Exp Brain Res (2001) 138:100–109
DOI 10.1007/s002210100687

RESEARCH ARTICLE

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Cytotoxic lesions of the hippocampus increase social investigation but do not impair social-recognition memory

Received: 3 August 2000 / Accepted: 11 January 2001 / Published online: 28 February 2001

Abstract A number of studies have implicated the hippocampal formation in social-recognition memory in the rat. The present study addressed this issue directly by assessing the effects of cytotoxic lesions confined to the hippocampus proper, encompassing the four CA subfields and the dentate gyrus, on this behavioural task. Ibotenate-induced hippocampal lesions led to locomotor hyperactivity and a marked spatial working-memory impairment on the elevated T-maze. In addition, they also led to increased social investigation. However, despite these clear effects, there was no effect of the lesions on social-recognition memory. These results suggest that the hippocampus proper does not subserve social-recognition memory; but does not, however, preclude the possibility that other areas of the hippocampal formation (e.g. entorhinal cortex or subiculum) may support this memory process.

Keywords Hippocampus · Cytotoxic lesions · Social-recognition memory · Hyperactivity · Spatial learning

Introduction

Hippocampal damage causes profound amnesia in humans (Scoville and Milner 1957). In animals, hippocampal damage impairs performance on a variety of memory tasks (see Squire 1992; Jarrard 1993). Although a great many of these paradigms entail substantial training, there are some memory-testing procedures that rely on spontaneous behaviours and, thus, require minimal pretraining. One example of this is social-recognition memory in rats.

When an adult male rat is exposed to a juvenile male rat, there follows a period of intense social investigation from the adult towards the younger animal. If, after a short time interval (e.g. 30 min), the adult rat is then re-exposed to the same juvenile, then the amount of time spent investigating the younger animal is reduced. In contrast, if the adult rat is paired with a second, different juvenile after a half-hour delay, then the level of social investigation remains constant across both exposures. The reduction in investigatory behaviour on re-exposure to the same animal reflects a discriminative memory process for establishing individual identity (Thor and Holloway 1982). This social-recognition memory process is based on olfactory cues (Sawyer et al. 1984), decays as the interval between exposures increases (Thor and Holloway 1982) and is sensitive to interference (Thor and Holloway 1982; Dantzer et al. 1987).

Although a number of studies have examined the effects of pharmacological interventions on social-recognition memory (e.g. Perio et al. 1989), the neuroanatomical substrate underlying this memory process remains unclear. Evidence from a number of studies points towards a role of the hippocampal formation in social-recognition memory. For example, both Fournier et al. (1993) and Terranova et al. (1994) have found that lesions of the medial septum, an area of the brain that has strong reciprocal projections to and from the hippocampal formation, impair recognition. Along similar lines, Maaswinkel et al. (1996) have shown that transection of the fimbria, which carries a number of fibre projections both to and from the hippocampus, including septal afferents and efferents, also impairs social-recognition memory (but see also Petrulis et al. 2000). In addition, Terranova et al. (1994) found that social-recognition memory was disrupted following carotid artery occlusion. The authors attributed this disruption after cerebral ischemia to neuronal loss in the CA1 subfield of the hippocampus. The

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notion that the hippocampus mediates social memory is also supported by Lemaire et al. (1994), who showed that transection of the perforant path, which provides the afferent input to the hippocampus from the entorhinal cortex, impairs social memory but spares performance on an olfactory-discrimination test.

Taken together, these studies suggest a role of the hippocampus in social-recognition memory. The aim of this current study was to assess this hypothesis directly by examining the effects of selective, cytotoxic hippocampal lesions on social-recognition memory in the rat. These lesions spare fibres of passage and vasculature and cause minimal damage to adjacent and overlying cortical structures (Jarrard 1989). Lesioned and sham-operated animals were also tested on a spatial non-matching to place (or rewarded alternation) task on the elevated T-maze in addition to social-recognition memory. It is well established that rats with hippocampal damage exhibit robust and reliable deficits on spatial tasks such as the T-maze (O'Keefe and Nadel 1978; Rawlins and Olton 1982; Aggleton et al. 1992; Jarrard 1993). Performance on this task is extremely sensitive to hippocampal dysfunction and, thus, provides an ideal check on lesion efficacy (Bannerman et al. 1999). Similarly, there are numerous demonstrations that locomotor activity is increased after hippocampal lesions (e.g. Kimble 1963; Teitelbaum and Milner 1963; Bannerman et al. 1999). Locomotor activity was therefore assessed in photocell activity cages as a further index of hippocampal dysfunction.

Materials and methods

Subjects

Male Lister hooded rats (n=26) were obtained from Harlan Olac Ltd. and housed in pairs, except just prior to and during social-memory testing (see below). A 12 h light/dark cycle was maintained (0700–1900), with all behavioural testing conducted during the "light" phase. The pre-operative weights of the animals just prior to surgery ranged between 290 and 370 g. One group of rats (n=15) received complete bilateral cytotoxic lesions of the hippocampus (HPC). A second group of rats (n=11) acted as sham-operated controls.

Following surgery, locomotor activity was assessed followed by spatial learning on the elevated T-maze. Two weeks prior to social memory testing, the adult rats were housed individually in the experimental room in transparent perspex cages (450×240×200 mm) with ad libitum access to food and water (except during testing). The juvenile animals (male, Lister hooded) were housed in groups of five on a separate cage rack in the same experimental room. The experiments described were conducted in accordance with the U.K. Animals, Scientific Procedures Act (1986), under project license number PPL 30/1505.

