Conditioning with spatio-temporal patterns:

Constraining the contribution of the hippocampus to configural learning

Natasha M. Dumigan, Tzu-Ching E. Lin, Mark A. Good and *Robert C. Honey

School of Psychology, Cardiff University, Park Place, Cardiff, CF10 3AT, UK

Revision submitted in May 2017 to:

Neurobiology of Learning and Memory

Keywords: rat, limbic system, context, time

*Corresponding author.

e-mail address: honey@cardiff.ac.uk (R.C. Honey).

ABSTRACT

The conditions under which the hippocampus contributes to learning about spatio-temporal configural patterns are not fully established. The aim of Experiments 1-4 was to investigate the impact of hippocampal lesions on learning about where or when a reinforcer would be delivered. In each experiment, the rats received exposure to an identical set of patterns (i.e., spotted+morning, checked+morning, spotted+afternoon and checked+afternoon); and the contexts (Experiment 1), times of day (Experiment 2), or their configuration (Experiments 3 and 4) signalled whether or not a reinforcer would be delivered. The fact that hippocampal damage did not disrupt the formation of simple or configural associations involving spatio-temporal patterns is surprising, and suggests that the contribution of the hippocampus is restricted to mediated learning (or updating) involving spatio-temporal configurations.

1. Introduction

The hippocampus plays a central role in episodic memory in humans (e.g., Tulving, 2002; Tulving & Markowitsch, 1998). The suggestion that this role is at least partly conserved in nonhuman animals receives support from a variety of sources (e.g., Aggleton & Brown, 1999; Eacott & Norman, 2004). For example, Iordanova, Good and Honey (2008) showed that rats could learn that the whereabouts of two auditory stimuli (a tone and a series of clicks) depended on the combination of the times of day and the contexts in which they were placed. Thus, in the morning a tone was presented in one context (A; a chamber decorated with spotted wallpaper) and clicks were presented in another context (B; a chamber decorated with checked wallpaper); whereas in the afternoon the tone was presented in context B and the clicks in A. The fact that the rats had encoded the four configurations was revealed by pairing the tone with mild shock at midday in a third context (C; an undecorated chamber), and then showing that the rats were more fearful in the context+time of day configurations in which the tone had originally been presented (i.e., context A in the morning and context B in the afternoon). This effect was abolished in rats that had pretraining excitotoxic lesions of the hippocampus, and also by inactivation of the hippocampus during the test (Iordanova, Burnett, Aggleton, Good & Honey, 2009; see also, Iordanova, Burnett, Good, & Honey, 2011a; see also, Ergorul & Eichenbaum, 2004; Li & Chao, 2008). There was no effect of the same manipulations of hippocampal function in simple discriminations where the context (A or B) predicted the nature of the auditory stimulus (irrespective of the time of day), or the time of day indicated which auditory stimulus would be present (irrespective of the context in which rats were placed; for a review, see Honey, Iordanova & Good, 2014).

There are alternative theoretical bases for predicting that the hippocampus will play a selective role in learning what will happen where and when. For example, the procedures in which there were deficits were configural in nature: the combination of contexts and times of day was critical to learning where the auditory stimuli would be presented (Rudy & Sutherland, 1989; Sutherland & Rudy, 1989, 1995; O'Reilly & Rudy, 2001; see also, Alvarado & Rudy, 1995). Also, the nature of the stimuli that need to be combined had clear episodic content (which auditory stimulus was presented where and when; see Aggleton & Pearce, 2001; see also, Thorpe, Bates, & Wilkie, 2003). However, these analyses apply somewhat less readily to a final observation using this procedure: Disruption of synaptic transmission and NMDA receptor-dependent plasticity processes in the hippocampus during the stage in which the tone was paired with shock (in context C at midday) also abolished the critical effect during the final test; and this manipulation did not affect simple conditioning to the auditory stimuli (Iordanova, Good & Honey, 2011b). This observation suggests that the hippocampus might be involved in mediated learning (Hall, 1996; Holland, 1981) involving configurations rather than in configural learning *per se*. According to this analysis, the presentation of the tone during aversive conditioning trials evoked the stored configurations involving the tone (i.e., spotted+morning+tone and checked+afternoon+tone) and these evoked memories become linked to the memory of shock. This analysis is consistent with recent investigations of the conditions under which the hippocampus is involved in sensory preconditioning effects involving audio-visual stimuli (Lin, Dumigan, Good, & Honey, 2016; for a review, see Lin & Honey, 2016; see also Wimmer & Shohamy, 2012).

The alternative analyses of the results presented by Iordanova et al. (2009, 2011a, 2011b) make contrasting predictions about the impact of hippocampal lesions on direct learning about spatio-temporal patterns, in which configurations or their components predict whether a reinforcer will be delivered. If the hippocampus is involved in configural learning or specifically configural learning with spatio-temporal stimuli, then lesions to this structure should result in the same pattern of dissociations that was observed by Iordanova *et al*. (2009, 2011ab). However, if the hippocampus is involved in retrieval-mediated learning involving configural memories (Lin & Honey, 2016; Lin, Dumigan, Good, & Honey, 2016; see also, Lin, Dumigan, Recio, & Honey, 2016; Schlichting & Preston, 2016; Wimmer & Shohamy, 2012) then direct learning involving the same configurations might not be disrupted by hippocampal lesions. The experimental designs employed to assess these predictions are presented in Figure 1. In Experiments 1 and 2, the context in which animals were placed (spotted or checked) or time of day in which they were placed there (morning or afternoon) predicted the delivery of the reinforcer (food); whereas in Experiments 3 and 4, the combination of context and time of day predicted whether or not a reinforcer (food in Experiment 3 and shock in Experiment 4) would be delivered.

_____

Figure 1 about here

_____

2. Materials and Methods

2.1. Animals

Naïve Lister hooded rats were used (*Rattus norvegicus*) in all experiments. Sixteen rats were used in Experiment 1 (supplied by Harlan Olac Ltd, UK; mean weight = 306g) and

Experiment 2 (supplied by Harlan Olac Ltd, UK; mean weight = 317g). Twenty-seven rats were used in Experiment 3a (supplied by Charles River, UK; mean weight = 454g); and 32 rats were used in both Experiment 3b (supplied by Harlan Olac, UK; mean weight = 321g) and Experiment 4 (supplied by Harlan Olac Ltd, UK; mean weight = 370g). All rats were maintained at 85% of their *ad libitum* weights by giving them a restricted amount of food at the end of the day (≈ 1830 hrs). The rats were housed in pairs in a colony room that was illuminated between the hours of 8 a.m. and 8 p.m; and behavioral training began at, approximately, 09:30 each day.

2.2. Surgery

There were two groups of rats in each of the experiments: Sham and Hpc. In Experiments 1 and 2, there were 8 rats in both groups. In Experiment 3a, there were 13 rats in group Sham and 14 rats in group Hpc; and in Experiments 3b and 4 there were 16 rats in each group. All rats were first anaesthetised using an isofluorane-oxygen mix and then placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA). The bone of the skull above the region to be lesioned was removed, and rats in group Hpc (for Experiments 1-4) were infused with ibotenic acid (Biosearch Technologies, San Rafael, CA; dissolved in phosophate-buffered saline [pH 7.4] to provide a solution with a concentration of 63 mM) through a 2-µl Hamilton syringe held with a microinjector (Kopf Instruments, Model 5000). Table 1 shows the coordinates where the tip of syringe was positioned relative to bregma and associated volumes that were injected. A total of 15 infusions per hemisphere were made with an infusion rate of 0.05 µl/min and diffusion time of 2 min. After each injection, the needle was left in position for 2 min to allow diffusion of the ibotenic acid and to limit the spread of the drug into overlying cortical areas. Rats in group Sham received the same

surgical preparation with the exception that the dura was perforated with a 25-gauge Microlance3 needle (Becton Dickinson, Drogheda, Ireland), but no fluid was infused into the brain. During recovery, the rats were handled and weighed daily and food restriction and behavioural testing did not commence for a minimum of 14 days post-surgery when rats had established their preoperative weights.

