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**THE INFLUENCE OF PRE-EXPERIMENTAL EXPERIENCE ON SOCIAL
DISCRIMINATION IN RATS (*RATTUS NORVEGICUS*)**

OLIVER H.P. BURMAN¹ & MICHAEL MENDEL

University of Bristol, U.K..

Centre for Behavioural Biology
Department of Clinical Veterinary Science
Langford House
Langford
Bristol
BS40 5DU
UK

¹Corresponding Author

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2 Oliver H. P. Burman.

4 Correspondence concerning this article should be addressed to Oliver H.P. Burman, Division
of Animal Health & Husbandry, Department of Clinical Veterinary Science, University of

6 Bristol, Langford House, Langford BS40 5DU, U.K..

8 Email: oliver.burman@bristol.ac.uk

10 We used laboratory rats of known relatedness and contrasting familiarity to assess the
potential effect of pre-experimental social experience on subsequent social recognition. We
12 used the habituation/discrimination technique, which assumes that multiple exposures to a
social stimulus (e.g. soiled bedding) ensure a subject discriminates between that 'habituation'
14 stimulus and a 'novel' stimulus when both are introduced simultaneously. We observed a
strong discrimination if the subjects had different amounts of pre-experimental experience
16 with the donors of the two stimuli, but a weak discrimination if the subjects had either equal
amounts of pre-experimental experience or no experience of the stimuli. Pre-experimental
18 social experience does, therefore, appear to influence decision-making in subsequent social
discriminations. Implications for recognition and memory research are discussed.

20

Recent research has demonstrated that many common elements of the 'background' environment of a captive-housed animal, such as housing (e.g. Wurbel 2001), ultrasound emission (e.g. Sales 1991) and general husbandry (e.g. Burman & Mendl 2000), may influence the subsequent performance of the subjects in experimental studies. It has also become apparent that differences between strains of laboratory species (e.g. rats: Andrews 1996) and/or their degree of relatedness to one another (e.g. Nevison et al. 2000) may also influence research results. Another potential source of variation between subject animals that has been less explored, however, is social experience.

Animals used as subjects in behavioural research are often obtained from either recognised outside sources or wild caught/studied in the wild, and, because of this, they can have unknown degrees of both relatedness and familiarity when tested. Whilst relatedness can be specified to a certain extent, e.g. out-bred versus inbred rat strains (problems of discrimination can occur between inbred individuals, Nevison et al. 2000), it is often unclear as to exactly how familiar each animal is with the other animals in the group, prior to testing. Animals may have lived together for long periods, i.e. since weaning, have only recently encountered one another, or have never actually met each other at any stage. Some animals will therefore be very familiar with one another, whilst others remain totally unfamiliar.

There is evidence that previous social experience, in terms of agonistic interaction, may influence subsequent performance in aggressive encounters, with the probability of winning these future encounters affected by previous interactions (e.g. Stamps & Krishnan 1994; Barclay 2001). We were interested in whether previous social experience could also influence other, non-agonistic, measures of social behaviour. The ability of animals to discriminate, recognise and remember conspecifics is an important area of study in behavioural biology (e.g. Halpin 1986). Investigation in this area often assumes social discrimination on the basis of a discrepancy in familiarity between specific individuals (i.e. the social recognition test: e.g. Thor & Holloway 1982, Dantzer et al. 1987; the

50 habituation/discrimination technique: e.g. Johnston & Bullock 2001; the social discrimination
52 procedure: e.g. Engelmann et al., 1992; playback studies: e.g. Falls & Brooks 1975). When
these methods are used, pre-experimental social experience (e.g. Swaisgood et al., 1999) may
therefore have the potential to influence the results of subsequent experiments.

54 The habituation/discrimination technique is a commonly used procedure in studies of
social recognition memory (e.g. Halpin 1986; Brown et al. 1987; Johnston 1993; Johnston &
56 Jernigan 1994; Johnston & Bullock 2001). It relies upon the discrimination between
individuals on the basis of, at the simplest level, a difference in relative familiarity. This test
58 involves the repeated introduction of one social 'habituation' stimulus (e.g. a live conspecific:
e.g. Bluthé & Dantzer 1990; an anaesthetised conspecific: e.g. Kruczek 1998; an odour cue:
60 e.g. Sawyer et al. 1984) to a subject animal in order to encourage a habituation of stimulus
investigation. The original 'habituation' stimulus and a 'novel' social stimulus are then
62 introduced simultaneously, and stimulus investigation observed. Discrimination between the
two stimuli, as indicated by a preference to investigate the 'novel' social stimulus, is taken to
64 indicate that recognition of the original stimulus has occurred, whereas no such discrimination
suggests recognition failure.

