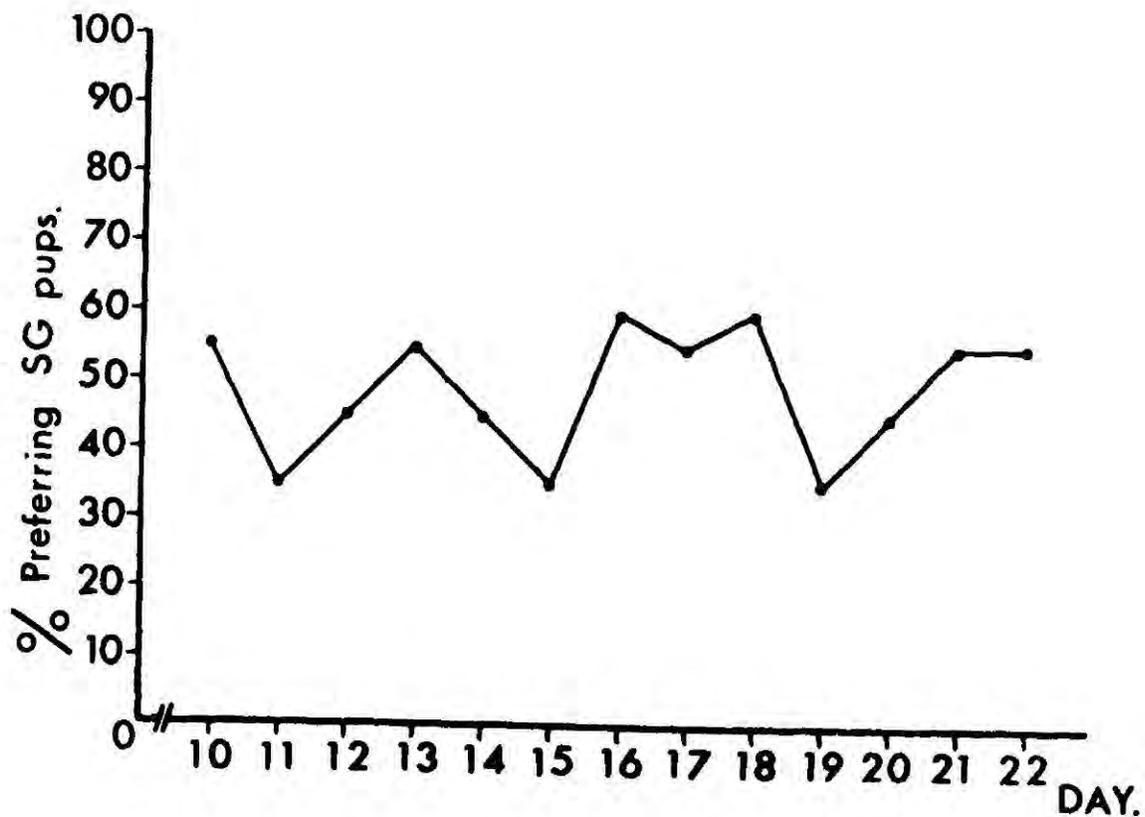
FIGURE 3.10
The percentage of pups preferring the socially and
genetically related litter (SG) on each day of testing
with no odour cues.

whether litter stimuli provide different cues from individuals, rats were tested for their preference for an individual sibling (SG) over an individual unrelated and unfamiliar pup (S̃G̃). The procedure was identical to that used in experiment 3.5, with the exception that it was not litters which were placed in the goal boxes but individual pups. Sixteen pups from two litters were tested from days 10-22 on their preference for an individual sibling (SG) as opposed to a single unfamiliar individual (S̃G̃).

3.9.1

Results

The results (see fig. 3.11) indicate that even when presented with single pups as stimuli, rat pups are still able to discriminate between siblings and non-siblings exhibiting a preference for their siblings on days 10-14 but a reversal of this on days 18-22 preferring their unfamiliar pups. The F.E.P. (see table 3.4) indicates there is a significant difference in the proportion of pups preferring their sibs in the two time periods (p=0.0425). At days 10-14 although eleven out of sixteen pups showed a preference for their siblings this did not reach significance (binomial test N=16, x=5, p=0.105, 1-tailed), similarily at days 18-22 although more pups preferred the unfamiliar individual, eleven out of sixteen, this did not reach significance (binomial test N=16, x=5, p=0.105, 1-tailed).

It may be that single pups present a weaker stimulus
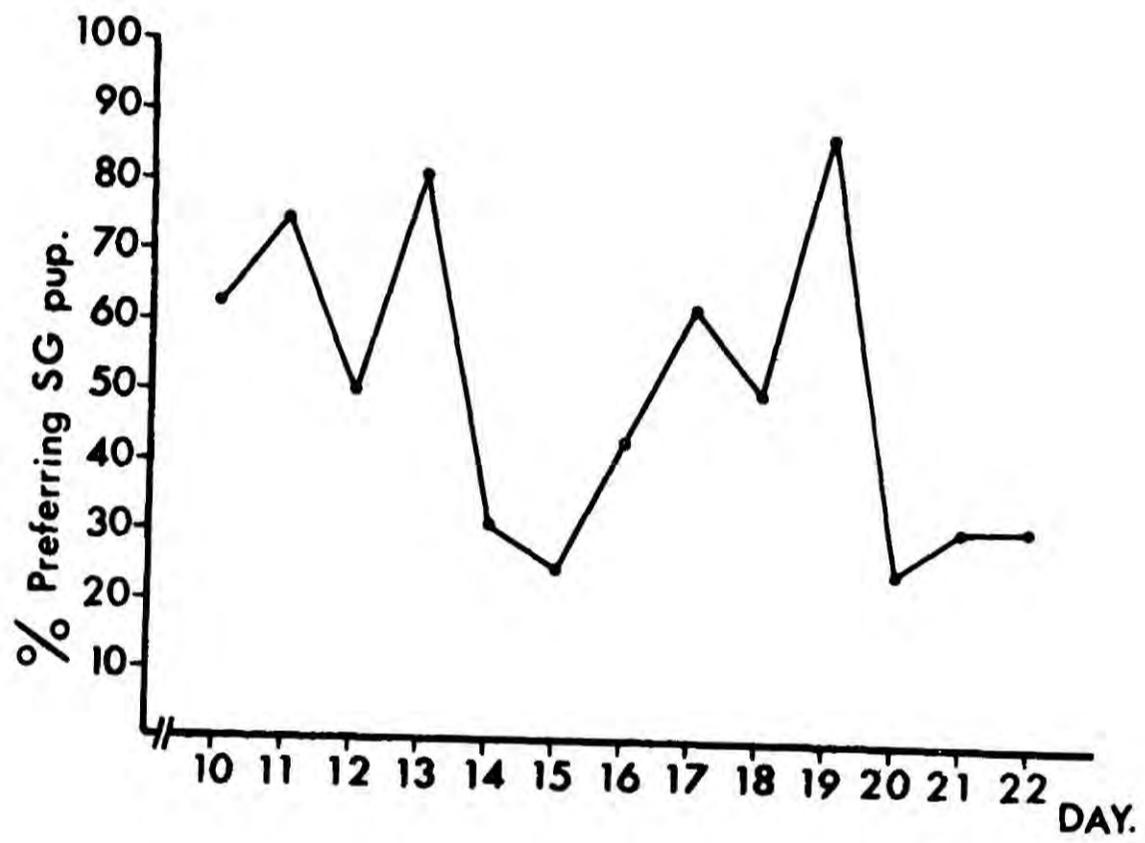
FIGURE 3.11
The percentage of pups preferring the socially and genetically related individual (SG) on each day of testing.

than does a litter and this may account for the weaker preference shown by the pups in this experiment. To investigate this possibility, the number of times each pup preferred its sibling on days 10-14 and on days 18-22, was compared by means of an independent t-test with the number of times pups preferred its sibling litter on days 10-14 and on days 18-22 (from expt. 3.5). Although the pups on days 10-14 showed a weaker preference for siblings if the stimulus was a single pup (mean, 3 out of 5) than if it was a litter (mean, 3.625 out of 5), this difference was not significant (t=1.5357, df=30, $0.2 > p > 0.1$). On days 18-22 pups discriminating siblings from unfamiliars showed a weaker preference for the unfamiliar if the stimulus was an individual (mean preference for siblings, 2.25 out of 5) than if the stimulus was a litter (mean preference for sibling litter, 0.5 out of 5) and this difference is significant (t=5.2175, df=30, $p < 0.001$). It does seem therefore that the weaker preference shown by pups presented with single pups as stimuli rather than litters is due to a weaker stimulus presented by the single pup.

The results from this experiment indicate that pups can recognise single siblings as well as a litter of siblings.

3.10

SIBLING RECOGNITION IN THE RAT

The results of these experiments indicate that rats do possess the ability to recognise their siblings and

that both social and genetic mechanisms play a role in the acquisition of this ability.

The cue used in this discrimination is of an olfactory nature. Olfactory stimuli are known to carry information about the rats' social status (Krames et al., 1969), group membership (Krames and Shaw, 1973), sex (Brown, 1977), sexual condition (Drewett and Spiteri, 1979; Gilman and Westbrook, 1978; Stern, 1970) and about its individuality (Carr et al., 1976; see also Chp. 2), and now kinship may be added to the list. Behavioural observations of the pups in the maze confirmed the results of experiment 3.10. Pups at the choice point were often observed to raise their heads and sniff whilst moving their heads between each arm of the maze. The results of experiment 3.4 revealed that pups can detect the stimuli presented by a litter on day 4 and experiment 3.8 indicated that even as early as day 2 and probably earlier the pups can detect and respond to the litter stimuli. At this age both visual and auditory senses are not functioning, thus any cue must be of an olfactory nature. The results indicate that by day 2 the pup can perceive the complex olfactory stimuli presented by their littermates, confirming other studies (Astic and Saucier 1982; Small, 1899; Smith and Spear, 1980), which indicate the pups' olfactory system is functioning at or near birth and that it can respond to complex biological odours as well as pure chemical stimuli.

Experiments 3.3 and 3.8 indicate that the pups find litter stimuli attractive. The difference between the two experiments may be due to a different response measure used in both experiments. In experiment 3.8 the pup was left for two minutes and then the trial ended. Thus in experiment 3.8 the pup may at first be attracted to the litter, however familiarity with this stimuli soon decreases its attractiveness such that after initial attraction the pup explores the maze and goes to the unfamiliar clean goal box. However the clean goal box soon loses its novelty as there is nothing there, so that the pups once again prefer the familiar goal box and the pup alternates between the two ends.

Experiment 3.6 confirmed the findings of experiment 3.1, that pups can recognise their littermates, and experiment 3.7 confirmed the findings of experiment 3.2 that siblings can recognise each other even after a period of separation. Experiment 3.5, using natural siblings, again indicated that pups do possess the ability to discriminate and recognise their siblings. The question now arises of how the pups come to recognise their siblings.

3.10.1
The Acquisition Of Sibling Recognition

In experiment 3.5, individuals have the opportunity to use both social and genetic mechanisms to acquire sibling recognition. In experiments 3.1 and 3.6, however,

individuals were denied the use of the genetic mechanism by rearing together unrelated (r=0.0) individuals. Thus individuals in this condition must have learned some olfactory characteristic of their littermates to enable recognition. Individuals may have learned the individual signatures of littermates and responded to these, but it is more likely that in a litter individuals come to possess a group odour. All the individual odours become mixed to form a single litter odour and it is this the pup learns and reponds to. A final possibility to consider is that the pups were labelled by the mother. All pups suck from the mother and in doing so the mother may deposit her odour on the pups and individuals achieve recognition of their littermates through this. This would not however explain the results of experiment 3.9 where pups had been separated from the mother for 15 days prior to testing and were still able to recognise their unrelated littermates.

In experiments 3.2 and 3.7, individuals were denied the use of a social mechanism by rearing unrelated individuals apart and individuals therefore had to rely upon a genetic mechanism. First, the possibility of acquisition through a social mechanism should be considered. Siblings had been together for 4 days in experiment 3.2 and 2 days in experiment 3.7 prior to separation, so sibling recognition may have been acquired in this period and remained stable for the duration of the experiment. The results obtained from experiments 3.1 and 3.6 would argue against this. Here pups at first exposed

to pups for the same amount of time as those in the S̃G condition and subsequently exposed to unfamiliar pups learned the characteristics of these unfamiliar pups, indicating that any learning in the first 2 or 4 days is modified by subsequent exposure. Thus for pups in the S̃G condition, if they did acquire their siblings' characters in the first few days, when cross-fostered with unrelated pups learning these new littermates' characteristics will occur which will alter that originally learned and make acquistion of siblings' characteristics within these 2/4 days impossible.

The learning of siblings' characteristics could be accomplished by an individual learning its own odour (cf. Dawkins, "armpit effect", 1982) and using this to recognise its siblings. The fact that siblings when separated from all kin can recognise their siblings is indicative of a genetically determined identifier. As all individuals were fed the same diet, variation due to the environmental factors in the identifier was minimal and any difference between identifiers due to genetic factors. Thus it would be possible for an individual to learn its own odour and because this is similar to that possessed by its kin, it could use this to discriminate sibs from non-sibs. Previous experiments have also suggested such a mechanism for kin recognition where individuals reared in isolation may have learned their own odour and used this to recognise kin, for example, Blaustein and O'Hara (1981), Waldman (1981), Wu et al. (1980). However in

these instances individuals were reared in isolation where the salience of their own body odour is greater. In this experiment individuals were reared with 6 or 7 littermates (expt. 3.2 and 3.7 respectively) plus the mother, and as such it is likely that the pups own odour becomes diluted and mixed with odour from other pups to such an extent that its individuality is reduced and self-recognition improbable. Pups may learn their own odour from licking the body but the pups used in this experiment were not observed to lick their bodies until day 14/15, a time at which they already exhibit the ability to recognise siblings. Thus self odour recognition may enhance the individual's ability to recognise its siblings but is unlikely to initiate it.

It is unlikely that any social mechanism is involved in the acquisition of sibling recognition by SG pups. The ability of these pups to recognise their siblings can be attributed to a genetic mechanism, of which there are two possibilities.

First, the pups may learn the characteristics of their siblings in utero, for example, from the mother's amniotic fluid. It has been shown that rat pups do have the ability to learn in utero (Stickrod et al., 1982) with pups showing a preference after birth for a chemical (apple juice) to which they were exposed prenatally. Thus the possibility exists for pups to learn prenatally and retain this postnatally, although no studies have yet

demonstrated the ability to learn conspecific odours prenatally. The learning of these more complex stimuli may prove more difficult than simple chemical stimuli. Indeed there may be a different mechanism involved in the acquisition of biological and botanical odours as 6-hydroxydopamine reduces rats' preferences for biological but not botanical odours (Marasco et al., 1979).

Secondly, the ability of pups to recognise their siblings could be due to 'recognition genes'. Yamazaki et al. (1976) studied mating preferences in the mouse and found that genes in the major histocompatibility complex (M.H.C.) are involved in mouse choice of sexual partner and argued that genes in the M.H.C. determine both female smell and the male recognition system. Although males may use their own odour in such preferences to compare to the females the the possibility exists that in the mouse the M.H.C. is directly involved in the ability to recognise ones kin. Similarily in the rat there may be some genes which determine kin recognition. As with the mouse, the most likely source for this is the M.H.C. which is responsible for self-cellular recognition (Dausset, 1981a,b) and this may provide the necessary genetic material to enable kin recognition.

One or both of these mechanisms could contribute to genetic acquisition of sibling recognition in the rat. The important point is that no matter what mechanism is used the pups are born with a representation of their

genetic kin. If recognition genes are used the pups, as they are related (r=0.5), will have a similar representation of their sibs. If it is learned in utero then all pups as they are bathed in the mothers amniotic fluid will learn a similar representation of siblings from this.

3.11
CONCLUSION

The experiments in this chapter provide evidence that pups do possess the ability at an early age to recognise their siblings, and that sib recognition may be acquired through either socially and/or genetically mediated mechanisms. As siblings can recognise one another, the way is open for them to gain advantages in their inclusive fitness by responding differentially to sibs and non-sibs and this is investigated in chapters 8-10.

CHAPTER 4

MOTHER-OFFSPRING RECOGNITION

IN THE RAT

4.1

INTRODUCTION

By caring for their offspring individuals can increase their offsprings' chances of survival and thus increase their own inclusive fitness, ensuring a better chance of maintaining their genes in the next generation (Klopfer, 1981). Parents could therefore be expected to maximise their inclusive fitness and should respond differentially to their own and alien offspring. To enable this parents must have some means of recognising their own offspring.

As the female's investment in her offspring is greater than the male's, both prenatally (Trivers, 1972) and postnatally due to her role as the caregiver (Barnett, 1975; Rosenblatt and Lehrman, 1963; although see Rosenblatt, 1969) it may be expected that she would be more likely to recognise her young than the male. In this chapter therefore, I investigate the ability of mother rats to recognise their offspring.

Although mothers may be expected to be specific to their own pups, in certain species, for example, pipistrelle bat mothers, <u>Pipistrellus pipistrellus</u>, allow

other pups to be cross-fostered onto them and allow other offspring to suckle (Kleiman 1969). One reason for this may be that if the offspring are competing for a non-restrictive resource, then the need for specificity may be lessened (see later for fuller explanation of possible reasons for non-discriminatory mothering). It may therefore be expected that differential responsiveness would be shown only in situations where either the pup is endangered or where a particular resource is scarce. For this reason the task used here to assess mother-infant recognition is that of retrieval. Pups who are dislodged, or move away from the nest are in danger not only from predation and attack from conspecifics but also from heat loss and nutritional deficit. It is important therefore that pups be retrieved quickly. Thus, because of the dangers to the pups, mothers would be expected to retrieve their own offspring faster than alien offspring.