_____

Table 1 about here

_____

2.3. Apparatus

Experiments 1-3 were conducted in the apparatus described in Dumigan, Lin, Good and Honey (Experiment 1; 2015). Briefly the chambers (23.0cm × 24.5cm × 21.0cm, L × W × H; supplied by Camden Instruments Ltd., UK) were arranged in a 2 × 2 array, and were constructed from three aluminium walls, an aluminium ceiling, and a plastic wall that served as the door to the chamber. The ceilings and walls of the top pair of boxes in the array were decorated with spotted wallpaper (black circles on a white background; see Honey & Watt, 1999), whereas the walls and ceiling of the lower two chambers were decorated with black and white checked wallpaper. Food pellets (45mg; supplied by P. J. Noyes, Lancaster, NH) could be delivered into a food access to which was guarded by a hinged plastic flap. Food-well entries were automatically recorded when the plastic flap was pushed approximately 3 mm. A series of stainless steel rods, 0.50 cm in diameter and 1.5 cm apart (centre-to-centre), served as the chamber floor. The chamber was locally illuminated by a single 15-V, 24-W jewel light positioned in the centre of the ceiling, and

received ambient illumination through the open doors of the sound-attenuating shells in which the chambers were placed.

Experiment 4 used a set of eight operant chambers also described in detail in Dumigan *et al*. (Experiment 2; 2015). Briefly, the chambers (30.5 cm × 26 cm ×2 0 cm; Test chamber 80004-D001; supplied by Campden Instruments Ltd., Loughborough, England) were arranged in a 4 × 2 array and positioned in sound-attenuating boxes. Each chamber had two aluminium sidewalls, a transparent Perspex back wall and a transparent Perspex ceiling, and the front walls were also constructed from transparent Perspex and served as the doors to the chamber. As in Experiment 1-3, the walls of the upper row of boxes were decorated with spotted wallpaper and those of the lower row were decorated with checked wallpaper. The chambers were illuminated by a 3-W light bulb positioned centrally and at 13.5 cm above the floor of the left aluminium wall. There was a stainless steel grid floor, constructed from 19 bars (diameter 0.47 cm, spacing from bar centre to bar centre 1.07 cm) to which a 0.5-s. 0.64 mA electric shock could be delivered using a scrambled shocker (Campden Instruments Ltd. Model HSCK1000). The level of rat activity in the chambers was measured using an ambulatory monitor (Campden Instruments Ltd. Model 80004 AM), which consisted of a horizontal strip attached to the back wall of the chamber and another to the front wall. These strips, positioned 3.0 cm above the grid floor, contained three infrared light sources and photo beam detectors that were located 3.0 cm from the left hand wall, in the centre of the chamber, and 3.0 cm from the right hand wall. Detection of the presence of the rat in the area covered by a photobeam followed by detection of the absence of the rat in this area, as the rat moved, was recorded as a value of 1. The number of times this occurred for each of the three beams

gave a single value for the total movement made by the rat in the chamber. A computer (Mark II Control Unit) controlled the apparatus, operated the programs (using Behavioural Net Controller Control 1.0), and recorded ambulatory movement data (all equipment was supplied by Campden Instruments Ltd.).

2.4. Behavioral procedure

Appetitive conditioning. On the first day of Experiments 1-3, rats were trained to retrieve food pellets from a food well in an undecorated chamber during two 10-min sessions. These sessions were conducted between 13:30 and 15:30. In the first session, the flaps in front of the food wells were fixed in a raised position to allow rats ready access to the 20 food pellets delivered on a VT 60s schedule; and in the second session these flaps were lowered and rats had to move them to gain access to 20 food pellets delivered according to the same schedule. On each of the following days of training, rats were placed in the two contexts (spotted and checked) in the morning and the same two visual contexts in the same sequence in the afternoon (spotted and then checked for half of the rats, and checked and then spotted for the remainder). For a given training day, the order in which the contexts were presented for a given rat was consistent, but across days the order was pseudo-random with the constraint that the same order could only be used on two consecutive days. Morning sessions took place between 09:30 and 11:30, and afternoon sessions took place between 4:30 and 6:30. Context placements lasted 5 min, and there was a 5 min interval between the pairs of sessions in the morning and in the afternoon. Rats were placed in a holding cage outside the testing room in these intervals.

In Experiment 1, the context in which rats were placed was relevant to the discrimination, but the time of day was not. Thus, in the morning two food pellets were

delivered to the food well every 30s (resulting in a total of 20 pellets delivered per session) when rats were placed in one of the contexts (e.g., spotted), but no food was delivered when they were placed in the other context (e.g., checked). This procedure was repeated in the afternoon. In Experiment 2, the time of day was relevant to the discrimination, but the context was not. Thus, food pellets were delivered to the food well when the rats were placed in both contexts at one time of day (e.g., morning) and in neither context at the other time of day (e.g., afternoon). The context that was reinforced in Experiment 1 and time of day that was reinforced in Experiment 2 was fully counterbalanced in groups Sham and Hpc. In Experiment 3, the combination of the context and time of day was relevant to the discrimination. In the morning sessions, food pellets were delivered in one of the contexts (e.g., spotted) and not in the other (e.g., checked), but in the afternoon sessions the contexts in which food and no food were delivered was reversed.

For all rats in both experiments, after the end of each pair of sessions the rats were returned to the colony room and replaced in their holding cages. Training continued for 20 days in Experiments 1 and 2, and for 24 days in Experiment 3. The rate of food well entries in the 30-s reinforcer-free period at the start of reinforced and nonreinforced sessions was used to assess discrimination learning. Food well entries were not recorded from the first two days in every cycle of four training days because on these days two additional food pellets were present in the food well at the start of each reinforced session. This arrangement was intended to reduce the likelihood that rats would learn that no food pellets were presented in the first 30s of any trial. A discrimination ratio (DR) was calculated from these response rates by dividing the rate of entries during reinforced sessions by the combined rate of entries during reinforced and non-reinforced sessions.

Using this ratio, scores above 0.50 indicate that rats were more likely to have entered the food well on reinforced trials than on nonreinforced trials. For Experiments 1 and 3, one DR was calculated for the morning and one for the afternoon, and for Experiment 2, one DR was calculated for the spotted context and one for the checked context. In all three experiments, a mean of the two DRs for each day was calculated to provide a single DR for each rat.