66

The habituation/discrimination technique, like other methods used to assess
68 recognition (see above), depends upon the creation of a newly established difference in
familiarity either between two individuals or their cues. It assumes that only this most
70 recently gathered information will influence subsequent subject behaviour in the
discrimination test. However, this newly gathered information may be influenced, or
72 prevailed over, by social experience already gained before the start of the
habituation/discrimination technique. Thus, stimuli selected for testing on the assumption that
74 they are equally familiar/unfamiliar to the subject may actually differ because of pre-
experimental social experience, resulting in a misleading behavioural response by the subject
76 and therefore inadvertent misinterpretation.

78 Allowing a period of acclimatisation (e.g. one or two weeks) for subject animals in
new social groups to get used to their new social environment before any experimental work
80 commences, may remove the effects of any residual short-term affiliations. But social
memory for more long-term relationships, particularly those between related individuals, may
82 be expected to persist for a greater length of time (e.g. Hepper 1987). This study was
therefore designed to investigate whether differences in familiarity, based on pre-
84 experimental social experience, can have a residual effect on subsequent decision-making
when short-term social memory in laboratory rats is assessed.

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METHOD

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Subjects, housing, and care

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The subjects were adult male (N=16) and female (N=16) Lister hooded rats (Harlan
92 UK Ltd, Bicester, UK), six months old at the start of testing. The rats were housed
individually during the experiments in standard laboratory cages (33 × 50 cm and 23 cm
94 high), with sawdust litter. Food (Harlan Teklad Laboratory Diet) and water were freely
available. The rats were housed in the same room in which they were tested, in a controlled
96 environment (19° C ± 1, 46% RH), on a reversed lighting schedule (lights off 08:30 – 20:30)
with dim light (10 W) allowing visibility for the researcher.

98

Pre-experimental experience

100

The rats had been used previously in a foraging experiment and were bred on site. For
102 this reason the relatedness and familiarity of the rats to one another was known. The fathers of
rats used in that study were all brothers (r=0.5), the mothers all sisters (r=0.5), and the fathers

104 and mothers unrelated. The resulting offspring could therefore be classified in terms of
relatedness as either full-siblings ($r=0.5$), 'half-siblings' ($r=0.375$), or 'cousins' ($r=0.25$).
106 They were actually more closely related than genuine half-siblings ($r=0.25$) and genuine
cousins ($r=0.125$), because in addition to sharing a father, their mothers were sisters. For this
108 study only 'cousins' were used, in order that relatedness was constant for all the subjects.

110 Following weaning (siblings were reared together) the sexes had been separated into
groups of six individuals for two months. These groups were then split into two groups of
112 three for an additional three months. Thus, by the start of the current study, particular
individuals had been housed together for a total of five months (continuously since weaning),
114 whilst, in contrast, not having ever cohabited with some of the other rats. Thus, all the
subjects (see 'Odour stimuli') selected for use in this study were equally related ('cousins'),
116 but differed in pre-experimental experience, with subjects either highly familiar (five months
of group housing) or unfamiliar (no group housing) with one another.

118

Procedure

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Rats were housed individually for four days prior to the start of testing to allow
122 acclimatisation. Because the rats acted as both subjects and stimulus odour donors (see
'Odour stimuli'), individual housing was necessary to allow the collection of an individual
124 odour. The rats, tested in their home cages, were presented on five separate occasions with the
same 'habituation' odour stimulus for a period of 5min, each exposure separated by an inter-
126 exposure interval of 15min. After the fifth exposure to the 'habituation' stimulus there was a
further 15-min inter-exposure interval before the subject rats were exposed, simultaneously,
128 to the same 'habituation' stimulus and a 'discrimination' stimulus obtained from a different
individual, again for a 5-min period. Four rats were tested each day, with treatment balanced
130 for order.

