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Research Paper

Putting objects in context: A prefrontal–hippocampal–perirhinal cortex network

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Abstract

When we encounter an object, we spontaneously form associations between the object and the environment in which it was encountered. These associations can take a number of different forms, which include location and context. A neural circuit between the hippocampus, medial prefrontal cortex and perirhinal cortex is critical for object-location and object-sequence associations; however, how this neural circuit contributes to the formation of object-context associations has not been established. Bilateral lesions were made in the hippocampus, medial prefrontal cortex or perirhinal cortex to examine each region contribution to object-context memory formation. Next, a disconnection lesion approach was used to examine the necessity of functional interactions between the hippocampus and medial prefrontal cortex or perirhinal cortex. Spontaneous tests of preferential exploration were used to assess memory for different types of object-context associations. Bilateral lesion in the hippocampus, medial prefrontal cortex or perirhinal cortex impaired performance in both an object-place-context and an object-context task. Disconnection of the hippocampus from either the medial prefrontal cortex or perirhinal cortex impaired performance in both the object-place-context and object-context task. Interestingly, when object recognition memory was tested with a context switch between encoding and test, performance in the hippocampal and medial prefrontal cortex lesion groups was disrupted and performance in each disconnection group (i.e. hippocampus + medial prefrontal cortex, hippocampus + perirhinal cortex) was significantly impaired. Overall, these experiments establish the importance of the hippocampal-medial prefrontal-perirhinal cortex circuit for the formation of object-context associations.

Keywords

Object-context memory, hippocampus, medial prefrontal cortex, perirhinal cortex, disconnection analysis

Introduction

Recognition memory, the ability to recognise that a stimulus, such as an object, has been encountered before, is not a unitary process as distinct types of information can be used to form judgements of prior occurrence. These judgements can be based on the relative familiarity of an object or alternatively can be made based using an association of an object and the spatiotemporal context in which it was encountered. Context has been defined as the integrated representation of various components of the available sensory information (Robertson et al., 2015), and it is suggested that contextual features in the environment are distinct from precise spatial locations. Thus, associations can be made between the stimulus and its spatial location (object-in-place memory) and also between a stimulus and context in which it was encountered (object-context memory) or indeed associations may be formed between the object and both the location and environment it is encountered in (object-place-context memory). An important question is whether different types of object associative memory share the same neural substrates.

Investigations into the neural basis of object-in-place memory have revealed the importance of the medial prefrontal cortex (mPFC), hippocampus (HPC) and perirhinal cortex (PRH). In addition, disconnection of the HPC from either the mPFC or PRH also impairs object-in-place memory indicating the importance of functional interactions between these regions (Barker and Warburton, 2011). In contrast, there has been less investigation of neural networks for object-context and object-place-context memory, although recent studies have shown that disconnection of the PRH from the postrhinal cortex significantly impaired object-context memory (Heimer-McGinn et al., 2017) and disconnection of the mPFC from the lateral entorhinal cortex impaired object-place-context memory (Chao et al., 2016). Disconnection studies have also shown that interactions between inferotemporal cortex, frontal cortex and the HPC are important for object-place-context judgements in Macaque monkeys (Browning et al., 2007; Wilson et al., 2007, 2008).

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There is evidence that the HPC and PRH may play dissociable roles in object-place-context and object-context memory. Lesion in the HPC impaired object-place-context memory without impairing object-context memory (Langston et al., 2010; Langston and Wood, 2010; but see Mumby et al., 2002, for object-context deficit). In contrast, lesion in the PRH impaired object-context memory without affecting object-place-context memory (Eacott and Norman, 2004; Norman and Eacott, 2005). Lesions in the mPFC in mice impaired object-context memory formation (Spanswick and Dyck, 2012), but the contribution of mPFC to object-place-context memory formation has not been studied. Therefore, the mPFC-HPC-PRH network may be selectively engaged in object-place-context or object-context associations.

Single-Item recognition does not normally depend upon the HPC (reviewed in Brown and Warburton, 2015; Winters et al., 2008); however, in one study, when a context switch was introduced between encoding and retrieval, animals with HPC lesions were unable to discriminate between a novel and familiar object (O'Brien et al., 2006). This finding suggests that there is a complex relationship between object memory and context memory; thus, here we explored this relationship by examining the role of the mPFC, HPC, PRH and mPFC-HPC-PRH circuit in performing object recognition memory judgements with a context switch.

The present study tested two hypotheses: first, that the HPC, mPFC and PRH are critical for the formation of object-context or object-place-context associations; second that the HPC functionally interacts with either the mPFC or PRH to form object-context or object-place-context associations. To test the first hypothesis, recognition memory in animals with bilateral lesions in the HPC, mPFC and PRH cortex was compared using a battery of context-dependent object recognition memory tasks. The second hypothesis was tested by making unilateral lesions of the HPC and either the PRH or mPFC in the same or opposite hemisphere (disconnection lesions). If hippocampal-cortical regions are functionally interdependent, then animals with contralateral lesions should be more impaired than animals with ipsilateral lesions; if the regions operate independently, then there will be no difference in performance between the contralateral and ipsilateral lesion groups.

Materials and methods

Subjects

All experiments were conducted in male pigmented rats (Dark agouti [DA] strain, weighing 230–250 g at the start of experiments, Bantin and Kingman). The animals were housed in pairs under a 12/12 h light/dark cycle, light phase 18.00–6.00 h. Behavioural testing was conducted during the dark phase of the cycle. Food and water were available ad libitum throughout the experiment. All animal procedures were conducted in accordance with the United Kingdom Animals (Scientific Procedures) Act (1986) and associated guidelines. All efforts were made to minimise any suffering and the number of animals used. The animals used in the current experiments had previously been run through a series of spontaneous preferential exploration tests (see Barker and Warburton, 2011).

Surgery

Rats were divided into two cohorts that were tested independently. Rats in the first group were subdivided into four groups consisting of animals with lesions in bilateral PRH, bilateral mPFC, bilateral hippocampal (HPC) and surgical controls (SHAM). Rats in the second group were divided into four groups and received one of the following combinations of lesions: (1) unilateral PRH lesion combined with a unilateral HPC lesion in the opposite hemisphere (PRH + HPC Contra), (2) unilateral PRH lesion and unilateral HPC lesion in the same hemisphere (PRH + HPC Ipsi), (3) a unilateral mPFC lesion combined with an HPC lesion in the opposite hemisphere (mPFC + HPC Contra) and (4) a unilateral mPFC lesion combined with an HPC lesion in the same hemisphere (mPFC + HPC Ipsi). Animals with ipsilateral lesions served as controls for animals with lesions in the contralateral hemispheres.

Each rat was anaesthetised with isoflurane (induction, 4%; maintenance, 2%–3%) and secured in a stereotaxic frame with the incisor bar set at the appropriate level (for the PRH or mPFC lesion surgery, the bar was set at +5 mm above the interaural line; for the HPC lesion surgery, the incisor bar was set so as to achieve flat skull (approximately –3.5 mm)). The scalp was then cut and retracted to expose the skull. Craniotomies were then made directly above the target regions, and the dura cut to expose the cortex. Lesions in the PRH or mPFC were made by injecting 0.09 M N-methyl-D-aspartate (NMDA) (Sigma) dissolved in phosphate buffer, pH 7.2. Lesions in the HPC were made by injecting 0.06 M NMDA. All the injections were made through a 1 μL Hamilton syringe into the appropriate sites in the hemisphere.