Aversive conditioning. The procedure is described in detail in Dumigan *et al*. (Experiment 2, 2015). On the first two days, each rat was placed in an undecorated operant chamber for approximately 20 min with the lights illuminated for 20-min. These sessions, conducted between 12 p.m. and 2 p.m., were followed by 24 days of training. On each day of training, rats were placed in the two contexts (spotted and checked) in the morning and afternoon in the same sequence (spotted and then checked for half of the rats, and checked and then spotted for the remainder). As in Experiment 3, across days the sequence in which rats were placed in the two contexts varied according to a pseudo-randomised sequence. Each exposure to a context was 3 min and there was an interval of 5 min between the exposures to the spotted and checked contexts at a given time of day. In the morning, in one context (e.g., spotted) two mild electric shocks were delivered through the grid floor of the chamber, one after the first minute and another after the second minute of the session. No shocks were delivered in the other visual context (e.g., checked). In the afternoon this arrangement was reversed (e.g., shocks were delivered in the checked but not in the spotted context). Unlike in Experiment 2, there were no sessions on which the reinforcer (in this case shock) was presented immediately after the rats entered the chamber, because such presentations disrupt fear conditioning (Fanselow,

1986). Details of the procedure that have not been mentioned were the same as for Experiment 3. Acquisition of the discrimination was assessed using a discrimination ratio: activity during first 30s of the session *without* shocks (e.g., checked+morning) divided by the combined activity during the first 30s of both sessions at that time of day (e.g., spotted+morning, and checked+morning). Using this measure, scores above 0.50 indicate rats are more active in sessions where no shocks will be delivered than in those where shocks will be delivered. In addition, we assessed the impact of the shocks themselves by measuring activity in the 30-s period that immediately followed shock presentation, and during the same time periods in the corresponding sessions where no shocks were delivered. This assessment allowed the impact of shock to be assessed in the two groups (see Fanselow, 1982).

2.5. Histological analysis.

After the completion of behavioral testing, rats received a lethal overdose of sodium pentobarbitone (Euthatal). They were then transcardially perfused, first with 0.9% saline followed by 10.0% formal-saline. The brains were removed and then placed in 10.0% formal-saline for 24 hours, and transferred to phosphate-buffered (0.1 M) 25.0% sucrose solution for 24 hours. Each brain was frozen, sectioned coronally using a sliding microtome, and the resulting 40µm sections were collected on gelatine-coated slides. These slides were left to dry in the oven of 30°C for 24 hours and the sections were stained with cresyl violet and examined using a microscope. Histological borders of hippocampal lesions were verified using the boundaries from Paxinos and Watson (2005).

2.6. Statistical analysis.

ANOVA was used to assess the changes in discrimination ratios across the course of training, and to assess the raw rates of responding from which these ratios were derived. In Experiment 4, ANOVA was also used to assess the impact of footshock on activity levels across the course of training. The rejection level that was adopted for all statistical analyses was $p < .05$.

3. Results

3.1. Histology

Inspection of the cell loss in the rats with hippocampal lesions in Experiments 1 and 2 revealed that 13 of the rats had a minimum of 50% total cell loss in the hippocampus (mean 73%), with a minimum of 70% cell loss in the septal region (mean 92%). The other 3 rats had less than 32% total cell loss in the hippocampus and were excluded from the analysis, leaving 6 rats in group Hpc in Experiment 1, and 7 rats in group Hpc for Experiment 2. The histology from the rats with acceptable lesions in Experiments 1 and 2 is depicted in Figure 2.

_____

Figures 2-4 about here

_____

Inspection of the cell loss in the rats with hippocampal lesions in Experiment 3a revealed that 12 of the rats had a minimum of 50% total cell loss in the hippocampus (mean 87%), with a mean of 98% cell loss in the septal region. The other two rats had large lesions such that sufficient transfer of brain slices onto slides was unsuccessful,

meaning that precise measurement of lesion size was not possible, and behavioural data from these rats were excluded from the analysis. However, the inclusion of the behavioral scores from these rats did not affect the pattern of results that was observed. In addition to the intended damage to the hippocampus, all rats had some limited damage to the overlying cortical regions, as seen in previous studies using this lesion method (e.g., Iordanova *et al*., 2009, 2011a; Lin et al., 2016). In all rats (other than the two already excluded) this damage was considered to be within satisfactory limits in so far as the damage was minimal and/or confined to one hemisphere. Inspection of the cell loss in the rats with hippocampal lesions in Experiment 3b revealed that 13 of the rats had a minimum of 50% total cell loss in the hippocampus (mean 69%), with a mean of 92% cell loss in the septal region. The other 3 rats had less than 42% total cell loss in the hippocampus and so were excluded from the analysis, leaving 13 rats in group Hippocampal for Experiment 3b. The histology from the rats with acceptable lesions in Experiments 3a and 3b is shown in panels A and B of Figure 3.

3.2. Experiment 1: Context relevant and time of day irrelevant

Figure 5 depicts the results of Experiment 1. The discrimination ratios increased across training blocks in both groups Sham and Hpc. ANOVA confirmed that there was a significant main effect of block ($F(4, 48) = 6.05$, $p < .001$), no effect of group ($F < 1$) and no interaction between block and group ($F(4, 48) = 1.91$, $p = 0.124$). Analysis of the rates of responding from which these ratios were derived confirmed that there significant main effect of reinforcement ($F(1, 12) = 59.72$, $p < .001$), no main effect of block ($F(4, 48) = 1.7$, $p > .16$) and no main effect of group ($F(1, 12) < 1$). There was a significant interaction

between reinforcement and block ($F(4, 48) = 5.49$, $p < .01$), no interaction between block and group ($F < 1$) and no three-way interaction ($F(4, 48) = 1.108$, $p > .36$).

Figure 5 about here

3.3. Experiment 2: Time of day relevant and context irrelevant

The lower panels of Figure 5 depicts the results of Experiment 2. The left-hand panel reveals that the discrimination ratios increased across training blocks in both groups Sham and Hpc. ANOVA confirmed that there was a significant main effect of block ($F(4, 52) = 9.71$, $p < .001$), no effect of group ($F(1, 13) = 1.54$, $p > .23$) and no interaction between block and group ($F < 1$). ANOVA conducted on the rates of responding from which these ratios were derived revealed a significant main effect of reinforcement ($F(1, 13) = 20.05$, $p < .01$), a significant main effect of block ($F(4, 52) = 5.00$, $p < .005$) and no main effect of group ($F < 1$). There was a significant interaction between reinforcement and block ($F(4, 52) = 10.24$, $p < .001$), no interaction between block and group ($F(4, 52) = 1.07$, $p > .37$) and no three-way interaction ($F < 1$).

3.4. Experiment 3: Context and time of day relevant (appetitive)

The results from Experiments 3a and 3b were combined for the purpose of analysis. The mean discrimination ratios and numbers of food well entries for groups Hpc and Sham in Experiments 3a and 3b are shown in Figure 6. The discrimination ratios for both groups increased over the course of training. ANOVA revealed a significant effect of block ($F(4, 200) = 11.55$, $p < .001$), no effect of group ($F < 1$) and no interaction between these factors ($F < 1$). This pattern of results is similar to that reported in Dumigan et al. (Experiment 1,

2016). ANOVA conducted on the rates of responding from which the ratios were derived confirmed that there was a significant main effect of reinforcement ($F(1, 50) = 32.42$, $p < .001$), a significant main effect of block ($F(4, 200) = 7.24$, $p < .001$) and a significant interaction between these two factors ($F(4, 200) = 6.49$, $p < .001$). There was no significant effect of group ($F(1, 50) = 4.01$, $p > .05$), no significant interaction between group and reinforcement ($F(1, 50) = 1.31$, $p > .05$), no significant interaction between group and block ($F < 1$) and no significant three-way interaction between reinforcement, block and group ($F < 1$). This analysis of the abolute levels of activity also revealed a similar pattern to that reported in Dumigan et al. (Experiment 1, 2016).