132 ***Treatments***

134 The four treatment groups were decided by selecting either a previously familiar
cage-mate (rats had been housed together for 5 months) or an unfamiliar individual (rats had
136 never been housed together) as the odour donors for the ‘habituation’ and ‘discrimination’
stimuli (see Table 1). Four male rats and four female rats were allocated randomly to each
138 treatment as subjects. If pre-experimental experience of the odour donors is sufficient to
interfere with the subject’s ability to choose between two stimuli, then one would expect rats
140 from the different treatments to show differing degrees of discriminative ability. If pre-
experimental experience does not have any effect, however, there should be no difference
142 between the treatments in their ability to accurately discriminate.

144 * Table 1 *

146 ***Odour stimuli***

148 Odour cues consisted of 10cm³ of four day-old soiled bedding from the home cages
of donor rats presented in spherical wire mesh containers (total volume 20cm³) secured to the
150 cage wall. These containers allowed the rats to investigate the odour stimulus without
disturbing it. All odour cues were collected at the same time immediately prior to testing, with
152 the result that both the familiar odour stimulus and that of the novel individual were the same
‘age’ when introduced for the discrimination test. The containers holding the odour cues were
154 changed and disinfected between each encounter to prevent odour deposition by the subject
rats. During the first five encounters, the odour cue was placed centrally at one end of the
156 home cage (16.5 cm from either side). For the discrimination test, one of the odour cues was
placed centrally on the left of the home cage and the other on the right (both 25cm from either
158 end), and this was balanced across treatments to control for possible side preference.

We used soiled bedding as the olfactory stimulus, rather than the conspecifics
160 themselves, to avoid the possible aggression that can arise in a direct interaction (cf. Burman
& Mendl 1999). Olfactory cues play a major role in rodent social behaviour (Brown &
162 MacDonald 1985), with individual identity signalled via glandular secretions (e.g. Johnston &
Bullock 2001) or urinary excretion (e.g. Hurst et al., 2001). In addition to the role of the
164 major histocompatibility complex in signalling individuality (e.g. Brown et al., 1987), rodent
urine contains major urinary proteins (MUPs) that have been found to play an increasingly
166 important role in individual recognition (Hurst et al. 2001).

168 Major urinary proteins also bind and release volatile pheromones that themselves can
influence biological processes such as reproduction (e.g. Biasi et al., 2001) and aggression
170 (e.g. Novotny et al., 1985). It therefore appears that olfactory cues are able to represent the
identity of individual animals - as demonstrated by the substitutability of urine or soiled
172 bedding as social stimuli for live animals in recognition tests (e.g. Sawyer et al., 1984).

174 ***Behavioural observations***

176 Investigation of the odour stimuli was recorded directly using an event recorder
(Psion Organiser II) with Noldus Observer software (Noldus Information Technology 1993),
178 and also by video recorder. Use of a video camera allowed data to be collected without
disturbing the rats during the test. Investigation included sniffing, licking, and/or the subject's
180 nose being held within 1cm of an odour container. One trained observer recorded stimulus
investigation to ensure consistency throughout the study. This observer was unaware which
182 rat belonged to each particular treatment, and whether an odour was the 'habituation' or the
'discrimination' stimulus in the discrimination test.

184

Data analysis

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Data from each of the treatments were analysed to determine whether: (i) habituation
188 occurred over the first five encounters with the 'habituation' odour stimulus; (ii) the subject
rats were able to discriminate between the 'habituation' and 'discrimination' stimuli. We
190 analysed the data using Minitab, Version 12 (Minitab Inc. 1996), all probability values were
two-tailed. Parametric statistical tests were used as the data were normally distributed and of a
192 similar variance.