Surgery

All rats were anaesthetised with tribromoethanol (Avertin – 0.29 g/kg) and placed in the stereotaxic frame (Kopf) with the head level between bregma and lambda. An incision of the scalp was made along the midline and the appropriate portion of the bone overlying the neocortex was removed.

The procedure used to destroy the cells of the hippocampus has been described elsewhere (Jarrard 1989). In short, rats received 24 injections of ibotenic acid (10 mg/ml; Tocris Neuramin, UK; see Table 1) dissolved in phosphate-buffered saline (pH 7.4). Injections (0.05–0.10 µl) were made with a 1-µl Hamilton syringe mounted on the stereotaxic frame over approximately 30–60 s. Upon completion of each infusion, the syringe needle was left in position for a further 60–120 s to allow for diffusion of the neurotoxin away from the injection site. Six rats in the sham-operated control group underwent a similar surgical procedure with the exception that no neurotoxin was injected and the needle tract was limited to the overlying cortex. The remaining five animals also underwent a similar surgical procedure, but the needle was not lowered into the cortex and the dura remained unbroken.

Table 1 Stereotaxic co-ordinates for injections of ibotenic acid (10 mg/ml) for cytotoxic lesions of the hippocampus (from Bregma)

AP	ML	DV (from brain surface)	Volume (µl)
–2.4	±1.0	–3.4	0.05
–3.0	±1.4	–3.4	0.05
–3.0	±1.4	–2.6	0.05
–3.0	±3.0	–3.0	0.10
–4.0	±2.6	–3.3	0.05
–4.0	±2.6	–2.3	0.05
–4.0	±3.7	–3.0	0.10
–4.8	±3.9	–7.0	0.10
–5.6	±4.1	–3.8	0.05
–5.6	±5.1	–5.8	0.05
–5.6	±5.1	–4.9	0.05
–5.6	±5.1	–4.0	0.05

On completion of the surgery, all animals were sutured and a topical antibiotic powder (P.E.P 2% powder; Intervet Laboratories, UK) was sprinkled over the wound. All animals also received a sub-cutaneous injection of antibiotic (Baytril 2.5%; Bayer, Ireland). Animals were allowed at least 2 weeks to recover before the start of behavioural testing.

Locomotor-activity testing

Locomotor-activity was measured in a set of specially designed hanging wire cages (39×25×23 cm; Modular Systems and Developments, U.K.), in which two horizontal photocell beams were located along the long axis of each cage (1.5 cm above the floor and 13 cm apart). Activity data was collected on an Acorn Archimedes RISC PC 600 computer using specialised software (Arachnid Activity Monitor; Paul Fray Ltd.), which provides a record of the number of beam breaks at both the front and the back of the cage independently, the total number of beam breaks, and the number of crossovers. The rats were placed individually into the wire activity cages and tested in the dark for an 8-h period.

Spatial learning on the elevated T-maze

Rats were put on a restricted feeding schedule and maintained at 85% of their free-feeding weight. Spatial learning was assessed in a discrete-trial rewarded alternation task, using an elevated wooden T-maze (for apparatus and testing procedure, see Bannerman et al. 1999). Rats were run one trial at a time with an ITI of approximately 10 min, for a total of six trials per session. The rats were tested for a total of ten sessions, giving a total of 60 trials.

Social-recognition memory testing

Social-memory testing was conducted in the home cages of the adult subjects and followed the protocol of Lemaire et al. (1994). Approximately 1 h prior to the start of behavioural testing, the ju-

venile rats (male Lister hooded rats; 40–50 g) were housed individually in order to isolate their odours. All adult rats received each of three different social-memory tests, conducted at least 48 h apart, according to a fully counterbalanced design. The three trials comprised of (1) a 30-min delay condition; (2) a zero-delay condition; and (3) a test during which a second, different juvenile was paired with the adult after a 30-min delay. All behavioural testing was conducted with the experimenter "blind" with respect to the lesion condition of the animals.

30-min delay condition

A juvenile rat was placed into the cage of the adult animal for 5 min, and the amount of time the adult spent investigating the younger animal was recorded. Investigative behaviour involved the adult rat sniffing or grooming the conspecific or closely following the path of the younger animal as it moved around the cage. After 5 min, the juvenile was removed and returned to its holding cage. Thirty minutes later, the same juvenile was returned to the adult's cage and a second 5-min observation period commenced. Social recognition manifests itself as a reduction in investigative behaviour between the first (T1) and second (T2) 5-min observation periods.

Different-juvenile condition

This test was identical to the 30-min delay condition with the exception that, during the second observation period (T2) a second, different juvenile (chosen from a different group cage) was placed in with the adult. Under these conditions, one would *not* expect a reduction in investigative behaviour between T1 and T2.

Zero-delay condition

As before, a novel juvenile rat was placed into the cage of the adult animal, and the amount of social investigation recorded. On this occasion, however, the observation period lasted for 10 min. The data was then analysed in two 5-min blocks (T1 and T2) as before.