Figure 6 about here

3.5. Experiment 4: Context and time of day relevant (aversive)

Inspection of Figure 7 shows that the discrimination ratios increased across training in groups Sham and Hpc in Experiment 4. ANOVA confirmed there was a main effect of block ($F(7, 175) = 4.26$, $p < .001$), no effect of group ($F < 1$) and no interaction ($F(7, 175) = 1.35$, $p > .23$). This pattern of results is similar to that reported in Dumigan et al. (Experiment 2, 2016). Absolute levels of activity were lower in group Hpc compared to group Sham, but both groups showed a consistent difference in responding between reinforced and nonreinforced trials. ANOVA revealed a main effect of block ($F(7, 175) = 4.02$, $p < .001$), a main effect of reinforcement ($F(1, 25) = 5.50$, $p < .05$) and a main effect of group ($F(1, 25) = 9.56$, $p < .01$). There was a significant interaction between block and reinforcement ($F(7, 175) = 2.57$, $p < .05$) and no other interactions (largest $F(7, 175) = 1.70$,

$p > .11$). This analysis of the abolute levels of activity reveals a similar pattern to that reported in Dumigan et al. (Experiment 2, 2016). The observation that discrimination performance was relatively modest probably reflects the fact that the impact of the footshock changed over the course of training, as is evident from an analysis of post-shock activity (see below).

Figures 7 and 8 about here

Figure 8 shows the post-shock activity during the 30-s periods after the delivery of shock and for the corresponding periods on nonreinforced trials. In both groups, there was a general increase in activity across blocks, and activity in the 30-s following the delivery of shock was greater than in corresponding periods on nonreinforced trials. It is also evident that, after the first block of training, the second shock resulted in a more pronounced increase in activity than the first; which is consistent with the first shock resulting in a sensitization effect. Across training, the difference between reinforced and nonreinforced epochs reduced for the first period (1), but not for the second period (2); which is consistient with a conditioned dimunition of shock-induced activity to the first shock (see Wagner, 1981). This description of the pattern of results depicted in Figure 8 is supported by the results of an ANOVA, which confirmed that there was a significant main effect of the presence of shock ($F(1, 25) = 92.24$, $p < .001$), an effect of shock number ($F(1, 25) = 68.84$, $p < .001$), an effect of block ($F(7, 175) = 10.24$, $p < .001$) and no effect of group ($F(1, 25) = 2.61$, $p > .12$). There were also interactions between shock and shock number ($F(1, 25) = 80.41$, $p < .001$), between shock and block ($F(7, 175) = 9.58$, $p < .001$), and

between shock number and block ($F(7, 175) = 4.65$, $p < .001$). There was a three-way interaction between shock, shock number and block ($F(7, 175) = 4.10$, $p < .001$). There were no significant two- or- three-way interactions where group was a factor (largest $F(7, 175) = 1.43$, $p > .19$) and the four-way interaction was not significant ($F(7, 175) = 1.14$, $p > .34$).

4. Discussion

Theoretical treatments of the role of the hippocampus in learning and memory abound. Recent treatments have focused on the idea that the hippocampus might be involved in episodic memory processes, aspects of which would be impossible to study in species other than humans (Tulving & Markowitsch, 1998). However, some rudimentary components of episodic memory are empirically tractable in nonhuman species that readily encode, learn about and retrieve episodic information. For example, rats can learn that an auditory stimulus (e.g., a tone) is presented in a specific place (e.g., a spotted chamber) at a given time (e.g., in the morning; Iordanova *et al*., 2008). This can be revealed through pairing the tone with footshock and showing that a fear response is provoked when the remaining components of the original configuration (the spotted chamber in the morning) are presented. This behavioral effect that is clearly dependent on the hippocampus (e.g., Iordanova *et al*., 2009, 2011ab). The involvement of the hippocampus in this behavioral effect could reflect its role in a number of mnemonic processes: configural learning in general (e.g., Rudy & Sutherland, 1989; Sutherland & Rudy, 1989, 1995; O'Reilly & Rudy, 2001), configural learning involving spatio-temporal information (see Aggleton & Pearce, 2001), or mediated learning (or updating) involving evoked configural memories (Lin & Honey, 2016; Lin, Dumigan, Good, & Honey, 2016; see

also, Lin, Dumigan, Recio, & Honey, 2016). The result of the experiments presented here are inconsistent with accounts which suppose that the hippocampus plays (i) a general role in configural learning, or (ii) a more constrained role in configural learning about spatio-temporal information.

The results of Experiments 1 and 2 confirm that rats with lesions of the hippocampus can learn where (Experiment 1) or when (Experiment 2) food will be delivered. Given the fact that performance in these procedures can be supported by elemental processes, the findings from these experiments simply confirm those from the analogous procedures described by Iordanova *et al*. (2009, 2011a, 2011b); while providing a basis for comparison with the procedures used in Experiments 3 and 4. The procedures used in Experiments 3 and 4 require configural integration of where and when. In contrast to the results described by Iordanova *et al*. (2009, 2011a, 2011b) there was no indication that configural learning in these procedures was disrupted by lesions to the hippocampus. Given the fact that the lesions in Experiments 3 and 4 were similar to those reported by Iordanova et al. (2009, 2011) and used the same protocol as Lin et al (2016), our new results suggest that the origin of the effects observed by Iordanova *et al*. (2009, 2011a, 2011b) reflected an impairment in learning about retrieved memories of configurations.

The principal difference between the procedures used in Experiments 3 and 4 and those used by Iordanova *et al*. is the fact that the configural memories were directly activated in Experiments 3 and 4 and were activated by the tone during aversive conditioning. The idea that the hippocampus is involved in mediated configural learning receives converging support from recent studies of sensory preconditioning using audio-visual compounds. Lin *et al*. (2016) gave rats preexposure to two audio-visual compounds

(AX and BY), and then received trials on which X was paired with shock and Y was not. Rats then received test trials with AX and BX, and with AY and BY. Rats with sham lesions were more likely to show fear to AX than BX, but showed similar levels of fear to AY and BY. This pattern of results suggests that during conditioning with X the retrieved representation of AX became linked to shock. This effect was abolished in rats with lesions of the hippocampus, but an anologous effect that did not require configural processes was not disrupted by the same lesions.

It is also worth noting that our preferred interpretation, in terms of mediated learning, is also consistent with the results of Wimmer and Shohamy (2012). They used a sensory preconditioning procedure that involved three stages: training sensory associations involving visual stimuli (e.g., AX), changing the value of one of the components (X), and then assessing whether this change is reflected in the remaining component (A). The sensory preconditioning effect during the test was correlated with hippocampal activity (as assessed using fMRI) during the phase in which the value of the components was changed (i.e., the mediated learning stage), but was unrelated to activity during initial training or test.

The results presented here add to a body of work suggesting that the involvement of the rodent hippocampus in configural learning is more constrained than would be anticipated on the basis of some models of hippocampal function. The results of primary theoretical significance is the absence of an effect of lesions to the hippocampus on configural processing of spatio-temporal information (Experiments 3 and 4). These null results were evident in procedures that allowed many opportunies for an effect to be observed, at different levels of performance, and with different reinforcers. One

alternative explanation for these null results is that other neural systems can assume the role ordinarily played by the hippocampus (in configural learning) when this structure is damaged; but this explanation leaves unanswered the following simple question: Why do these other systems not assume the role played by the hippocampus in the related procedures employed by Iordanova et al. (2009, 2011a, 2011b; see also Lin et al., 2016)? Instead, we have proposed one way to reconcile the results of Experiments 3 and 4 with those reported using the same spatio-temporal information in which there was a clear and reliable impact of hippocampal lesions on perfromance (i.e., Iordanova et al., 2009, 2011a, 2011b). This reconciliation involves an anatomical separation between configural learning based upon spatio-temporal configurations that are directly activated by their corresponding stimuli, and mediated configural learning involving otherwise equivalent memories that have been evoked by a component of those configurations. This separation receives independent support from the observation that configurations involving a stimulus and the trace memory of the same stimulus can enter into separate configural associations (Lin & Honey, 2010) as well as other evidence from rats (Lin et al., 2016) and humans (Wimmer & Shohamy, 2012).

