Hippocampus is necessary for spatial discrimination using distal cue-configuration

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Abstract

The role of the hippocampus in processing contextual cues has been well recognized. Contextual manipulation often involves transferring animals between different rooms. Due to vague definition of context in such a paradigm, however, it has been difficult to study the role of the hippocampus parametrically in contextual information processing. We designed a novel task in which a different context can be parametrically defined by the spatial configuration of distal cues. In this task, rats were trained to associate two different configurations of distal cue-sets (standard contexts) with different food-well locations at the end of a radial arm. Experiment 1 tested the role of the dorsal hippocampus in retrieving well-learned associations between standard contexts and rewarding food-well locations by comparing rats with neurotoxic lesions in the dorsal hippocampus with controls. We found that the hippocampal-lesioned rats were unable to retrieve the context-place paired associations learned before surgery. To further test the role of the hippocampus in generalizing altered context, in Experiment 2, rats were trained in a task in which modified versions of the standard contexts (ambiguous contexts) were presented, intermixed with the standard contexts. Rats were able to process the ambiguous contexts immediately by using their similarities to the standard contexts, whereas muscimol inactivation of the dorsal hippocampus in the same animals reversibly deprived such capability. The results suggest that rats can effectively associate discrete spatial locations with spatial configuration of distal cues. More important, rats can generalize or orthogonalize modified contextual environments using learned contextual representation of the environment.

When an animal learns a certain event in a memory task, not only the target event but also its associated background environment (i.e., context) are learned simultaneously. This can be demonstrated by showing that the learned target behavior is best expressed in the original context (Good and Honey, 1991; Hirsh, 1974; Kim and Fanselow, 1992; Phillips and LeDoux, 1992). It has been proposed that the capability of associating context or performing contextual discrimination is dependent on the hippocampus (Good et al., 1998; Good and Honey, 1991; Hirsh, 1974; Holt and Maren, 1999; McDonald et al., 1997). In many prior studies where an event should be associated with its context, an animal was often tested in a room where the target event took place and then transferred to a novel room (or different location in the same room) to show that the target event produced learned behavior conditionally in the original room but not in the novel room (Dellu et al., 1997; Good and Bannerman, 1997; Good et al., 1998; Good and Honey, 1991; Kim and Fanselow, 1992; Lee et al., 2004; Moses et al., 2007; Mumby et al., 2002; Piterkin et al., 2008; Rajji et al., 2006; Winocur and Olds, 1978). A critical assumption associated with this paradigm is that various cues (e.g., olfactory cues, distal cues, tactile cues, etc.) that are co-present as an ensemble in the room may serve as a unique context.

However, few studies, if any, have tested the *parametric relationship* between the extent of changes in the distal contextual cues and the changes in the animal's behavioral response to the altered contextual environment. Parametric manipulation in the current study refers to the manipulation of a single parameter (e.g., distance between distal cues) associated with contextual cues by varying degrees in a quantifiable manner. Some prior studies testing configural theories of context (Davidson et al., 1993; Gallagher and Holland, 1992; Rudy and Sutherland, 1989) manipulated the combinations of individual cues, but the combinatorial manipulations were not performed parametrically along a single parameter. Other studies indeed manipulated cue stimuli parametrically. For example, Shapiro et al. (1997) rotated and/or switched distal cue cards and local cues parametrically in some of their experiments as they recorded hippocampal neural activity in association with cue manipulation. Rats in the Shapiro et al. study performed a working memory task in a plus maze. Similarly, Lee et al. (2004) rotated distal cues and local cues in opposite directions using the parameter of angular distance between the distal and local cue sets. Principal neurons in CA1 and CA3 were recorded in the Lee et al. study as rats foraged for food on a ring track. Leutgeb et al. (Leutgeb et al., 2005) manipulated the shape of the recording chamber by varying degrees and recorded hippocampal neurons while the rats foraged for food in the chamber. In these and other electrophysiological studies (Anderson and Jeffery, 2003; Hayman et al., 2003), however, the animals were not required to change their behavioral responses according to the parametric manipulations of cues in the environment and the behavioral task remained unchanged despite parametric manipulation of contextual cues. In the current study, we have designed a novel task in which a context is defined by the spatial configuration of two sets of distal cues (the configuration being manipulated by varying the angular distance between the cue sets). In our study, therefore, all features (e.g., individual cues, spatial relationships among cues within a cue set, maze room, etc.) other than spatial configuration of the distal cue-sets remained the same across different contexts. More important, animals were required to associate different locations in the maze with different distal cue configurations. This enabled us to directly associate behavioral results with the configuration of distal cues. In Experiment 1, we tested whether rats can associate two discrete locations in space with distal cue configurations.