4.2

THE ADAPTIVENESS OF MOTHER-OFFSPRING RECOGNITION

By caring for her offspring the mother can ensure the individuals develop normally. I shall briefly consider below how the mother may affect the development of her offspring, concentrating mainly on the rat.

The effect of the mother upon the infant's development commences whilst the offspring are still in the womb. Pregnant rat mothers if stressed produce

infants which are slower to explore unfamiliar environments than non-stressed females (Dakhlof et al., 1978; Thompson et al., 1962; Ward, 1972). The uterine environment may be important for the development of kin recognition (see Chp. 1, section 1.4.3.2), enabling infants to learn about their kin whilst only kin are present.

After birth, the mother provides all the physical requirements, food, warmth, etc., which are necessary for normal development and survival. However mothers have to supply more than just the physical requirements, they also have to provide 'psychological' requirements. Individuals reared in isolation from conspecifics but given the necessary physical requirements show deficiences in later behaviour (e.g., Harlow, 1965). The mother's presence may be necessary to offset this. Most offspring remain with the mother for longer periods than nutritionally required. Rats in the wild are weaned at about day 27/28 (Leon and Moltz, 1972) but can survive without the mother from day 15 (pers. obs.). Early separation leads to behavioural changes in monkeys, Hinde and Spencer-Booth (1971) and, rats separated at 15 days from the mother become much more susceptible to gastric erosion due to stress than normal (Ackerman et al., 1975,1978). The pups' growth is affected by the mother's presence, for after separation from the mother the growth hormone levels in the infant decline (Butler et al., 1968; Kuhn et al., 1978). The mother influences the time of feeding of young rats (Levin

and Stern, 1975), the type of food eaten (Galef and Henderson, 1972) and the site of feeding (Galef, 1971).

The mother protects the pups. Neonates are prone to cannibalisation by males, and females show increased aggressiveness when lactating (see Svare, 1981) and will attack males who come too close to the nest.

This brief review illustrates that the mother contributes significantly to her offspring's physical and behavioural development. The importance of the mother to her offspring's development suggests the mother may show specificity to her own young to ensure they develop as best as possible. Such favouritism would increase the chances of her own offspring reproducing successfully and thus increase the inclusive fitness of the mother by ensuring the propogation of her genes in future generations.

4.3 MOTHER-OFFSPRING RECOGNITION: LITERATURE REVIEW AND COMMENTARY

Many animal species show some form of parental care for their offspring, and thus one may expect parents to act nepotistically to their offspring, indicating the ability to recognise their offspring. This ability may be determined by the sociality of the species concerned. Solitary living animals, who are unlikely to meet other members of the species, may have little need to recognise

their own offspring as they are unlikely to meet any others. However in group living animals where there is a likelihood of meeting other young, the demand for offspring recognition may be greater.

In this review I shall consider the ability of mothers to recognise or respond differentially to their own offspring. Romanes (1883a) in plotting his scale of evolution of intelligence places the ability to recognise offspring at level 20 and postulates that animals possessing this ability are as intelligent as 10 week old humans. The ability to recognise offspring is attributed by Romanes to insects and higher order taxonomic groups. In "Animal Intelligence" (1883b), Romanes reports that spiders are capable of maternal affection, that earwigs will retrieve their eggs, and that several species of snake show parental affection of young when hatched . These observations are the only reports I have been able to find of the ability of invertebrates, amphibians and reptiles to recognise offspring and the following review reports only studies on fish, birds and mammals. The ability of rats to recognise their young has been omitted from this review and will be considered later.

4.3.1

Fish

MacGinitie (1939) reports that parent blind gobies, Typhlogobius californiensis, will not eat their offspring but will eat those from other broods, indicating that

these fish have the ability to recognise their own young.
Brown bullheads, _Ictalurus nebulosus_, can distinguish the
presence of strange young in their own brood (Breder,
1939). Using experimental techniques, Kuhme (1963) and
McKaye and Barlow (1976) have demonstrated that the dwarf
cichlid, _Nannacara anomala_, and the cichlid, _Cichlasoma
citrinellum_, respectively, can distinguish their own young
from others. In both cases the parents exhibited a
preference for a water outlet from a tank containing their
own brood to that of an unfamiliar brood. In an
investigation of the cues used by the cichlid fish, _C.
citrinellum_, to recognise its own brood, when the nares of
the fish were blocked, recognition disappeared indicating
that recognition is based on chemical cues (McKaye and
Barlow 1976). A similar conclusion was reached by Myberg
(1975) where females of the cichlid fish, _Cichlasoma
nigrofasciatum_ were able to recognise their own brood,
using visual cues to orient to the young but olfactory
cues to recognise them.

4.3.2
Birds

There is evidence to suggest that mothers can
recognise their young prior to hatching, that is, they can
recognise their eggs. In the ostrich, _Struthio camelus_,
eggs are placed in a communal nest and these are guarded
by the major hen. She can only incubate 20 eggs
successfully and there are often 30-40 eggs in the nest.
To overcome this the major hen pushes out surplus eggs and

Bertram (1979) reports that she avoids pushing out her own eggs. Similar recognition of eggs has been reported by Rothstein (1975) where so called "rejector" species, birds which reject foreign eggs from the nest, have been shown to recognise their own eggs: these include the cedar waxwing, Bombycilla cedrorum; the western kingbird, Tyrannus verticallis; the robin, Turdus migratorius; the catbird, Dumetella carolinensis; the brown thrasher, Toxostoma rufum; the eastern kingbird, Tyrannus tyrannus and the blue jay, Cyanocitta cristata. The village weaverbird, Ploceus cucullatus, has also been shown to possess the ability to recognise its own eggs (Victoria, 1972).

Investigators examining the parent-offspring behaviour of birds have also concluded that parents have the ability to recognise their own offspring. The creche behaviour of the Royal tern, Stern maxima, suggests that the parents can recognise their chicks as the parents feed only their chicks (Buckley and Buckley, 1970,1972). Recognition of their chicks is apparently accomplished both visually and vocally (Buckley and Buckley, 1970). Similar observations have been reported by Rowley (1980) where galah cockatoo, Cacatua rosekapilla, parents recognise their own young and refuse to feed chicks other than their own, and by Thompson and Emlen (1969) concerning the Adelie penguin, Pygoscelis adeliae which comes to recognise its chick by day 8-17 and thereafter refuses to feed chicks other than its own. In the penguin

a complicated procedure is established for recognition, not only involving spatial and vocal recognition, but also "feeding chains" whereby once identified the chick has to follow the parents for some way to get food. This complex behaviour apparently ensures by means of redundancy that the adult delivers food only to its own young (Thompson and Emlen, 1969).

The ability of mothers to recognise their young has been reported for the herring gull, Larus argentatus, (Goethe, 1937); the sooty tern, Sterna fuscata, (Watson and Lashley, 1915) and the jackdaw, Corvus monedula, (Lorenz, 1938). The ring billed gull, Larus delawerensis, has been shown to recognise its chicks after 7-9 days post-hatching and uses individual variability in physical appearence to achieve this (Miller and Emlen, 1975).

4.3.3
Mammals

Alaska fur seal, Callorhinus ursinus, females nurse only their own young and will not accept other pups to suckle (Bartholomew, 1952,1959). Similarily the Stellar sea lion, Eumetopias stelleri, responds only to its young (Bonnet, 1928; Mathison et al., 1962) and was observed to sort through the young in the harem to find its own pup (Rowley, 1929). The sea lion, Otaria flavescens SHAW, can recognise its offspring 24 hours after birth and uses both acoustic and olfactory cues in this ability (Vaz-Ferreira and Achaval, 1979). The galapagos fur seal, Arctocephalus

galapogoensis, and the galapagos sea lion, Zalophus
californianus(=wollebacki), can both recognise their pups
(Eibl-Eibesfeldt, 1955; Trillmich, 1981), the calls of
the pups of both species containing enough
inter-individual variability to allow recognition.

The grey seal, Halichoerus grypus, recognises its own
young by means of olfaction and rejects other alien young
(Fogden, 1971), in this species the first ten minutes
after birth may be important in the establishment of
recognition of offspring (Burton et al., 1975).
Similarly the harp seal, Pagophilus groenlandicus, is
also able to recognise its own pup, using visual and
auditory cues to locate the pup and olfaction for
recognition (Terhune et al., 1979).

The mothers of the Northern elephant seal, Mirounga
angustirostris, whilst tolerant of alien young and
allowing them to nurse (Klopfer and Gilbert, 1966) can
recognise the distress vocalisations of their own pups
from those of other pups (Petrinovich, 1974) and retrieval
tests indicate that the cows can discriminate their own
pups from alien pups (Klopfer and Gilbert, 1966). The
sea-elephant, Mirounga leonina, remains close to its calf
and was observed to bite at alien pups which came too
close (Laws, 1956).

Behavioural observation of the dolphin, Tursiops
truncatus, indicates that at the end of feeding the

mothers return and retrieve their own young (Tavolga and Essapian, 1957).

Amongst ungulates, mother-infant recognition has been studied extensively in two species, the sheep and the goat. Lindsay and Fletcher (1968) found that Border Leicester and Merino ewes could recognise their lambs in a T-maze and thought sight was the main sense used in recognition. Morgan et al. (1975) took a different line and using Border Leicester and Merino ewes concluded that recognition depended entirely on smell with both hearing and sight used in the location of the lamb. In a series of studies investigating the role of various senses in the recognition of lambs by ewes in Merino sheep, mothers were found to prefer lambs similarly coloured to their own (Alexander and Shillito, 1978) both the head and tail regions being used by the mother to provide cues for close recognition of their lambs (Alexander, 1978). From these results they concluded that Merino ewes can recognise their lambs from auditory and visual cues alone, but that correct olfactory cues were required for sucking to be permitted (Alexander and Stevens, 1981). Soay sheep as with Merino sheep rely on olfactory cues for recognition at close quarters (Baldwin and Shillito, 1974). Border Leicester, Jacob and Soay can also recognise their lambs vocalisations, mothers bleating more in response to a recording of their own lamb than that of an alien lamb (Walser et al., 1981). In sheep, olfactory recognition of offspring may be accomplished very soon after birth: 20

minutes or more spent licking a lamb is associated with subsequent rejection of alien young (Smith et al., 1966).

Similarly for goats, whilst tolerant of strange kids and allowing them to suck, mothers were observed to butt strange young but never their own (Hafez and Scott, 1962). Toggenberg goats can recognise their kids (Klopfer and Gamble, 1966) by using olfactory cues and recognition of their young may develop very rapidly after birth, in the first 5 minutes (Klopfer and Klopfer, 1968), although later studies suggest that recognition is not accomplished so rapidly (Gubernick et al., 1979).

Pigs are able to recognise their own litter by olfactory cues (Hafez et al., 1962) and cattle can also recognise their calves, recognition being based on olfactory, visual and auditory cues (Hafez and Schein, 1962). The barren ground caribou, Rangifer tarandus groenlandicus, is capable of recognising its own young (Lent, 1966) and warns off strange calves (Pruitt, 1960). The reindeer, Rangifer tarandus, recognises its offspring by its distinctive vocalisations (Espmark, 1971,1975), whereas the bontebok, Damaliscus dorcas dorcas, recognises its young by olfactory means (David, 1975). Behavioural observations of many other ungulate species also indicate that they possess the ability to recognise their offspring, for example, zebras, Equus burchelli, (Wackernagal, 1965), Equus quagga, (Klingel and Klingel, 1966); red deer, Cervus elephas, (Darling, 1937,1938);

elk, _Cervus_ _canadensis_ _nelsonii_, (Altman, 1963); Wildebeest, _Gorgon_ _taurinus_, (Estes, 1963; Talbot and Talbot, 1963) and bison, _Bison_ _bison_, (McHugh, 1958).

In carnivores, lionesses, _Panthera_ _leo_, although tolerant to all cubs in the pride, prefer their own cubs (Schenkel, 1966). Mice, _Mus_ _musculus_, are capable of recognising their own young, parents spending longer sniffing an alien pup than their own; such recognition based upon olfactory and probably gustatory cues (Ostermeyer and Elwood, 1983). Guinea pig, _Cavia_ _porcellus_, mothers prefer to remain in the proximity of their own litter rather than an alien litter of the same age (Porter et al., 1973).

One group of mammals which have been well studied is that of bats. In the nursery roost of the bat, _Anthrozus_ _pallidus_, each female nurses only her own young (Beck and Rudd, 1960), and females of the lump-nosed bat, _Corynorhinus_ _rafinesquei_, have been observed searching through the juveniles for their own young (Pearson et al., 1952). By banding the individuals they found females did in fact find their own young and reject alien young. Big brown bat, _Eptesicus_ _fuscus_, females retrieve young which had fallen to the floor and recognise their own young (Davis et al., 1968). Kleiman (1969) investigating maternal behaviour in three bats concluded that the noctule, _Nyctalus_ _noctula_, and the serotine, _Eptesicus_ _serotinus_, recognise their own offspring and do not accept

alien young, whereas the pipistrelle, Pipistrellus
pipistrellus, although suckling other young, retrieved
only her own young. Such recognition is primarily due to
smell: in the serotine, the muzzle gland and in the
noctule, the glands in the corner of the mouth may be
important in producing in individual odours (Kleiman
1969).

Richardson's ground squirrels, Spermophilus
richardsonii, show aggression to alien young but not to
their own young (Michener and Sheppard, 1972).

The infant calls of the vervet monkey, Cercopithecus
aethiops, if played back to their mother or an unfamiliar
lactating female elicit a quicker response from the
mother, who approaches the sound source more often and
more quickly than control mothers (Cheney and Seyfarth,
1980). Pigtail macaque, Macaca nemestrina, mothers when
placed with strange infants show scant regard for them,
and the mother's behaviour seems infant-specific in that
she responds only to her infant (Jensen and Tolman, 1962).
The mother's maternal specificity to the infant develops
in the first 2-3 weeks and from day 17 onwards is
increasingly indifferent to infants other than her own
(Jensen, 1965). Recognition of the young in this species
is not based upon auditory cues as the mothers did not
respond differentially to calls of their own infants as
opposed to alien infants (Simons et al., 1968). Stumptail
macaques, Macaca speciosa, show significantly more

touching of the pictures of their own infants, than either pictures of familiar or unfamiliar infants, indicating that visual cues are responsible for infant recognition in this species (Swartz, 1977). Squirrel monkeys, Saimiri sciureus, became more active on hearing the calls of their own infants than when hearing those from an unfamiliar infant (Kaplan et al., 1978). Galago mothers, Galago crassicaudatus, and, G. senegalensis, require some days after birth before they can discriminate their own young from others, both smell and vocal stimulation enabling recognition to take place (Klopfer, 1970).

The ability of humans to recognise their own offspring is well known, but there has been little research to plot the development of this ability. Cases of the wrong babies given to the wrong parents in hospitals with no realisation of this by parents indicates that learning is involved in this ability.

4.3.4
Summary And Critique

The studies reported above fall into two catagories: first, studies relying on behavioural observations, in which observation of mothers responding differentially to different individuals is taken as evidence of maternal recognition, and second, experimental studies, in which the mother is presented with discreet choices and her behaviour noted. A major problem with behavioural observation studies concerns the question of the

relatedness of the mother and offspring. The recording of behaviour in which the mother rejects one individual but accepts another relies on the premise that the individual accepted is actually her offspring. In species where the young are left whilst the parents feed, and are apparently sought out on the parents' return, marking the young has been employed to ensure that the mothers do return to their own pups (e.g., Alaska fur seal, Bartholomew, 1952; lump-nosed bat, Pearson et al., 1952).

Different species of animals can be classified according to their reaction to alien young, as either acceptors or rejectors, in both cases they may recognise their own young. Rejector species will accept no young other than their own to nurse (e.g., Richardson's ground squirrel, Michener and Sheppard, 1972; noctule bat, Kleiman, 1969), whereas acceptors will accept alien young to suckle, although still capable of discriminating between their own and alien young (e.g., the elephant seal, Klopfer and Gilbert, 1966).

The ability of mothers to recognise infants has been demonstrated in a wide range of vertebrate species and the development of this ability is likely to be reflected in the individual's life style. In species which live in large groups, where the presence of young other than their own is common place, one would expect a rapid period for the acquisition of the young's characteristics, for example, in the harp seal, the first few minutes after

birth are important for the establishment of offspring recognition (Burton et al., 1975), similarly in goats (Klopfer and Klopfer, 1968). However in species in which the the young are secluded and unlikely to meet other individuals, the establishment of offspring recognition may take longer, see for example, the galah cockatoo. In this species the young do not meet other young until they leave the nest on day 46 and recognition is not established until day 40 (Rowley, 1980).

4.4
AN INVESTIGATION OF MOTHER-OFFSPRING RECOGNITION
IN THE RAT

In the experiments described here I investigate the ability of rats to recognise their offspring. In a comprehensive investigation of the laboratory rat's maternal behaviour, Wiesner and Sheard (1933) concluded that rats are unable to discriminate between their own and alien young. In a later study Beach and Jaynes (1956) criticised the technique used by Wiesner and Sheard and concluded that under the circumstances used the "female had no opportunity to display preferential tendencies in retrieving" (p. 179). Beach and Jaynes used a more sensitive technique and recorded the time taken by mothers to retrieve their own young and alien young. They found that mothers retrieved their own pups faster than alien young and concluded that the mothers could recognise their own pups. In my investigations of mother-infant recognition I too have used a retrieval task.