Acknowledgements

This research was supported by a grant from the Biotechnology and Biological Sciences Research Council (UK) and a School of Psychology studentship awarded to Natasha M. Dumigan.

References

Aggleton, J. P., & Brown, M. W. (1999). Episodic memory, amnesia, and the hippocampal-anterior thalamic axis. *Behavioural and Brain Sciences, 22*, 425- 489.

Aggleton, J. P., & Pearce, J. M. (2001). Neural systems underlying episodic memory: insights from animal research. *Philosophical Transactions of the Royal Society of London Series B - Biological Sciences, 356*, 1467-1482.

Alvarado, M. C., & Rudy, J. W. (1995). A comparison of kainic acid + colchichine and ibotenic acid induced hippocampal formation damage on four configural tasks. *Behavioral Neuroscience*, *109*, 1052-1062

Eacott, M. J., & Norman, G. (2004). Integrated memory for object, place and context in rats: a possible model of episodic-like memory? *Journal of Neuroscience, 24*, 1948-1953.

Ergorul, C., & Eichenbaum, H. (2004). The hippocampus and memory for "what", "where" and "when". *Learning and Memory, 11*, 397—405.

Fanselow, M. S. (1982). The postshock activity burst. *Animal Learning & Behavior, 10*, 448-454.

Fanselow, M. S. (1986). Associative vs. topographical accounts of the immediate shock freezing deficit in rats: Implications for the response selection rules governing species specific defensive reactions. *Learning & Motivation, 17*, 16-39.

Hall, G. (1996). Learning about associatively activated stimulus representations: Implications for acquired equivalence and perceptual learning. *Animal Learning & Behavior, 24*, 233-255.

Holland, P. C. (1981). Acquisition of representation-mediated conditioned food aversions, *Learning and Motivation, 12*, 1-18.

Honey, R. C., Iordanova M. D., & Good, M. (2014). Associative structures in animal learning: Dissociating elemental and configural processes. *Neurobiology of Learning and Memory, 108*, 96-103.

Honey, R. C., & Watt, A. (1999). Acquired relational equivalence between contexts and features. *Journal of Experimental Psychology: Animal Behavior Processes, 25*, 324-333.

Iordanova, M. D., Good, M. A., & Honey, R. (2008). Configural learning without reinforcement: Integrated memories for correlates of what, where and when. *Quarterly Journal of Experimental Psychology, 61*, 1785-1792.

Iordanova, M., Burnett, D. J., Aggleton, J. P., Good, M., & Honey, R. C. (2009). The role of the hippocampus in mnemonic integration and retrieval. *European Journal of Neuroscience, 30*, 2177-2189.

Iordanova, M. D., Burnett, D., Good, M., & Honey, R. C. (2011a). Pattern memory involves both elemental and configural processes: Evidence from the effects of hippocampal lesions. *Behavioral Neuroscience, 125*, 567–577.

Iordanova, M. D., Good, M., & Honey, R. C. (2011b). Retrieval-mediated learning involving episodes requires synaptic plasticity in the hippocampus. *Journal of Neuroscience, 31*, 7156–7162.

Li, J-S., & Chao, Y-S. (2008). Electrolytic lesions of dorsal CA3 impair episodic-like memory in rats. *Neurobiology of Learning and Memory, 89*, 192-198.

Lin, T.E., & Honey, R.C. (2010). Analysis of the content of configural representations: The role of associatively evoked and trace memories. *Journal of Experimental Psychology*: *Animal Behavior Processes*, *36,* 501-505.

Lin, T.E., & Honey, R.C. (2016). Learning about stimuli that are present and those that are not: Separable acquisition processes for direct and mediated learning. In R.A. Murphy and R.C. Honey (Eds.) *The Wiley Handbook on the Cognitive Neuroscience of Learning* (pp. 69-85). Oxford: Wiley-Blackwell.

Lin, T.E., Dumigan, N.M., Good, M.A., & Honey, R.C. (2016). Novel sensory preconditioning procedures identify a specific role for the hippocampus in pattern completion. *Neurobiology of Learning and Memory*, *130*, 142-148.

Lin, T.E., Dumigan, N.M., Recio, S.A., & Honey, R.C. (2016). Mediated configural learning in rats. *Quarterly Journal of Experimental Psychology* (in press).

O'Reilly, R. C., & Rudy, J. W. (2001). Conjunctive representations in learning and memory: principles of cortical and hippocampal function. *Psychological Review, 108*, 311-345.

Paxinos, G., & Watson, C. (2005). *The rat brain in stereotaxic coordinates* (5th ed). San Diego: Academic Press.

Rudy, J. W., & Sutherland, R. J. (1989). The hippocampal formation is necessary for rats to learn and remember configural discriminations. *Behavioural Brain Research, 34*, 97-109.

Rudy, J. W., & Sutherland, R. J. (1995). Configural association theory and the hippocampal formation: an appraisal and reconfiguration. *Hippocampus, 5*, 375- 389.

Schlichting, M.L., & Preston, A.R. (2016). Hippocampal-medial prefrontal circuit supports memory updating during learning and post-encoding rest. *Neurobiology of Learning and Memory, 134*, 91-106.

Sutherland, R. J., & Rudy, J. W. (1989). Configural association theory: The role of the hippocampal formation in learning, memory, and amnesia. *Psychobiology, 17*, 129-144.

Thorpe, C. M., Bates, M. E., & Wilkie, D. M. (2003). Rats have trouble associating all three parts of the time-place-event memory code. *Behavioural Processes, 63*, 95–110.

Tulving, E. (2002). Episodic Memory; From Mind to Brain. *Annual Review of Psychology, 53*, 1-25.

Tulving, E., & Markowitsch, H. (1998). Episodic and declarative memory: role of the hippocampus. *Hippocampus, 8*, 198–204.

Wagner, A.R. (1981). SOP: A model of automatic memory processing in animal behavior. In N.E. Spear & R.R. Miller (Eds.) *Information processing in animals: Memory mechanisms* (pp 5-48). Hillsdale, NJ: Lawrence Erlbaum Associates.

Wimmer, G.E., & Shohamy, D. (2012). Preference by association: How memory mechanisms in the hippocampus bias decisions. *Science*, *338*, 270-273.

Table 1

AP	ML	DV	Volume (μl)
-5.5	±4.2	-7.6	0.10
		-3.9	0.10
	±5.5	-6.8	0.10
		-5.8	0.10
		-5.0	0.10
-4.7	±4.0	-7.5	0.10
		-3.5	0.05
	±4.5	-8.0	0.10
-3.9	±2.2	-3.7	0.10
		-3.0	0.10
	±3.5	-2.7	0.10
-3.1	±1.4	-4.0	0.10
		-3.0	0.10
	±3.0	-2.7	0.10
-2.4	±1.0	-3.8	0.05

Note: AP, ML and DV indicate the coordinates in relation to bregma from anterior to posterior (AP), from medial to lateral (ML) and from dorsal to ventral (DV).