Defining context as in the current study allows us to investigate how animals respond to varying degrees of changes in context and how the hippocampus processes contextual information in those situations, because only a single dimension (i.e., angular distance between distal cue-sets) is parametrically manipulated while all other dimensions of the environment remain the same. Computational models suggest that the hippocampal CA3 can perform generalization or pattern completion to treat the representation of a cue-altered environment similarly to the representation of its original environment. The CA3 network can also perform an opposite operation called orthogonalization or pattern separation to disambiguate less similar contexts into orthogonal representations (O'Reilly and McClelland, 1994; Rolls and Kesner, 2006). Physiological studies have shown that such computations may occur in the hippocampus (Lee et al., 2004; Leutgeb et al., 2005; Leutgeb et al., 2004; Vazdarjanova and Guzowski, 2004). Critically, however, animals in those physiological experiments were not required to use the contextual information to make mnemonic choices in the environment. If the computational functions of the hippocampus subserve the animal's capability for generalizing and orthogonalizing environmental contexts, inactivation of the hippocampus should disrupt such capability. In Experiment 2, we tested this by altering the spatial configuration of distal cues in the environment and temporally inactivating the hippocampus with muscimol.

Materials and Methods

Subjects

Fourteen male Long-Evans rats (300-400 g) were used in Experiment 1 and eight rats (300 – 400 g) were used in Experiment 2. They were housed individually in standard rodent cages and maintained on a 12-hour light-dark cycle. All behavioral experiments were conducted during the light phase of the light-dark cycle. During behavioral testing, they were food-deprived to 80% of their free-feeding weight but allowed to access water *ad libitum*.

Behavioral apparatus

An elevated, radial arm maze (Fig. 1) made of black Plexiglas was positioned in a circular curtained arena (260 cm in diameter). Each arm of the maze was 68 cm long and 10 cm wide. Adjacent arms were separated by 25.7°. All arms radiated from a circular center stage (48 cm in diameter) and a start box (20 x 25 x 30) with an opaque vertical door was located in the center stage. Access to an arm was controlled by an experimenter using a transparent guillotine door (10 x 25 cm) installed in the entrance of the arm. At the end of each arm, there was a rectangular *choice platform* (23 x 30 cm) in which three food wells (each 2.5 cm in diameter) were aligned, separated by transparent Plexiglas partitions.

The circular testing arena was created by gray curtains (*background curtains*). Overlaid with the background curtains were two rectangular curtains (each 135 x 299 cm) that could be moved independently in the foreground along a circular curtain track running in parallel with and immediately adjacent to the circular track installed for the background curtains in the ceiling (Fig. 1). The two curtains in the foreground will be called *cue-curtains* throughout the study. Each cue-curtain contained three distinctive visual cues. Therefore, six distinct visual distal cues were used throughout the study. For the left cue-curtain, two poster boards with cartoon characters (e.g., Elmo and Dora) attached and a soccer ball-shaped board were used as distal cues. For the cue-curtain on the right side, a checkerboard, a dragon-shaped kite, and a heart-shaped decorative object were attached (Fig. 1B). The cue-curtains and background curtains were identical in color and texture and it was very difficult even for an experimenter to visually dissociate the cue-curtains from the background curtains. A digital CCD camera was installed in the ceiling (above the center of the maze) to record behavioral sessions and two loudspeakers were located underneath the center stage of the maze to provide white noise during behavioral sessions.

Handling, familiarization, and shaping

After naïve animals (300-325 g) arrived in the colony, they were handled for about a week. During handling, rats were trained to eat sugar-coated cereal (Froot-Loops, Kellogg's) in an open space outside the behavioral testing room. Once the rats had readily consumed multiple pieces of cereal, the familiarization session was initiated. During familiarization, small pieces of cereal were scattered throughout the maze with the doors for all arms opened. Animals were encouraged to move freely and consume cereal rewards during a period of 35 minutes for two days in a row.