For the PRH and HPC lesions, each injection was made gradually over a 3-min period and the needle was left in situ for a further 3-min before being withdrawn; for the mPFC lesions, each injection was made over a 4-min period and the needle left in situ for a further 4 min (due to greater volume of fluid). For the PRH lesions, the anterior–posterior (AP), lateral (LAT) and dorsoventral (DV) stereotaxic coordinates were calculated relative to bregma. For the mPFC and HPC lesions, the AP and LAT coordinates were calculated relative to bregma, and the DV coordinates were calculated relative to the top of the cortex. The coordinates used and the volume of neurotoxin injected are shown in Tables 1–3. In the disconnection lesion group, the left (LAT+) and right (LAT–) hemispheres were targeted in different animals. Sham control lesions of these structures were made using the procedure described above, but in these cases, the injection needle was lowered to the level of the target structure and left in place for the appropriate length of time before being removed.

At the completion of surgery, the skin was sutured and an antibiotic powder (Acramide; Dales Pharmaceuticals) was applied. All animals then received a single administration of 5 mL of glucose saline subcutaneously and systemic analgesia intramuscularly (0.05 mL Temgesic; Reckett and Colman). All animals were allowed to recover for at least 10 dsys before habituation to the testing arena began.

Histology

At the end of the experiment, each rat was anaesthetised with Euthetal (Rhone Merieux) and perfused transcardially with phosphate buffered saline (PBS) followed by 4% paraformaldehyde. The brain was postfixed in paraformaldehyde for a minimum of 2 h before being transferred to 30% sucrose in 0.2 M phosphate buffer and left for 48 h. Coronal sections were cut at 50 μm on a cryostat and stained with cresyl violet.

Table 1. Lesion coordinates for the HPC relative to bregma.

	AP	LAT (±)	DV	Volume of 0.06 M NMDA
1	−2.1	1.1	−3.5	100 nL
2	−2.5	1.0	−3.7	100 nL
3	−2.5	2.2	−3.6	100 nL
4	−3.0	1.2	−3.4	100 nL
5	−3.0	2.5	−3.5	100 nL
6	−4.0	2.5	−3.0	100 nL
7	−4.0	4.1	−4.0	150 nL
8	−4.0	5.5	−5.1	150 nL
9	−4.5	2.5	−3.4	150 nL
10	−4.5	4.5	−4.0	150 nL
11	−5.2	4.5	−4.2	150 nL
12	−5.6	4.6	−6.6	150 nL
13	−6.0	4.2	−3.8	150 nL

HPC: hippocampus; AP: anterior–posterior; LAT: lateral; DV: dorsoventral; NMDA: N-methyl-D-aspartate.
In the animals which received a unilateral lesion, the left (+LAT) and right (LAT−) hemispheres were targeted in different animals.

Table 2. Lesion coordinates for the mPFC relative to bregma.

	AP	LAT (±)	DV	Volume of 0.09 M NMDA
1	+2.7	0.7	−4.5	280 nL
2	+2.7	0.7	−2.2	280 nL
3	+4.0	0.7	−3.5	280 nL
4	+4.0	0.7	−2.0	280 nL

mPFC: medial prefrontal cortex; AP: anterior–posterior; LAT: lateral; DV: dorsoventral; NMDA: N-methyl-D-aspartate.

Table 3. Lesion coordinates for the PRH relative to bregma.

|	AP	LAT (±)	DV	Volume of 0.09 M NMDA
1	−1.2	5.8	−9.3	180 nL
2	−3.2	6.1	−9.5	180 nL
3	−4.7	6.2	−9.1	180 nL

PRH: perirhinal cortex; AP: anterior–posterior; LAT: lateral; DV: dorsoventral; NMDA: N-methyl-D-aspartate.

To determine the extent of damage and the total area of tissue remaining in each of the structures that contained a lesion, the remaining area of the target structure was measured (Leica Qwin V3) in every fourth section between the following AP coordinates relative to bregma: mPFC +4.70 to +2.2 mm, HPC −1.9 to −6.3 mm, PRH −4 to −7.8 mm. The size of lesion was determined by comparing the total area remaining of each structure in each lesioned animal to the equivalent area in the sham-operated animals (100 − ((lesion area/sham area) × 100)) and average lesion sizes for each group were determined. Additional sections were studied under the light microscope to identify incidental damage outside the targeted regions (see the 'Results' section).

Behavioural apparatus

Exploration occurred in an open-topped arena (50 cm × 90 cm × 100 cm, H × W × D) made of wood, the walls inside the arena were surrounded with a cloth to a height of 1.5 m so that no external cues could be seen during the experiment and the floor of the arena was covered with sawdust. An overhead camera and a DVD recorder were used to monitor and record the animal's behaviour for subsequent analysis. The stimuli presented were objects constructed from Duplo blocks (Lego); varied in shape, colour and size (9 cm × 8 cm × 7 cm to 25 cm × 15 cm × 10 cm) and were too heavy for the animals to displace.

Contexts

Objects were presented in two different contexts within the same arena. The arena remained in the same position within the experimental room; thus, both contexts in which the animals encountered the objects occupied the same location within the testing room. Context A comprised grey walls and black curtains surrounding the arena (this context had previously been used for object recognition memory testing as described in Barker and Warburton, 2011), and context B comprised white walls with small black spots, and white curtains surrounding, the floor (sawdust) did not change between contexts, the floor of the arena remained constant in contexts A and B, so any associations made by the animals were between the objects and distal wall cues.

Behavioural testing

Pretraining. After being handled for a week, the animals were habituated to Context A (the grey wall/black curtain) without stimuli for 10 to 15 min daily for 4 days before the commencement of behavioural testing. Animals were subsequently run through tests of object recognition, object location, object-in-place and temporal order memory (reported in Barker and Warburton, 2011). Following the completion of these experiments, the animals were habituated to Context B (white wall with black spots/white curtain) for 10 to 15 min daily for 4 days before the commencement of the context memory behavioural experiments.