Figure legends

Figure 1. Schematics for the experimental designs used in Experiments 1-4. All rats received presentations of the same four context+time of day patterns. Whether the patterns would be reinforced or nonreinforced was predicted by the context (spotted or checked; Experiment 1), time of day (morning or afternoon; Experiment 2), or the configuration of context and time of day (Experiments 3 and 4).

Figure 2. The maximum (light grey) and minimum (dark grey) extent of the lesions for rats from group Hpc in Experiments 1 and 2 (pooled). The coronal sections are at specific distances (in mm) from Bregma (adapted from Paxinos & Watson, 2005).

Figure 3. The maximum (light grey) and minimum (dark grey) extent of the lesions in rats from group Hpc in Experiments 3a and 3b. The coronal sections are at specific distances (in mm) from Bregma (adapted from Paxinos & Watson, 2005).

Figure 4. The maximum (light grey) and minimum (dark grey) extent of the lesions in rats from group Hpc in Experiment 4. The coronal sections are at specific distances (in mm) from Bregma (adapted from Paxinos & Watson, 2005).

Figure 5. Mean discrimination ratios (±SEM) in two-day blocks for groups Sham and Hpc in Experiment 1 (upper panel) and Experiment 2 (lower panel).

Figure 6. Mean discrimination ratios (±SEM) in two-day blocks for groups Sham and Hpc in Experiment 3a (upper panel) and Experiment 3b (lower panel).

Figure 7. Mean discrimination ratios (±SEM) in three-day blocks for groups Sham and Hpc in Experiment 4.

Figure 8. Mean activity levels (±SEM) in the 30 s periods after the first (1) and second (2) shock during reinforced sessions and the corresponding periods in the nonreinforced periods.

Figure 1

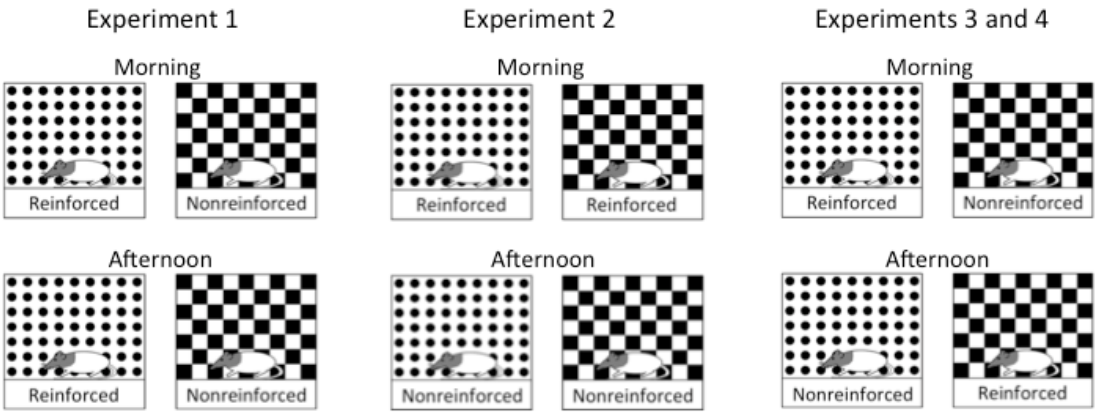

Experiment 1
Morning
Reinforced
Nonreinforced
Afternoon
Reinforced
Nonreinforced
Experiment 2
Morning
Reinforced
Reinforced
Afternoon
Nonreinforced
Nonreinforced
Experiments 3 and 4
Morning
Reinforced
Nonreinforced
Afternoon
Nonreinforced
Reinforced

Figure 2

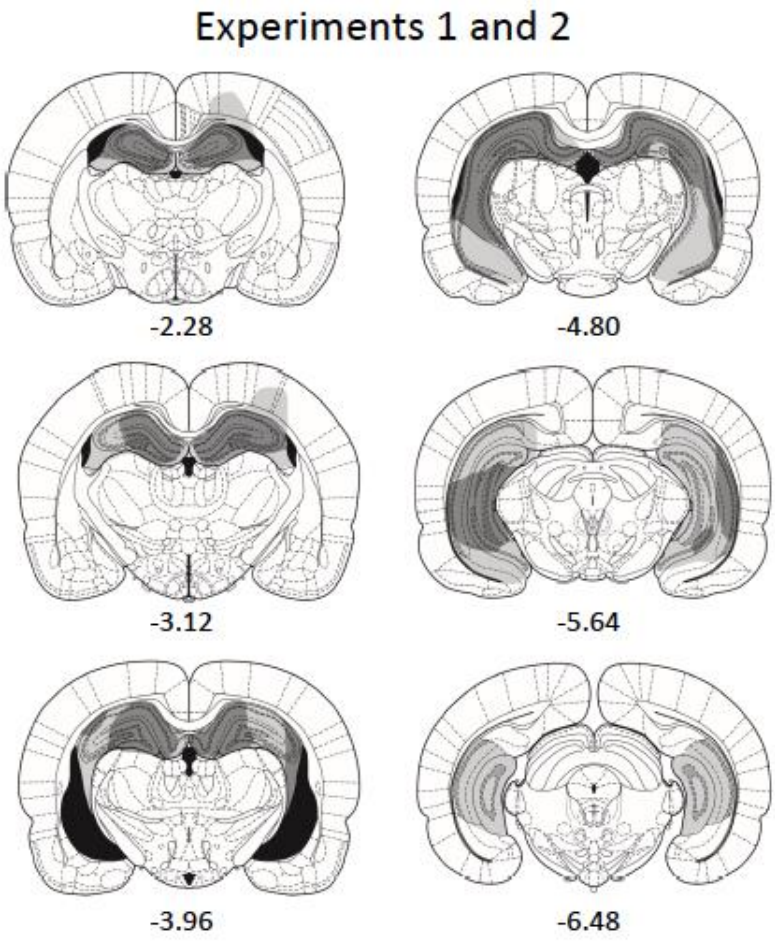


Figure 3

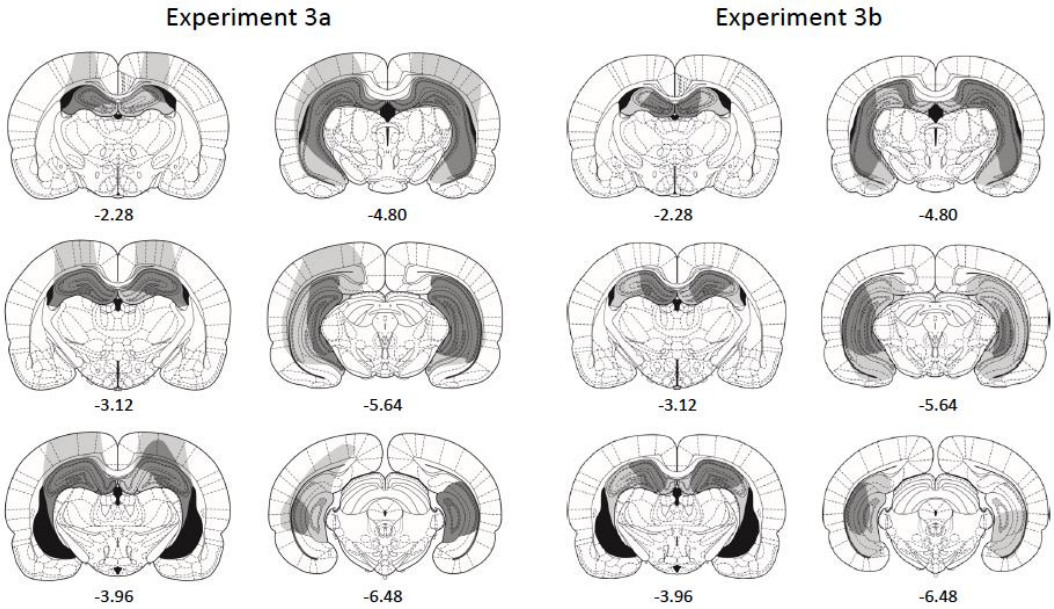

Experiment 3a
Experiment 3b
-2.28
-4.80
-2.28
-4.80
-3.12
-5.64
-3.12
-5.64
-3.96
-6.48
-3.96
-6.48

