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

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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 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’ 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 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 social experience does, therefore, appear to influence decision-making in subsequent social discriminations. Implications for recognition and memory research are discussed.

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 habituation/discrimination technique: e.g. Johnston & Bullock 2001; the social discrimination 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.

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 & 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 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: 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 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 indicate that recognition of the original stimulus has occurred, whereas no such discrimination suggests recognition failure.

The habituation/discrimination technique, like other methods used to assess 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 recently gathered information will influence subsequent subject behaviour in the discrimination test. However, this newly gathered information may be influenced, or prevailed over, by social experience already gained before the start of the habituation/discrimination technique. Thus, stimuli selected for testing on the assumption that 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 and therefore inadvertent misinterpretation.

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 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 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-experimental social experience, can have a residual effect on subsequent decision-making when short-term social memory in laboratory rats is assessed.

METHOD

Subjects, housing, and care

The subjects were adult male (N=16) and female (N=16) Lister hooded rats (Harlan 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 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 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.

Pre-experimental experience

The rats had been used previously in a foraging experiment and were bred on site. For 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 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). 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 study only ‘cousins’ were used, in order that relatedness was constant for all the subjects.

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 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), 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’), but differed in pre-experimental experience, with subjects either highly familiar (five months of group housing) or unfamiliar (no group housing) with one another.

Procedure

Rats were housed individually for four days prior to the start of testing to allow 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 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-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, 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 for order.

Treatments

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 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 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 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 between the treatments in their ability to accurately discriminate.

* Table 1 *

Odour stimuli

Odour cues consisted of 10cm^3 of four day-old soiled bedding from the home cages of donor rats presented in spherical wire mesh containers (total volume 20cm^3) secured to the 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 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 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 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 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 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 & 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 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 important role in individual recognition (Hurst et al. 2001).

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 (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 bedding as social stimuli for live animals in recognition tests (e.g. Sawyer et al., 1984).

Behavioural observations

Investigation of the odour stimuli was recorded directly using an event recorder (Psion Organiser II) with Noldus Observer software (Noldus Information Technology 1993), 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 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 rat belonged to each particular treatment, and whether an odour was the ‘habituation’ or the ‘discrimination’ stimulus in the discrimination test.

Data analysis

Data from each of the treatments were analysed to determine whether: (i) habituation 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 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 similar variance.

RESULTS

The habituation encounters

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), 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 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 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 reduced between encounters 2 & 5, and 3 & 5 (means ± SE: 39.1 ± 2.5 (encounter one); 30.5 ± 2.8 (encounter two); 24.6 ± 4.1 (encounter three); 19.8 ± 3.5 (encounter four); 12.9 ± 1.9 (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 (means ± SE: 21.1 ± 1.6 (encounter one); 13.1 ± 2.1 (encounter two); 16.6 ± 1.9 (encounter three); 9.4 ± 1.5 (encounter four); 6.4 ± 1.0 (encounter five) (see Fig.1). Male rats also investigated the odour stimuli significantly more than female rats for four out of the five encounters.

* Figure 1 *

The discrimination tests

In order to determine whether subject rats had successfully discriminated between the ‘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) 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 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 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 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 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)).

* Figure 2 (a-c) *

DISCUSSION

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.

Following habituation to the repeated presentation of the same ‘habituation’ odour stimulus, rats were simultaneously introduced to the ‘habituation’ odour and a ‘discrimination’ odour stimulus. Whilst treatments two and three strongly preferred to 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 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 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 increased discrimination when there was a difference in pre-experimental familiarity between the ‘habituation’ and ‘discrimination’ stimuli, regardless of which particular stimulus-type was either previously familiar or unfamiliar.

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 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 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 results of this study because both sexes were allocated equally to the different treatments.

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), 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 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 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 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 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 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. 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 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 discrimination learning (e.g. Channell & Hall 1981; Saksida 1999).

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 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 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 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 achieved via the recognition of increasingly complex arrays of cues representing identity - because individuals may be equally ‘familiar’ - (e.g. Barnard & Burke 1979), discrimination 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 contribute towards the discrimination observed between ‘non-threatening’ conspecifics holding neighbouring territories, and ‘threatening’ strangers – the so-called ‘dear-enemy’ effect (Fisher 1954).

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 established relationships between social stimuli created by the experimental procedure of the habituation/discrimination technique. If these newly established relationships had taken 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 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 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’ environment (e.g. Sales 1991; Burman & Mendl 2000; Wurbel 2001) can influence the behavioural response of subjects in subsequent experiments, resulting in potentially misleading observations.

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 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 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 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 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 be advantageous to the conspecifics involved and implies the existence of social memory.

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 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 (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 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 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 unlikely that long-term social memory is affected in the same way.

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 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 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. 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 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 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 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 to be used in a social discrimination test is increased, so their discriminability is enhanced.