In the rat, retrieval behaviour usually consists of the mother carrying the infant by the scruff of the neck (see plate 4.1) from one place to another, either back to the nest or to a new nest. Such behaviour is important to the young ensuring they receive constant care and attention and must be efficient if they are to survive. Studies of retrieval behaviour indicate that it declines about 13-14 days postpartum (Rosenblatt, 1969; Rosenblatt and Lehrman, 1963) and it is a stereotyped pattern under multisensory control (Barnett, 1975). Two cues have been demonstrated to be important in the initiation of this behaviour. Young rats when cold emit an ultrasonic signal (Okon, 1972) and this stimulates retrieval behaviour (Allin and Banks, 1971). Olfactory cues are also required for the initiation of pup retrieval (Smotherman et al., 1974). It seems that retrieval behaviour is under the control in the main, of these two cues: ultrasound used as a beacon in directing the mother to the pup and olfactory cues to recognise the pup once located.

To see if rats possessed the ability to recognise their offspring, mothers were presented with a group of their own offspring and a group of alien (unrelated, r=0.0, and unfamiliar) young, and the time taken to retrieve these recorded. It was hypothesised that if the mothers could recognise their own young these would be retrieved more quickly than alien young. The possibility of recognition being achieved by a socially mediated mechanism was examined by cross-fostering a genetically

PLATE 4.1
Mother retrieving one of her young.

unrelated (r=0.0) litter onto the mother after removing her own pups, soon after birth, and then later examining the retrieval behaviour for these only socially related pups and alien pups. Young rats were separated from their mothers soon after birth to investigate the possibility of the mother acquiring offspring recognition through a genetically mediated mechanism. Mothers were later observed and the time to retrieve her pups from which she had been separated soon after birth (i.e., genetically related r=0.5, but socially unrelated) and alien pups recorded. It was hypothesised that in all three cases the mother should retrieve 'her pups' faster than alien pups.

4.4.1
Subjects

Twelve adult Sprague-Dawley females, born in the Department of Psychology, University of Durham, from rats supplied by B & K (Animal Suppliers) Ltd. were used. The females at 140-160 days of age were placed individually in Bowman's cages with an adult male Sprague-Dawley rat also supplied by B & K Ltd. All females were nulliparous. The males were left in the cages until signs of pregnancy were detected and then removed. All males and females were unrelated (r=0.0) to each other. Food and water were freely available throughout the experiment. The rats were kept on a 12 hour light/dark cycle, darkness starting at 1200 hours. Cages were checked twice daily for litters and the day of birth termed day 0. The females were then divided into three groups of 4, all females in each group

giving birth on the same day. Females were left until day 2 and then moved into much larger cages (32"x16.5"x15") so retrieval behaviour could be observed more easily. Pups were culled leaving 8 per litter and rearranged into 2 rearing conditions on day 2.

Rearing Condition 1: Mothers were left with their own pups for the duration of the experiment. Four mothers were placed in this condition, all having given birth on the same day. This rearing condition created the experimental condition whereby pups were both socially (S) and genetically (G) related to the mother, the SG condition, the natural relationship of the mother to her offspring.

Rearing Condition 2: Mothers were separated from their own litter and given a same aged litter to rear. Two groups of 4 mothers comprised this condition, each group of mothers giving birth on the same day. The mothers and pups remained separated for the duration of the experiment. This rearing condition provided two experimental conditions. First the $S\widetilde{G}$ condition, in which pups were socially (S) related to their 'mother' but not genetically ($\widetilde{G}$) related (r=0.0). Second the $\widetilde{S}G$ condition, in which mothers had had no social experience with their pups ($\widetilde{S}$) but were genetically (G) related (r=0.5).

$\widetilde{S}\widetilde{G}$ pups were pups who were neither socially ($\widetilde{S}$) nor genetically ($\widetilde{G}$) related to the mother.

4.4.2

Apparatus

All tests were carried out in the mothers home cage, in which she had been since day 2. This cage (see fig. 4.1) was constructed of three solid aluminium sides, with a half inch mesh front and top. Sawdust was placed in the cage when the mother was first placed in the cage on day 2 and was left for the duration of the experiment. Plastic gloves were used when handling the pups to avoid any contamination with human odour.

4.4.3

Procedure

An identical procedure was used for all three experiments, therefore experiment 4.1 will be described in detail and only points of difference mentioned for the others.

4.4.3.1

EXPERIMENT 4.1: The ability of mothers to recognise and retrieve their own pups (SG) and alien pups ($\widetilde{SG}$)

In this experiment mothers were tested for their ability to retrieve their own pups, with whom they were both socially and genetically related, and unfamiliar pups, with whom they were neither socially nor genetically related. All mothers were given four trials, 2 with their own pups and 2 with the unfamiliar pups at hourly intervals. Two of the mothers had to retrieve first their
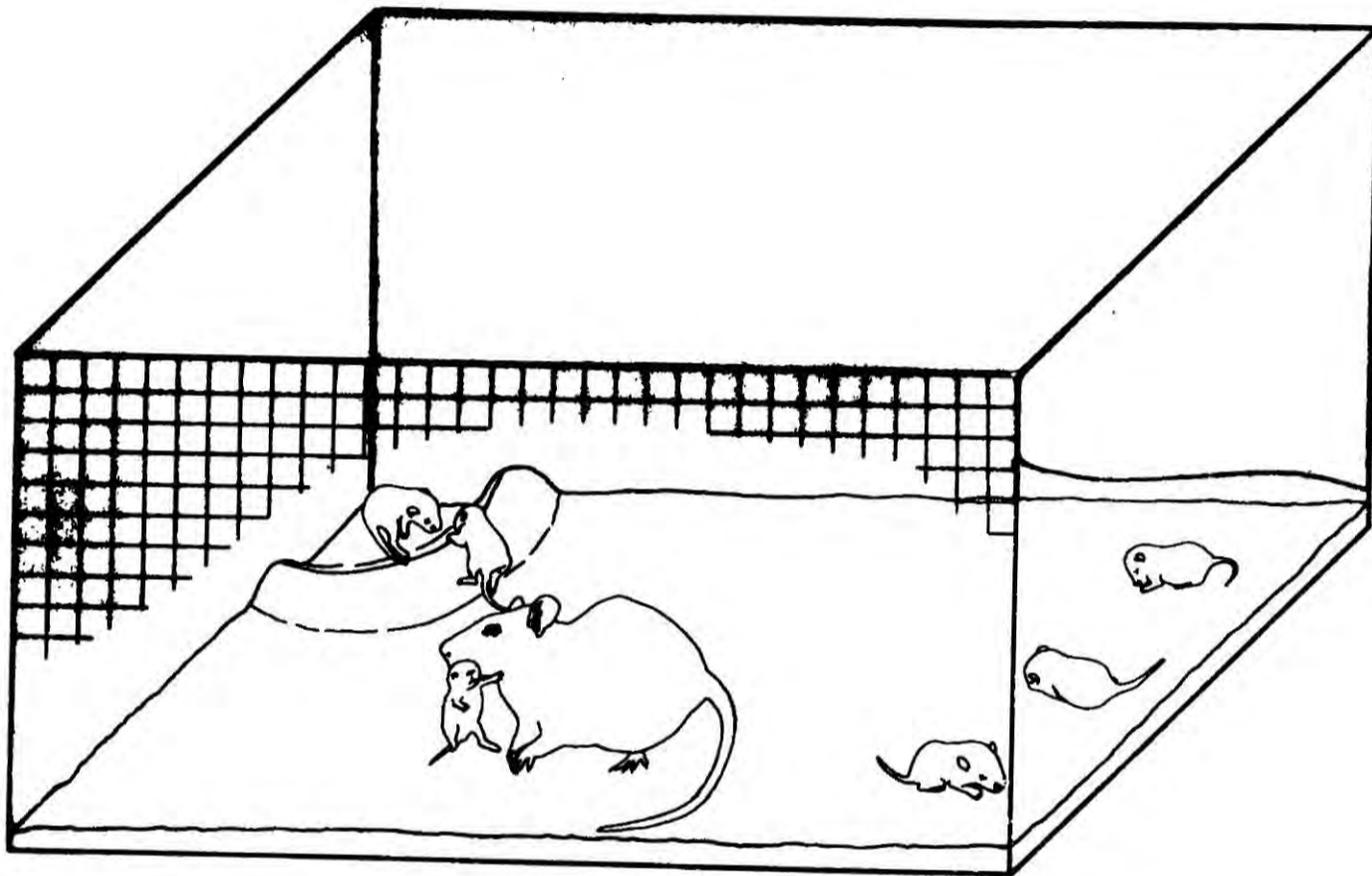
FIGURE 4.1
Diagrammatic representation of testing situation. Note
pups placed equidistant apart at opposite end of the cage
to the mother's nest.

own pups, then unfamiliar pups, own pups and finally unfamiliar pups. The other two mothers had to retrieve pups in the reverse counterbalanced order: unfamiliar, own, unfamiliar, own. All tests were carried out under red light and in the first half of the dark cycle, between 1200 and 1800 hours.

In each trial the procedure was the same. The mother was removed from the cage and placed in an individual holding cage. The litter was then removed from the from the cage and similarly placed in a separate holding cage. The unfamiliar pups were likewise held in a holding cage. Six pups from the appropriate litter were then placed in the mother's cage. One was placed on the nest and the other 5 placed at the opposite end from the nest, approximately 1" from the side wall at 3" intervals (see fig. 4.1). The time taken to retrieve each pup to the nest was recorded. The trial ended when all pups had been retrieved and returned to the nest, if no retrieval behaviour was forthcoming after 5 minutes the trial was stopped. The pups were then returned to their appropriate mother and left for 1 hour until the next trial, using unfamiliar pups if the mother's own pups had been used previously, or the mother's own pups if unfamiliar pups had been used. Four mothers were tested in this way and the time taken to retrieve their own and the unfamiliar pups for each trial recorded.

4.4.3.2

EXPERIMENT 4.2: The ability of mothers to recognise and retrieve their 'own' pups (S$\widetilde{G}$) and unfamiliar pups ($\widetilde{SG}$)

In this experiment mothers were tested for their ability to retrieve pups which they had been exposed to since day 2 but which were not genetically related and alien pups. The procedure used was identical to that described for experiment 4.1. Four mothers were tested and the time taken to retrieve their 'own' and unfamiliar pups recorded.

4.4.3.3

EXPERIMENT 4.3: The ability of mothers to recognise and retrieve their own ($\widetilde{S}$G) and unfamiliar pups ($\widetilde{SG}$)

In this experiment mothers were tested for their ability to retrieve their own offspring (genetically related, r=0.5) but from whom they had been separated since day 2 (socially unrelated), and alien pups. The procedure was identical to that described for experiment 4.1. Four mothers were tested in this experiment and the time taken to retrieve their own and unfamiliar pups recorded.

4.4.4

Analysis And Results

Due to the small numbers (n=4) the primary analysis pooled the results from the three experiments. The time taken for each rat to retrieve all five pups was obtained

for each trial for their 'own' and alien pups. The mean
of these was also obtained to give the mean time to
retrieve 'own' and alien pups for each individual (see
table 4.1). Rat No. 2, in experiment 4.3 ($\widetilde{S}$G), when
presented with alien pups attacked these on both trials
and for the pups' safety the trial was stopped
immediately. This mother was not observed to attack her
own pups. This differential behaviour was taken as an
indication of the mother's ability to recognise her own
pups, and for statistical analysis a time of 300 seconds
was used as the time to retrieve alien pups on both
trials, this being the cutoff point for a trial if no
retrieval had taken place. All other mothers exhibited
retrieval behaviour. Mothers were classified according to
whether they retrieved their 'own' or alien pups more
quickly on trial 1, trial 2 and for the mean time to
retrieve pups on trial 1 and 2. A binomial test (see
Siegel, 1956) was then performed upon the number of
mothers retrieving their 'own' and the alien pups fastest
on   trial 1, trial 2 and the mean time for both   trials.
The results indicate that significantly more mothers
retrieved their 'own' pups more quickly than alien pups on
trial 1 (N=12, x=2, p=0.038, 2-tailed), trial 2 (N=12,
x=0, p<0.003, 2-tailed) and for the mean time   for
retrieving pups on both trials (N=12, x=1, p=0.006, 2
tailed).

To see whether there was any difference between the
retrieval behaviour of mothers with differently related

TABLE 4.1

Summary of the time (in seconds) taken by the mother to retrieve all 5 pups (own/alien) in each experiment for trial 1 and 2 and the mean time to retrieve 5 own and alien pups (trials 1 and 2).

	RAT	TRIAL 1 OWN	TRIAL 1 ALIEN	TRIAL 2 OWN	TRIAL 2 ALIEN	MEAN TRIALS 1/2 OWN	MEAN TRIALS 1/2 ALIEN
EXPT. 4.1 SG	1	62	256	26	127	44.0	191.5
	2	57	140	52	54	54.5	97.0
	3	45	284	90	377	67.5	330.5
	4	31	65	24	75	27.5	70.0
EXPT. 4.2 SG̃	1	33	197	22	45	27.5	121.0
	2	50	98	28	185	39.0	141.5
	3	45	61	35	200	40.0	130.5
	4	101	77	61	75	81.0	76.0
EXPT. 4.3 S̃G	1	50	44	41	82	45.5	63.0
	2	67	300	59	300	63.0	300.0
	3	33	51	27	45	30.0	48.0
	4	45	170	30	110	37.5	140.0

pups (SG, SG̃, S̃G) an analysis of variance (ANOVA) was performed (see Winer, 1962). The effects of mother-offspring relatedness (SG, SG̃, S̃G), between subject factor, type of pup to be retrieved (own, alien) and trial (1/2), within subject factors, was examined. There was no significant main effect of rearing condition, trial, nor any interaction effect (see table 4.2). The only significant result was that of type of pup to be retrieved, here mothers retrieved their own pup (mean time to retrieve 5 own pups, 46.417 secs.) significantly faster (F=13.6421, df 1,9, p=0.0052) than alien pups (mean time to retrieve 5 alien pups, 142.417 secs.) supporting the results of the binomial test.

The results from these analyses indicate that mothers retrieve their 'own' pups faster than alien pups, and that there is no significant difference between the retrieval behaviour of mothers related to offspring socially and genetically, just socially or just genetically.

Further analysis was then carried out with the results split back to their original experimental groups. Graphs were drawn of the mean cumulative time for the mother to retrieve their 'own' and alien pups (see figs. 4.2, 4.3, 4.4). Observation of these graphs supports the previous results of a difference between the time to retrieve alien pups and 'own' pups, alien pups being retrieved slower. The difference is largely due to the mothers being slower to retrieve the first two alien pups

TABLE 4.2

The effect of mother-offspring relatedness (SG,SG̃,S̃G), pup (own,alien), and trial (1/2) on the retrieval behaviour of rats.

Summary of Analysis of Variance

SOURCE	DF	SS	MS	F	PROB
SUBJ	11	107909.6667			
RL	2	6685.5417	3342.7708	0.2972	0.7527
ERROR	9	101224.1250	11247.1250		
PR	1	110592.0000	110592.0000	13.6421	0.0052
PR x RL	2	5754.8750	2877.4375	0.3549	0.7142
ERROR	9	72960.1250	8106.6806		
T	1	768.0000	768.0000	0.2608	0.6260
T x RL	2	338.3750	169.1875	0.0575	0.9445
ERROR	9	26501.6250	2944.6250		
PR x T	1	65.3333	65.3333	0.0343	0.8509
PRxTxRL	2	2185.0417	1092.5208	0.5741	0.5865
ERROR	9	17126.6250	1092.9583		
WITHIN	36	236292.0000			