Figure 4

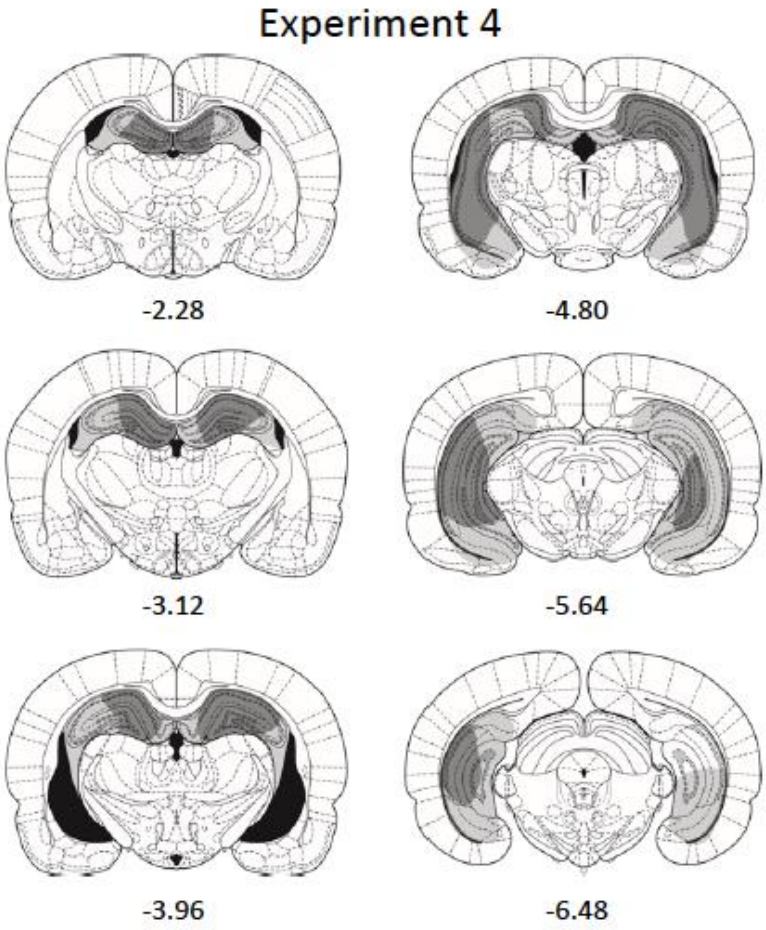


Figure 5

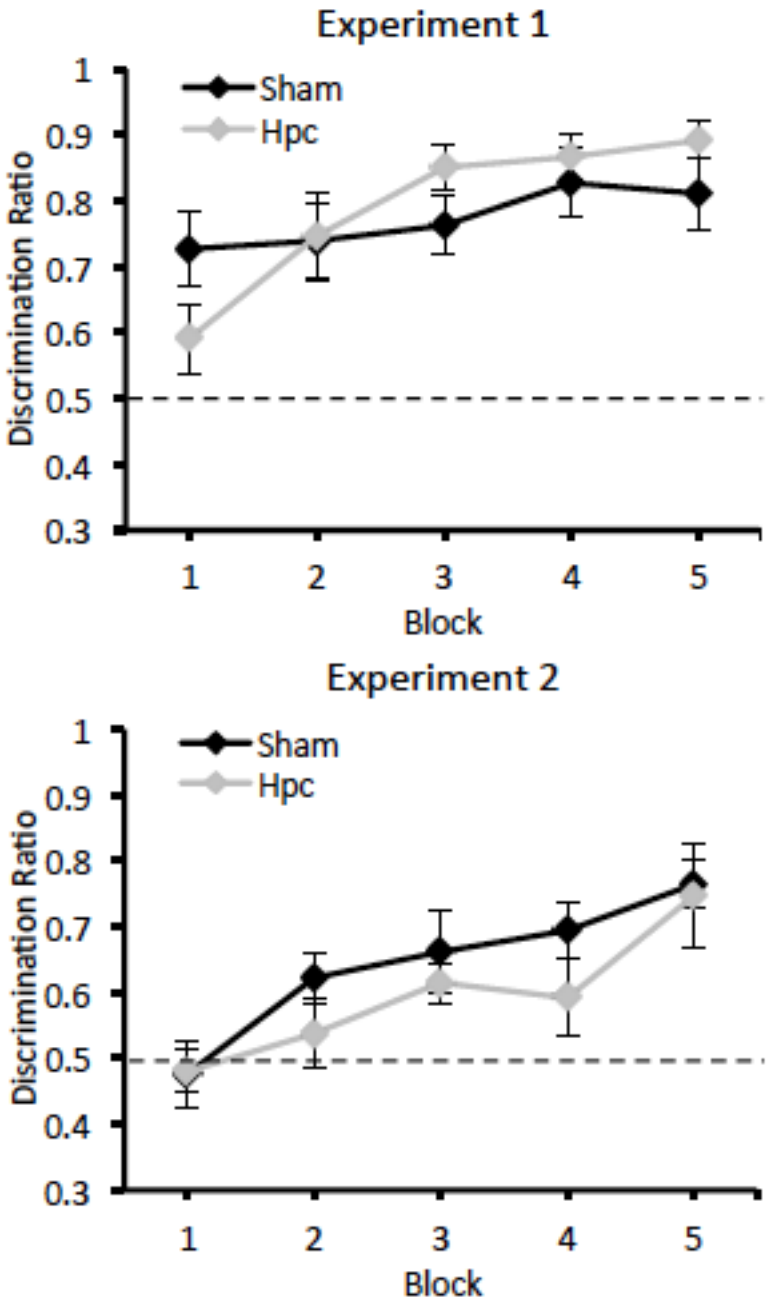

Experiment 1
Sham
Hpc
Discrimination Ratio
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
1
2
3
4
5
Block
Experiment 2
Sham
Hpc
Discrimination Ratio
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
1
2
3
4
5
Block