After this, rats were trained to retrieve food rewards only from the center arm. Specifically, the rat was placed in the maze's start box with the door closed. When the start-box was opened, the rat entered the center arm of the maze because all the other arms were inaccessible and cereal reward was only provided in the middle arm. A quarter piece of cereal reward was hidden in either the left or right food well in the choice platform. Each food well was covered with a small metal disc (3.5 cm in diameter) and the rat was required to displace one of the discs to obtain a reward. Each disc was made by modifying a metal washer by filling its center hole with hot glue. The animal, after obtaining the cereal piece, was gently guided back to the start box and was allowed to consume the reward only in the start-box. With two days of shaping (32 trials per day), rats voluntarily returned to the start-box after they found reward with minimal guidance by the experimenter. As shown in our previous study (Lee and Solivan, 2008), this self-returning protocol is helpful for controlling the amount of exposure to the maze environment after the animal makes a choice.

Pre-surgical training

For pre-surgical training, only the center arm of the maze was used; all other arms were inaccessible throughout the study. In each trial, while the animal was in the start box, the experimenter manipulated the spatial configuration of distal cue-sets by adjusting the angular distance between the cue-curtains. Throughout the study, the configuration of distal cues is called *Context A* when the medial edges of the two cue-curtains were aligned along the imaginary midline of the center arm (thus 0° angular separation from each other; Fig. 1, left). When the two cue-curtains were separated from each other by 80°, the configuration of distal cue-sets was denoted *Context B* (Fig. 1, right). The order in which two different contexts was presented was pseudo-randomized (i.e., no more than three consecutive trials of the same context condition presented and different context conditions were given for each of the initial four trials) throughout the study and each context was presented in 16 trials during each day's session (32 trials in total; intertrial interval of 25-30 s). After the experimenter prepared a context, the rat was released from the start box and allowed to enter the arm. In the choice platform, the animal was required to displace one of the discs overlying the left and right food wells (center food well was never used). In Context A, choosing the left food well was rewarded, whereas the choice of the right food well was rewarded in Context B (Fig. 1). Selection of the wrong food well was punished by not allowing the animal to correct its behavior and making the rat return to the start box to end the trial. The two discs were randomly switched between left and right food wells across trials to prevent the animals from paying attention to subtle differences that might be present in the discs. Probe trials were inserted occasionally to test the possibility that the rats might use odor cues. The probe trials included trials in which neither food well was baited, but the rat still displaced the correct disc in those trials. In addition, when a food reward was accidentally placed in the wrong food well in some trials, the rats still displaced the correct disc in the absence of the food reward underneath the disc.

It took 2 to 5 weeks for the rats to meet the pre-surgical performance criterion (median = 22 d). Once the rat passed the performance criterion (≥ 75% correct choices in both contexts for two consecutive days), in Experiment 1, the animal was randomly assigned to either control (CTRL) or dorsal hippocampus lesion (HP-LES) group, whereas the animal was assigned to either saline (SAL) or muscimol injection (MUS) group in Experiment 2.

Surgery

In both Experiment 1 and Experiment 2, each animal was deeply anesthetized with isoflurane in an induction chamber. The animal was then injected with ketamine (60 mg/kg) and xylazine (8 mg/kg) intraperitoneally and was placed in a stereotaxic instrument. Then an incision was made along the midline of the scalp.

Experiment 1

Seven animals received axon-sparing neurotoxic lesions created by the injection of ibotenic acid into the dorsal hippocampus (HP-LES group); the other seven rats received control lesions in the same area (CTRL group). Multiple injections of either ibotenic acid (HP-LES) or saline (CTRL) were administered to the dorsal hippocampus. Four burr holes were drilled in the skull and injections were made using the following coordinates: (1) 3.1 mm posterior to bregma, 1.8 mm lateral to midline, and 3.7 mm ventral from the skull surface, (2) 4.3 mm posterior to bregma, 3.2 mm lateral to midline, and 3.8 mm ventral from the skull surface. During the injection of either phosphate-buffered saline for the CTRL group or ibotenic acid (10 mg/ml, .3 µl/site at 10 µl/h) for the HP-LES group, an injection needle (30G cannula) was left in place for one minute before and after each injection to ensure proper diffusion of the drug in the target site. Injections were made by a Hamilton syringe (10 µl) operated by a microinjection pump (Cole-Parmer, Vernon Hills, IL). For the HP-LES group, diazepam (20 mg/kg, i.p.) was injected postoperatively to prevent possible seizure activity. A heating pad was used for all animals after surgery to maintain body temperature until the animal was fully awake and mobile.