RL = Mother-Offspring relatedness (SG,SG̃,S̃G).
PR = Type of pup to be retrieved (own,alien).
T = Trial (1,2).
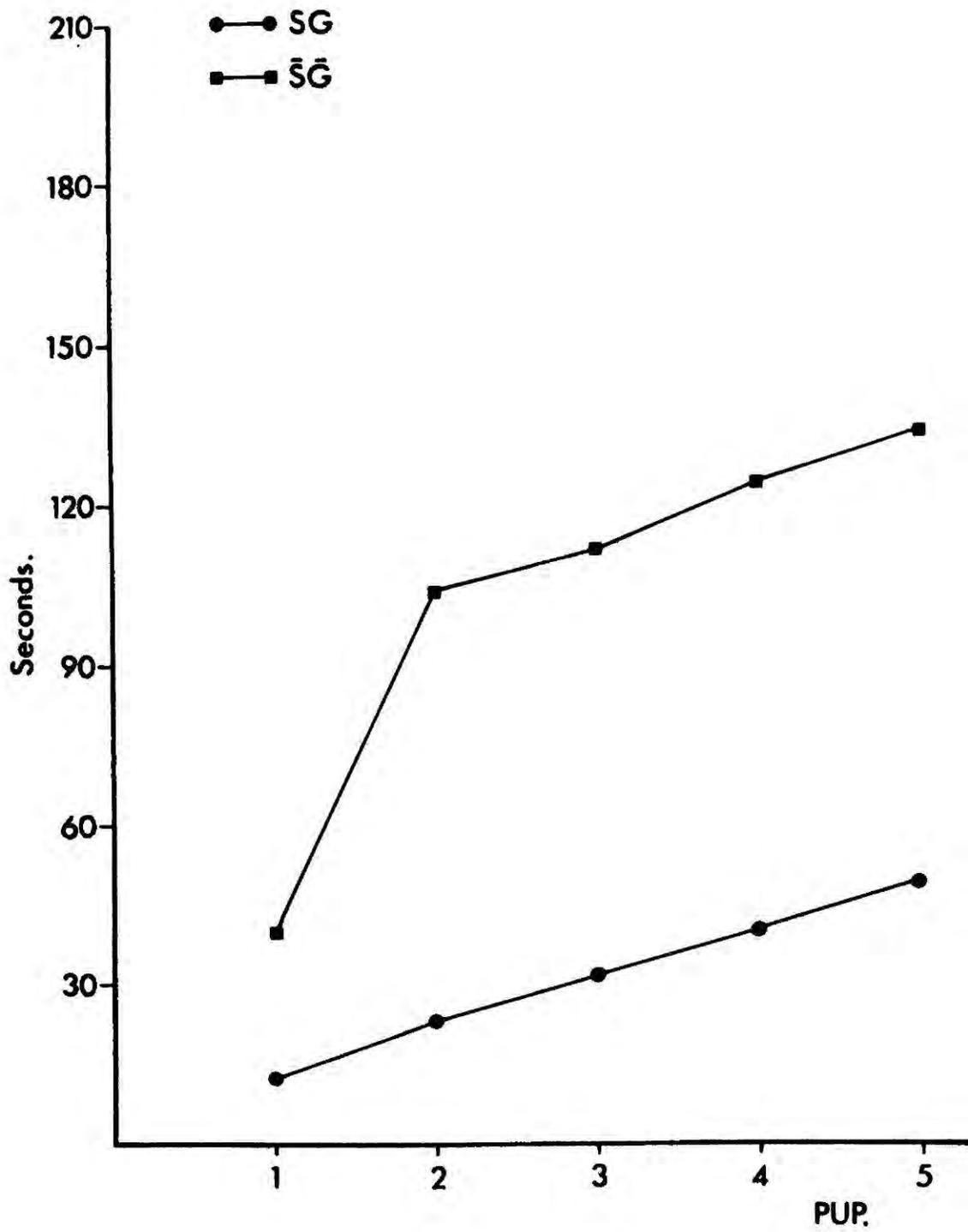
FIGURE 4.2
Mean cumulative time (in seconds) for mothers to retrieve their own (SG) and alien (S̄Ḡ) pups to the nest.
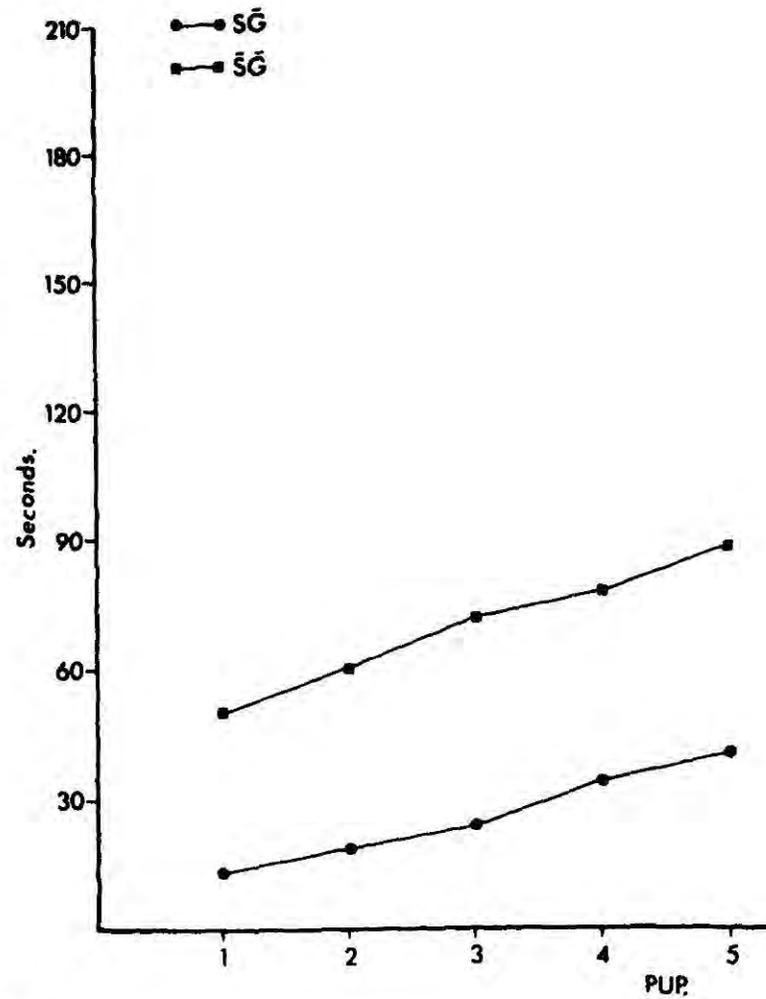
FIGURE 4.3
Mean cumulative time (in seconds) for mothers to retrieve their 'own' (S͠G) and alien pups (S͠G) to the nest.
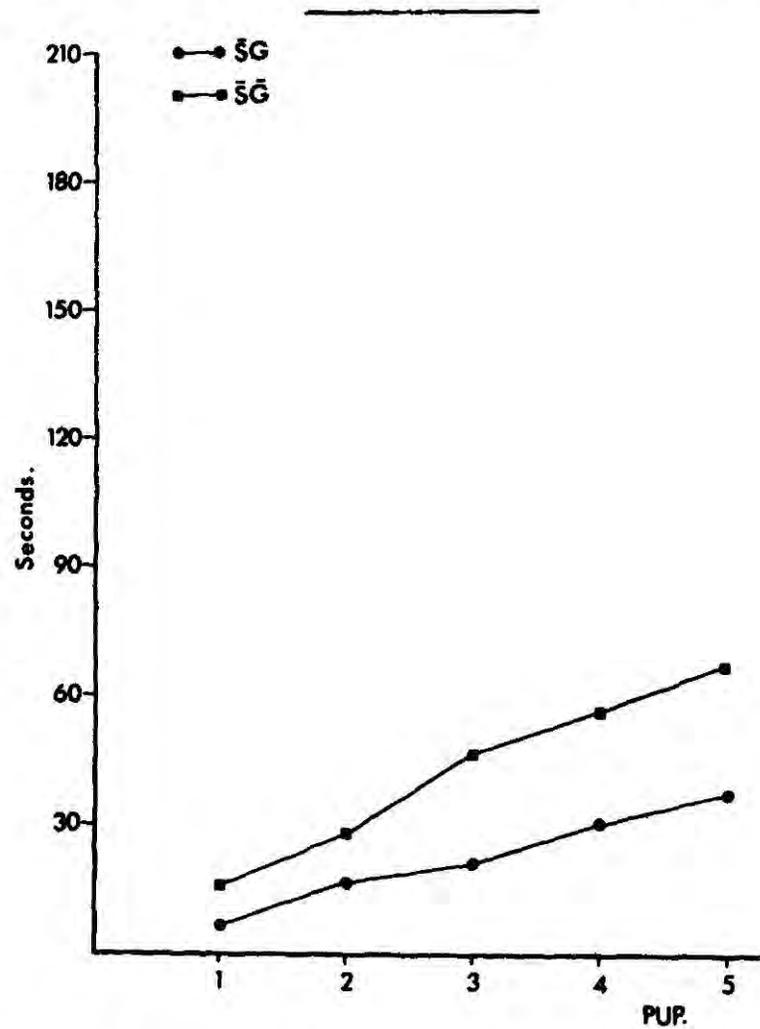
FIGURE 4.4
Mean cumulative time (in seconds) for mothers to retrieve their own (S͠G) and alien pups (S͠G) to the nest.

than the first two pups of their own which is due to a greater amount of investigation exhibited by mothers when presented with alien pups than when presented with their own pups.

An ANOVA was performed for each experiment to examine the effects of pup relatedness (own/alien) and trial (1/2), see Winer (1962). Although no factors reached significance, in all three experiments the factor of pup relatedness did approach significance (see table 4.3).

These experiments demonstrate that mothers retrieve their own offspring faster than alien young of the same age, and that either social or genetic experience with the pups is enough to enable this behaviour to occur.

4.4.5

Discussion

The results indicate that mothers can recognise their offspring, and that this recognition can be achieved through social experience with them, that is, via a socially mediated mechanism, or via a genetically mediated mechanism. These results support the study of Beach and Jaynes (1956) who similarly found mother-infant recognition, and differ from those of Wiesner and Sheard (1933) who claimed rats did not possess the ability to recognise their offspring.

TABLE 4.3

The effect of pup (own/alien) and trial (1,2) on the retrieval behaviour of mothers.

Summary of Analysis of Variance for Experiments 4.1-4.3

SOURCE	DF	SS	MS	F	PROB
EXPT. 4.1					
SUBJ	3	51621.1875			
PR	1	61380.0625	61380.0625	5.5535	0.0989
ERROR	3	33157.6875	11052.5625		
T	1	826.5625	826.5625	0.1935	0.6880
ERROR	3	12813.1875	4271.0625		
PR x T	1	742.5625	742.5625	0.6007	0.5026
ERROR	3	3708.1875	1236.0625		
WITHIN CELLS	12	112628.2500			
EXPT. 4.2					
SUBJ	3	603.6875			
PR	1	19810.5625	19810.5625	7.7656	0.0675
ERROR	3	7653.1875	2551.0625		
T	1	7.5625	7.5625	0.0018	0.9677
ERROR	3	12292.1875	4097.3958		
PR x T	1	1501.5625	1501.5625	0.3647	0.5901
ERROR	3	12350.1875	4116.7292		
WITHIN CELLS	12	53615.2500			
EXPT. 4.3					
SUBJ	3	48999.2500			
PR	1	35156.2500	35156.2500	3.2806	0.1674
ERROR	3	32149.2500	10716.4167		
T	1	272.2500	272.2500	0.5850	0.5026
ERROR	3	1396.2500	465.4167		
PR x T	1	6.2500	6.2500	0.0176	0.8982
ERROR	3	1068.2500	356.0833		
WITHIN CELLS	12	70048.5000			

PR = Type of pup to be retrieved (own,alien).

T = Trial (1,2).

4.4.5.1
Comparison of Expt. 4.1 with Beach and Jaynes (1956)

A number of differences may be noted between the retrieval behaviour of the mothers in the Beach and Jaynes study and with the mothers in this experiment. Firstly the onset of retrieval behaviour started much earlier in the Beach and Jaynes study (approximately 6 seconds) for both own and alien young whereas in this experiment it commenced at 19 seconds for own pups and 72.25 seconds for alien young. A major point of difference between the two studies is the time difference between the retrieval of the first alien and first own pup. In this experiment the mother's first own pup was returned faster that the first alien pup (see fig. 4.2), whereas in the Beach and Jaynes study both first pups were retrieved in a similiar time. A final difference between this study and that of Beach and Jaynes is that the overall time to retrieve all 5 alien pups was slower in this experiment (172.25 secs.) than in Beach and Jaynes (approx. 105 secs.). These differences may be due to a number of different factors. The size of the test cages in this experiment was 32"x16.5"x15" whereas Beach and Jaynes used a cage "three feet square and one foot deep" (p. 178), also Beach and Jaynes placed their mothers in this cage 5 days prepartum whereas the mothers in this experiment were placed in the cage 2 days postpartum. The mothers in this experiment may have been tested later than those of Beach and Jaynes, and although retrieval behaviour does not decline until day 13-14 (Rosenblatt and Lehrman, 1963) this may account

for the difference observed in the onset of retrieval behaviour. Also a greater period of time with the pups may allow the mothers in this experiment to be better discriminators than those used by Beach and Jaynes.

A point of similarity between the two experiments concerns the rate of retrieval of alien pups. In both cases after the first pup had been retrieved, there was a large gap before retrieving the second pup, but after this both alien and 'own' pups were retrieved at a similar rate.

Although there are minor differences between the two studies, both support the notion that mother rats can recognise their offspring, and experiments 4.2 and 4.3 reinforce and extend these findings, by demonstrating that offspring recognition may be accomplished through both socially mediated and genetically mediated mechanisms.

4.5
MOTHER-OFFSPRING RECOGNITION IN THE RAT

The cue used in the recognition process is most probably of an olfactory nature. Previous experiments, Beach and Jaynes (1956) and Smotherman et al. (1974) found that individuals deprived of their olfactory sense fail to discriminate between their own and alien pups. Although no olfactory deprivation experiments were carried out here it is highly probable, as with previous experiments, (expt. 3.10, Chp. 3, section 3.8), that

olfactory cues are used in the recognition process. Observation of the mothers behaviour during the experiment indicated that the mothers sniffed the pups when they had located them prior to retrieval.

4.5.1
The Acquisition Of Mother-Offspring Recognition

In experiment 4.2 (S$\tilde{G}$ vs $\tilde{S}$$\tilde{G}$) where the pups have only been together since day 2, some learning of the characteristics of the pups must have occured. The mother spends much time licking the pups, not only to stimulate micturation but also generally cleaning the pups, such behaviour would provide a good opportunity for learning her offspring's characteristics. A similar explanation would apply for experiment 4.1 (SG vs $\tilde{S}$$\tilde{G}$). Another possibility in these two experiments is that the mother has deposited her own odour on the pups and uses this to recognise her pups. Beach and Jaynes (1956) postulate that " 'own' young are distinguished on the basis of nest odours to which the female herself has contributed" (p. 180). This is a distinct possibilty as all tests were carried out in the mother's home cage. The pups in the SG and S$\tilde{G}$ conditions were present in the nest and could have acquired the nest odour. Thus when located at the periphery of the cage, her own pups would have smelled similar to the nest whereas the alien pups would have been discrepant and recognition or rejection may be based on such a discrepancy.

The above explanations do not work for experiment 4.3 (S̃G vs S̃G̃). Here pups have been separated from their mother since day 2 and thus any acquisition of the pups' characteristics by the mother must have occured in these two days. As I have previously discussed (see Chp. 3, section 3.10.1) this possibility is unlikely. The possibility that recognition is accomplished by a discrepancy between nest odour and pup odour is also unlikely. Both alien and own pups have not been in the nest and have therefore not contributed to the nest odour. Thus both alien and own pups are likely to be discrepant from the nest odour. Similarly the hypothesis that recognition is accomplished by the mother depositing her odour on the pups is not possible as the pups have been separated from their mother since day 2.

It appears that the mother does not learn the characteristics of her young in the S̃G case postnatally. However one further possibility should be considered and that is that at the start of the experiment, the mothers already know who or what their kin are. The mother and offspring are related, r=0.5, that is, they share on average half of their genes in common. As the odour is in part genetically determined the odour of both mother and offspring will be similar. It may therefore have been possible for the mother to learn her own odour and apply this to her offspring. Mothers when pregnant lick the nipple line and also the genital and pelvic region more often (Roth and Rosenblatt, 1967; see plate 4.2) and this

PLATE 4.2
Lactating female Sprague-Dawley rat licking nipple line.

may present the mother with the opportunity to learn the characteristics of her offspring by self-odour learning. Recognition of her offspring could be accomplished by learning the 'kin' odour from other genetically related individuals. I have already demonstrated that siblings can recognise each other, thus the mothers may have acquired a representation of kin from their sibs. The mother's siblings are related to her (r=0.5) and to her offspring (r=0.25), any representation of kin learned from her siblings will be similar to that possessed by her offspring. The mother may therefore, prior to the birth of her offspring have a representation of her kin, including her offspring and this enables the mother to recognise her offspring. This possibility could apply to both experiment 4.1 as well as experiment 4.3.

4.6

CONCLUSION

The results from these experiments demonstrate that mothers do have the ability to recognise their offspring, that this recognition does not require the presence of the pups after birth, and that recognition of pups exposed to the mothers postnatally can be achieved by the mother. The results also confirm a prediction of kin selection theory, that in certain situations individuals would be expected to respond differentially to kin and non-kin. In this experiment mothers retrieve their own offspring

faster than alien young thereby decreasing the risk of predation and death due to heat loss, lack of food in their young. By this nepotistic behaviour the female increases the chances of her offspring surviving and therefore the behaviour benefits the mother's inclusive fitness.

CHAPTER 5

OFFSPRING-MOTHER RECOGNITION

IN THE RAT

5.1

INTRODUCTION

At birth the distribution of costs incurred by the mother and her offspring are uneven, the mother having invested heavily in the offspring whereas the young have invested nothing in their mother. This could lead to the pups being non-discriminating in their care seeking. The need to recognise the mother is not imposed on the young at first by themselves but is required by the mother. Although some mothers may be undiscriminating and allow alien young to suck, for example, the pipstrelle bat, Pipistrellus pipistrellus, (Kleiman 1969), most species will reject alien young and not allow them to suck (see Chp. 4, section 4.3). For this reason the young may have to be discriminating and recognise their mother to ensure they do receive adequate care and to prevent attack from alien mothers. Further in development other advantages may accrue from recognising the mother, for example, protection and avoidance of inbreeding. This chapter investigates the ability of offspring to recognise their parents.

5.2

OFFSPRING-MOTHER RECOGNITION:

LITERATURE REVIEW AND COMMENTARY

The review will not consider reports of offspring's inability to recognise their mother. Many reports of such an inability stem from behavioural observations of the offspring's response to adults including its mother, for example, Davis et al. (1968) report that young of the big brown bat, Eptesicus fuscus, will attempt to grab any adult female to suck. Fogden (1971) reports that pups of the grey seal, Halichoerus grypus, are unable to recognise their mother because they attempt to suck indiscriminately, similarly , Bartholomew (1952) discounts the ability of pups of the Alaska fur seal, Callorhinus ursinus, to recognise their parents as they too are indiscriminate when sucking. Such observations may indeed reflect the inability of young to recognise their mothers but further tests would have to be carried out to assert this. A lack of preference does not logically entail a failure to discriminate and all that can be concluded from these observations is that the young show no preference on sucking.