194

RESULTS

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The habituation encounters

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To investigate the levels of stimulus investigation over the first five encounters we
carried out a repeated measures General Linear Model (GLM) with sex (male/female),
200 treatment (1-4) and encounter (1-5) as factors. Male rats investigated the odour stimuli more
than females ($F_{1,24}=41.3$, $P<0.001$), however there was no overall effect of treatment. There
202 was a highly significant effect of encounter ($F_{4,96}=20.8$, $P<0.001$) and an interaction between
sex and encounter ($F_{4,96}=2.48$, $P<0.05$). Post-hoc examination of this interaction revealed that
204 for male rats there was a reduction in investigation (Tukey's Pairwise Comparison $P<0.05$)
between encounters 1 & 3, 1 & 4, and 1 & 5, and that investigation was also significantly
206 reduced between encounters 2 & 5, and 3 & 5 (means \pm SE: 39.1 \pm 2.5 (encounter one); 30.5
 \pm 2.8 (encounter two); 24.6 \pm 4.1 (encounter three); 19.8 \pm 3.5 (encounter four); 12.9 \pm 1.9
208 (encounter five) (see Fig.1). For females there was a significant reduction in investigation
between encounters 1 & 2, 1 & 4, and 1 & 5, and also between encounters 3 & 4, and 3 & 5
210 (means \pm SE: 21.1 \pm 1.6 (encounter one); 13.1 \pm 2.1 (encounter two); 16.6 \pm 1.9 (encounter
three); 9.4 \pm 1.5 (encounter four); 6.4 \pm 1.0 (encounter five) (see Fig.1). Male rats also

212 investigated the odour stimuli significantly more than female rats for four out of the five
encounters.

214

* Figure 1 *

216

The discrimination tests

218

In order to determine whether subject rats had successfully discriminated between the
220 'habituation' and 'discrimination' odour stimuli in the sixth 'test' encounter we utilised a
repeated measures GLM with sex (male/female), treatment (1-4) and odour (familiar/novel)
222 as factors. Again, male rats investigated the stimuli significantly more than the females
($F_{1,24}=6.3, P<0.05$) (see Fig.2 (a & b)), and there was no overall effect of treatment. There
224 was a highly significant effect of odour, with the 'discrimination' stimulus being investigated
more than the 'habituation' stimulus ($F_{1,24}=30.55, P<0.001$). However, there was also an
226 interaction between treatment and odour ($F_{3,72}=3.79, P<0.05$). Post-hoc analysis of this
interaction (paired *t* tests) revealed that there was a non-significant trend towards a preference
228 to investigate the 'discrimination odour' in both treatment one ($T=-1.95, N=8, P=0.093$) and
treatment four ($T=-2.12, N=8, P=0.071$). However, there was a strongly significant preference
230 to investigate the 'discrimination' odour in treatments two ($T=-3.69, N=8, P<0.01$) and three
($T=-3.54, N=8, P<0.01$) (see Fig. 2 (c)).

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* Figure 2 (a-c) *

234

DISCUSSION

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238 The results of this study suggest that pre-experimental social experience can over-ride more
recently gathered information about conspecifics, with rats discriminating better between
stimuli of contrasting, rather than identical, pre-experimental familiarity.

240

Following habituation to the repeated presentation of the same 'habituation' odour
242 stimulus, rats were simultaneously introduced to the 'habituation' odour and a
'discrimination' odour stimulus. Whilst treatments two and three strongly preferred to
244 investigate the 'discrimination' stimulus, treatments one and four showed only a non-
significant trend for such a preference. Treatments one and four shared the common feature
246 that the 'habituation' and the 'discrimination' stimuli were the same in terms of pre-
experimental familiarity, i.e. either both were previously unfamiliar (treatment one) or both
248 were previously familiar (treatment four). In contrast, in treatments 2 and 3, one stimulus was
previously familiar, and the other was unfamiliar. These results suggest that the rats showed
250 increased discrimination when there was a difference in pre-experimental familiarity between
the 'habituation' and 'discrimination' stimuli, regardless of which particular stimulus-type
252 was either previously familiar or unfamiliar.

254 In spite of the relatively small number of subjects used, we also observed a strong
difference between the sexes in the levels of investigation directed towards the odour stimuli
256 in this experiment, with males investigating the stimuli almost twice as much as females. This
confirms the results of previous studies (e.g. Bluthé & Dantzer 1990) in which female rats
258 were found to show a reduced persistence in the investigation of social stimuli, perhaps due to
hormonal differences (Bluthé & Dantzer 1990). This sex difference had little effect on the
260 results of this study because both sexes were allocated equally to the different treatments.