Experiment 2

Guide cannulae (23G, 5 mm long; Plastics One, Roanoke, VA) coupled with stylets (1 mm protrusion from the tip of the guide cannula) were implanted bilaterally targeting the dorsal hippocampus. After four small holes were drilled in the periphery of the skull for positioning anchoring machine screws (0-80), two burr holes for cannulae were made in the skull using the following coordinates: 3.8 mm posterior to bregma, 2.6 mm lateral to midline. After the cannulae were implanted, the gaps between the cannulae and the edges of the burr holes in the skull were filled with medical silicon (WPI, Sarasota, FL). Bone cement (Zimmer, Warsaw, IN) was used to finalize the implantation. The same post-surgical care as described above for Experiment 1 was administered. After surgery, each rat was randomly assigned to either the saline-injection (SAL) group or the muscimol-injection (MUS) group.

For both Experiment 1 and 2, one week of recovery was allowed. All protocols conformed to the NIH Guide for the Care and Use of Laboratory Animals and the Institutional Animal Care and Use Committee.

Post-surgical testing

Experiment 1

Post-surgical testing was conducted in Experiment 1 to test the retrieval of previously learned paired associations between contexts and food-well locations after surgery. Rats were tested for two days in Context A and Context B as in the pre-surgical training period.

Object discrimination task

To demonstrate that the rats used in the current study were normal when performing reward-based association for non-contextual items (e.g., objects), some rats (4 from CTRL and 5 from HP-LES) were trained with a simple object-discrimination task as in our previous study

(Lee and Solivan, 2008) after Experiment 1 was completed. The center arm of the radial maze was also used in this task but all visual cues on the cue-curtains and two discs were removed from the maze. Instead, two different toy objects (i.e., toy egg and Barney figure, Fig. 3C) were used with a metal washer attached at the bottom of each object. Object location was switched between the left and right food wells in a counterbalanced manner throughout the task. The Barney figure was always rewarded. In this task, therefore, the rat was not required to process contextual information to obtain reward. The rats were trained for two consecutive days (32 trials per day).

Experiment 2

Experiment 2 was conducted to achieve two objectives: One was to test the involvement of the hippocampus in retrieving previously learned context-location associations using reversible inactivation with muscimol within subjects. The other aim was to investigate whether the hippocampus was critical in generalizing and disambiguating similar distal cue-configurations when context was modified. Originally, we aimed to test hippocampal-lesioned animals with the paradigm used in Experiment 2. However, we found that the hippocampal-lesioned animals could not discriminate even the most divergent contexts such as Context A and Context B, as shown by the results in Experiment 1. Since this eliminated the baseline performance to which the performance of rats in ambiguous contexts could be compared, in Experiment 2, we pre-trained the rats with the most divergent contexts (Contexts A and B) first. Afterwards, by reversibly inactivating the hippocampus while introducing ambiguous contexts, we attempted to differentiate the role of the hippocampus in variants of the original contexts. The overall drug-injection and task schedules for Experiment 2 are summarized in the table in Figure 6A.

For the first aim mentioned above, rats were tested with standard contexts (i.e., Context A and Context B) for four days. The first two days included injection-free testing sessions. Afterwards, the rats were injected with saline (.3 μl, 10 μl/h) bilaterally during the remaining two days. For each drug injection in Experiment 2, one minute was allowed before and after placement of the injection needle (30G) in the target site to ensure proper diffusion of the drug. Behavioral testing was conducted 20 minutes after drug injection.