A similiar criticism may be levelled against certain experimental studies rejecting the idea of infant-mother recognition. Porter et al. (1973) in an investigation of mother-young attachment in the guinea-pig, Cavia porcellus, used a 2 choice discrimination task and found that young guinea-pigs spent equal amounts of time with

the mother and a novel lactating female. Likewise Barnett (1977) finds that fry of the cichlid fish, Cichlasoma citrinellum, show no preference in a T-maze for their own mother when compared to a novel mother. Neither study, however, can be used to demonstrate lack of maternal recognition, only a failure of preference. A different test may well reveal evidence of maternal recognition.

In comparison to work investigating the ability of mothers to recognise their young there has been little work aimed at the offspring's ability to recognise their mother and what has been done has mainly concentrated on the infant's ability to recognise the mother and neglected the abilities of older offspring. Romanes in "Animal Intelligence" (1883b), places recognition of offspring on level 20 in his hierachy of intellectual abilities, but does not include offsprings recognition of their parents. As with previous reviews I shall consider the available data in taxonomic groups. Rats will be excluded from this general review and considered later.

5.2.1
Invertebrates

Only one study has investigated the ability of invertebrate young to recognise their parents. Cockroach, Byrsotria fumigata, young were observed to congregate under their own mother in preference to one of the same size and age with similarly aged offspring (Liechti and Bell, 1975). The authors speculate that such recognition

is mediated by a contact pheromone excreted in the mother's faeces.

5.2.2
Fish

Again there is only one study which may indicate the ability of the fry of the cichlid fish, Etroplus maculatus BLOCH, to recognise its parents. When presented with models of the parents the fry only respond to the one exhibiting pelvic fin flickering, indicating this to be of importance in parental recognition (Cole and Ward, 1970). However whether the fry can use this to discriminate between their own and other parents, or just between individuals fin flickering or not, is unclear.

5.2.3
Amphibians And Reptiles

I could find no references to work which demonstrates the existence of maternal recognition in these two taxonomic groups.

5.2.4
Birds

One of the most comprehensive studies of infant-mother recognition has been carried out by Beer (1969,1970) on the laughing gull, Larus atricilla. By 6 days of age laughing gull chicks can identify their parents using the individual characteristics of adult gull calls (Beer, 1969). Moreover, the chick's responsiveness

to different aspects of their parents' calls changes over time: at first they are responsive to the "crooning call", but older chicks respond preferentially to the "ke-hah" call, such a change being linked to changes in the growing chick's social situation and the differing characteristics of the various calls (Beer, 1970).

Royal tern, Sterna maxima, chicks are attracted to the calls of their parents (Buckley and Buckley, 1970) and common tern, Sterna hirundo, chicks at 2-4 days old respond to the played back calls of their parents but not to the calls of other parents (Stevenson et al. 1970). Recognition of parents takes longer to achieve in the Galah cockatoo, Cacatua rosecapilla: not until 5 weeks old do the chicks begin to recognise their parents and refrain from begging food from other adults (Rowley, 1980).

Evans (1980) investigated how young ring-billed gulls, Larus delawarensis, acquire recognition of their parents and concluded that the birds recognition and responsiveness to its parents' calls is based on learning reinforced by the parents feeding of the chicks. Young of the common murre, Uria aalge, learn to recognise the call of their parents in the first few days after hatching and react selectively to these calls, and evidence suggests that the young murres learn certain aspects of their parents' calls whilst still in the egg (Tschanz cited in Wilson, 1975).

Evidence that young can recognise the mother may be drawn from the phenomenon of imprinting (see Bateson, 1966,1971,1981; Immelman and Suomi, 1981). A number of avian species have been demonstrated to be imprintable (see Hess, 1973, especially chapters 3,4). Although Lorenz (1935) proposed imprinting to be used in species recognition, later researchers have proposed it may also serve other functions: mating partner selection (Bateson, 1978a,b,1982) and more importantly for our purposes that of parental recognition (Bateson, 1966,1981).

5.2.5

Mammals

In the sea lion, _Otaria flavescens_, the young are able to recognise their mother within the first 24 hours after birth, such recognition being accomplished by both acoustic and olfactory cues (Vaz-Ferreira and Achaval, 1979). Both Stellar sea lion, _Eumetopias stelleri_ and Californian sea lion, _Zalophus gillespei_ (=californianus), pups are reported to be able to recognise the calls of their mothers (Bonnet, 1928). In a study of the mother-infant behaviour of the bottle nosed dolphin, _Tursiops truncatus_, Tavolga and Essapian (1957) report that infants show a preference for their mothers and conclude that these individuals do possess the ability to recognise their mothers.

Schneirla et al. (1963) studying the domestic cat, _Felis sp._, report that kittens can distinguish their own

mothers shortly after birth. Lion mothers, <u>Panthera</u> <u>leo</u>, on rejoining the litter call their cubs with a soft roar, which stimulates the cubs to run to their mother (Schenkel, 1966). These pups may be able to distinguish their mother by this call or may simply be stimulated by an approaching sound.

The problems in interpreting the results of preference tests are illustrated by studies examining infant-mother recognition in the guinea-pig, <u>Cavia</u> <u>porcellus</u>. Using a preference test Porter et al. (1973) report that guinea-pig young show no preference for their mother over another lactating female. However King (1956) examining the social relationships of guinea-pigs under semi-natural conditions observed guinea-pig young and reported that the young although following all members of the colony around, spend longer following the mother, providing evidence of recognition. Infant agutis, <u>Dasyprocta</u> <u>aguti</u>, are able to recognise their mothers within 3 days of birth (Roth-Kolar, 1957). Rowell (1960) observed the reactions of golden hamster, <u>Mesocricetus</u> <u>auratus</u>, pups to their mothers when their eyes opened and found that they reacted differently to the appearance of their mother than to strange females. Young of the field mouse, <u>Microtus</u> <u>arvalis</u>, similarly react differently to their own mother and a strange lactating female (Frank, 1952), resisting retrieval attempts by all females save their mother. Infant degus, <u>Octodon</u> <u>degus</u>, spend more time with a novel lactating female than with their own mother (White

et al., 1982) although whether the infants can recognise their mother or are responding to novelty is unclear. Nestlings of the wild rabbit, Oryctolagus cuniculus, learn to recognise the odour derived from the secretion of their mother's anal gland and show a different facial expression to the odour from their mother than to that of a strange female (Mykytowycz and Ward, 1971).

Lambs given a choice in a T-maze between their own mother and a lactating female prefer their own mother (Lindsay and Fletcher, 1968), but the cues used to recognise the mother could not be isolated in this study. Later studies attempting to isolate the cue(s) involved in recognition found that Merino lambs could recognise their dams by visual cues at about 3 weeks old but not at 1 week (Alexander and Walser, 1978). Walser and Hague (1980) investigated the bleats from Clun forest, Dalesbred, Jacob and Border Leicester sheep and found that each individual ewe had a call different from that of any other and the lambs seemed to use the whole call rather than one particular aspect of it to recognise the dam. Calves of cattle are able to recognise their mother by sound (Hafez and Schein, 1962) and reindeer young, Rangifer tarandus, are capable of recognising their mother by her individual call (Espmark, 1971). Buffalo, Bison bison, calves are able to recognise their own mother and move directly to her when separated (McHugh, 1958). Similarly bontebok, Damaliscus dorcas dorcas, calves can recognise their mother by sight and run to her from as much as 100 metres

(David, 1975). Wildebeest, Gorgon taurinus, calves initially cannot recognise their mother but after 24 hours exposure to her recognition is accomplished (Estes, 1963). Red deer, Cervus elephas, young are reported by Burckhardt (1958) to be able to recognise their mother, and an infant of the African elephant, Loxodonta africana africana, was observed to remain close to a particular individual, which presumably was its mother (Douglas-Hamilton, 1972).

Infant langurs, Presbytis entellus, at 2 weeks old recognise their mothers by visual cues and will orient to them from 15-20 feet (Jay, 1963). Infant squirrel monkeys, Saimiri sciureus, if socially reared prefer their mother to an unfamiliar female, infant or empty cage, but if the infants were reared with their mother and without sibs no preference was observed (Kaplan and Schusterman, 1972). Whether they had lost the ability to recognise the mother or just the preference for her is unclear. Pigtail macaque, Macaca nemestrina, infants are not mother specific at birth but soon learn to recognise their mother and come to respond only to her (Jensen and Tolman, 1962). Young stumptail macaques, Macaca speciosa, are able to recognise their mother, preferring her to familiar or unfamiliar mothers (Swartz, 1977), similarly bonnet macaques, Macaca radiata, prefer their own mother to a familiar or unfamiliar adult (Alpert, 1978). Juvenile rhesus monkeys, Macaca mulatta, when played the "coo-type" vocalisation of their mother and an unfamiliar mother show increased vocalisation and locomotor activity to their own

mother's calls but not to those of the unfamiliar mother (Hansen, 1976).

In humans, the abilty of infants to recognise their mother appears quite early in life. If given a choice of a breast pad from their mother and one from another mother by 6 days of age the babies prefer their own mother's breast pad (Macfarlane, 1975). Infants given a non-nutritive nipple to suck, which if sucked in different ways produced either their mother's voice or that of a strange female, by 3 days of age sucked more to obtain their mother's voice than that of a strange female (DeCasper and Fifer, 1980).

5.2.6

Summary And Critique

It becomes obvious from this review that the vast majority of studies concerned with offspring-mother recognition have concentrated on either birds or mammals with very few studies considering the possession of this ability by members of other taxonomic groups, fish, reptiles and amphibia.

The studies reviewed also demonstrate another interesting fact that nearly all, with one exception in juveniles (Hansen, 1976), have used infants to investigate offspring-mother recognition. Infants may be well protected by the mother and have little opportunity to leave her and therefore may have little need to recognise

the mother. Instead they may use this period to acquire
or cement maternal recognition for use later in life, for
example, when juvenile or subadult they may be threatened
by older conspecifics and may need the mother for
protection. Also later in life when sexually mature,
recognition of parents may be necessary to avoid
inbreeding. Most studies concerning infant recognition do
not consider whether this ability is retained later in
life, assuming that once established it will persist for
as long as necessary. Consideration of the offspring's
ability to recognise the mother outside of the infancy
period may lead to a wider range of species being
attributed with the ability of offspring to recognise their
parents.

The techniques used to study maternal recognition
fall into two catagories: behavioural observation and
preference tests. Behavioural observations, for example,
Schenkel (1966), Vaz-Ferreira and Achaval (1979), study
individuals in their natural habitat and differential
responsiveness by the offspring to their own and alien
mothers is taken as evidence of the ability to recognise
their mothers. There are a number of problems with this:
the question of kinship may be in doubt (see Chp. 3,
section 3.3.7), the technique only allows the
establishment of offspring-mother recognition, negative
results may be due to lack of preference rather than lack
of recognition. Finally, the technique has been used at a
gross level, for example, to study who offspring attempt

to suck. More detailed observation may provide better evidence of the ability to recognise the mother, see Rowell (1960) who observed the reactions of hamsters to their mother upon eye opening.

Preference tests, for example, Kaplan and Schusterman, 1972; Lindsay and Fletcher, 1968; White et al., 1982), involve giving an individual a choice between different stimuli, usually 2 choices (e.g., Macfarlane, 1975; Swartz, 1977), but sometimes greater (e.g., Kaplan and Schusterman (1972) use 4 choices). This technique allows better control of the test stimuli, especially kinship, but as with behavioural observation lack of a preference cannot be used to claim lack of recognition ability.

The best studies (e.g., Beer, 1970) involve the use of carefully controlled stimuli, as in playback experiments (e.g., Espmark, 1971) coupled with close observation of the infant's response to the various stimuli (e.g., Rowell, 1960), where problems concerning kinship, preference measures and what the individuals are discriminating may be carefully controlled.

Although at present offspring-mother recognition has been reported for relatively few species as compared to mother-infant recognition, the use of older offspring and more refined techniques will undoubtedly lead to more species being found to possess this ability.

5.3
INFANT-MOTHER RECOGNITION IN THE RAT

Lactating female rats emit a pheromone (maternal pheromone) which is highly attractive to young rats (Leon and Moltz, 1972). Although rats will orient to these cues as early as day 2/3 (Altman et al., 1973), orientation and movement towards the cues from a distance is not really seen until day 14, and it eventually wanes around day 27 (Leon and Moltz, 1972; see Leon 1980 for a review of maternal pheromone including production). The function of the maternal pheromone seems to be to attract young pups back to the mother, from whom they still require some nursing and protective care. It may also protect the young from necrotizing enterocolitis and enhance brain development (Moltz and Lee, 1981). Although the response wanes circa day 27, coinciding with weaning in the rat (Leon and Moltz, 1972), pups are attracted to the lactating females earlier than day 14, lactating females being preferred to virgin females between days 8-26 (Holinka and Carlson, 1976) and 10 day old pups approach mothers in preference to a non-lactating female (Nyakas and Endroczi, 1970). Similarly 10 day old pups after separation from their mother for 2 hours exhibit acceleration of heart rate when presented with maternal odour but no effect was noted on presentation of odour from a non-lactating female (Compton et al., 1977). Schapiro and Salas (1970) report that as early as day 2 maternal odour inhibits ongoing activity of rat pups. However, this activity decrease is probably due to the

pups raising their heads to sniff the odour and stopping activity to do so. It is not clear whether the pups were responding to the odour as maternal or just an odour, as odour from a non-lactating female was not presented, it does however indicate that pups can detect the odour presented by their mother at 2 days old.

Whilst there have been many studies demonstrating that rats prefer the odour of their mother to that of a non-lactating female (e.g., Compton et al., 1977; Holinka and Carlson, 1976; Leon and Moltz, 1971; Nyakas and Endroczi, 1970), the ability of pups to recognise their mother has received scant attention. Leon and Moltz (1971) demonstrated that it does not have to be the pups' own mother that attracts the pups, since the pups are attracted by any lactating female. In a 2 choice experiment Leon and Moltz (1971) presented pups with a choice of their mother and a strange lactating female, and found that pups showed no preference for their own mother, only 53% of the pups preferring their mother (N=60). This study suggests that rat pups show no preference for their own versus an unfamiliar mother. However pups were aged 16 days old at the time of testing, and previous research has shown that at this age pups exhibit no preference for familiar or unfamiliar odours (Carr et al., 1979b). Thus if the pups could discriminate between the two, it might be expected that the pups would show no preference.

Brown and Elrick (1983) using Long-Evans rats found

in a 2 choice preference test that 18-20 day old pups preferred the odour from their mother's faeces to that from the faeces of a strange lactating female. This preference only occured if the pups were reared with their mother alone, rearing with their dam and sire destroyed this preference. Although demonstrating the ability to recognise their own mother's faeces it does not answer the question of whether they can recognise their mother. The mother may present a different set of cues to that of only her faeces and pups should be examined for their ability to respond differentially to the mothers (own/alien) as a whole before maternal recognition can be truly attributed to these individuals.

Thus although studies have attempted to demonstrate the ability of young to recognise their mother, this ability has yet to be conclusively proved.

5.4
AN INVESTIGATION OF OFFSPRING-MOTHER RECOGNITION
IN THE RAT

Rat pups were tested for their ability to recognise their mother between the ages of 5-22 days, an age when mothers have been demonstrated to be capable of recognising their pups (Chp. 4) and pups to recognise their siblings (Chp. 3). Pups were tested in a T-maze for their preference between their own mother and an unfamiliar lactating female with pups of the same age. As with sibling and offspring recognition the possibility of

acquiring maternal recognition through a socially mediated mechanism was investigated by examining pups' preferences for a mother with whom they had only experienced postnatally and an unfamiliar mother. The possibility of maternal recognition acquired through genetically mediated mechanism was examined by testing pups for their preference between their mother from whom they had been separated soon after birth and an unfamiliar lactating female.

It was hypothesised that because the mother can recognise her offspring and because the pup needs care and protection from the mother, the pups should possess the ability to recognise their mother and respond preferentially to her.

To ensure that if no preference was exhibited by the pups this was not due to the pups' inability to detect the stimuli, the mother's odour, pups were tested for their ability to detect maternal odour as early as day 1.

5.4.1
Subjects

Rat pups born in the Department of Psychology, University of Durham from adult outbred Sprague-Dawley rats supplied by B & K (Animal Suppliers) Ltd. were used. Care was taken in breeding to ensure that all adult rats were unrelated (r=0.0). All cages were inspected twice daily and if a litter was found it was noted and that day

termed day 0 in the rats life. On day 2 litters were placed in one of two rearing conditions.

Rearing Condition 1: Two litters were used in this condition, each being culled to eight members. Both litters were born on the same day. Each litter was left as at birth, ensuring pups in each litter were both socially (S) and genetically (G) related to their respective mothers. A third litter was added to this condition consisting of eleven pups to test for pup attraction to maternal odour.

Rearing Condition 2: Seven litters were used in this condition. Each was culled to 7 members and all litters were born on the same day. Each litter was numbered such that litter one contained rats all marked 1, litter 2 all marked 2 and so on. These original seven litters were then rearranged to form 7 new litters each containing one from each of the seven litters (each new litter contained rats marked 1,2,3,4,5,6,7).

Thus each new litter contained only one of the mother's original pups and litters were left like this for the duration of the experiment. Rearing condition 2 provided two experimental conditions. First the $S\widetilde{G}$ condition, in which pups were socially (S) but not genetically ($\widetilde{G}$) related to their 'mother' and second the $\widetilde{S}G$ condition in which pups were not socially ($\widetilde{S}$) related to their mother but were genetically (G) related.

Unfamiliar mothers and pups (S̃G̃) were neither socially (S̃) nor genetically (G̃) related to one another.