262 Our main results indicate that, if a particular rat, e.g. 'rat A', has already experienced
one of the two stimuli to be used in a forthcoming discrimination test (i.e. treatments 3 & 4),
264 then it performs better/learns faster in that test than either 'rat B', who has never previously

experienced either stimulus (treatment 1), or 'rat C', for whom both the stimuli are equally
266 familiar (treatment 4). This may be because, unlike 'rat B' and 'rat C', at the start of the
discrimination test 'rat A' has already established a clear mechanism for discriminating
268 between the two stimuli, i.e. a contrast in familiarity. The discrimination test in this study can
be thought of as consisting of two separate processes. One that requires that the subject
270 discriminates between the two stimuli (i.e. it determines that one of the stimuli is the
'habituation' stimulus), and another, that requires the subject to target a response towards one
272 of the stimuli (i.e. to investigate the 'novel' stimulus) (cf. Shettleworth 1998). Pre-exposure to
one of the to-be-used stimuli may allow the former process (the discrimination) to be
274 accomplished immediately at the start of testing – due to the contrast in familiarity, i.e. a
long-term memory is already formed and is readily accessed to facilitate the discrimination.
276 This 'head-start' may result in the observed improvement in performance of 'experienced'
rats because, unlike for the other rats, only one of the two processes (the response) now
278 remains to be achieved. This finding reflects results from the psychology literature on
perceptual learning that suggest pre-exposure to non-social stimuli can facilitate subsequent
280 discrimination learning (e.g. Channell & Hall 1981; Saksida 1999).

282 That rats should appear to perform better at discriminating between stimuli of
contrasting familiarity is not, in itself, unexpected. Even for non-social objects, differences in
284 relative familiarity provide the opportunity for discrimination (e.g. Steckler et al. 1998; Dix &
Aggleton 1999). For a territorial group-living animal like the rat (Barnett 1963), there will be
286 an immediate need to be able to distinguish between individuals on the basis of familiarity,
e.g. between individuals belonging to one's own group and those belonging to a completely
288 different social group, in order to identify, and behave appropriately towards, intruders (e.g.
Popik & van Ree 1998). Whilst discrimination between group-members is likely to be
290 achieved via the recognition of increasingly complex arrays of cues representing identity -
because individuals may be equally 'familiar' - (e.g. Barnard & Burke 1979), discrimination
292 between group-members and strangers is likely to be based on a more simple, and therefore

more immediate, process - such as a discrepancy in familiarity. Such a mechanism may also
294 contribute towards the discrimination observed between 'non-threatening' conspecifics
holding neighbouring territories, and 'threatening' strangers – the so-called 'dear-enemy'
296 effect (Fisher 1954).

298 However, what we might not have expected, was that the influence of social
relationships formed prior to the start of the experiment appeared to prevail over the newly
300 established relationships between social stimuli created by the experimental procedure of the
habituation/discrimination technique. If these newly established relationships had taken
302 precedence over the pre-experimental social experience then, despite the contrast - or lack of
contrast - in pre-experimental familiarity, all the treatments should have demonstrated similar
304 levels of discrimination, but this was not the case. In other words, the observed results
occurred as a consequence of the contrast in familiarity created by the pre-experimental social
306 experience, rather than as a result of the new experimental procedure. This suggests that pre-
experimental social experience, in common with other elements of the 'background'
308 environment (e.g. Sales 1991; Burman & Mendl 2000; Wurbel 2001) can influence the
behavioural response of subjects in subsequent experiments, resulting in potentially
310 misleading observations.

312 It appears that during the five months of group housing prior to the start of the
experiment, 'long-term' relationships established between group-mates continued to impact
314 upon subject behaviour following separation, and following the creation of newly established
'short-term' relationships. When rats are mixed with unfamiliar conspecifics, they soon form
316 a stable dominance hierarchy determined by predominately non-injurious aggression (e.g.
Hurst et al. 1996). The formation of a hierarchy avoids the need for continuous reassessment
318 and confrontation between individuals within a small stable group (e.g. Pagel & Dawkins
1997; Whitfield 1998). Although this behaviour is compromised to an extent by the
320 constraints of captivity – for instance there is no way for subordinate animals to escape

interaction with the dominant individual (e.g. Hurst et al. 1996) – such behaviour is likely to
322 be advantageous to the conspecifics involved and implies the existence of social memory.