After the first 4 days of testing, to achieve the second aim, the two groups were further tested for 8 more days but two novel contexts (i.e., ambiguous contexts) were also presented, intermixed with the standard contexts. The ambiguous contexts (Context A' and Context B') were constructed as follows (Fig. 4): When the cue-curtains were separated by 14° (thus +14° deviation from Context A), the configuration of distal cues was named Context A' (due to its similarity to Context A). When the cue-curtains were separated by 66° (thus -14° deviation from Context B), the cue-configuration was called Context B'. In Context A', the same food well (i.e., left food well) rewarded in Context A was rewarded, and the same was true for Context B' (i.e., right food well was rewarded as in Context B). The task, therefore, was designed to be learned faster if the rats generalized similar spatial relationships among distal cues (e.g., Context A and A') and associated them with a common, rewarding spatial location (e.g., left food well) in the environment. Each behavioral session was composed of 32 trials and each context was presented eight times during the session. As in Experiment 1, the same context condition was not presented more than three times consecutively and different context conditions were given for the first four trials. During the first four days when the four contexts were presented in a mixed fashion, muscimol (30 mg/ml; .3 µl, 10 µl/h) was bilaterally injected in the MUS group, whereas saline was injected in the SAL group. For the remaining four days, saline was administered to both groups after three days of rest. The three days of rest were allowed to ensure that learning capability was tested without any residual effect that might have been present after the prolonged injections of muscimol for four days.

Probe task with partial cues

After rats finished Experiment 2, probe trials were performed to test the animals' reliance on distal cue configuration. Specifically, when 32 trials were given in a session, eight of those trials were used as probe trials and the rest were composed of normal trials. Eight probe trials were composed of two trials for each context condition (i.e., Context A, A', B, and B') and the order of presenting the trials was pseudo-randomized. For a probe trial, two of the three distal cues in a cue-set associated with each cue-curtain were removed while all the other experimental manipulations were held constant. Conversely, this manipulation left a single distal cue per cue-curtain. We expected that rats would have difficulty in judging the configural relationship between distal cues when only two cues were present in the environment. If rats had learned the task by using alternative cues such as the positions of cue-curtains themselves (although it was very difficult to notice the boundaries of the cue-curtains against the background curtains), the probe manipulations should not have affected performance. Rats were tested in this probe-containing version of the task for a single day with only saline injections for both SAL and MUS groups.

Histology

Histological verifications of lesions (Experiment 1) and cannula positions (Experiment 2) were conducted after the completion of all behavioral tests. A lethal dose of sodium pentobarbital (Euthasol, Henry Schein, Melville, NY) was administered to the rat, and 0.9% saline and 10% formaldehyde solutions were infused transcardially. Brains were stored in 10% formalin-30% sucrose solution at 4°C for 48 hours. The brains were frozen, cut in coronal sections (40 µm) on a sliding microtome (Thermo Fisher Scientific, Waltham, MA) and later stained with thionin (Sigma-Aldrich, St. Luis, MO).

In Experiment 1, the relative amount of damage in the hippocampus was measured using computer software. Detailed procedures can be found in previous studies (Gilbert et al., 2001; Lee and Kesner, 2003a; Lee and Kesner, 2003b; Lee and Solivan, 2008). In Experiment 2, the cannula positions were verified by identifying the tips of injection cannulae. Also, the diffusion range of the drug was visually verified by assessing the diffusion range of fluorescent muscimol (BODIOY TMR-X Muscimol; Molecular Probes, Eugene, OR). Before the animal was sacrificed, the same amount of fluorescent muscimol was bilaterally injected (.3 µl per cannula, 10 µl/h) to the target loci following the protocol used during the testing periods. The unstained sections adjacent to the thionin-stained sections (labeled with fluorescent muscimol) were then examined with a yellow-orange fluorescent filter (572 nm wavelength).

Results

Histological results

Experiment 1

Figure 2 shows representative sections to illustrate the extent of neurotoxic lesions in the dorsal hippocampus in the HP-LES group. There was no damage in the dorsal hippocampus in the CTRL group, whereas the dorsal hippocampus was mostly eliminated by ibotenic acid in the HP-LES group. On the basis of volumetry, 91% of principal cell layers in the dorsal hippocampus were removed in the HP-LES group as compared to the CTRL group. In both groups, extrahippocampal areas such as the entorhinal cortex and perirhinal cortex were normal under microscopic examination.

Experiment 2

It was verified that the injection cannula tips were placed within the hippocampus in all animals (Fig. 5A). In addition, fluorescent photomicrographs showed that the injection parameters used for muscimol injections resulted in fairly localized spread of muscimol in the dorsal hippocampus (Fig. 5B).

Behavioral results

Two days of behavioral testing were grouped into one block to increase the statistical power throughout the analyses used in the current study.