Object-place-context task. The task comprised two acquisition phases separated by a 5-min delay and a recognition test 1 h after the second acquisition phase (see Figure 1(a)). In the first acquisition phase, two different objects (A1 and B1) were presented in one of the contexts (A or B counterbalanced between rats). Animals were placed into the arena and allowed to explore the two objects for 4 min. At the end of the first acquisition phase, animals were returned to the home cage for the 5-min inter-acquisition delay. In the second acquisition phase, the same two objects (A2 and B2) were presented in the second context, but the position of each object was swapped (see Figure 1(a)); the animals were placed into the arena and allowed to explore the objects for 4 min. At the end of the second acquisition phase, animals were returned to their home cage for the acquisition-test delay of 1 h. In the test phase (3-min duration), animals were presented with two identical copies of one of the objects presented in the sample phases (either A3 and A4 or B3 and B4) in the context used in the first acquisition phase. Intact object-place-context memory was demonstrated by preferential exploration of the object in a novel context-location association versus the copy of the object in a familiar context-location association (indicated by the arrow in Figure 1(a)). The position of each object in each context, the object presented at test and the order in which contexts were
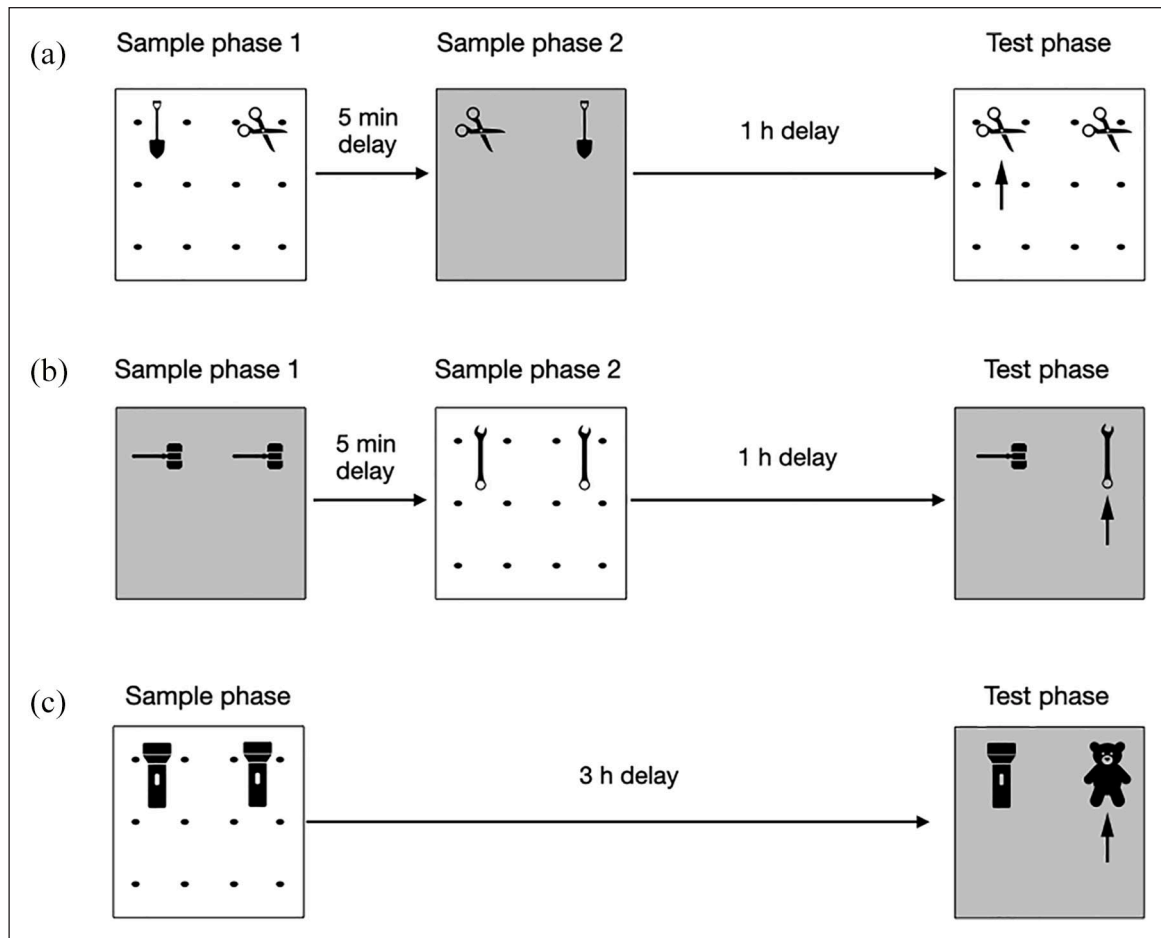
Figure 1. Outline of the three object-context memory tasks: (a) object-place-context task, (b) object-context task and (c) object recognition in different contexts task. Arrow in test phase indicates novel stimulus in each task.

presented was counterbalanced between and within each lesion condition.

Object-context task. The task comprised two acquisition phases separated by a 5-min delay and a recognition test 1 h after the second acquisition phase (see Figure 1(b)). In the first acquisition phase, two identical copies of an object (A1 and A2) were presented in one of the contexts (A or B counterbalanced between rats). Animals were placed in the arena and allowed to explore the objects for 4 min. At the end of the sample, phase animals were returned to the home cage for the 5-min inter-acquisition delay. In the second acquisition phase, animals were presented with two copies of a different object (B1 and B2) in the second context; animals were placed in the arena and allowed to explore the objects for 4 min. Animals were returned to the home cage for the 1-h acquisition-test delay. In the test phase, animals were presented with one copy of each of the objects presented in the acquisition phases (A3 and B3) in the context used for the first acquisition phase. Animals were placed into the arena and were allowed to explore the objects for 3 min. Intact object-context memory was demonstrated by a preferential exploration of the object which had not previously been encountered in the test context (i.e. in a novel object-context association, indicated by the

arrow in Figure 1(b)). The order in which the objects were presented, the context used for the first acquisition phase and the position of the objects in the test phase was counterbalanced between and within each lesion condition.

Object recognition in different contexts task. The task consisted of one acquisition phase and a recognition test separated by a 3-h delay. In the acquisition phase, animals were presented with two identical copies of an object (A1 and A2) in one of the contexts (Figure 1(c)). Animals were placed into the arena and allowed to explore the objects until either 40 s of object exploration had been completed or 4 min had passed. At the end of the acquisition phase, animals were returned to the home cage for the 3-h delay. In the test phase, animals were presented with one copy of the object presented in the acquisition phase (A3) and a novel object (B3) in the second context, that is, a different context to where the sample phase occurred, animals were placed into the arena and allowed to explore the objects for 3 min. Intact object recognition memory was demonstrated by a preferential exploration of the novel object over the familiar object (indicated by the arrow in Figure 1(c)). The object presented in the acquisition phase, the context used in the acquisition phase and the position of the objects at test were counterbalanced between and within each lesion condition.

Behavioural measures and statistical analysis

All measures of exploration were made with the experimenter blind to the lesion status of each animal. Exploratory behaviour was defined as the animal directing its nose towards the object at a distance of <2 cm. Any other behaviour such as looking around while sitting or resting against the object was not considered as exploration. Any subjects that failed to complete a minimum of 15 s of object exploration in either sample phase or 10 s of object exploration in the test phase were excluded from the analysis. No animals were excluded from the data due to insufficient object exploration.

Discrimination between the objects was calculated using a discrimination ratio (DR), calculated as the absolute difference in the time spent exploring the novel and familiar objects divided by the total time spent exploring the objects. The DR takes into account individual differences in the total amount of object exploration (Dix and Aggleton, 1999; Ennaceur and Delacour, 1988).