Odour-detection test

Having completed the three social-memory tests, the olfaction of the animals was then assessed (see Carr et al. 1976; Lemaire et al. 1994). The adults were exposed for 5-min, in their home cages, to two small boxes (85×68×20 mm) containing soiled bedding from either the cage of a juvenile (novel odour) or from the adult's own cage (familiar odour). The amount of time the adult rat spent sniffing each box was recorded. Control animals tend to spend much more time investigating the box containing the novel odour of the juvenile than the box containing their own odour. The left/right arrangement of the boxes in the cage was counterbalanced to avoid any spatial bias.

Histology

At the end of behavioural testing, the rats were injected with Euthetal (200 mg/kg sodium pentobarbital) and perfused transcardially with physiological saline and 10% formol saline. Their brains were then removed and placed in formol saline solution. Subsequently the brains were placed in a sucrose-formalin solution for 24 h, frozen and then sectioned horizontally (50 μm). All brain sections were then stained with cresyl violet.

Data analysis

There were no differences between the two sham-operated groups. In order to simplify the subsequent analyses, the two sham groups were combined to give one control group of n=11. All behavioural data were subjected to an ANOVA. Furthermore, social-recognition memory data was also expressed as the "ratio of investigation duration" (RID; Perio et al. 1989), which was calculated for each animal as the amount of investigation during the second observation period (T2) divided by the amount of investigation during the initial observation period (T1). The lower the RID value, the better the recognition, whereas an RID value of 1 corresponds to a complete lack of recognition. The RID values were subsequently analysed using a non-parametric Wilcoxon Mann-Whitney test. This form of analysis has been commonly used for social recognition data (see, for example, Perio et al. 1989; Lemaire et al. 1994). To make the figures more legible, however, the data are expressed and displayed as the mean±SEM rather than the median and interquartile range (see Fig. 3B).

Results

Histology

Examination of the histology from rats with complete hippocampal lesions revealed that only the hippocampal subfields showed substantial cell loss; there was only minimal damage to adjacent structures such as the subiculum (see Figs. 1 and 2). There was complete loss of pyramidal and granule cells in the dorsal part of Ammon's horn and the dentate gyrus, respectively, in all of the lesioned animals (Figs. 1C and 2). In addition, most of the ventral hippocampus was destroyed in the majority of the lesioned rats (Figs. 1D and 2). In four of the animals, there was complete pyramidal and granule cell loss along the entire septotemporal extent of the hippocampus (Fig. 2, left column). Nine of the lesioned rats exhibited near complete ventral hippocampal cell loss, with just some sparing of the most posterior portions of both the CA1 subfield and the dentate gyrus (Fig. 2, centre column). The two remaining lesioned subjects did, however, display considerable sparing of the ventral hippocampus (Fig. 2, right hand column).

In a number of the lesioned animals (10/15), damage was also detectable in adjacent areas of subiculum, particularly at the interface between this region and the CA1 subfield. The greater part of the subiculum was always spared; cell loss was very restricted and limited to ventral areas (Figs. 1 and 2). There was no clear evidence of damage to the entorhinal cortex in the lesioned animals, although there was some evidence of a limited amount of damage to the overlying cortex in a small number of the animals in the lesion group. All animals from the hippocampal lesion group were included in the behavioural analysis (n=15).

Locomotor-activity testing

Hippocampal lesioned animals were considerably more active than sham-operated controls during the 8-h test