324 For animals group-housed over a period of five months, it is unlikely that long-term
memories concerning the identity of the group-mates are immediately extinguished due to
326 separation from those group-mates. Even very brief periods of social experience can result in
the formation of a durable social memory. Mice (e.g. Kogan et al., 2000) and guinea-pigs
328 (e.g. Beauchamp & Wellington 1984) appear to remember other individuals for up to one
week after only 2min of experience, and hamsters for at least 10 days after 25min of
330 experience (Johnston 1993). Nor is it likely that long-term memories are disrupted by the
uptake of new information about the identity of novel conspecifics. Whilst very short-term
332 social memory does appear to be interfered with retroactively by the introduction of a novel
conspecific (e.g. Thor & Holloway 1982; Dantzer et al. 1987, Burman & Mendl 2000), it is
334 unlikely that long-term social memory is affected in the same way.

336 Thus, the greater the amount of social experience prior to subsequent experimental
testing, then the more likely that this pre-experimental experience will influence subject
338 behaviour as this information becomes increasingly important for the subject to retain, for the
reasons mentioned above. The habituation/discrimination technique assumes that the subjects
340 will respond solely on the basis of the newly created difference in relative familiarity (see
Halpin 1986). Yet, if such information has already been gained prior to the experiment (e.g.
342 Swaisgood et al., 1999), then the subject may also consider this information before a decision
about the appropriate behavioural response is made. Perhaps information in an established
344 long-term memory store is more easily retrieved, and/or takes precedence over, newly
acquired information in a short-term memory store (temporary). In this way, as was seen in
346 this study, a behavioural response may still be dictated by previously established social
experience rather than by the current experimental procedure. This may have implications for
348 social discrimination research if pre-experimental social experience is not controlled. The

results of this study also suggest that, as the contrast of familiarity between the social stimuli
350 to be used in a social discrimination test is increased, so their discriminability is enhanced.

352

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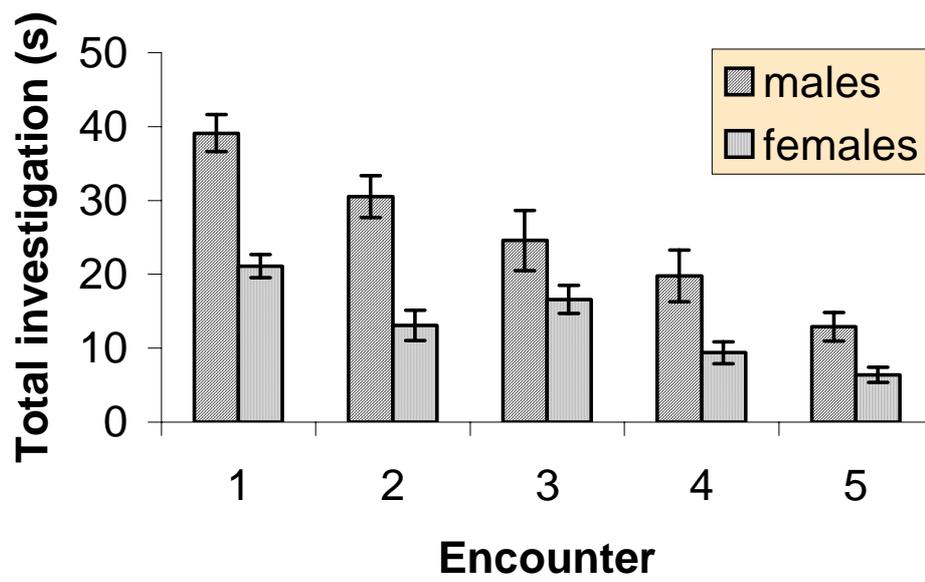
| Treatment | 'Discrimination' stimulus | 'Habituation' stimulus |
|-----------------|----------------------------------|----------------------------------|
| Treatment One | Unfamiliar ¹ | Unfamiliar ¹ |
| Treatment Two | Previously familiar ² | Unfamiliar ¹ |
| Treatment Three | Unfamiliar ¹ | Previously familiar ² |
| Treatment Four | Previously familiar ² | Previously familiar ² |

All animals are equally related (cousins)