Performance in each task was compared statistically using a between-subjects one-way analysis of variance (ANOVA) followed by post hoc tests with a Bonferroni correction for multiple comparisons where appropriate. Performance of the animals in the bilateral lesion groups (SHAM, HPC, PRH, mPFC) was compared statistically against each other, while the performance of animals with unilateral combined lesions in either the HPC + mPFC (Contra and Ipsi) or HPC + PRH (Contra and Ipsi) was compared. Additional analyses examined whether individual groups had discriminated between the objects, using a one-sample t-test (two-tailed) versus 0. Overall object exploration levels in the sample and test phases were compared statistically using either a two-way mixed design ANOVA with sample phase as a within-subject factor and lesion as a between-subjects factor (sample phase exploration in object-place-context task and object-context task) or a one-way between-subjects ANOVA with lesion as factor (sample phase exploration in object recognition task in different contexts and exploration in all test phases).

Results

Histology

Lesion histology was previously reported in detail in Barker and Warburton (2011); histological results are summarised below.

Bilateral mPFC lesion group

All animals ($n=12$) received significant lesions in the prelimbic and infralimbic cortex (mean $79\% \pm 1.1\%$, minimum lesion size (min) 72%, maximum lesion size (max) 86%), 2 animals had unilateral minor sparing in the posterior prelimbic cortex and 10 animals had minor sparing in the most posterior region of the infralimbic cortex. All animals had additional minor damage in the medial orbital cortex, anterior cingulate cortex and motor cortex, and six animals also had minor damage to lateral septum.

Bilateral PRH lesion group

All animals ($n=10$) received significant bilateral lesions in the PRH (mean $84\% \pm 4.0\%$, min 58%, max 98%), and four animals had unilateral sparing in the most anterior portion of PRH. All animals had additional damage in temporal association cortex (area Te2) and minor damage in the dorsal region of lateral entorhinal, somatosensory and visual cortex. Nine animals had minor damage to piriform cortex and eight animals had minor damage to auditory cortex.

Bilateral HPC lesion group

All animals ($n=10$) had almost complete cell loss in the dorsal HPC (CA1, CA2, CA3) and dentate gyrus (DG), three animals had unilateral sparing of the medial DG and four animals had bilateral sparing of medial DG. Damage in the ventral HPC was less complete, eight animals had sparing of the ventral tip of the HPC and in two animals, the ventral HPC was largely spared. Two animals had minor bilateral damage in the ventral subiculum. Mean lesion size was $58\% \pm 5.8\%$ of HPC (min 40%, max 93%). All animals had some damage to the overlying cortical regions, including primary somatosensory cortex, visual cortex and posterior parietal cortex.

HPC + PRH Contra

All animals had major unilateral cell loss in the dorsal HPC; in five animals, there was some sparing of DG; and in one animal, there was sparing of both the DG and CA1. Four animals had major cell loss in the ventral HPC, and the remaining six animals had only moderate cell loss. Five animals had minor damage in the dorsal subiculum and seven animals had minor damage in the ventral subiculum. Mean lesion size was $54\% \pm 4.3\%$ of HPC (min 35%, max 68%).

All animals had significant unilateral cell loss in the PRH (mean $85\% \pm 3.6\%$, min 63%–99%) and one animal had sparing in the anterior portion of the PRH. All animals had additional damage in area TE (in four animals, the damage was minor) and dorsal lateral entorhinal cortex. Some animals had minor damage in ventral auditory cortex ($n=2$), piriform cortex ($n=1$) and postrhinal cortex ($n=2$).

In all animals, there was minor bilateral damage to primary somatosensory cortex and visual cortex, and there was also minor damage to posterior parietal cortex; in one animal damage was bilateral, and in all others damage was unilateral.

HPC + PRH Ipsi

All animals ($n=9$) had unilateral major cell loss in the dorsal HPC, four animals had minor sparing in the DG and two animals had some sparing of the medial region of the CA1 and DG. Six animals had major cell loss in the ventral HPC with sparing restricted to the ventral tip, and in three animals, damage to the ventral HPC was minor. Four animals had minor damage in the dorsal subiculum and six animals had minor damage in the ventral subiculum. Mean lesion size was $61\% \pm 2.3\%$ of HPC (min 52%, max 70%).

All animals had significant damage to PRH (mean $86\% \pm 2.4\%$, min 74%, max 94%) and two animals had sparing in the posterior PRH. In all animals, there was damage in area TE and minor damage in dorsal lateral entorhinal cortex. Four animals had minor damage in the ventral auditory cortex and two animals had minor damage to postrhinal cortex.

All animals suffered additional unilateral damage to cortical regions overlying the HPC and PRH. In eight animals, there was

major unilateral damage to the posterior region of somatosensory cortex; however, the anterior regions were spared. One animal had minor damage to primary somatosensory cortex. All animals had unilateral damage to visual cortex (minor $n=8$, major $n=1$) and posterior parietal cortex (minor $n=4$, major $n=5$).

HPC + mPFC Contra

In all animals ($n=10$), there was extensive unilateral cell loss in the dorsal HPC, four animals had minor sparing in the DG and two animals had sparing in the medial CA1 and DG. Cell loss in ventral HPC was less extensive; three animals had major cell loss in ventral HPC with sparing only at the ventral tip and seven animals only had minor damage to ventral HPC. Three animals had minor damage in the dorsal subiculum and four animals had minor damage in the ventral subiculum. Mean lesion size was $57\% \pm 3.7\%$ of HPC (min 49%, max 71%). All animals suffered damage to the cortical tissue overlying the HPC, including minor damage to primary somatosensory cortex, visual cortex and posterior parietal cortex.

In all animals, there was significant unilateral cell loss in the prelimbic and infralimbic cortices (mean $74\% \pm 4.5\%$, min 51%, max 85%), three animals had sparring in the anterior–dorsal region of prelimbic cortex and there was minor sparring of the most posterior part of infralimbic cortex. All animals had additional minor unilateral cell loss in the anterior cingulate cortex and secondary motor cortex, seven animals had minor cell loss in the medial orbital cortex, eight animals had minor loss in the lateral septum, two animals had minor cell loss in the striatum and one animal had minor loss in the nucleus accumbens.

HPC + mPFC Ipsi

In all cases ($n=10$), there was extensive cell loss in the dorsal HPC, three animals had minor sparing in the DG and five animals had minor sparring of the medial CA1 and DG. Cell loss in the ventral HPC was less extensive; two animals had major cell loss in the ventral HPC with sparing only at the ventral tip and eight animals only had minor cell loss in the ventral HPC. Three animals had minor damage in the dorsal subiculum and two animals had minor damage in the ventral subiculum. Mean lesion size was $54\% \pm 4.3\%$ of HPC (min 35%, max 68%). All animals had unilateral cell loss in the cortical regions overlying the HPC; there was minor cell loss in primary somatosensory cortex, visual cortex and posterior parietal cortex.

All animals had significant unilateral cell loss in mPFC (mean $69\% \pm 2.8\%$, min 55%, max 82%) and four animals had minor sparing in the anterior–dorsal portion of the prelimbic cortex. All animals had additional unilateral minor cell loss in the anterior cingulate cortex and secondary motor cortex, four animals had minor unilateral cell loss in the medial orbital cortex and three animals had minor unilateral cell loss in the lateral septum.