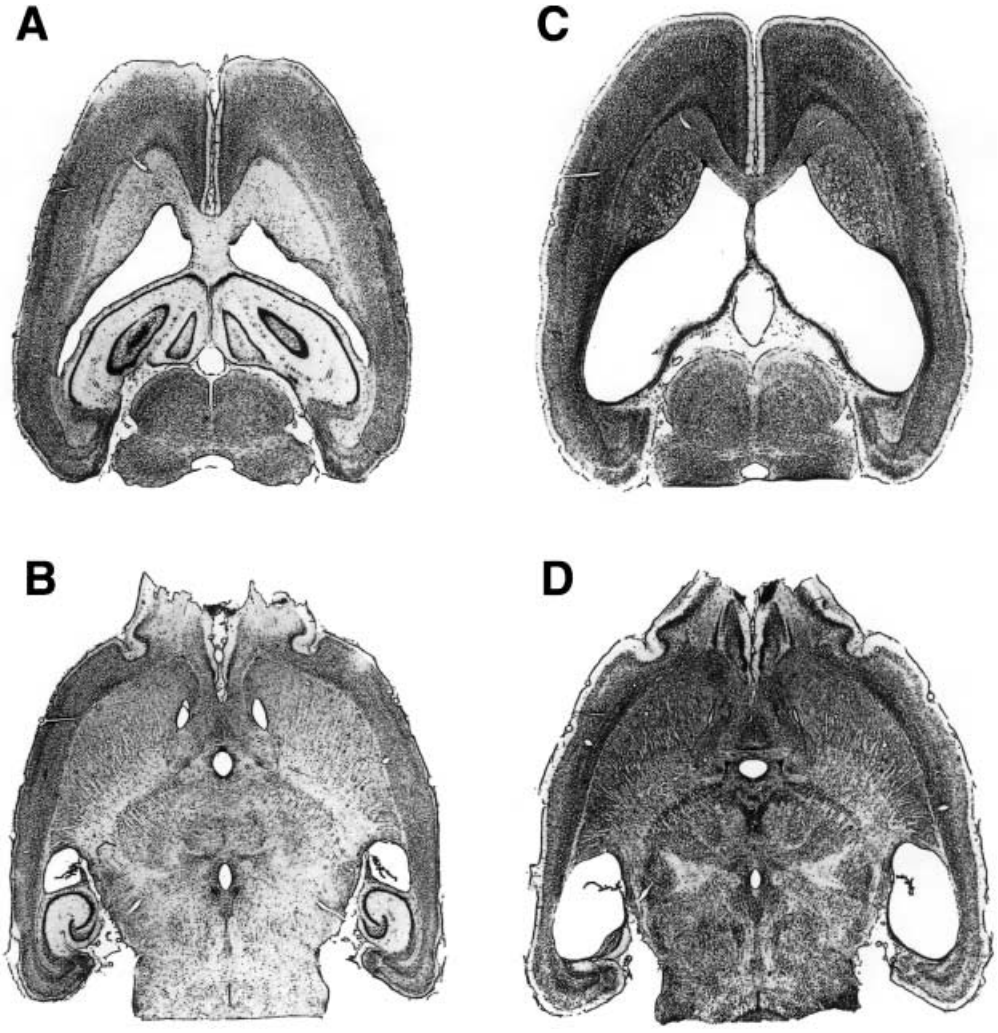


Fig. 1 Photomicrographs of horizontal sections showing dorsal (**A**) and ventral hippocampus (**B**) in a sham-operated animal. Typical cell loss is illustrated for both dorsal (**C**) and ventral hippocampus (**D**) from a representative lesioned rat

session in the photocell activity cages (total number of beam breaks; sham=2081±163; HPC=3527±407). An ANOVA of the total number of beam breaks made during this session revealed a significant group difference [$F(1,24)=8.44$; $P<0.01$].

Spatial performance on the elevated T-maze

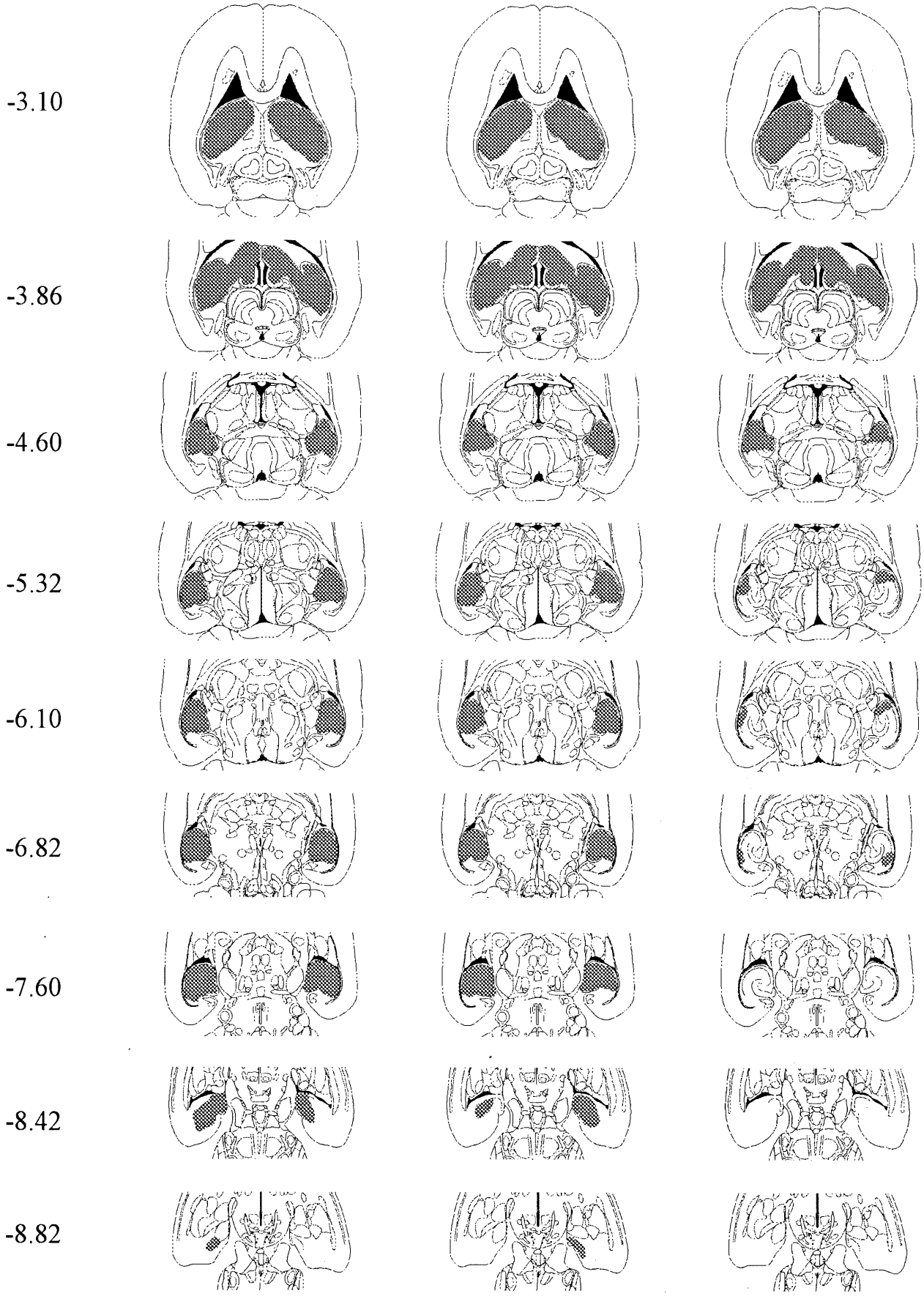
Rats in the hippocampal lesion group displayed a substantial spatial learning impairment during non-matching to place testing on the elevated T-maze. Whereas the control group performed the task extremely efficiently (91±1% correct), the hippocampal lesioned animals displayed near chance levels of performance (52±4% correct). In fact, all fifteen lesioned rats exhibited near chance levels of performance across the 60 trials of spatial non-matching to place testing (range=48.3–55.0%). Analysis revealed a significant difference between the two groups [$F(1,24)=973.6$; $P<0.0001$].