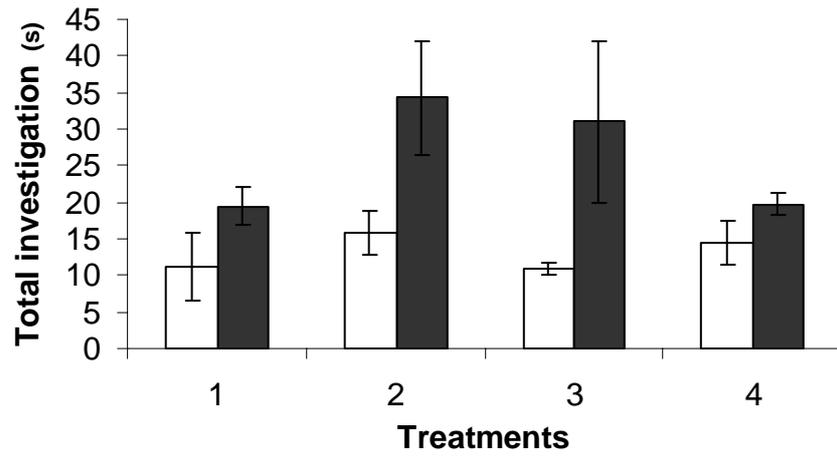
¹Subject and odour donor have never cohabited

²Subject and odour donor cohabited for five months prior to the start of this experiment

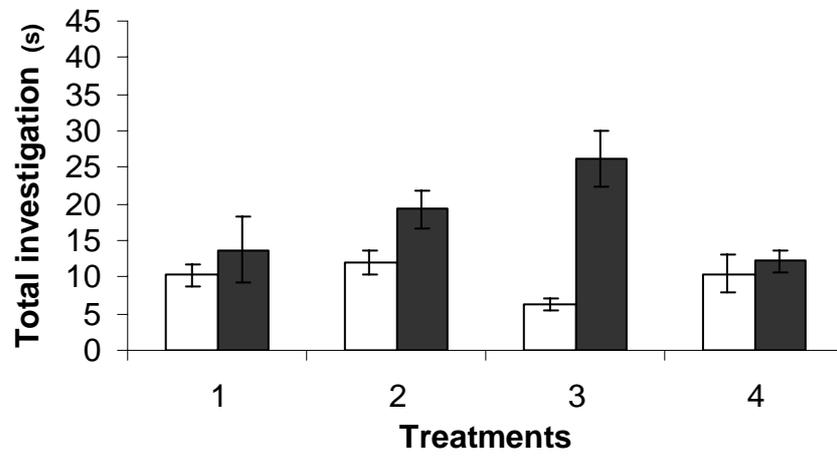
Table 1: Description of treatments



A



B



C

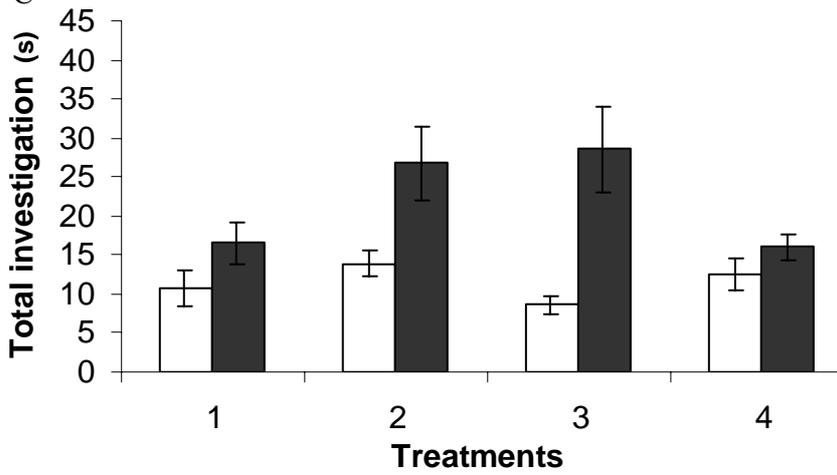


Figure 1: Mean (\pm SE) total investigation (s) of the 'habituation' stimulus during the five initial habituation encounters. Data for the different treatments are pooled, but shown separately for the male rats (hatched), and for the female rats (dotted).

Figure 2 (a-c): Mean (\pm SE) total investigation (s) of the 'habituation' stimulus (white) and the 'discrimination' stimulus (black) both introduced simultaneously in the sixth encounter for the four different treatments. (a) shows data for the male rats only, (b) for the female rats only, and (c) for the males and females pooled together.