# Social discrimination of cage-mates and non-cage-mates by rats

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### ABSTRACT

The ability to discriminate between group-mates and non-group-mates likely underpins the occurrence of affiliative and aggressive behaviour towards 'in-group' and 'out-group' individuals. Here we present two experiments aimed at testing the ability of rats (*Rattus norvegicus*) to discriminate between cage-mate (CM: animals residing in the subject's home cage) and non-cage-mate (NCM) conspecifics. In experiment 1, rats were trained to discriminate between different exemplars of CM and NCM using a lever pressing task employing symmetrical reinforcement. Subjects did not reach performance criterion, but they did show some evidence of discrimination between the two types of stimuli. In experiment 2, we employed a digging task to determine if rats can discriminate between odour cues from CM and NCM presented simultaneously on two sand-filled bowls. Subjects reached performance criterion on the first pair of odours, and on three more different pairs of CM and NCM odours. The results of a reversal task, using a fifth pair of odours, indicate that the rats were using a common factor to discriminate between social cues from CM and NCM conspecifics, rather than learning each pair independently. Possible candidates include a group-specific odour cue, or the development of a CM/NCM category concept.

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## 1. Introduction

The ability to discriminate individuals as belonging to a 'familiar/unfamiliar' or 'group-member/non-group-member' category may allow animals to respond to conspecifics appropriately whilst reducing the cognitive demand associated with remembering numerous individuals (Coulon et al., 2011; Wilkinson et al., 2010; Zayan and Vauclair, 1998). In a recent study Wilkinson et al. (2010) investigated the ability of pigeons to discriminate between 'familiar' and 'unfamiliar' individuals using a category concept. They used 2D images of group-mates as the 'familiar' stimuli, and images of individuals from another group, housed in a different compartment (no visual cues available), as the 'unfamiliar' stimuli. After learning the initial discrimination, two of the six experimental birds successfully discriminated between images of novel exemplars of familiar and unfamiliar birds suggesting that pigeons are able to categorise conspecifics using the concept of familiarity (Wilkinson et al., 2010). A similar study by Coulon et al. (2011) suggests that heifers are also able to discriminate between 2D images of 'familiar' and 'unfamiliar' conspecifics using a categorisation approach.

Captive farm and laboratory animals are often moved between groups or temporarily separated from their group-mates for husbandry, experimental or production reasons. In these circumstances, the ability to discriminate between 'group-members' (familiar) and 'non-group-members' (unfamiliar) may mediate the responses shown which, in species such as rodents and pigs, can include damaging aggressive behaviour. The length of time it takes for a conspecific to be categorised as a group member when introduced into a group and to be re-categorised as a non-group member following separation from the group, are important to understand if we are to manage social groups effectively and to minimise damaging aggression, especially when temporarily separated group-mates are reunited.

The aim of this study was to initiate investigation into these issues in an important laboratory species, the rat, by studying whether rats are able to make a general discrimination between group-members and non-group members. Because the ecological relevance of 2D visual images may be limited (Bovet and Vauclair, 2000), especially for a species like the rat which appears to rely more on olfaction than vision (Burn, 2008), we were also interested in establishing a method that uses whole animal stimuli or olfactory cues. The latter are likely to be ecologically relevant for rats and are often encountered under natural conditions in the absence of the whole individual (Beynon and Hurst, 2004). We also wanted to develop a method that involves presentation of just one stimulus on any one trial with the requirement that the subject responds to indicate whether it is a group member or non-group member. Such

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a method would ultimately allow us to present cues from individuals who had previously been removed from the subject's group to see whether the subject still categorised them as a group member, and hence to track decay of social familiarity memory across time.

Commonly used techniques for investigating social discrimination in rats and other rodents capitalise on their natural behavioural responses to social stimuli which include greater investigation of novel as opposed to familiar/recently encountered stimuli (Thor and Holloway, 1982; Dantzer et al., 1987; Engelmann et al., 1995; Johnston, 1993). For example, repeated presentation of the same social stimulus (e.g. separated by 15 min inter-exposure-intervals) results in habituation of investigatory behaviour towards the stimulus. However, if the subject is then presented with a different social stimulus (habituation/dishabituation test), an increase in investigation is interpreted as evidence that it is able to discriminate the new stimulus from the old one (e.g. Thor and Holloway, 1982; Johnston, 1993; Burman and Mendl, 1999, 2000). Likewise, if it is presented with both the familiar stimulus and a different stimulus (habituation/discrimination test), greater investigation of the novel stimulus indicates successful social discrimination (e.g. Johnston, 1993; Engelmann et al., 1995; Todrank and Heth, 1996; Burman and Mendl, 2002, 2003, 2004, 2006).