Behaviour

Object-place-context

Recognition during test phase

Bilateral lesion group. Performance in the object-place-context task was significantly impaired in the HPC, PRH and
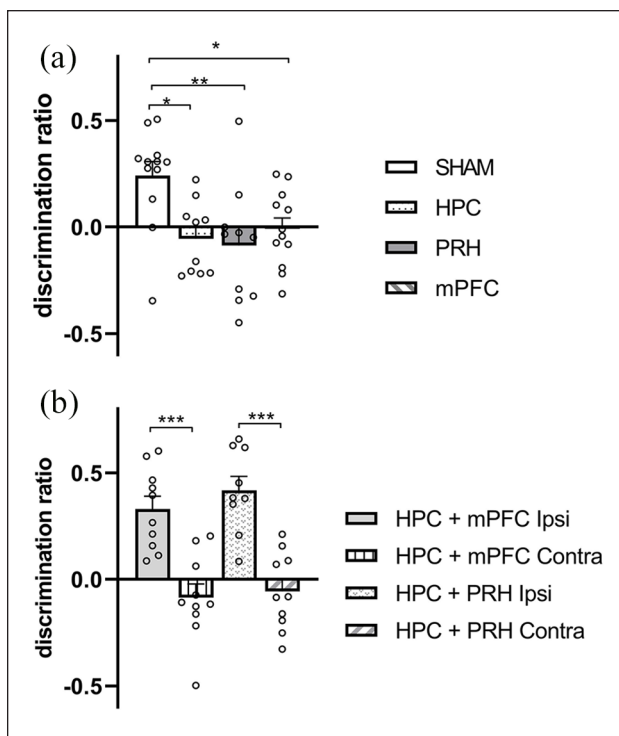
Figure 2. Performance of the experimental groups in the object-place-context task. (a) Performance of the sham lesioned animals (SHAM), bilateral hippocampal (HPC), perirhinal (PRH) or medial prefrontal cortex (mPFC) lesion groups. (b) Performance of animals with combined lesions in the hippocampus and medial prefrontal cortex in the same hemisphere (HPC + mPFC Ipsi) or opposite hemisphere (HPC + mPFC Contra), and animals with combined lesions in the hippocampus and perirhinal cortex in the same hemisphere (HPC + PRH Ipsi) or opposite hemisphere (HPC + PRH Contra). Data presented for each group are mean + SEM (bar) and individual animals' performance (open circles). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

mPFC bilateral lesion groups (see Figure 2(a)) compared to the sham group; thus, a one-way between-subjects ANOVA revealed a significant effect of lesion group, $F_{(3, 40)}=5.37$, $p=0.003$, and post hoc analysis revealed that the performance of the HPC ($p=0.017$), PRH ($p=0.007$) and mPFC ($p=0.045$) groups was significantly worse than the performance of the sham group. There were no significant differences between any of the lesion groups. Further analyses confirmed that the SHAM group showed significant discrimination between objects, that is, spent a greater amount of time exploring the object in the novel location context compared to the object in the familiar location context, $t_{(11)}=3.65$, $p=0.004$. In contrast, the HPC, $t_{(9)}=-1.04$, $p=0.327$; PRH, $t_{(9)}=-0.98$, $p=0.351$; and mPFC, $t_{(11)}=-0.18$, $p=0.857$, lesion groups failed to show such discrimination.

Disconnection lesion group. Disconnection of the HPC from either the mPFC or PRH significantly impaired performance in the object-place-context task (see Figure 2(b)). One-way between-subjects ANOVA revealed a significant main effect of lesion group, $F_{(3, 35)}=17.60$, $p=0.0001$, and post hoc analysis revealed that the performance of the HPC + mPFC CONTRA group was significantly worse than that of the HPC + mPFC IPSI

Table 4. Object exploration levels in each of the sample phases and test phase of the object-place-context and object-context task in the bilateral lesion group and disconnection lesion group.

Task		Object-place-context			Object-context		
Group	Condition	Exploration in sample phase 1 (s)	Exploration in sample phase 2 (s)	Exploration in test phase (s)	Exploration in sample phase 1 (s)	Exploration in sample phase 2 (s)	Exploration in test phase (s)
Bilateral lesion	Sham	29.4 ± 2.6	26.7 ± 2.7	18.7 ± 2.3	30.2 ± 2.5	27.7 ± 2.7	19.4 ± 1.9
	HPC	43.3 ± 5.2	31.9 ± 4.0	22.6 ± 2.4	45.7 ± 4.6	48.5 ± 7.3	24.4 ± 1.7
	PRH	26.9 ± 2.5	23.8 ± 1.8	18.3 ± 1.9	35.2 ± 3.2	33.8 ± 3.7	25.2 ± 3.0
	mPFC	37.8 ± 4.3	26.2 ± 3.3	19.2 ± 1.9	40.3 ± 4.5	33.6 ± 4.3	27.3 ± 3.0
Disconnection lesion	HPC + mPFC Ipsi	38.7 ± 4.4	33.5 ± 3.4	32.8 ± 3.9	33.7 ± 2.5	36.8 ± 3.7	23.4 ± 2.4
	HPC + mPFC Contra	55.2 ± 8.0	44.0 ± 6.1	36.5 ± 4.3	51.4 ± 5.8	41.9 ± 5.8	25.9 ± 2.2
	HPC + PRH Ipsi	44.4 ± 6.9	35.9 ± 4.8	32.2 ± 2.9	40.2 ± 5.0	36.9 ± 5.4	19.3 ± 2.2
	HPC + PRH Contra	41.8 ± 4.2	39.1 ± 5.4	26.5 ± 3.9	39.5 ± 3.1	32.3 ± 3.3	22.9 ± 2.1

HPC: hippocampus; PRH: perirhinal cortex; mPFC: medial prefrontal cortex; SEM: standard error of the mean.
SHAM, $n = 12$; HPC lesion, $n = 10$; PRH lesion, $n = 10$; mPFC lesion, $n = 12$; HPC + mPFC Ipsi, $n = 10$; HPC + mPFC Contra, $n = 10$; HPC + PRH Ipsi, $n = 10$; HPC + PRH Contra, $n = 9$. Data presented as mean ± SEM.

group ($p = 0.0002$) and that the performance of the HPC + PRH CONTRA group was significantly worse than the HPC + PRH IPSI group ($p = 0.0001$). Additional analysis revealed that the HPC + mPFC IPSI, $t(9) = 5.52$, $p = 0.0004$, and HPC + PRH IPSI, $t(9) = 6.42$, $p = 0.0002$, groups showed significant discrimination between the object in the novel context-location and the object in the familiar context-location object, while the HPC + mPFC CONTRA, $t(9) = 1.33$, $p = 0.215$, and HPC + PRH CONTRA, $t(9) = 1.00$, $p = 0.343$, groups failed to discriminate between the objects.