Social-recognition memory testing

Lesions of the hippocampus produced an increase in investigative social behaviour, but did not impair social-recognition memory (see Fig. 3). There was evidence of social-recognition memory (in terms of a reduction in investigative behaviour) in both the sham and lesioned animals, during both the zero- and 30-min-delay conditions. In contrast, when a second, different juvenile was put into the adult's cage at T2, the adult animal showed a comparable level of investigative behaviour to that observed during T1.

30-min delay condition

An ANOVA of the investigation times at T1 and T2 during the 30-min delay trial (Fig. 3A) revealed a significant overall effect of group [sham vs. lesion; $F(1,24)=5.82$; $P<0.05$], a significant overall effect of time [T1 vs. T2; $F(1,24)=16.33$; $P<0.0005$], but no group × time interaction ($F<1$; $P>0.20$). The presence of a significant main effect of time in the absence of a significant group by time interaction indicates that both groups of animals dis-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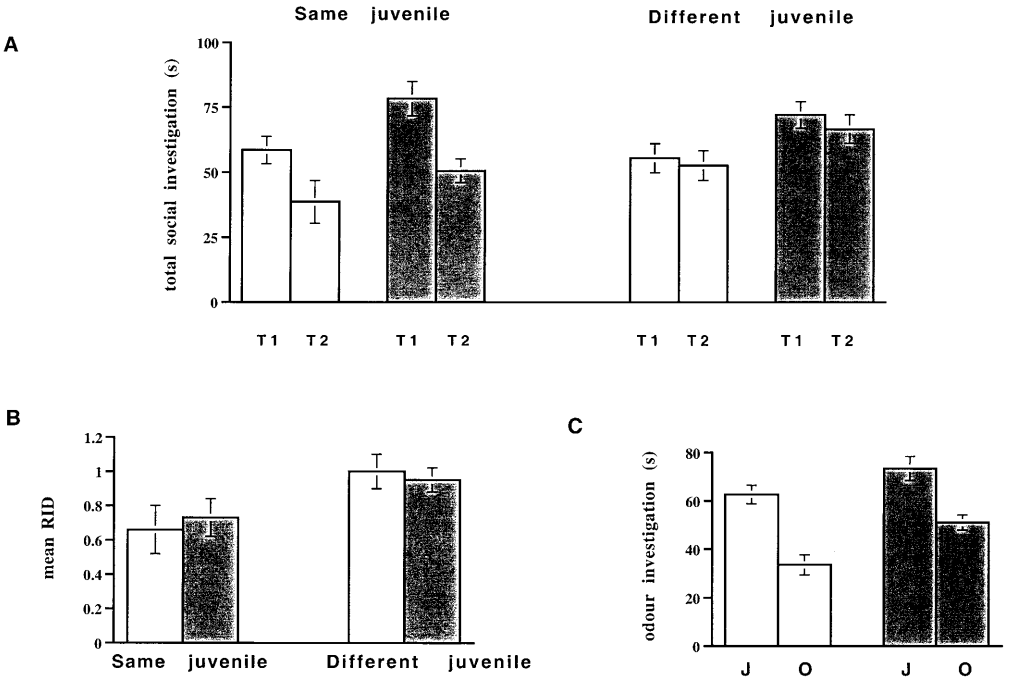

Fig. 3A–C The effects of selective hippocampal lesions on social-recognition memory in the rat. **A** The mean amount of time (seconds ±SEM) spent engaged in social investigation during 5-min trials by sham-operated (*white*) and hippocampal-lesioned (*grey*) animals. Following an initial exposure to a juvenile animal (*T1*), the rats were paired with either the same (*left*) or a different (*right*) juvenile during a second exposure (*T2*) after an interval of 30 min. **B** Mean ratio of investigation duration (*RID*; ±SEM) for sham-operated (*white*) and hippocampal-lesioned (*grey*) animals for trials involving either the same juvenile (*left*) or a different juvenile (*right*) during the second exposure (T2). **C** The mean amount of time (seconds ±SEM) spent sniffing the bedding from either the cage of a juvenile (*J*) or from the adult's own cage (*O*) during a 5-min olfaction test by sham-operated (*white*) and hippocampal-lesioned (*grey*) animals

played recognition memory for the juvenile at T2. This interpretation was confirmed by additional analyses of simple main effects, which showed that there was significant recognition memory (i.e. a significant reduction in investigative behaviour between T1 and T2) for both the sham [$F(1,24)=4.91$; $P<0.05$] and lesion groups [$F(1,24)=13.14$; $P<0.005$; see Howell 1997]. Analysis of the RID values calculated for the 30-min delay condition also revealed no evidence of a social-recognition memory impairment ($z=-0.57$; $P>0.20$; Fig. 3B).