Figure 6

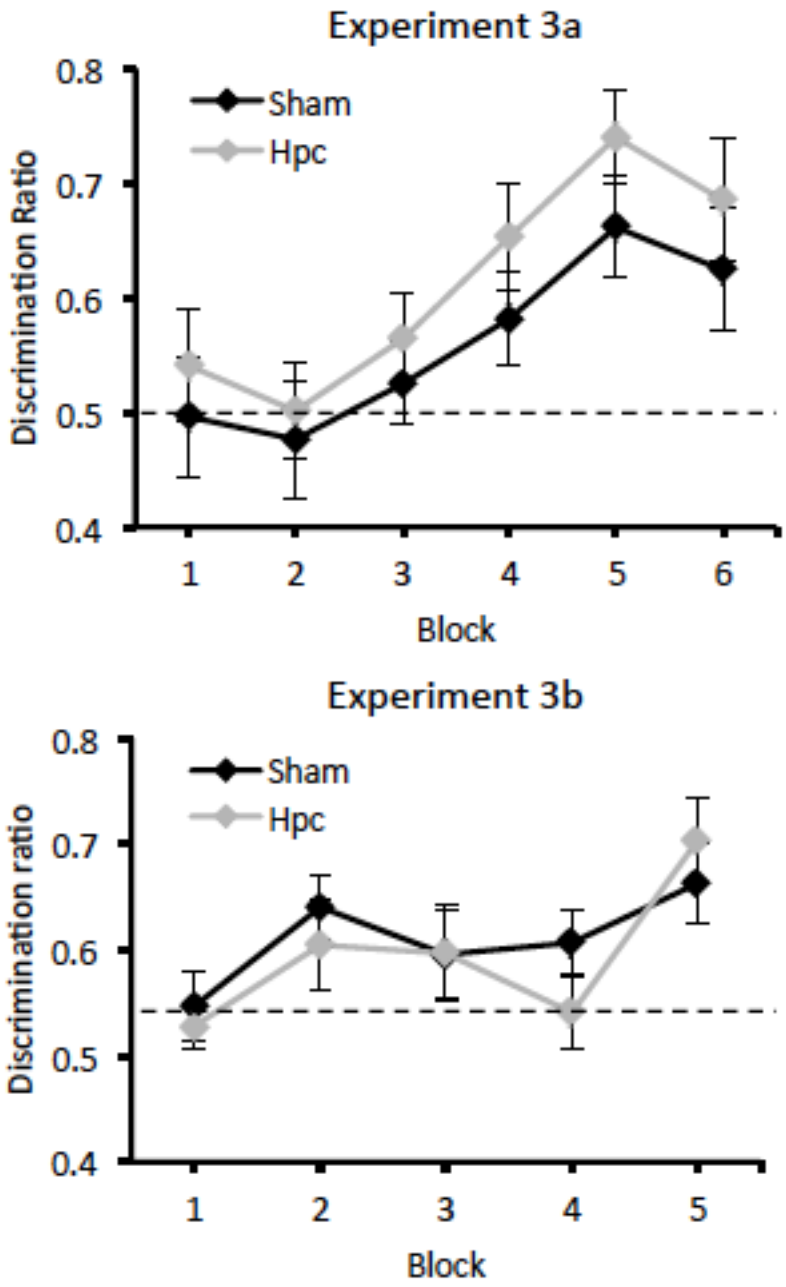

Experiment 3a
Sham
Hpc
Discrimination Ratio
0.8
0.7
0.6
0.5
0.4
1
2
3
4
5
6
Block
Experiment 3b
Sham
Hpc
Discrimination ratio
0.8
0.7
0.6
0.5
0.4
1
2
3
4
5
Block

Figure 7

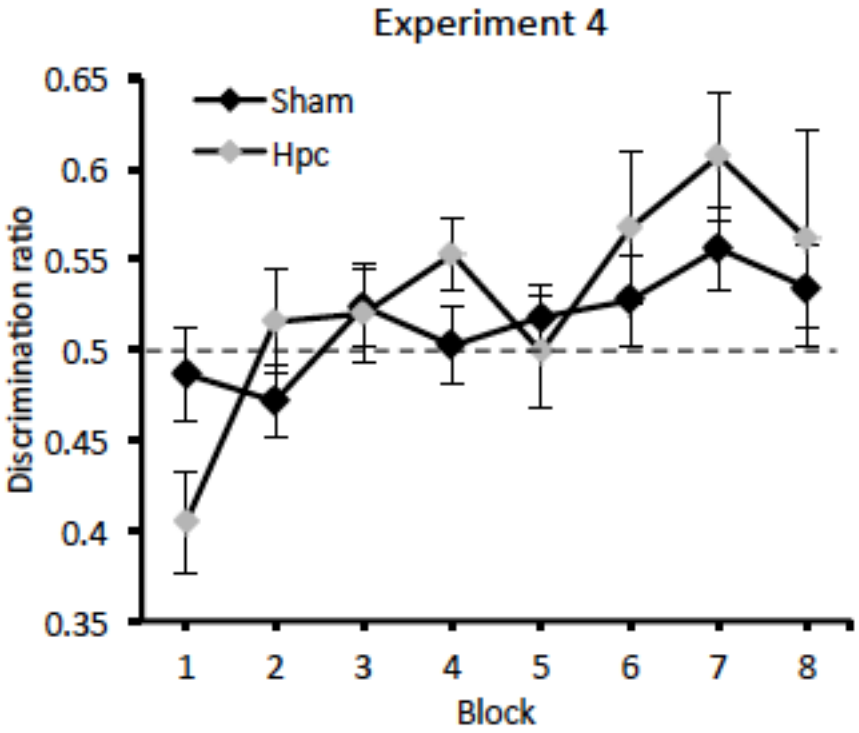
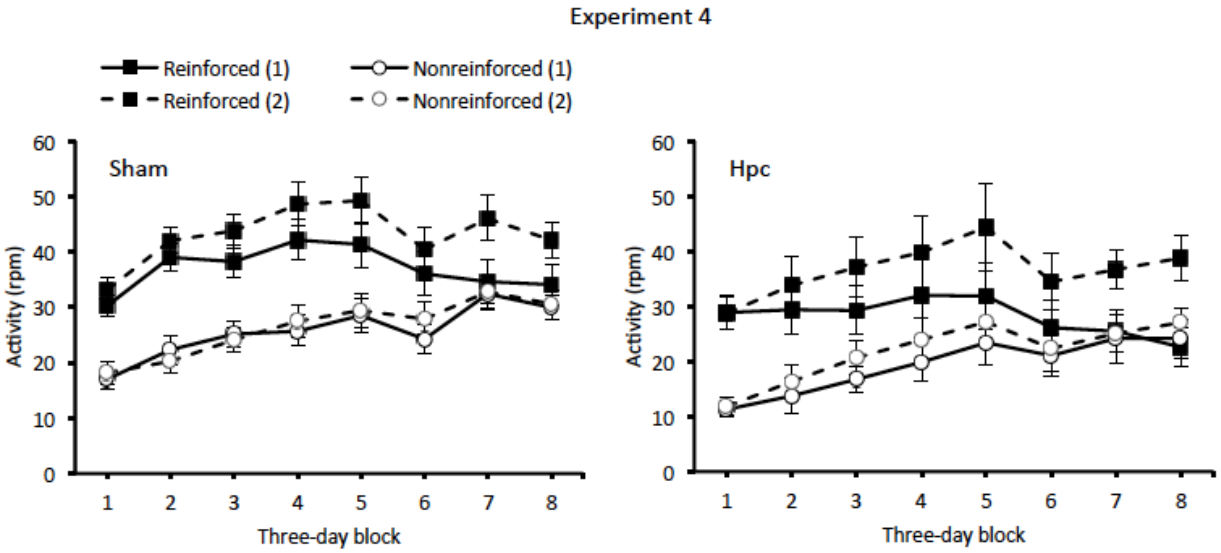

Experiment 4
Sham
Hpc
Discrimination ratio
0.65
0.6
0.55
0.5
0.45
0.4
0.35
1
2
3
4
5
6
7
8
Block

Figure 8


Experiment 4
Reinforced (1)
Nonreinforced (1)
Reinforced (2)
Nonreinforced (2)
Sham
Hpc
Activity (rpm)
Three-day block
0
10
20
30
40
50
60
1
2
3
4
5
6
7
8