Pups were marked daily with a black Carioca felt-tip pen from day 2 until day 12 (marking took the form of writing the appropriate number on the back of the rat). From day 13 onwards all rats were marked with alcoholic Picric acid every three days.

Rats were kept in plastic Bowman's cages (16"x12"x7") with food and water freely available on a 12 hour light/dark cycle (darkness starting at 1200 hours).

5.4.2
Apparatus

Pups were tested in the same apparatus as that used to test for sibling recognition. Again the three different sizes were used to accomodate the growing pups, the only difference being that the large goal box (7"x7"x7") was used throughout the experiment (see Chp. 3, section 3.5.2, for fuller description of this apparatus).

All pups were placed in the apparatus individually for 2, 30 minutes sessions prior to experimental testing; similarly all mothers were placed in the goal box for 2, 15 sessions prior to the start of the experiment to familiarise them with the apparatus.

5.4.3

Procedure

A number of experiments were carried out to investigate the preferences of infant rats for their socially and/or genetically related mothers. The procedure used was similar to that described for sibling recognition and fuller details will be found there (Chp. 3, section 3.5.3).

5.4.3.1

EXPERIMENT 5.1: The preference of infant rats for their mother (SG) or an unfamiliar (S̃G̃) lactating female

Rat pups were tested for their preference between the mother with whom they had been with since birth, and an unfamiliar lactating female with pups of the same age. Mothers were placed in each goal box on tissue paper, with the fans on and left for 10 minutes. The test pups were held in their home cage until testing. The test pup was placed in the start tube with its head at the choice point and left until it crawled to one side or other. Preliminary observations had indicated that on arrival at the mesh the mothers attempted to pull the pup through and it was decided to use as the response the side to which the rat crawled to prevent any harm to the pup. The trial terminated as soon as the pup's head touched the mesh. After each trial the maze was throughly cleaned and for half the pups each day the maze was rotated through 180 degrees prior to testing. Sixteen pups were tested from 2

litters, each pup was tested once daily, from day 5 to day 22. All tests were carried out at the same time each day, each litter of SG pups experiencing the same 2 mothers each day.

5.4.3.2

EXPERIMENT 5.2: The preference of rat pups for their socially related mother (S$\widetilde{G}$) over an unfamiliar ($\widetilde{SG}$) lactating female

Pups were tested for their preference between their mother with whom they had been since day 2 and an unfamiliar mother with pups of the same age to assess the possibility of acquiring maternal recognition through a socially mediated mechanism. The procedure was the same as that described for experiment 5.1. Twelve pups were tested from 4 litters from day 5 until day 22.

5.4.3.3

EXPERIMENT 5.3: The preference of infant rats for their genetically related mother ($\widetilde{S}$G) over an unfamiliar ($\widetilde{SG}$) lactating female

Pups were tested in this experiment for their preference between their mother from whom they had been separated since day 2 and an unfamiliar mother with pups of the same age to assess the role of a genetically mediated mechanism of maternal recognition. The procedure was the same as that described for experiment 5.1. Twelve pups were tested from 4 litters from day 5 until day 22.

5.4.3.4

EXPERIMENT 5.4: Development of pup attraction to social stimuli

Neonates were tested for their preference for their own mother (socially and genetically related) as opposed to a clean goal box. Eleven individuals from a single litter were used. This experiment used pups younger than the previous experiments testing pups from day 1 until day 8. As the pups in this experiment were very young and had a little developed ambulatory response, the crawling response was supplemented by recording head orientation. As with experiments 3.4, 3.8 (see chapter 3) if the pups head crossed the line and remained for 15 seconds with no crawling response, then the side the pup was facing recorded as the positive response.

5.4.4
Analysis And Results

The number of pups crawling to their 'own' (SG,$\widetilde{SG}$,$\widetilde{S}G$) mother on each day was expressed as a percentage and plotted graphically (see figs. 5.1, 5.2, 5.3). Observation of these graphs indicates pups prefer their natural mother (SG) for the duration of the experiment, whereas pups either socially ($\widetilde{SG}$) or genetically ($\widetilde{S}G$) related, do not show such a preference.

To examine whether there was any difference between these three groups each pup was classified as to whether
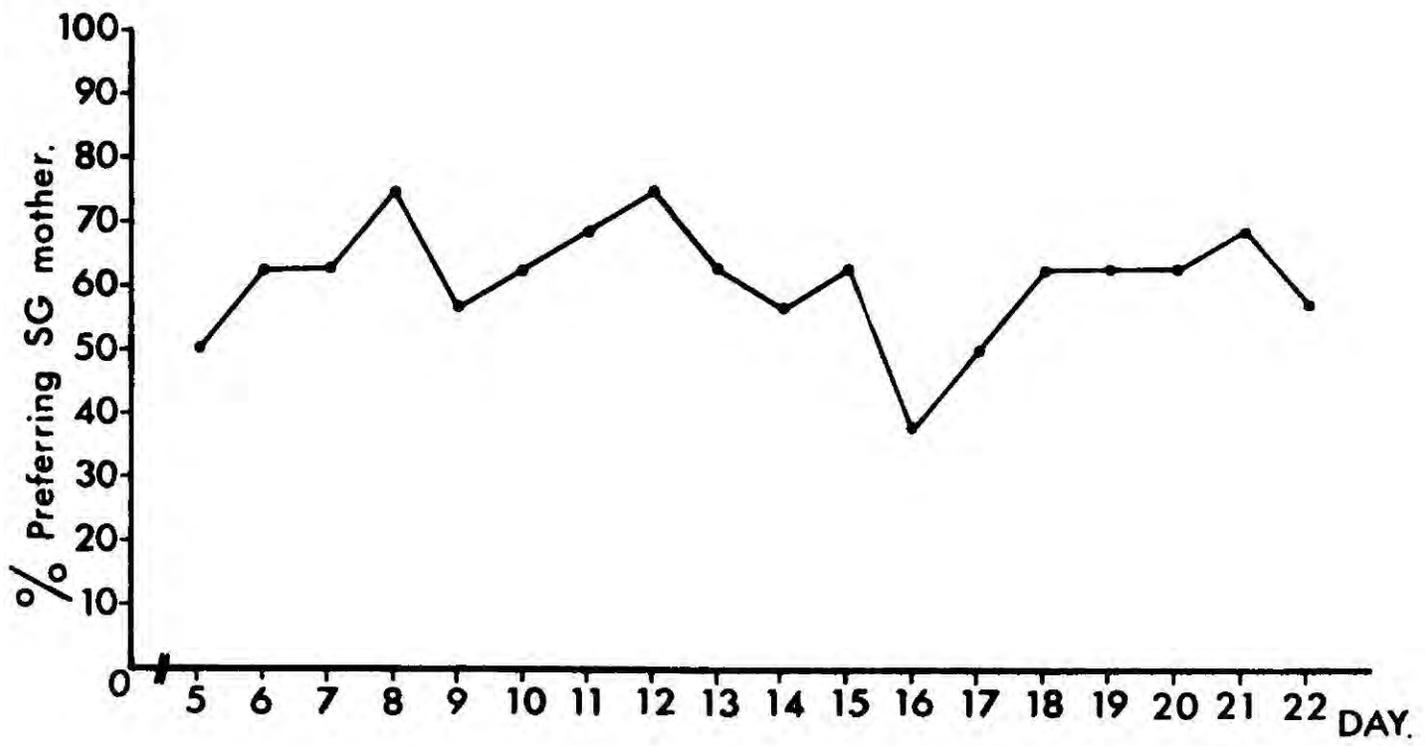
FIGURE 5.1
The percentage of pups preferring the socially and
genetically related mother (SG) on each day of testing.
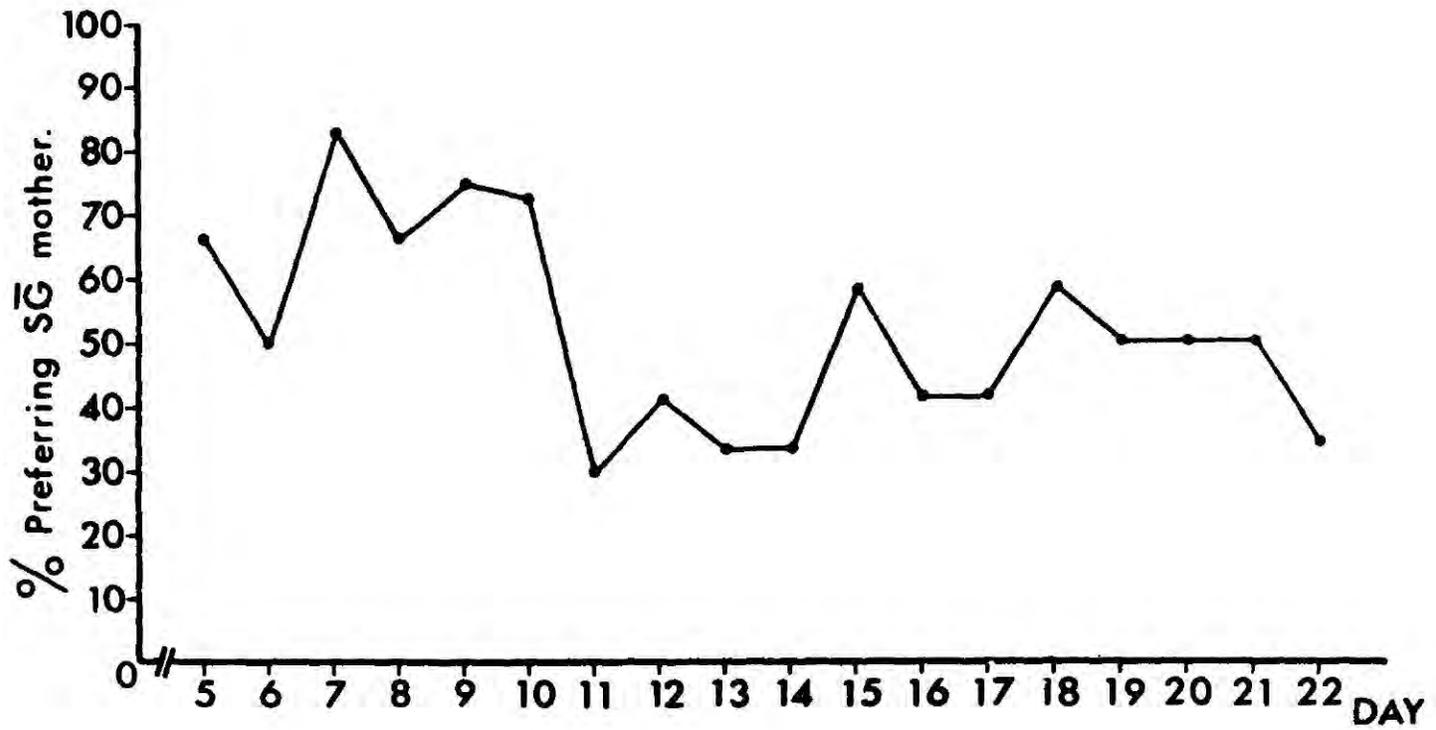
FIGURE 5.2
The percentage of pups preferring the socially related
mother (SḠ) on each day of testing.
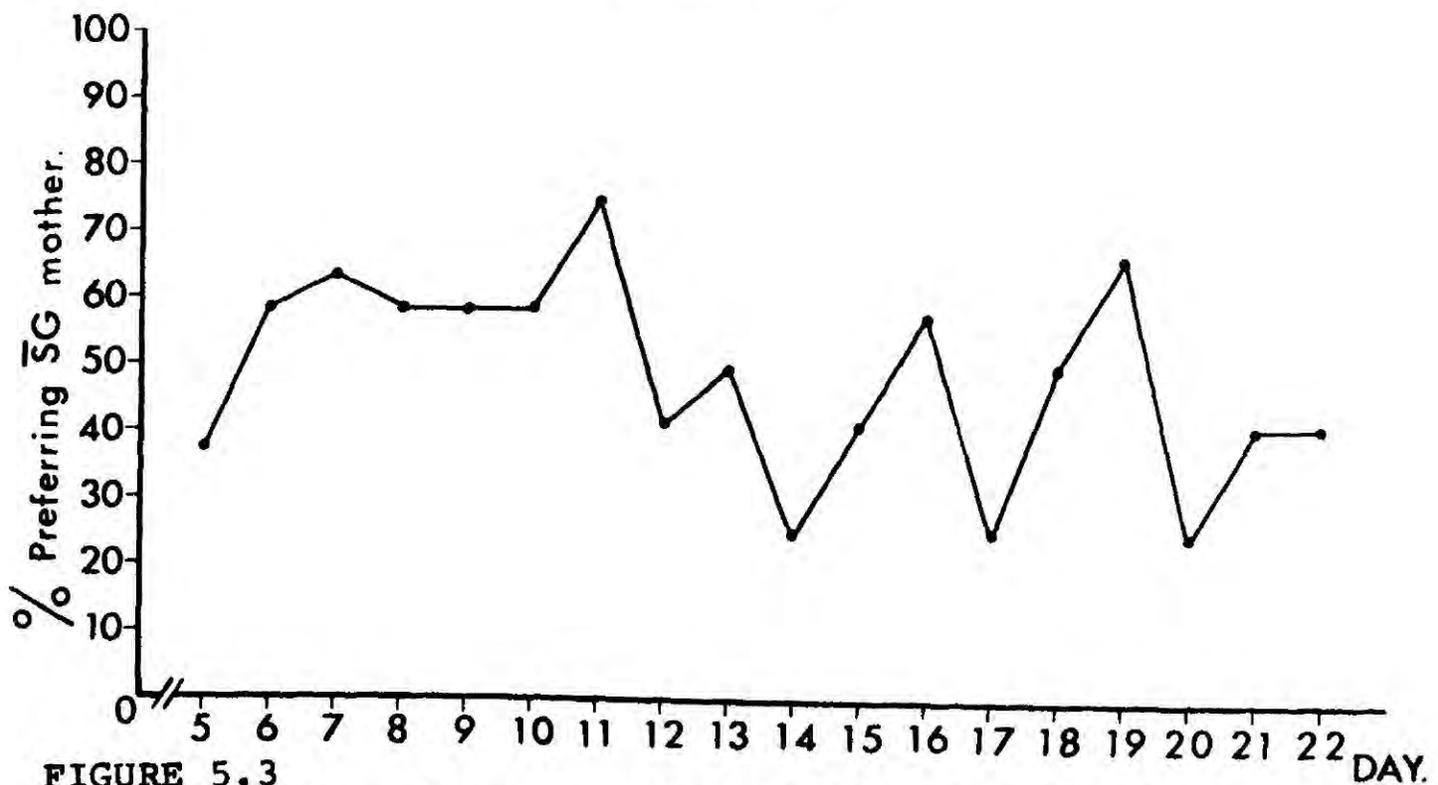
FIGURE 5.3
The percentage of pups preferring the genetically related
mother (S̄G) on each day of testing.

it exhibited an overall preference to its 'own' mother or the alien mother, on the basis of all its responses in the experiment. In the case of ties, the first tie was attributed against the hypothesis (i.e., the individual was taken as preferring the alien mother), the next for the hypothesis (i.e., the individual taken as preferring their own mother). This produced a 3x2 contingency table (see table 5.1) of relatedness to the mother (SG, $S\widetilde{G}$, $\widetilde{S}G$), and overall preference ('own' or alien) on which the x^2 was performed. The test indicated there was a significant difference expressed by the groups in their preference for the 'own' and alien mother (x^2 =6.1552, df=2, 0.05>p>0.02). Binomial tests (see Siegel, 1956) indicate that pups showed an overall preference for their own natural mother (SG), N=16, x=3, p=0.022, 2-tailed, but did not show an overall preference for their only socially related mothers ($S\widetilde{G}$), N=12, x=5, p=0.774, 2-tailed, nor for their genetically related mothers ($\widetilde{S}G$), N=12, x=5, p=0.774, 2-tailed.

These results indicate that pups do show a preference for their natural mothers, but this is not based on social or genetic relatedness alone. It may be that as with sibling recognition, the pups show a changeover in preference over time, and thus no overall preference would be shown. To examine this possibility the F.E.P. test (see Siegel, 1956) was performed for each experiment. The number of rats preferring the familiar and unfamiliar mother on days 10-14 and days 18-22 was calculated and a

TABLE 5.1

Summary of the number of pups showing an overall preference for the 'own' and alien mother between days 5-22 for experiments 5.1 - 5.3.

		EXPERIMENT		
		5.1	5.2	5.3
		SG	SG̃	S̃G
MOTHER PREFERRED OVERALL	'OWN'	13	5	5
	ALIEN	3	7	7

$$x^2=6.1552, \ df=2.$$
$$0.05>p>0.02.$$

2x2 contingency table of time (days 10-14, 18-22) and mother preferred (familiar SG,$\widetilde{SG}$,$\widetilde{SG}$, unfamiliar $\widetilde{\widetilde{SG}}$) was drawn up.

The F.E.P. (see table 5.2) indicates that in no case was there any significant changeover in preferences from days 10-14 to 18-22, SG:0.25>p>0.2, s$\widetilde{G}$:0.25>p>0.2, $\widetilde{SG}$:0.3>p>0.25.

Experiment 5.4 examined the ability of pups to respond to a lactating female vs an empty goal box. The number of pups, expressed as a percentage, responding preferentially to the mother each day is presented in figure 5.4. Observation of this graph indicates from the first day of testing more pups responded preferentially to the mother. Each pup was classified as showing an overall preference for the mother or empty goal box for the duration of the experiment and a binomial test performed (see Siegel, 1956). The results indicate that pups did show an overall preference for their mother (N=11, x=0, p<0.01, 2-tailed). These results replicate and further those obtained in experiments 3.4 and 3.8, here pups 1 day old have a functioning olfactory system and are able to detect conspecific stimuli.