Inspection of Fig. 3A and the presence of a significant main effect of group in the absence of a significant group × time interaction indicates, however, that the lesioned animals spent significantly more time investigating the juvenile rat during the initial 5-min exposure (T1). Consequently, lesioned animals had more exposure to the younger animal than controls during T1 and, therefore, may have had more opportunity to learn about the conspecific. This opportunity for increased learning in the hippocampal lesion group could conceivably have masked any mnemonic impairment in these animals. To investigate this possibility, additional statistical tests were performed. Regression analyses showed that there was no significant correlation between the duration of investigation during T1 and T2 for either the sham [$r(10)=0.39$; $P>0.20$] or hippocampal-lesioned animals [$r(14)=0.09$; $P>0.20$]. In addition, both sham and lesion groups were divided into subgroups ("high" and "low") on the basis of the amount of time spent investigating the juvenile during T1. In both cases, the median values were excluded from the analysis to ensure equal subgroup sizes. The amount of time spent investigating the juvenile during T2 by the *high* and *low* subgroups were then compared. Subsequent *t*-tests performed on the T2 investigation times showed that there were no differences between *high* and *low* subgroups for either sham (*high*=42.7; *low*=37.5; $t<1$; $P>0.20$) or hippocampal-lesioned rats (*high*=48.6; *low*=46.6; $t<1$; $P>0.20$). Finally, a further comparison was made between the complete sham group (T1=58.5) and the *low* hippocampal subgroup (T1=56.0). An ANOVA including both T1 and T2 data revealed a significant effect of time [$F(1,16)=5.31$; $P<0.05$], but neither a significant group effect nor a significant group × time interaction ($F<1$; $P>0.20$ for both comparisons). On the basis of these additional analyses, therefore, it seems unlikely that the absence of a hippocampal lesion deficit is due to an increase in time spent investigating the juvenile during T1, which might lead to an increased opportunity for learning, thus masking any mnemonic deficit. It is worth noting that there was no obvious histological correlate of the "high" and "low" investigators in the hippocampal lesion group.

◀ **Fig. 2** Reconstructions of hippocampal lesions. Maximal (*left column*), representative (*centre column*) and minimal (*right column*) extent of the lesions in horizontal sections between plate 116 (–3.10 mm from the brain surface) and plate 93 (–8.82 mm from the brain surface) of Paxinos and Watson (1998)

Zero-delay condition

Examination of the investigation times at T1 and T2 for the zero-delay trial revealed a similar pattern of results. There was a significant effect of group [$F(1,24)=5.61$; $P<0.05$], a significant effect of time [$F(1,24)=40.81$; $P<0.0001$], but no group × time interaction ($F<1$; $P>0.20$). Analysis of the RID values calculated for the zero delay condition also revealed no group difference ($z=-1.32$; $P>0.05$).

Different-juvenile condition

Analysis of the data obtained from the "different juvenile" condition (Fig. 3A) again showed a significant group effect [$F(1,24)=4.97$; $P<0.05$], but neither a significant effect of time [$F(1,24)=1.22$; $P>0.20$] nor a group × time interaction ($F<1$; $P>0.20$). The RID values calculated for both groups when a different juvenile was put into the adult's cage at T2 were higher than those observed when the same juvenile was replaced at T2 (Fig. 3B). The RID values approached a value of 1, indicating that the different juvenile was correctly identified as unfamiliar. There were no group differences ($z=-0.42$; $P>0.20$).

Odour-detection test

During the odour-detection test, the hippocampal-lesioned animals spent more time investigating the odour boxes than the sham-operated controls (Fig. 3C). Both groups, however, spent more time sniffing the box containing the bedding from the juvenile's cage. ANOVA revealed a significant overall effect of group [$F(1,24)=12.57$; $P<0.005$], a significant effect of the odours [$F(1,24)=35.01$; $P<0.0001$], but no group × odour interaction ($F<1$; $P>0.20$). The significant main effect of odours in the absence of a significant group × odour interaction indicates that both sham and lesioned animals showed a preference for the juvenile's odour. Indeed, subsequent analysis of simple main effects confirmed that both groups spent significantly more time sniffing the juvenile's bedding than their own [sham: $F(1,24)=19.38$; $P<0.001$; HPC: $F(1,24)=15.65$; $P<0.001$; see Howell 1997].

Discussion

Selective, cytotoxic lesions of the hippocampus proper, encompassing both the pyramidal cells in the four CA subfields and the granule cells in the dentate gyrus, did not impair social-recognition memory. They did, however, result in a general increase in investigative behaviour, which was observed throughout testing, during both the social memory and olfaction tests. In addition, hippocampal-lesioned animals displayed a pronounced hyperactivity in photocell activity cages and a robust spatial working-memory impairment on the elevated T-maze, confirming that the lesions were sufficient to cause severe hippocampal dysfunction. This study suggests that the hippocampus proper does not mediate social-recognition memory in the rat, but does not preclude the possibility that other areas of the hippocampal formation (e.g. entorhinal cortex or subiculum) may support this memory process.