Exploration in sample and test phases. Table 4 shows the mean levels of exploration completed in sample phases 1, 2 and the test phase. A two-way ANOVA with sample phase and lesion as factors found no significant interaction between sample phase and lesion in either lesion group (bilateral lesion group, $F(3, 40) = 2.81$, $p = 0.051$; disconnection lesion group, $F(3, 35) = 0.51$, $p = 0.676$) or main effect of lesion (bilateral lesion group, $F(3, 40) = 2.79$, $p = 0.053$; disconnection lesion group, $F(3, 35) = 1.32$, $p = 0.284$). However, there was a significant main effect of sample phase in both groups (bilateral lesion group, $F(1, 40) = 23.55$, $p = 0.0001$; disconnection lesion group, $F(1, 35) = 6.91$, $p = 0.013$) which reflected a greater level of exploration in sample phase 1 across all experimental conditions. Analysis of the amount of exploration completed in the test phase revealed no significant differences in either lesion group (bilateral lesion group, $F(3, 40) = 0.82$, $p = 0.492$; disconnection lesion group, $F(3, 35) = 1.11$, $p = 0.358$).

Object-context

Recognition during test phase

Bilateral lesion group. Performance in the object-context task was significantly impaired following bilateral lesions in the HPC, PRH or mPFC (see Figure 3(a)). One-way between-subjects ANOVA revealed a significant main effect of lesion group, $F(3, 40) = 12.81$, $p = 0.0001$, and post hoc analysis revealed

that the performance of animals in the HPC, PRH and mPFC groups was significantly worse than the performance of animals in the SHAM group (HPC, $p = 0.0002$; PRH, $p = 0.001$; mPFC, $p = 0.0001$). There were no significant differences in performance between any of the lesion groups. Additional analysis confirmed that the SHAM group showed significant discrimination between the objects out-of-context and in-context, $t(11) = 6.21$, $p = 0.0001$, while the HPC, $t(9) = 0.82$, $p = 0.432$; PRH, $t(9) = 0.11$, $p = 0.916$; and mPFC, $t(11) = 1.831$, $p = 0.094$, lesion groups failed to discriminate between the objects.

Disconnection lesion group. Disconnection of the HPC from either the PRH or mPFC significantly impaired performance in the object-context task (see Figure 3(b)); thus, a one-way between-subjects ANOVA revealed a significant main effect of lesion, $F(3, 35) = 26.79$, $p = 0.0001$. Post hoc analysis revealed that the performance of the HPC + mPFC CONTRA group was significantly worse than the performance of the HPC + mPFC IPSI group ($p = 0.0001$) and that the performance of the HPC + PRH CONTRA group was significantly worse than the HPC + PRH IPSI group ($p = 0.0001$). Additional analysis revealed that the HPC + mPFC IPSI, $t(9) = 9.23$, $p = 0.0001$, and HPC + PRH IPSI, $t(9) = 7.12$, $p = 0.0001$, groups showed significant discrimination between the object out-of-context and in-context, and the HPC + mPFC CONTRA, $t(9) = -1.05$, $p = 0.319$, and HPC + PRH CONTRA, $t(9) = -0.53$, $p = 0.609$, groups failed to discriminate between the objects.

Exploration in sample and test phases. Table 4 shows the mean levels of exploration completed in sample phases 1, 2 and during the test phase for all groups. A two-way ANOVA with sample phase and lesion as factors found no significant interaction between sample phase and lesion in either lesion group (bilateral lesion group, $F(3, 40) = 0.92$, $p = 0.441$; disconnection lesion group, $F(3, 35) = 2.24$, $p = 0.101$). In the bilateral lesion group, there was no significant main effect of sample phase, $F(1, 40) = 0.91$, $p = 0.345$; however, there was a significant main effect of lesion, $F(3, 40) = 4.11$, $p = 0.012$. Post hoc analysis revealed
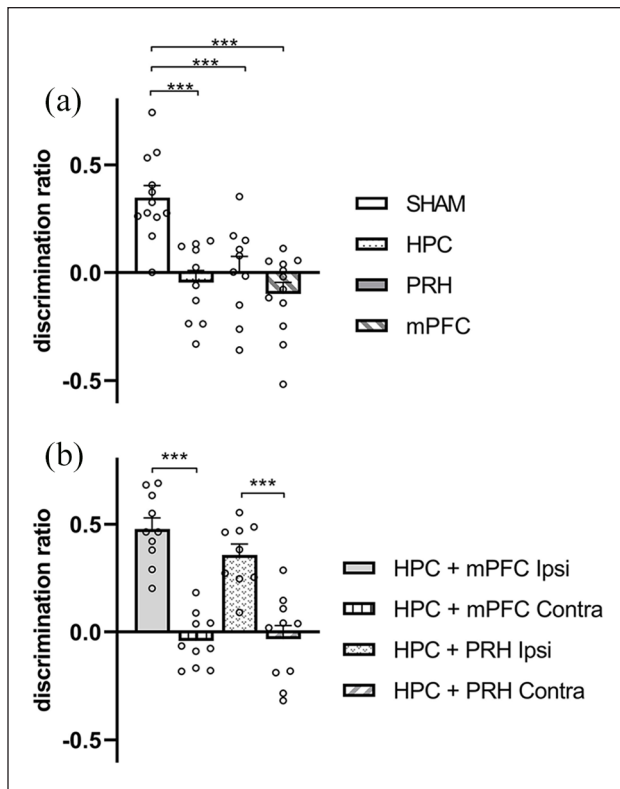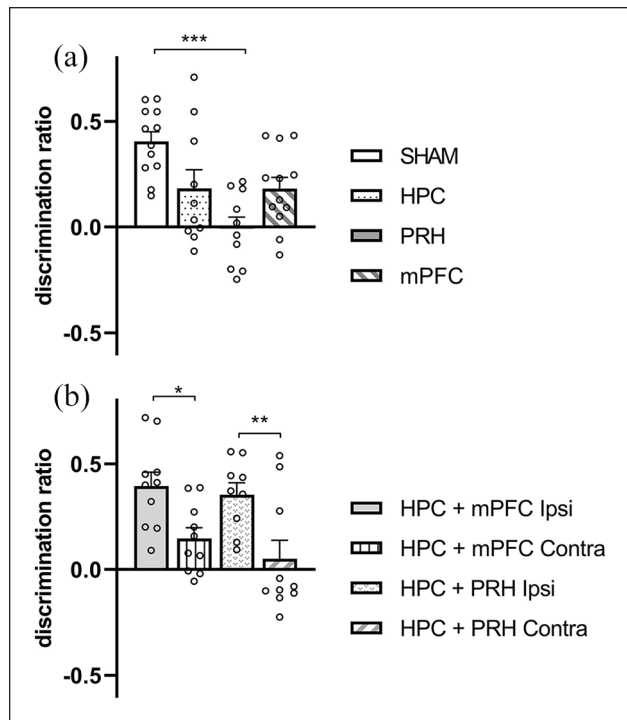
Figure 3. Performance of the experimental groups in the object-context task. (a) Performance of the sham lesioned animals (SHAM), bilateral hippocampal (HPC), perirhinal (PRH) or medial prefrontal cortex (mPFC) lesion groups. (b) Performance of animals with combined lesions in the hippocampus and medial prefrontal cortex in the same hemisphere (HPC + mPFC Ipsi) or opposite hemisphere (HPC + mPFC Contra) and animals with combined lesions in the hippocampus and perirhinal cortex in the same hemisphere (HPC + PRH Ipsi) or opposite hemisphere (HPC + PRH Contra). Data presented for each group are mean + SEM (bar) and individual animals' performance (open circles). ***p < 0.001.