5.4.5

Discussion

The results indicate that pups who are both genetically and socially related to the mother prefer her

TABLE 5.2

Summary of the number of pups showing an overall
preference for their own and alien mother at 10-14 and
18-22 days of age for experiments 5.1 - 5.3 with Fisher
probability.

	SIDE PREFERRED	TIME PERIOD (DAYS)		FISHER PROBABILITY
		10-14	18-22	
EXPT. 5.1	SG	10	12	p=0.2259
	S̃G̃	6	4	
EXPT. 5.2	SG̃	3	5	p=0.2369
	S̃G	9	7	
EXPT. 5.3	S̃G	6	5	p=0.2932
	S̃G̃	6	7	
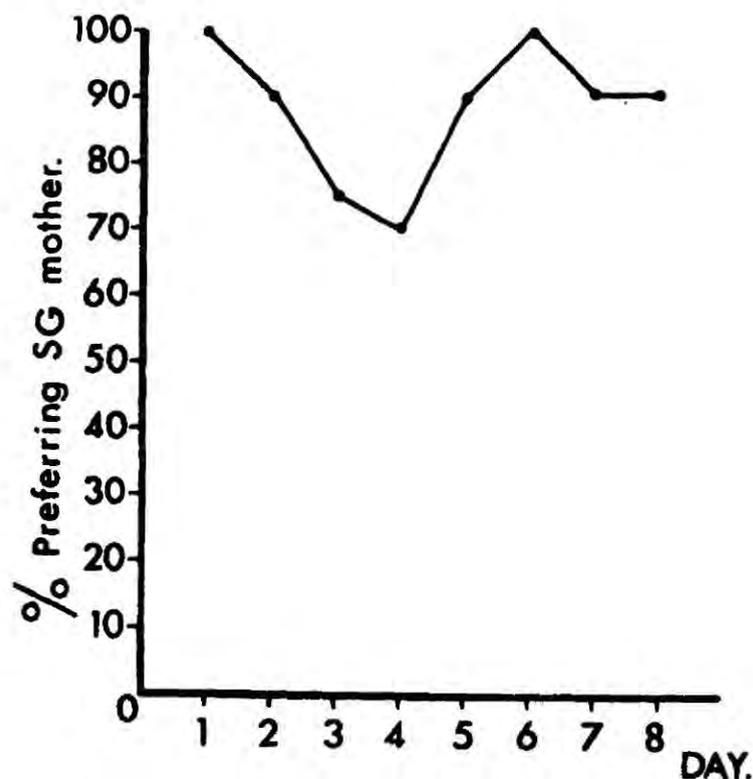
FIGURE 5.4
The percentage of pups preferring their mother (SG) on
each day of testing.

to an unfamiliar lactating female, whereas those pups only socially related or only genetically related to their 'mother' show no preference for her over an unfamiliar mother. From these results it can be said that the infant rats can recognise their mother and it seems that both socially and genetically mediated mechanisms are necessary for the acquisition of this ability. Although pups in experiments 5.2 and 5.3 exhibited no preference, one cannot conclude from this that these pups could not recognise their mother, just that they exhibited no preference. There are a number of possible reasons why a preference may not have been exhibited.

It may be that the pups cannot recognise their mother if only allowed access to either a socially or genetically mediated mechanism. However results from studies on sibling recognition (Chp. 3) would argue against this. In chapter 3 pups socially and genetically related to sibs could recognise them, as could pups only socially related and only genetically related. This would suggest that the pups are able to acquire the characteristics of their conspecifics via only a socially or only genetically mediated mechanism, and thus should be able to achieve maternal recognition by these means. It may be that the mothers do not provide salient cues which enable them to be identified by the pups as their own mother. However pups in experiment 5.1 did exhibit a preference, indicating that the mothers presented a cue salient enough for them to be identified as the pup's own mother. Also

pups in experiment 5.4 were able to detect the stimuli presented by the mother as early as day 1. It is therefore unlikely that the absence of preference was due to an inability to recognise the mother, and we must seek some other reason.

One possibility is that the pups are simply indifferent between the 2 lactating females. However as the pups in experiment 5.1 clearly showed a preference for their mother and it is unlikely that the pups in experiments 5.2 and 5.3 do not have a preference. It may be that no preference is being shown due to the fact that the stimuli are unattractive to the pups. However lactating females emit a pheromone which is highly attractive to the pups (Leon and Moltz, 1972) and there is no reason to suppose the females used in these conditions were any different and as such would be highly attractive to the rat pups.

No preference was shown probably for a combination of a number of reasons. Pups in experiment 5.1 had both pre- and post-natal experience with their mothers, whereas pups in experiment 5.2 had only post-natal experience and pups in experiment 5.3 only prenatal experience. This may lead to the representation of the mother being weaker for the pups in experiment 5.2 and 5.3, than those in experiment 5.1 (compare sibling recognition Chp. 3, section 3.6.5, where pups socially and genetically related to their sibs show a stronger response to each other, compared to sibs

either only socially or genetically related). Thus
although the pups in these experiments (5.2,5.3) can
recognise their mother they may not be as good as the pups
in experiment 5.1 at recognising her. This may lead to
the situation of the pups in experiment 5.1 when presented
with two highly attractive stimuli (due to maternal
pheromone emmission) perceiving one as the mother above
the attractiveness of each mother. However in experiments
5.2 and 5.3 recognition of the mother is thwarted by the
attractiveness of the stimuli and the pups respond to the
mothers on the basis of maternal pheromone rather than
'motherness'. Thus it is probable that the pups in all
three experiments do have the ability to recognise their
mother but the task is not sensitive enough in experiments
5.2 and 5.3 to separate attractiveness to a lactating
female from attractiveness to their mother.

Identification of the mother as with sib recognition
and mother-infant recognition is most likely based on
olfactory cues (see Chp. 1, section 1.4.1). Observation
of the pups in the maze indicated that at the choice point
pups would raise their heads and sniff down both arms of
the maze before heading down one arm. However pups may
have been helped by the actions of the mothers in the
maze. Behavioural observation of the mothers in the goal
box suggested they could recognise their own pups in the
maze (supporting the findings of Chp. 4). On placing the
pup in the maze, both mothers sniffed at the infant, the
infant's mother continuing for a longer period than the

unfamiliar mother. Generally the pup's own mother appeared to pay more attention to the pup than the unfamiliar mother. Thus there may have been an interaction between the mother and her pup, whereby the mother being more active when presented with her pup, may draw the pup's attention to her as a more salient stimulus, inducing the pup to crawl to her.

In summary, the results indicate that pups socially and genetically related to their mother prefer their mother over an unfamiliar lactating female, and thus are able to recognise their mother. Pups only socially or only genetically related to their mother show no preference for her over an unfamiliar mother, but this does not necessarily mean they are unable to recognise their mother. Recognition is based upon olfactory cues, but an interaction effect between the mother and pup, with the mother paying more attention to her pup may also provide the pup with an indication of who their mother is.

One final question is how the pups acquire the recognition of their mother. Only experiment 5.1 (SG) will be considered as this provided the only definite indication of the pups' ability to recognise their mother. As the pups in experiment 5.1 achieved recognition, whereas pups in experiment 5.2 and 5.3 may not have done, it can be concluded that both social and genetic mechanisms were involved in the acquisition of this ability. If only one or the other factor (social/genetic)

had been involved, then the corresponding experiment allowing only that experience would be expected to show similiar results to experiment 5.1, as it did not it can be concluded that both social and genetic mechanisms were involved in the pups acquisition of maternal recognition.

5.5
OFFSPRING-MOTHER RECOGNITION IN THE RAT

One interesting fact to emerge from these experiments is that although the pups were able to recognise their mother, they did not show a reversal of preference over time. In the previous experiments on sibling recognition pups preferred their siblings on day 12, but by day 20 this preference had reversed, such that siblings preferred non-siblings (see Chp. 3). Likewise Carr et al. (1979b) report that pups when exposed to home cage and strange cage bedding, at 12 days prefer home cage bedding, at 16 days show no preference but at 20 days show a reversal in preference and prefer the strange cage bedding. From these results they conclude "that rat pups aged 12-20 days can recognise their own dam or nest, as opposed to a strange conspecific dam or nest" (p. 1150). My results whilst supporting the conclusion that pups can recognise their mother, do not indicate the reversal in preference as shown by Carr et al. (1979b). In Carr et al's. study approximately 67% of the pups preferred the familiar bedding on day 12 and in this experiment on day 12, 75% of the pups preferred the mother. On day 20 however only 38.3% of Carr et al's. pups preferred the familiar

bedding whereas 62.5% of pups in this experiment preferred their own mother. One of the most obvious reasons for this is that different stimuli were used, in this experiment actual mothers were used whereas Carr et al. used bedding. This implies that the mothers possess different stimulus qualities to either bedding or siblings, which both elicit a reversal of preference over time (see also criticism of Brown and Elrich, 1983, section 4.4). It may be that the mother because she is such an important stimulus for the pup, that is, she provides warmth, food, protection etc., retains her attractiveness over time. Another factor which may have kept the preference for their mother over the duration of the experiment is the interaction between the pup and its mother in the maze. As I have previously mentioned when the pup was placed in the T-maze the pups own mother showed much more interest in the pup than the unfamiliar mother, and this increased attention may have drawn the pup to the mother retaining the preference over time.

5.6

CONCLUSION

These experiments indicate that rat pups are able to recognise their mother from day 5 to day 22 and prefer their mother to an unfamiliar mother. This ability may ensure that should the pup stray from the nest it will return to its mother, thus gaining the care and attention it requires and also avoiding the possibility of attack by conspecifics. The ability to recognise their mother may

allow the pup to gain benefits accruing to them and the mother as predicted by kin selection theory.

CHAPTER 6

THE MECHANISMS OF KIN RECOGNITION

IN THE RAT

6.1

INTRODUCTION

The experiments reported in the previous chapters indicate the existence of a number of different routes through which individuals may accomplish kin recognition. Experiments in chapter 2 demonstrated that rats have the ability to recognise individuals, thus making possible the acquisition of kin recognition through recognising individuality, a social mechanism. Experiments in chapters 3-5 investigated the role of genetically and socially mediated recognition and evidence was found to support both of these mechanisms. I shall now consider further how such mechanisms may function, but first I shall discuss the modality of kin recognition and the nature of the identifier.

6.2

THE PERCEPTUAL SENSE OF KIN RECOGNITION

At the start of this thesis it was hypothesised that information concerning kinship would be transmitted via olfactory channels (Chp. 1, section 1.4.1). Subsequent experiments have indicated this to be so. Deprivation of olfactory cues (Expt. 3.10, section 3.8) destroyed the ability of individuals to recognise their siblings.

Although no olfactory deprivation experiments were performed for mother-infant, infant-mother, and individual recognition, behavioural observations indicated that, as with sibling recognition, olfactory cues were important in mediating these recognition processes. This supports previous experiments in the rat which have indicated olfaction to be the carrier of information concerning social status (Krames et al., 1969), group membership (Krames and Shaw, 1973), sex (Brown, 1977), sexual condition (Drewett and Spiteri, 1979; Gilman and Westbrook, 1978; Stern 1970), individuality (Carr et al., 1976), stress (Mackay-Sim and Laing, 1980), reward/frustration (Morrison and Ludvigson, 1970) and site of safe feeding areas and diet preferences (Galef, 1982a). Studies on other rodents also indicate the importance of olfaction in kinship recognition, for example, Spiny mice, Acomys cahirinus, (Porter et al., 1978), mice, Mus musculus, (Gilder and Slater, 1978; Kareem and Barnard 1982) and white-footed mice, Peromyscus leucopus (Grau, 1982).

One other significant finding to emerge from these studies is the ability of newborns to respond to their conspecifics' odour. Although studies have shown the olfactory system to be functioning at or soon after birth (see below), their have been few studies showing a response to a biological odour at an early age. One exception to this is the preferential response of infants to the odour of the mother's amniotic fluid facilitating

nipple attachment and sucking a few hours after birth (Blass and Teicher, 1980). However studies using odours to which the infants have not been exposed prenatally indicate infants are not responsive to these odours so soon after birth. Sniffing patterns are poorly maintained in rats less than 1 week old (Alberts and May, 1980) and this may account for the lack of findings indicating the ability to respond to conspecific odours in pups under 1 week old. Gregory and Pfaff (1971) report that pups 12 days old prefer home cage shavings over clean shavings but pups at 7 days old do not. Compton et al. (1977) demonstrate an increase in heart rate in pups 8 days old when presented with maternal odour. Sczerzenie and Hsiao (1977) report that pups 5-6 days old prefer the odour of home nesting material to clean shavings, Cornwell-Jones and Sobrian (1977) report a similiar preference at 3-4 days of age. Two day olds show a decrease in activity when presented with maternal odour (Schapiro and Salas, 1970), although this decrease in activity may be due to raising the head to sniff an odour rather than a response specific to maternal odour.

In two experiments reported here (3.8, 5.4, sections 3.6.4.4, 5.4.4 resp.), neonate rats responded preferentially to sibling litter odour and maternal odour on the first day of testing. Individuals responding preferentially to sibling litter odour on day 2 and responding preferentially to their mother's odour on day 1. Such results support and extend previous work

indicating neonates possess a functioning olfactory system
at birth which is capable of responding to conspecific as
well as other odours.

One further consideration is that of the site of
odour production. There are a number of possible sites
for odour production, for example, the integument
(sebaceous and sweat glands); salivary glands
(submandibular, submaxillary, sublingual); accessory
glands of the eye; foot pad; preputial gland; faeces;
urine, and vaginal secretions (Adams, 1980). A number of
these have been demonstrated to emit biologically
significant odours in the rat and other species. The
preputial gland of the rat yields a sex attractant (Adams,
1980) and female rats prefer the odour of the male's
preputial gland to that of its foot-pad, submaxillary,
sublingual, or coagulating glands (Gawienowski et al.,
1975). The submandibular gland of the rat produces a
chemical which also acts as a sex attractant (Materazzi,
1967). The faeces of lactating female rats serve as a
source of maternal pheromone (Leon, 1974). Cues
concerning individuality or kinship may be found in the
urine, as rats are capable of discriminating familiar and
unfamiliar putative urinary odours (Fass et al., 1978).
However the most likely source of olfactory information
concerning kinship and individuality is the integument,
that is, the skin glands. Eccrine sweat glands in the
plantar surface of the mouse foot secrete an individually
recognisable scent (Adams, 1980) and foot-pad glands in

the rat may do the same. The sebaceous glands are a very likely source of information concerning individuality and kinship. Skin lipids produced by the sebaceous glands contain enough variability between individuals and consistency within individuals to enable individual recognition (Nicolaides, 1974) and may allow kin recognition. Thus there are a number of possible sites of odour production which could produce information concerning kinship, the most likely source being the skin glands especially the sebaceous glands.

6.3
THE NATURE OF THE KIN IDENTIFIER

Two main issues were raised concerning the nature of the kin identifier. First, it was suggested that kin may be recognised by individual cues or by a common cue possessed by all kin. The previous experiments provide evidence for both mechanisms. Experiments in chapter 2 demonstrated the ability of rats to discriminate between individuals for a food reward, indicating that rats possess the ability to recognise individuals and that they produce individually identifiable odours. Individuals were capable of discriminating between siblings indicating that genetically related individuals, even though they may have a degree of commonality between their odours, can still be distinguished individually.

Evidence for a commonly possessed identifier may come from the $S\widetilde{G}$ condition in chapters 3-5, where individuals

were able to recognise littermates. There can be little
doubt that when huddling individuals will deposit their
odour on fellow huddlers and so individuals in the group
may come to possess a common odour made up of all the
individual odours. Moreover, individuals possessing the
same mother may smell alike through maternal odour being
deposited upon them. Definite evidence of a commonly
possessed cue comes from the ability of individuals to
recognise their siblings even though they had been
separated soon after birth, the SG condition. Such an
ability indicates a commonly possessed identifier. Thus
both individual and commonly possessed cues are available
to the individual to use to recognise its kin.

The second question concerns whether the identifier
is genetically or environmentally determined. Odours are
a mix of genetically determined factors and
environmentally determined ones, for example, diet. It
was argued that environmental cues may be transient and
introduce too much variability into an odour to allow
accurate identification. For this reason all
environmental influences during the experiment were
carefully controlled, rats were housed identically and all
fed the same diet, thus reducing the effect of
environmental factors on odour. The remaining variation
due to genetic factors was great enough to allow
individuals to be recognised (Chp. 2) and kin to be
recognised (Chps. 3-5). Thus the genetically determined
factors of odour production are sufficient to enable kin

and individual recognition.

6.4
THE ACQUISITION OF KIN RECOGNITION

6.4.1
Socially Determined Mechanisms Of Kin Recognition

The fact that individuals reared with unrelated conspecifics come to respond to these rearing partners preferentially (see expts. 3.1,3.6,4.2) indicates they have learned or acquired the characteristics of their littermates postnatally. Signs of recognition are present by day 10, and possibly earlier, so the acquisition of 'kin' characteristics must have taken place by this time. It was hypothesised that socially acquired kin recognition could take two forms: either recognition of individuality or recognition of a common kin identifier. Whichever is used, there are three possibilities to explain how these characteristics are acquired, the three catagories not being mutually exclusive:

6.4.1.1
Physiological Imprinting

This mechanism requires that the prevailing olfactory stimulus perceived by the individual imprints itself onto the nervous system of the organism and this allows a special selectivity of response to that odour. Young rats exposed from day 14 onwards to the chemical, cycloodanone, have been observed to exhibit consistent patterns of cell degeneration in the mitrial cells of the olfactory bulb

(Doving and Pinching, 1973), and exposure to a number of different odours produced patterns of mitrial cell degeneration specific to that odour (Pinching and Doving, 1974), indicative of a topographical representation of odours in the brain. However Leon (1980) used different conspecific odours and found no evidence of specific mitrial cell degeneration to particular odours. Thus although there is evidence for a topographical representation of pure chemical stimuli, there is no evidence to support this for conspecific odours. The above experimental techniques analysed events at a gross anatomical level and a finer analysis may be needed to expose the effects of more complicated odours (e.g., conspecific odour). Evidence of topographical representation may indicate cell growth and neural synapse patterns to be fashioned by the prevailing odour and allow a special selectivity of response to that odour.