Methods relying on spontaneous behaviour to study social memory are quick and easy to implement (Gheusi et al., 1997). However, a failure to show differential behaviour indicative of discrimination does not necessarily imply that the animal is unable to discriminate (Gheusi et al., 1997; Kristensen et al., 2001; Burman and Mendl, 2004). Such failures may result from uncontrolled changes in motivation or hormone levels altering spontaneous responses to stimuli, or the behaviour being recorded not reliably indicating preference or interest, or other issues with the testing regime (Schellinck et al., 2008). In addition, previous experience can affect the subject's spontaneous responses in tests (Burman and Mendl, 2003) – animals may be reluctant to approach dominant individuals to which they have lost a fight (Lai and Johnston, 2002), and a general preference for 'unfamiliar' over 'familiar' may be overridden by preferences for specific individuals (Hauser and Huber-Eicher, 2004). Furthermore, habituation/dishabituation tests are often used to assess the ability to discriminate between individuals. It is much more difficult to use them to determine whether animals can discriminate between classes of individuals, particularly categories such as cage-mate vs non-cage-mate that may differ in a variety of unknown ways which may or may not be revealed by differences in spontaneous behaviour.

Tests employing operant learning techniques can be used to address these issues. Although they are less naturalistic and hence more time consuming, testing is more controlled as animals are trained to perform specific arbitrary responses for a reward. Thus, for example, they can be trained to perform response A if individuals of one class are presented, or response B if individuals of another class are presented, allowing the researcher to investigate category-discrimination abilities. Responses A and B may be unbalanced (e.g. Go vs No-go; Heth et al., 1996; Gheusi et al., 1997), or balanced (e.g. left lever press vs right lever press; Go vs Go; Gheusi et al., 1994). The latter method prevents the test animal from only learning the characteristics of one stimulus animal as both are associated with a reward and require an active, rather than passive (more difficult to interpret) response. Our aim here was to use an active choice task of this sort to investigate whether rats can discriminate between relevant social categories such as cage mates vs non-cage mates, something which has received relatively little attention in previous studies.

Our approach differed from that of Wilkinson et al. (2010) and Coulon et al. (2011) in that test subjects were presented with one stimulus at a time and had to perform a different response (e.g. press left or right lever) to each stimulus type (cage-mate (CM):

rat that resided in the subject animal's home cage; non-cage-mate (NCM): rat that did not reside in the subject's home cage). On reaching a performance criterion on this task, we then aimed to examine whether subjects could generalise the learned responses to several new sets of stimuli. If so, we expected that performance would remain high throughout these new stimulus sets. To determine whether rats were making a general discrimination of CM from NCM rather than simply getting quicker at successive individual discrimination tasks (i.e. rapidly learning each stimulus-response-reward contingency without acquiring a general 'learning set'), we then planned to conduct a reversal task (e.g. switching the operant contingency from pressing left lever for CM to pressing right lever for CM). If the rats had simply learnt the general rule that in each task a left lever press to one stimulus is rewarded whilst a right lever press to the other stimulus is rewarded, performance in the reversal task should remain high. On the other hand, if they had learnt that, for example, rewarded left lever presses are associated with CM, whilst rewarded right-lever presses are associated with NCM, their initial performance should drop below chance levels followed by a gradual re-learning of the new contingency. Here we present two experiments using different methods and stimulus types.

## 2. Experiment 1: discrimination between cage-mates and non-cage-mates using symmetrical reinforcement

### 2.1. Methods

#### 2.1.1. Subjects, housing and care

Forty-eight female Lister Hooded rats (Harlan, UK) were purchased at three weeks of age. Four rats were taken from each of twelve different litters and littermates were placed in different cages (no siblings were housed in the same cage). They were housed in four groups of twelve in large (99 cm × 51.3 cm × 37 cm) enriched cages (sawdust substrate, shredded bedding, two wooden tunnels, red plastic shelter, wooden platform, climbing rope and freely accessible food) in a controlled environment (target temperature 19–23 °C). Lighting was on a reversed schedule (lights off 8 am–8 pm) and food (complete pelleted diet) and water were provided ad libitum. During the testing period, test subjects were placed in a holding cage (with another test conspecific from their group) with no food available for one hour prior to testing. This was to aid task motivation, as food pellets (Bio-Serv dustless precision pellets) were used as rewards during training and testing. Trials were performed during the dark phase, in a different room to that in which the animals were housed. A dim white light (25 W) was used during training to allow behavioural observation, and also to give rats the opportunity to use visual cues during discrimination/recognition. Rats were regularly handled prior to training. Two rats from each cage (eight in total) were used as test subjects, and the remaining rats were potential stimulus animals. The eight subject animals were taken from eight different litters.

#### 2.1.2. Apparatus

The training and design of the apparatus was adapted from Gheusi et al. (1994). The apparatus consisted of a turntable and an operant test chamber (Fig. 1a and b). The turntable comprised a rotating plastic base (85.5 cm diameter) split into six separate sections. The sides and top of the turntable were manufactured using galvanised wire mesh. A hole in the top of each section allowed up to six 'stimulus' rats to be placed on the turntable (one in each section). The rotatory movement of the apparatus allowed these rats to be presented as stimuli during a single session and minimised the amount of animal handling required. The stimulus turntable