Figure 4. Performance of the experimental groups in the object recognition in different contexts task. (a) Performance of the sham lesioned animals (SHAM), bilateral hippocampal (HPC), perirhinal (PRH), or medial prefrontal cortex (mPFC) lesion groups. (b) Performance of animals with combined lesions in the hippocampus and medial prefrontal cortex in the same hemisphere (HPC + mPFC Ipsi) or opposite hemisphere (HPC + mPFC Contra), and animals with combined lesions in the hippocampus and perirhinal cortex in the same hemisphere (HPC + PRH Ipsi) or opposite hemisphere (HPC + PRH Contra). Data presented for each group are mean + SEM (bar) and each individual animals' performance (open circles).
*p < 0.05, **p < 0.01, ***p < 0.001.

that the HPC group spent significantly more time exploring the objects in the sample phases than the SHAM group ($p = 0.008$). There were no other significant differences. Examination of exploration across the disconnection lesion groups revealed a significant main effect of sample phase, $F_{(1, 35)} = 5.15$, $p = 0.030$, which reflected a greater amount of exploration completed in sample phase 1 by all groups; however, there was no significant main effect of lesion, $F_{(3, 35)} = 1.73$, $p = 0.179$. Analysis of the amount of exploration completed in the test phase revealed no significant differences (bilateral lesion group, $F_{(3, 40)} = 2.13$, $p = 0.111$; disconnection lesion group, $F_{(3, 35)} = 1.50$, $p = 0.232$).

Object recognition in different context

Recognition during test phase

Bilateral lesion group. Performance in the object recognition in different contexts task was significantly impaired following lesion in the PRH. While performance in the HPC and mPFC lesion groups was reduced, it was not significantly

different to the SHAM group (see Figure 4(a)). One-way between-subjects ANOVA revealed a significant effect of lesion group, $F_{(3, 40)} = 7.68$, $p = 0.0004$, and post hoc analysis revealed that the performance of the PRH group was significantly worse than the performance of the SHAM group ($p = 0.0002$), but performance of the HPC and mPFC groups was not significantly different from either the SHAM (HPC, $p = 0.085$; mPFC, $p = 0.059$) or PRH (HPC, $p = 0.250$; mPFC, $p = 0.211$) group. Additional analysis revealed that both the SHAM, $t_{(11)} = 8.82$, $p = 0.0001$, and mPFC, $t_{(11)} = 3.36$, $p = 0.006$, groups showed significant discrimination between the novel and familiar objects, but the HPC, $t_{(9)} = 1.10$, $p = 0.300$, and PRH, $t_{(9)} = 0.14$, $p = 0.890$, groups did not.

Disconnection lesion group. Performance in the object recognition task in different context was significantly impaired following disconnection of the HPC from either the mPFC or PRH (see Figure 4(b)). Thus, a one-way between-subjects ANOVA revealed a significant effect of lesion, $F_{(3, 35)} = 6.09$, $p = 0.002$, and post hoc analyses revealed that the performance of the HPC + mPFC Contra group was significantly worse than performance in the HPC-mPFC Ipsi group ($p = 0.012$) and that performance in the HPC + PRH Contra group was significantly worse

Table 5. Object exploration levels in the sample and test phases of the object recognition in different contexts task in the bilateral lesion group and disconnection lesion group.

Group	Condition	Time taken to complete sample phase (s)	Exploration in sample phase (s)	Exploration in test phase (s)
Bilateral lesion	Sham	231 ± 9.0	24.5 ± 2.1	19.8 ± 2.0
	HPC	207 ± 13.3	34.3 ± 2.7	23.6 ± 1.8
	PRH	228 ± 11.2	24.8 ± 2.6	23.9 ± 1.7
	mPFC	234 ± 18.1	26.3 ± 2.5	15.7 ± 1.7
Disconnection lesion	HPC + mPFC Ipsi	199 ± 15.6	33.7 ± 2.4	25.6 ± 3.5
	HPC + mPFC Contra	170 ± 21.3	37.5 ± 1.4	37.8 ± 4.3
	HPC + PRH Ipsi	189 ± 23.7	34.8 ± 3.9	26.6 ± 6.3
	HPC + PRH Contra	197 ± 12.7	37.6 ± 1.3	27.3 ± 2.1

HPC: hippocampus; PRH: perirhinal cortex; mPFC: medial prefrontal cortex; SEM: standard error of the mean.
Data presented as mean ± SEM.

than the performance of the HPC + PRH Ipsi group ($p = 0.003$). There was no significant difference in performance between the HPC + mPFC IPSI and HPC + mPFC CONTRA groups ($p = 0.07$). Additional analysis confirmed that the HPC + mPFC IPSI, $t(9) = 6.06$, $p = 0.0002$; HPC + mPFC Contra, $t(9) = 2.85$, $p = 0.019$; and HPC + PRH IPSI, $t(8) = 6.29$, $p = 0.0002$, groups showed significant discrimination between the novel and familiar object, and the HPC + PRH Contra group, $t(9) = 0.58$, $p = 0.574$, failed to discriminate.

Exploration in sample and test phases. Table 5 shows the mean levels of exploration in the sample and test phases. Analysis of the exploration in the sample phase in the bilateral lesion group revealed no significant effect of lesion on the amount of time taken to complete the sample phase, $F(3, 40) = 1.34$, $p = 0.275$; however, there was a significant effect of lesion on the amount of exploration completed in the sample phase, $F(3, 40) = 3.43$, $p = 0.026$. Post hoc analyses revealed that the HPC group completed significantly more exploration in the sample phase than the SHAM group ($p = 0.04$); there were no significant differences between any of the other lesion groups. Analysis of the exploration in the sample phase in the disconnection lesion group revealed no significant effect of lesion on either the time taken to complete the sample phase, $F(3, 35) = 0.55$, $p = 0.649$, or the amount of exploration completed in the sample phase, $F(3, 35) = 1.05$, $p = 0.384$.

Analysis of the total amount of exploration completed in the test phase in the bilateral lesion group revealed a significant effect of lesion, $F(3, 40) = 4.43$, $p = 0.009$. Post hoc analysis revealed that the HPC and PRH groups completed significantly more exploration in the test phase than the mPFC group (HPC, $p = 0.026$; mPFC, $p = 0.019$); there were no significant differences between any of the other groups. Analysis of the amount of exploration completed in the test phase in the disconnection lesion group found no significant effect of lesion, $F(3, 35) = 2.08$, $p = 0.121$.

Discussion

The present study had two aims: first to assess the contribution of the HPC, mPFC and PRH to the formation of associations between objects and the contexts in which they were encountered and second to examine whether the HPC interacts with the mPFC and PRH in order to form object-context associations.