Physiological imprinting may function by altering the sensitivity of the system to odours to which it has previously been exposed. However pre-exposure of an individual to an odour does not affect its perception of that odour (Laing and Panhuber, 1980) nor does it affect its response to different odours (Dalland and Doving, 1981).

A final posibility to consider is that the receptor sites for perception of olfactory stimuli may become altered by exposure to an odour to become more responsive

to that odour.

In summary, the possibility exists for early exposure to an odour to alter the physiological system of the individual such that the individual becomes more responsive to that odour. Such a system is more likely to function for a commonly possessed kin identifier rather than for individually possessed identifiers, as this would entail differential responsitivity to only one odour rather than many.

6.4.1.2
Exposure Learning

Differential responding to kin and non-kin (i.e., kin recognition) may arise through exposure to a particular odour. The individual being presented with the odour may become familiar with this odour and respond differentially to it. Imprinting has been postulated to function similarly . Here exposure to a particular stimulus causes this stimulus to become more familiar and attractive whilst other stimuli, due to a narrowing of preferences, exert a much lesser effect (Bateson, 1981). Individuals may acquire recognition of kin through imprinting (e.g., Bateson, 1979).

Leon (1980) proposes a neurobehavioural model to explain how olfactory stimuli may become familiar/imprinted in rats. Basically he proposes that the young rat because of its immature olfactory system

cannot habituate to olfactory stimuli, thus the nervous system continues to respond to salient olfactory stimuli with little response decrement. This allows continuous stimulation by olfactory cues which enables the individual to become familiar with them. As the olfactory system matures, the ability to habituate increases and new odour stimuli no longer have prolonged access to the olfactory system and thus have less chance of becoming familiar.

In the rat pup, the salient olfactory stimuli up until the time of adult functioning of the olfactory system (i.e., the development of habituation, after about 2 weeks in the rat, Leon, 1980), will be the mother and littermates, kin. Thus infants have the opportunity to become familiar with their kin, either individually or through a commonly possessed cue, and may be able to achieve kin recognition by this mechanism. Pager (1974) provides some evidence for the functioning of such a mechanism, where presentation of an odour to young rats caused an increase in mitrial cell firing if they had been previously exposed to the odour but in adult rats no effect was observed.

There have been a number of examples of individuals reared with novel odours who later exhibit a preference for these. Rat pups coated with Yardleys red roses perfume from 2 to 30 days were more sexually responsive to same smelling rats (Marr and Gardner, 1965). Likewise rats exposed to either acetophenone or ethyl benzoate

rubbed on conspecifics on days 3-10 preferred individuals smelling the same as that odour with which they had been reared two weeks later (Marr and Lilliston, 1969). The effects of exposure may be long lasting. Echandia et al. (1982) reared individuals in an environment sprinkled with lemon from birth to 21 days of age and pups tested 120 days later still exhibited a preference for the lemon odour.

Although these experiments do indicate pups exhibit a preference for an odour to which they have previously been exposed, the role of conspecifics in the acquisition of these preferences indicates it is not due just to exposure. Galef and Kaner (1980) draw a distinction between simple exposure to an odour, an odour which is present in the environment but not emanating from conspecifics, and mother exposure or sibling exposure (I shall call these collectively conspecific exposure) where the odour emanates from a conspecific. They found no evidence of differential acquisition of an odour preference induced by either simple exposure or conspecific exposure, but pups receiving simple exposure were unable to maintain the preferences as long as pups experiencing conspecific exposure, and the simple exposure induced odour preference extinguished more rapidly than the conspecific induced preference (Galef, 1982b). These results suggest that although simple exposure is enough to initiate a preference, conspecific exposure is necessary for its maintenance, and some form of associative learning

may be involved in odour preference acquisition (see also Brake, 1981; Pederson et al., 1982).

Thus the possibility exists for the acquisition of kin recognition through exposure learning, possibly by some imprinting process, although care must be taken to separate the effects of simple exposure learning from conspecific exposure where some associative learning may be involved.

6.4.1.3
Associative Learning

In this catagory a particular stimulus takes on a certain value due to its association with other stimuli. In terms of kin recognition, the rats response to/learning of, the kin identifier is due to its association with other stimuli or events. In the previous section, olfactory induced preferences were maintained for longer if associated with a conspecific than if not associated with a conspecific (Galef, 1982; Galef and Kaner, 1980). It may be that the individual learns to recognise kin through a classical conditioning process. When huddling or sucking individuals may associate the positive reward gained from indulging in these activities, warmth, nutrition with the particular odour present (kin odour as pups huddle with sibs and feed from the mother) and come to learn this odour. In our experiments rats exhibited a preference for kin by day 10 and thus any conditioning must have occured by then.

There have been a number of experiments indicating the ability of neonates to form associations, for example, conditioned aversions. Eight day old rats exhibit avoidance reaction to an odour, lemon, if this odour had previously been paired with an aversive stimulus, lithium chloride, on day 2 (Rudy and Cheatle, 1977,1979) and at 7 days of age are capable of second order conditioning (Rudy and Cheatle, 1980). One day olds exhibit signs of selective associations (see Garcia and Ervin, 1968; Seligman, 1970; Seligman and Hager, 1972), being able to associate a taste with an illness and a texture with shock, but unable to associate taste with shock, or texture with illness (Gemberling and Domjam, 1982). The presence of conspecifics may also aid in the formation of associations, as 2 day old rat pups acquire an odour-illness aversion better when conspecifics are present than when not (Smith and Spear, 1980).

Neonates are also capable of appetitive learning. Three day old pups gain a preference for a novel odour which had previously been paired with oral infusions of milk (Johanson and Teicher, 1980). The aversiveness of odours is also open to change through conditioning. Cedar wood odour elicits an aversive reaction from pups but when paired with milk, three day old pups show decreased aversiveness to this odour (Johanson and Hall, 1982).

Neonates may be operantly conditioned. One day old pups will learn to probe upwards to a terrycloth paddle

for small infusions of milk, and will discriminate between two paddles, responding more to one giving milk (Johanson and Hall, 1979). Three day old pups will also learn to press a paddle for electrical stimulation of the brain (Moran et al., 1981).

These studies demonstrate the ability of neonates to acquire behaviours through associative learning and allow the possibility of the acquisition of kin recognition through this process. The ability to form associations may be necessary if the individuals are required to recognise different classes of kin, where individuals may be required to associate different degrees of familiarity with different conspecific individuals (see Bekoff, 1981).

Thus there are a number of possible mechanisms, physiological imprinting, exposure or associative learning open to the rat to acquire the representation of kin and allow them to respond differentially to kin and non-kin. These mechanisms are not mutually exclusive and may all play a role in the acquisition of kin recognition through social mechanisms.

It is perhaps worth adding here a note of caution pertinent to many studies of olfaction. In many experiments used to demonstrate the ability of neonates to respond to, or be conditioned by, olfactory stimuli the olfactory stimulus used has been a simple pure chemical, for example, Rudy and Cheatle (1977) used lemon, Marr and

Lilliston (1969) acetophenone and ethyl benzoate, Laing and Panhuber (1980) cyclohexanone, and these odours may elicit different reactions than conspecific odour. In a series of experiments investigating the role of 6-hydroxydopamine (6-OHDA) upon the preferences of rat pups for conspecific and botanical odours it has been found that 6-OHDA will act to reduce the response to conspecific but not botanical odours (Cornwell-Jones, 1981; Marasco et al., 1979; Sobrian and Cornwell-Jones, 1977). This may be indicative of a different physiological mechanism for botanical and conspecific odours, in their storage/reception, or of a different mechanism for the preference of the two odour classes. Other experiments provide behavioural evidence that the preference for conspecific odour may result from an inherent sensory afferent bias (Galef and Kaner, 1980).

It may be that there are 'two olfactory systems' for the processing of botanical and conspecific odour, and if so, experiments using simple odours might not reflect the situation with conspecific odour. Alberts and May (1980) however found no evidence of differential sensitivity in the olfactory system for biological or non-biological odours, and the difference may therefore be reflected not in the sensitivity of the system but perhaps in storage or the preference mechanism.

6.4.2
Genetically Determined Mechanisms Of Kin Recognition

The results from chapter 3 suggest that individuals are capable of recognising their siblings with little experience of them postnatally and in this case the acquisition of kin recognition must be through one or both of the two following mechanisms:

6.4.2.1
Recognition Genes

The ability of a gene(s) to enable kin recognition may arise through either a single gene with pleiotropic effects or a number of coadapted genes which would allow kin to be recognised with no experience of them. As previously discussed, evolution may favour a number of coadapted genes. The existence of such a 'supergene' or of a single gene to allow kin recognition is most likely to be found in the Major Histocompatibility Complex (M.H.C.).

The M.H.C. is a section of chromosome containing a number of gene loci which control the cell membrane alloantigens (Klein, 1975) are responsible for transplantation antigens (Scofield et al., 1982), the immune response (Dausset, 1981b) and more recently have been thought to serve the function of self-cellular recognition (Dausset, 1981a). Self-recognition is an essential property of all cells, and is necessary for the survival of all multicellular organisms (Dausset, 1981a),

to allow co-operation between cells and to prevent the immune response from attacking ones own cells. As such the M.H.C. has evolved to allow self-cellular recognition and may serve the secondary function of allowing individuals to recognise kin.

Self-recognition between cells takes place at the cell surface (Macfarlane-Burnet, 1976) and is a result of cells producing molecular structures which are recognised by other cells as self, that is, the same as produced by themselves. These two factors are the two functions proposed for recognition; a) the production of the kin identifier and b) the recognition of this. Such parallels between the function of the M.H.C. and the proposed recognition genes make this complex the most likely source of recognition genes.

Further support for this idea comes from studies indicating a role for the M.H.C. in determining mating preferences. Genetically identical male mice differing only at the H-2 complex (the M.H.C. of mice) were observed to choose females with the same or different H-2 complex dependent upon the two H-2 complexes offered as a choice (Yamazaki et al., 1976). Further studies demonstrated this preference to be linked with mating behaviour, for outside of the mating situation, in a 2 choice preference test, no preference for any H-2 type was exhibited (Yamazaki et al., 1979). These findings indicate that the gene for mating preference is situated

close to the M.H.C. Information concerning the H-2 type is carried by olfactory stimuli (Yamazaki et al., 1979). The results of these studies in mice indicate that the M.H.C. controls production of female odour and reception of these signals by the male by having closely linked genes for each (Yamazaki et al., 1976). However this may just reflect the task used, female stimulus and male choice, and reversal of roles may implicate the M.H.C. in male odour production and the female recognition system. Such evidence strongly implicates the M.H.C. as the site for possible recognition genes.

How could the M.H.C. influence odour production and recognition? I have previously discussed evidence for the existence of genetically determined odour and shall here concentrate upon genetically determined recognition. A number of possibilities are available. The olfactory bulb may be preprogrammed to respond preferentially to a particular odour, either through topographical representation of odours and neural wiring to connect these areas to those of behaviour, for example, mating etc., allowing the perception of a particular odour to induce a particular behaviour or preference. More likely however, considering the parallels between the functioning of the M.H.C. immune response and olfactory perception (Yamazaki et al., 1980), the M.H.C. may code for differential responsiveness at the level of the primary receptors.

It is widely accepted that odours are initially perceived by acting upon the cilia of the olfactory neurons arising from the terminal swelling (Dodd and Squirrel, 1980). What is not agreed is how such contact stimulates the neurone. Wright R.H. (1977) argues it is the vibration frequency of the chemical that determines its smell. Amoore et al. (1964) argue for the shape of the molecule as the critical factor, whereas Mozell (1971) argues that different chemicals will have different absorption rates which affects the quality of smell perceived. However odours are perceived, the cilia could be predisposed to respond preferentially to that of kin. Such a mechanism may be more feasible than differential responsiveness at the level of the olfactory bulb. Self-cellular recognition takes place at the cell surface (Macfarlane-Burnet, 1976), it may be that chemicals used in self-recognition may be similar to those released by individuals in kin identification and present the opportunity for genetic recognition at the level of the primary receptors, the surface of the cilia.

The ability to respond innately to biological odours has already been demonstrated in the rat. Pups respond preferentially to maternal odour (Leon, 1974), and individuals denied access to maternal pheromone still exhibit a preference for females emitting the pheromone to females not emitting the pheromone (Schumacher and Moltz, 1982), indicating an innate response to a biologically relevant odour. Foxes are natural predators of rats and

rats exhibit the ability to recognise this odour without experience of foxes. In a study to examine the electrophysiological and behavioural effects of lesions on the lateral and medial olfactory pathways, Cattarelli (1982a,b) presented rats with the odours of, amongst others, fox and lion. Both were unfamiliar to the rat, but the fox is a natural predator whereas the lion is not. In an alleyway running task, presentation of fox odour caused greater upset in running performance, slower start time, longer running time, more and longer stops than lion odour which exerted a similar effect to grouped conspecific odour. Similarly there was greater electrophysiological responsiveness to the odour of the fox than to that of the lion, the fox exhibiting a much greater waking influence than lion odour. This study provides evidence of an innate (genetically determined) response to a biologically significant odour, that of a predator.

In conclusion, although the existence of recognition genes has often been doubted (e.g., Dawkins, 1982; Holmes and Sherman, 1983) there is evidence to suggest that they do exist. The M.H.C., evolved primarily for self-cellular recognition, may provide the genetic basis for recognition of kin. Its similar construction to that proposed for recognition genes, a number of coadapted genic loci; its similar mode of functioning to the olfactory system, action at cell membrane and its involvement in producing genetically determined odours and recognition systems for

these odours argue strongly for its consideration as the site of genes enabling the recognition of kin.

6.4.2.2
Intra-uterine Learning

The second possibility for genetically mediated acquisition is that the pups learn about their kin whilst in the uterine environment from the amniotic fluid. It is known that rat fetuses ingest amniotic fluid (Lev and Orlic, 1972; Narayanan, 1971) and that both gustatory and olfactory receptors are present and appear to be functioning in the rat fetus near birth (e.g., Farbman 1965; Tucker, 1963). The necessary physiological mechanisms are present to allow such learning. Again learning may take a number of forms, physiological imprinting, exposure learning or associative learning.

The ability of pups to respond preferentially to odours/tastes experienced in utero has been demonstrated by Smotherman (1982a). Fetuses on day 20 of gestation exposed to apple juice injected into the amniotic fluid when tested at 60 days postpartum show a preference for apple juice in a two choice preference test. The ability of rat pups to learn associatively whilst fetuses has been demonstrated. Fetuses given apple juice followed by an injection of lithium chloride, show a decreased nipple attachment at 16 days to nipples coated with apple juice compared to individuals given apple juice + saline (Stickrod et al., 1982). Smotherman (1982b) using a

conditioned emotional response demonstrated what is learned is an odour aversion. Apple juice followed by lithium chloride was injected into the amnion surrounding 20 day old fetuses. On day 10 postpartum, pups were trained to approach a dam to suckle. Once approaching consistently apple juice odour was then introduced into the chamber. Then speed of approach decreased indicating that a conditioned aversion to the odour experienced in the uterus had been formed. These studies do demonstrate that rat fetuses can form associations in utero that will guide later behaviour.

In summary, the possibility of rats acquiring kin recognition through either the possession of recognition genes or through intrauterine learning has been demonstrated.

One finding which may cause problems for a genetically mediated system is that by 40 days pups which had been separated from their siblings postnatally and who had shown the ability to recognise such siblings at 22 days, no longer show the ability to recognise theie sibs. This poses little problem for kin recognition acquired through intra-uterine learning, as without further exposure to kin but with continual exposure to non-kin, the learning which occured during the gestation period may be extinguished and replaced by that acquired postnatally.

It is however more of a problem for the recognition

gene hypothesis, which proposes that individuals possess genes which enable recognition of kin. Such recognition genes may predispose an individual to recognise a particular odour, however this does not mean the system is necessarily permanent and cannot be altered. It has already been seen that exposure to particular odours causes individuals to exhibit a preference for this odour, and it may be that if no further experience is forthcoming then continual exposure to another odour will lead to a preference for that odour. This may parallel the M.H.C. and immune response, where the bodies cells can 'learn' the characteristics of foreign cells such that on the next meeting they are more able to deal with them (Klein, 1975; also the principle of vaccines). Continued exposure to a new odour may 'override' the olfactory bulb or may change the receptiveness of the end receptors (the nerve cells of the olfactory epithelium are capable of replacement by new cells, Dodd and Squirrel, 1980) and thus change the responsiveness of the olfactory system. A similar change in a programmed preference may be found for cedar wood odour. Cedar wood is a naturally aversive odour to rat pups, however by exposing pups to the odour or by pairing with milk, the natural aversiveness of this odour is reduced (Johanson and Hall, 1982).

In conclusion, although recognition genes may preprogramme an individual to respond preferentially to kin, lack of exposure to kin odour coupled with continual exposure to non-kin, may allow the response to kin odour

to extinguish and lead to preferential responsiveness to the non-kin odour, such an ability being adaptive to the individual.