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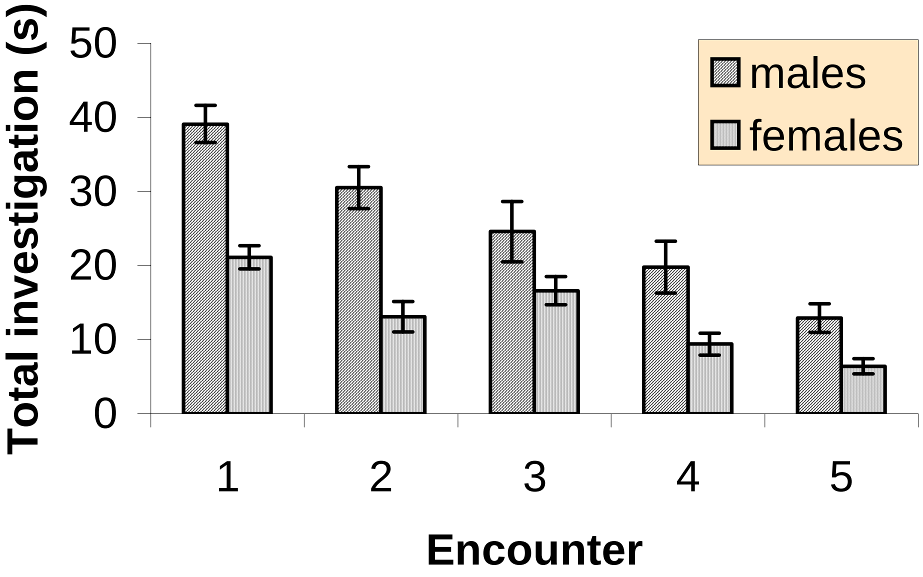
Treatment	**'Discrimination'** stimulus	**'Habituation'** stimulus
Treatment One	Unfamiliar[1]	Unfamiliar[1]
Treatment Two	Previously familiar[2]	Unfamiliar[1]
Treatment Three	Unfamiliar[1]	Previously familiar[2]
Treatment Four	Previously familiar[2]	Previously familiar[2]

All animals are equally related (cousins)

[1] Subject and odour donor have never cohabited

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

Table 1: Description of treatments


Total investigation (s)
50
40
30
20
10
0
males
females
1
2
3
4
5
Encounter

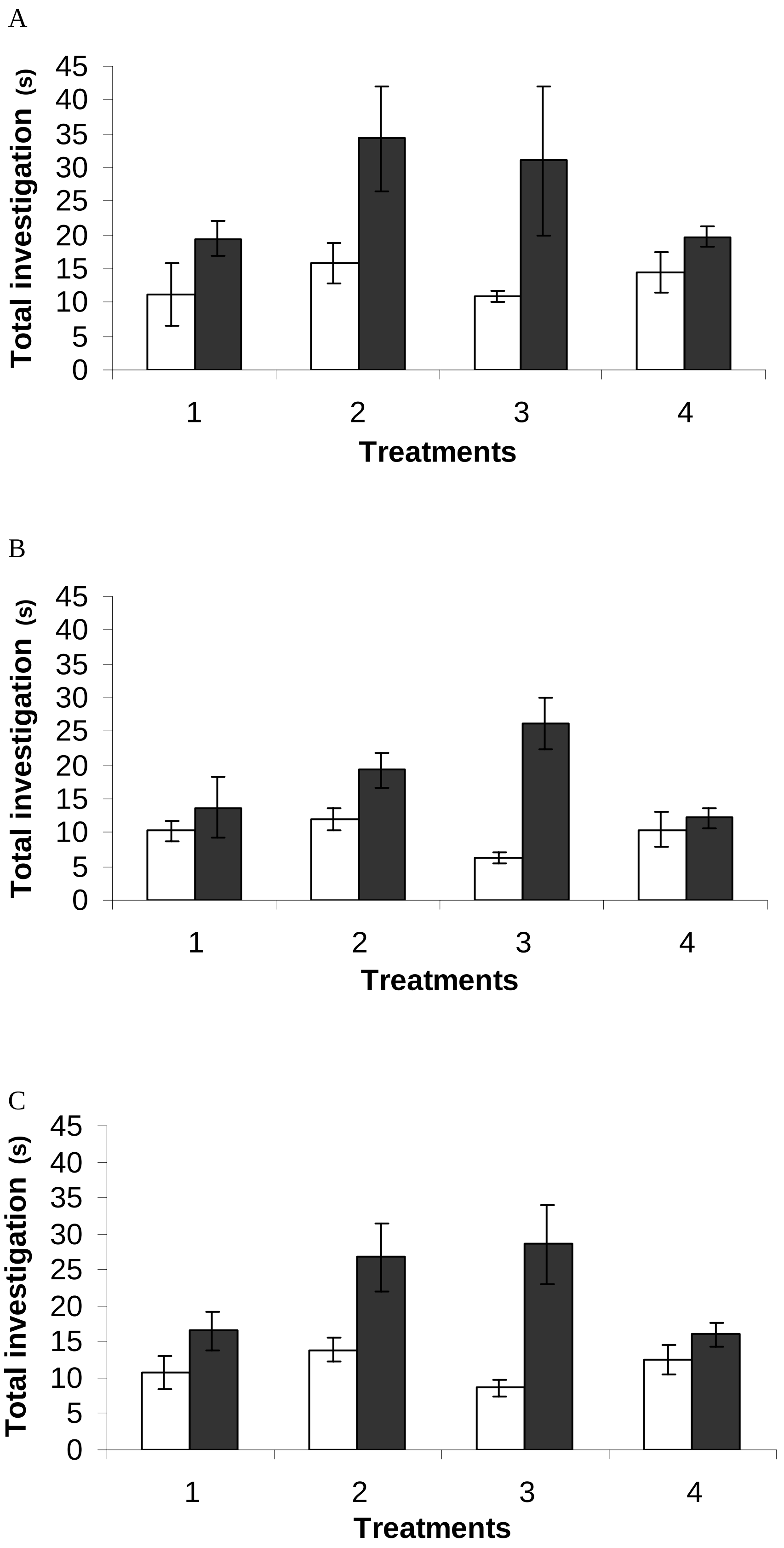

A
Total investigation (s)
0
5
10
15
20
25
30
35
40
45
1
2
3
4
Treatments
B
Total investigation (s)
0
5
10
15
20
25
30
35
40
45
1
2
3
4
Treatments
C
Total investigation (s)
0
5
10
15
20
25
30
35
40
45
1
2
3
4
Treatments

Figure 1: Mean (± 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 (± 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.