Selective cytotoxic lesions of the hippocampus do not impair social-recognition memory

A number of previous findings have suggested that the hippocampus may support social-recognition memory in the rat. For example, the fact that septal lesions (Fournier et al. 1993; Terranova et al. 1994), fimbria transections (Maaswinkel et al. 1996; but see also Petrulis et al. 2000), carotid artery occlusions (of which the resultant damage includes cell loss in the CA1 subfield; Terranova et al. 1994) and perforant path transections (Lemaire et al. 1994) all impair social-recognition memory is consistent with such a possibility. However, none of these studies directly assessed this hypothesis by actually examining the effects of hippocampal lesions on the social-recognition memory task. The role of the hippocampus in this behaviour has been inferred either from the effects of lesions, which remove selected inputs to the hippocampus (but which may also deafferent other brain areas), or from global manipulations (eg. carotid artery occlusions), which are also likely to disrupt additional brain systems, over and above any effects on the hippocampus.

The present experiment clearly shows preserved social-recognition memory in hippocampal lesioned animals. Both sham and lesioned rats showed an equivalent reduction in investigative time between the two exposures to the juvenile (T1 and T2), indicating recognition memory for the conspecific. Although it could be argued that the fact that the hippocampal-lesioned animals spent more time investigating the juvenile during T1 may have provided an increased opportunity for learning, which may, in turn, have masked any mnemonic deficit in the lesioned animals, the additional analyses performed suggest, however, that this was not the case. The reduction in investigative behaviour between T1 and T2 was not simply due to a generalised habituation to the testing procedure or to the environment. Indeed, when a second, different juvenile was placed in the testing cage after a 30-min delay (at T2), both sham and lesioned animals showed high levels of social interaction, comparable to those observed during the initial exposure to the first conspecific (T1).

Neither can the absence of a social-memory impairment in the hippocampal lesion group be readily explained in terms of significant hippocampal sparing. Examination of the histology obtained from lesioned animals shows that cell loss was extensive, encompassing all of the dorsal and, in most cases, a substantial propor-

tion of the ventral hippocampus. Furthermore, these lesions were sufficiently large to produce the characteristic behavioural effects associated with hippocampal damage. The lesioned animals displayed a pronounced increase in spontaneous locomotor activity in the photocell cages, a result which is in agreement with a number of previous studies (e.g. Kimble 1963; Teitelbaum and Milner 1963; Bannerman et al. 1999). The hippocampal lesion group also showed increased investigatory behaviour throughout all of the social and olfactory tests, although it is possible that this increase in investigative behaviour in the hippocampal lesioned animals simply reflects the general increase in activity levels.

Furthermore, all 15 lesioned animals displayed a robust and enduring spatial working-memory impairment on the elevated T-maze. Performance of this task is extremely sensitive to hippocampal dysfunction and, thus, provides an ideal method by which lesion efficacy can be assessed (Bannerman et al. 1999). The present study thus demonstrates a clear dissociation between the effects of hippocampal lesions on social-recognition memory and spatial working memory on the elevated T-maze, suggesting that these behaviours may be subserved by distinct and separate memory systems within the brain (Squire and Zola-Morgan 1983; Eichenbaum et al. 1994).

The absence of a social-memory impairment in the present study in rats with selective hippocampal lesions does, however, contrast with a recent report that mice with selective cytotoxic hippocampal lesions display a social-recognition memory impairment (Kogan et al. 2000). Although this apparent discrepancy between the two studies may reflect a species difference (rat versus mouse) or a difference in terms of the exact size and location of the lesions, it may also reflect a subtle, but potentially very important difference in the testing paradigms adopted. Kogan et al. kept their adult mice housed in group cages right up until 15-min before the start of the social memory test. In contrast, in most other studies, including the present experiment, the adults are isolated and housed individually for much longer prior to testing (2 weeks in the present study). Kogan et al. found that group housed adult mice displayed a much longer lasting social memory for the juvenile, which could last for up to 7 days, compared with the short-term social memory typically seen in isolated rodents, which lasts for 1–2 h. It is possible, therefore, that the housing conditions of the adult (social versus isolated) may influence the nature of the memory trace that is formed during any subsequent social encounter with a juvenile and that only the longer lasting memory trace is sensitive to hippocampal damage. Interestingly, Winocur et al. (1990) found that rats with hippocampal lesions displayed a deficit during social transmission of food preference, a different kind of social learning task, but only at delays of 2 days or longer. It should be noted, however, that under other conditions a 30-min delay is more than adequate to reveal a hippocampal impairment. For example, the spatial working-memory impairment observed on the elevated T-maze in the present study was evident with a minimal delay of approximately 15 s. It is also worth noting that a 30-min delay was sufficient in previous social-recognition memory experiments to reveal deficits with medial septal lesions (Fournier et al. 1993; Terranova et al. 1994) or perforant path cuts (Lemaire et al. 1994). As the aim of the present study was to determine whether or not those deficits were due specifically to hippocampal dysfunction, a similar testing protocol was adopted in which the adults were isolated well in advance of the social memory test and a 30-min delay interposed between T1 and T2. Further studies would be required to determine whether or not using a different experimental protocol, in which the adult animals remain group housed right up until just before testing, would reveal a longer-lasting social memory trace which is sensitive to selective hippocampal lesions.