Bilateral ablation of either the HPC, mPFC or PRH significantly impaired both object-place-context and object-context memory. Performance in the object recognition task with context switch was significantly impaired in animals with PRH lesions, but while memory performance in animals with HPC was disrupted (i.e. the mean DR was not significantly different from zero), the performance of this group was not significantly different to either the SHAM or PRH lesion group. In the second series of experiments, disconnection of the HPC from either the mPFC or PRH significantly impaired performance in the object-place-context, object-context and the object recognition task in different contexts task. Neither the bilateral lesions nor the unilateral disconnection lesions significantly altered overall object exploration behaviour; animals with HPC lesions tended to show greater levels of exploration, and increased activity following HPC lesion has been observed previously (Douglas and Isaacson, 1964; Maren et al., 1997). Overall changes in object exploration are unlikely to explain the observed deficits in performance.

That the HPC, mPFC and PRH are necessary for object-place context and object-context association tasks accords in part with previous work. It has previously been proposed that HPC is only critical for object-place-context associations (Langston et al., 2010; Langston and Wood, 2010), while the PRH is only critical for object-context associations (Eacott and Norman, 2004; Norman and Eacott, 2005). One possible reason for the divergence in findings is that the current study used a longer delay between sample and test than the previous studies (1 h compared to 2–5 min). Therefore, the deficits observed may be because an HPC- or PRH-independent memory formed is not sufficient to support memory performance at longer delays. Lesions of the PRH increase instability of CA1 place fields over time (Muir and Bilkey, 2001), demonstrating that lesions in one brain region can affect the stability of neural representations in another brain region. Another difference between the present study and the previous studies may be the nature of the context used. In this study, the floor of the arena used in contexts A and B was identical, while in previous studies the floor was changed. Thus, in previous studies, animals with HPC lesions may have formed an association between the object and local contextual cues, such as the floor in which the object is directly placed. When the floor material is not a predictor of context, as in the present study, object-context associations could involve distal cues, that is, the colour and pattern of the walls, a process more likely to engage the HPC

(Nadel and Wilner, 1980). In support of this suggestion, lesions of the HPC have been shown to spare performance in tasks where proximal cues are critical but significantly impair performance when distal cues are critical for task performance (Albasser et al., 2013; Hudson et al., 2003; Save and Poucet, 2000). It is less clear what effect the difference in context would have had on the PRH lesioned animals as it is not clear if PRH is preferentially influenced by proximal or distal cues (Burwell et al., 1998).

The present results confirmed the importance of the mPFC for object-context associations consistent with a previous study in mice showing that lesions focused on the anterior cingulate region of the mPFC cortex impaired object-context memory (Spanswick and Dyck, 2012). Given that lesions in the infralimbic/prelimbic as in the present study, and the anterior cingulate impaired performance, multiple prefrontal regions are clearly critical for the formation of object-context associations.

Here, results from the disconnection analyses demonstrate that the formation of an association between an object and its context depends on a functional interaction between the HPC and the mPFC and PRH. In addition, this functional interaction is not dependent upon a distinct spatial cue as both object-context and object-place-context were significantly impaired. Previous studies have shown that HPC-mPFC and HPC-PRH interactions are critical for object-place associations (Barker and Warburton, 2011), and the addition of object-context associations to the role of the circuit suggests that the network is critical for successfully binding together an object with a wide range of spatiocontextual cues. These results also build on previous work in the monkey showing the importance of frontal-inferotemporal cortex–HPC interactions for object-place-context associations (Browning et al., 2007; Wilson et al., 2007, 2008) and suggest a degree of conserved circuitry between the rat and monkey.

While this study has highlighted the importance of the interactions between the HPC, mPFC and PRH in the formation of object-context associations, they are not the only brain regions which are part of this neural network. Interactions between the PRH and postrhinal cortex have been shown to be critical (Heimer-McGinn et al., 2017); it is therefore reasonable to hypothesise that the postrhinal cortex may also play a key role through interaction with the HPC and mPFC during object-context associations, although this has not been explored. There is also compelling evidence that the lateral entorhinal cortex is critical to the formation of both object-place-context and object-context associations (Wilson et al., 2013a, 2013b) and functionally interacts with the mPFC (Chao et al. 2016). Based on this evidence, it is likely that the lateral entorhinal cortex is also part of the wider temporal lobe-frontal cortex network critical for associating objects with contexts.

While a hippocampal-medial prefrontal-PRH network is critical for multiple types of object associations, the way the network functions to form these associations may not be the same. The disconnection lesion approach used here cannot determine information flow between regions of interest and cannot reveal the importance of specific regional connections. Given there are both direct and indirect anatomical pathways between all three of these brain regions (Burwell et al., 1995; Delatour and Witter, 2002; Jay and Witter, 1991), to understand how the network functions more precise network manipulations will be critical. For example, selective deactivation of direct projections from layer II of lateral entorhinal cortex to the DG impaired object-place-context but not object-context associative memory (Vandrey et al., 2020). Therefore, the precise anatomical connections between the brain regions which are critical for the formation of contextual associations may be different.

When a context switch was introduced between the sample and test phase of a standard novel object recognition task, animals in which the HPC was disconnected from either the mPFC or HPC were impaired. Similarly, animals with bilateral HPC or mPFC lesions showed lower levels of discrimination between the novel and familiar stimulus. This result was somewhat surprising as item recognition does not normally depend on the HPC but rather on the PRH (Barker and Warburton, 2011). On the face of it, context information in an object recognition task is irrelevant to task performance; indeed, the SHAM animals showed significant discrimination between the novel and familiar stimulus. That a lesion in the HPC and HPC-mPFC or HPC-PRH disconnection altered performance suggests that processing of both object and contextual information may actually be important. Indeed, some theories of HPC function have emphasised its role in the flexible use of memory information (Eichenbaum, 2000; Eichenbaum et al., 1999) as it has been argued that animals in which hippocampal function is compromised may combine cues into a single representation while normal animals treat individual cues as distinct items (Eichenbaum et al., 1989). Thus, during the sample phase of an object recognition task, SHAM control animals form a representation of the object, and the association between the object and context, and when presented with the sample object in a different context they can separate the representation of the object from the context. In contrast, animals with HPC damage encode a single object + context representation; thus, when the object is presented in a different context, it is regarded as a novel representation, and discrimination is impaired, that is, the representation of the object was tied to the context, it was originally encountered in.

What role might the mPFC play in the context switch task? Deactivation of the mPFC has been shown to impair context-dependent neuronal firing in the HPC (Navawongse and Eichenbaum, 2013), and it has been suggested that the mPFC plays a role in top-down control of context-appropriate memory representations in the HPC via projections to the PRH and lateral entorhinal cortex (Eichenbaum, 2017). Animals with HPC-mPFC disconnection may therefore struggle to discriminate between the novel and familiar object when the context is changed as they were unable to appropriately disentangle the object and context representations formed during memory encoding. This pattern of deficits clearly demonstrates the importance of the network in the formation of distinct representations of object and association between them.

In summary, this study demonstrated the importance of a hippocampal-medial prefrontal-PRH network in forming associations between objects and the contexts they are encountered in. Further research to identify the other key elements of this network and the precise anatomical networks which support distinct forms of object-context association will be essential to fully understand the functioning of this network.

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