The absence of a social-memory impairment following hippocampal damage in the present study is not, however, necessarily inconsistent with current theories of hippocampal function. Social memory is essentially a simple olfactory recognition task (Sawyer et al. 1984), a task on which few, if any, of the currently influential accounts of hippocampal function would predict a deficit. A common element to these theories (e.g. spatial, contextual, configural, declarative, relational) is that the hippocampus is responsible for taking highly processed sensory information from all the sensory modalities and for forming a complex representation of this information, which can then be used flexibly by the animal to guide subsequent behaviour (Hirsch 1974; O'Keefe and Nadel 1978; Squire 1992; Rudy and Sutherland 1995; Eichenbaum et al. 1994). Indeed, Eichenbaum and colleagues have shown that the hippocampus is not required for the acquisition or formation of biases towards single olfactory stimuli, nor for maintaining memories for single odours during an olfactory recognition memory task (Otto and Eichenbaum 1992). In contrast, however, hippocampal lesions have been found to impair performance on more complex behavioural tasks that require the animals to use olfactory information in a more flexible manner or in combination with temporal information (Bunsey and Eichenbaum 1995, 1996; Dusek and Eichenbaum 1998). In view of such current thinking regarding the possible function(s) of the hippocampus, the absence of a social-memory impairment in the lesioned animals during this study was not unexpected.

A question still remains, therefore, concerning the anatomical substrate underlying social memory in the rat, because the present data are inconsistent with suggestions that social-memory impairments observed after septal lesions, carotid artery occlusions, fimbrial transections and perforant path transections result from a disruption of hippocampal function (Fournier et al. 1993; Lemaire et al. 1994; Terranova et al. 1994; Maaswinkel et al. 1996). Although the present data appear to rule out a role of the hippocampus proper in social-recognition memory, the current study does not preclude the involvement of other areas of the hippocampal formation, such

as the entorhinal cortex or subiculum, in these behaviours. There is now strong evidence that the pattern of behavioural effects that occur after selective entorhinal cortex lesions are *not* identical to those observed after selective damage to the hippocampus proper, suggesting that these two closely associated brain areas may in fact contribute to two separate memory systems within the temporal lobe or, at least, represent two functionally distinct components of the same memory system (see Eichenbaum et al. 1994; Pouzet et al. 1999).

If social-recognition memory was supported by the entorhinal cortex or subiculum, rather than by the hippocampus proper, this would be consistent not only with the current data (no behavioural impairment, given little, if any, damage to subiculum or entorhinal cortex), but also with previous studies that have implicated the hippocampal formation in this behaviour (Fournier et al. 1993; Lemaire et al. 1994; Terranova et al. 1994; Maaswinkel et al. 1996). For example, lesions of the medial septum, which have been shown to impair social-recognition memory (Fournier et al. 1993; Terranova et al. 1994), destroy the cholinergic projections not only to the hippocampus, but also to the entorhinal cortex, so that medial septal lesions decrease or completely abolish both acetylcholinesterase staining and theta rhythm in the entorhinal area (Mitchell et al. 1982). It is possible, therefore, that the deficits in social memory observed after septal lesions are due to a disruption of entorhinal cortex function rather than any effect on the hippocampus proper. Likewise, fimbrial transections will disrupt projections to and from the lateral entorhinal area in addition to disrupting hippocampal afferents and efferents. Thus, deficits in social memory after fimbrial cuts could also be due to entorhinal dysfunction.

Similarly, disruptions in social memory after cerebral ischemia could be due to neuronal loss or damage in entorhinal cortex or subiculum, rather than to cell loss in the CA1 subfield of the hippocampus (Terranova et al. 1994). Carotid artery occlusions, in addition to causing cell death in the hippocampus, also induce neuronal loss and damage in other parts of the brain (Pulsinelli et al. 1982a, 1982b; Ginsberg and Busto 1989; Nunn et al. 1994), including subicular (Smith et al. 1984) and entorhinal areas (Prehn et al. 1993; Sano et al. 1993; Thaminy et al. 1997; see also Dubois et al. 1988; Ikeda et al. 1994).

Likewise, the demonstration by Lemaire et al. (1994) that knife cuts of the perforant path impair social-recognition memory could equally be explained in terms of a disruption of either hippocampal or entorhinal function. By definition, sectioning of the perforant path not only disrupts hippocampal input, but also impedes a major component of the output from the entorhinal cortex. Such a knife cut is also likely to disrupt cholinergic projection fibres from the medial septal area to the entorhinal cortex and also prevent other entorhinal ouput pathways from reaching their intended target structures (Mitchell et al. 1982; Witter et al. 1989).

It seems possible, therefore, that previous data suggesting that the hippocampus proper supports social-recognition memory could equally well indicate a selective role for entorhinal cortex or subiculum underlying this behaviour. Consistent with this suggestion, Petrulis et al. (2000) have recently shown that female golden hamsters with aspiration lesions of the parahippocampal region (which included damage to entorhinal, perirhinal and temporal cortex) were unable to discriminate between the scents of individual male animals. The lesioned females were, however, able to discriminate between male and female odours and habituated to repeated presentations of the same odour. On the basis of these results, the authors suggested that the parahippocampal region was essential for individual odour discrimination.

To conclude, therefore, the present study suggests that the hippocampus proper is *not* essential for social-recognition memory in the rat. This experiment does not, however, preclude the possibility that other areas of the hippocampal formation (e.g. entorhinal cortex or subiculum) may support this memory process. This latter hypothesis is consistent both with earlier studies that had previously implicated the hippocampal formation in social-recognition memory (Fournier et al. 1993; Lemaire et al. 1994; Terranova et al. 1994) and also with current theories concerning the roles of the hippocampus proper and the parahippocampal region in olfactory memory processing (Eichenbaum 1998).

Acknowledgements This work was supported by a Wellcome Programme Grant (U.K. 039129/Z/93). The authors would like to thank Greg Daubney for his assistance with the histology.

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