Experiment 1

As shown in Figure 3A, hippocampal lesions produced a significant impairment in performance. A repeated-measures ANOVA was run with testing day (pre- and post-surgery) as a within-subject factor and surgical group (CTRL and HP-LES) as a between-subject factor. There was a significant effect of testing day ($F_{(1, 12)} = 12.4, p < .01$), surgical group ($F_{(1, 12)} = 106.1, p < .001$), and interaction between the two variables ($F_{(1, 12)} = 18.6, p < .01$). The results indicate that the CTRL group was able to retrieve learned associations between contexts and locations 1 week after surgery, whereas the HP-LES group was unable to do so. Pair-wise comparisons were used for post-hoc statistical testing unless specifically noted otherwise. Post-hoc comparisons showed that the HP-LES group was more impaired than the CTRL group after surgery ($F_{(1, 20)} = 21.0, p < .001$), whereas both groups showed similar performances before surgery ($F_{(1, 20)} < 1$, *n.s.*). Both groups showed lower performance post-surgically compared to their pre-surgical performance level. However, the post-surgical performance of the CTRL group (77.1%) was statistically equivalent to its pre-surgical performance (75%; *one-sample t-test*, $t_{(6)} = 1.5, p = .19$).

Hippocampal-lesioned animals tended to show perseverative behavior in choosing the food well on a particular side (e.g., left food well) regardless of distal contextual cues. When a response bias was calculated by taking the absolute value of the differences between left and right responses divided by the total number of trials (i.e., if the rat chose one side only, the index would be 1 and if the rat chose both sides equally, the index would be 0). Such a trend between the two groups was verified as shown in Figure 3B ($F_{(1,\,12)} = 4.7$, $p = .05$). Similar response bias was also observed in our previous study with hippocampal-lesioned rats in an object-place paired-associate task (Lee and Solivan, 2008).

The impairment observed in the HP-LES group cannot be explained by any non-specific deficit in associating external stimuli with reward (which might have been caused by hippocampal lesions) for the following reason. As shown in Figure 3C, hippocampal-lesioned rats were normal in associating an object (a non-contextual, elemental item) with reward ($t_{(7)} < 1$, *n.s.*). The results demonstrate that rats' general associative capacity using food reward was unaffected. Overall the results from Experiment 1 strongly suggest that the dorsal hippocampus is critical in retrieving associative representations between discrete locations in space and different contexts formed by distal cues in the environment.

Experiment 2

Experiment 2 further tested the involvement of the hippocampus in generalizing and disambiguating distal cue-contexts by introducing modified versions (Context A' and B') of standard contexts and inactivating the hippocampus with muscimol within subjects (Fig. 6A). When no injection was made in any of the groups (post 1) and when saline was given to both groups (post 2) (Fig. 6B), the two injection groups showed performances similar to each other and comparable to their pre-surgical performance levels (all p's > .05). Performance level remained at around 75%, which was our surgical criterion during pre-training. A repeated-measures ANOVA with testing day (pre-surgery, post 1, and post 2) as a within-subject factor and injection group (SAL and MUS) as a between-subject factor showed that there was no significant effect of testing day ($F_{(2, 12)} = 2.03$, $p = .17$), injection group ($F_{(1, 6)} < 1$, *n.s.*), and interaction between the two variables ($F_{(2, 12)} < 1$, *n.s.*). The results indicate that rats remembered the task well after the cannula-implantation surgery and vehicle injections in the hippocampus did not affect performance.

From post 3 onwards, two ambiguous contexts (A' and B') were presented with standard contexts A and B. For ambiguous contexts, rats were required to either generalize ambiguous contexts to the representation of the similar standard contexts (e.g., generalize Context A' to Context A) or disambiguate these from dissimilar contexts (e.g., disambiguate Context A' from Contexts B and B'). The role of the dorsal hippocampus was assessed by contrasting the SAL group's performance against the MUS group's performance during post 3 and 4. During this period, muscimol was bilaterally injected in the MUS group, but saline was injected to the SAL group (Fig. 6B). The SAL group maintained a high level of performance (75-85%) during this period, whereas the MUS group showed marked impairment in performance (near chance level). A repeated-measures ANOVA revealed that there was only a significant effect of group ($F_{(1, 6)} = 242.5$, $p < .001$), no effect of testing day (post 3 and post 4) ($F_{(1, 6)} = 2.5$, $p = .17$), and no significant interaction between the two ($F_{(1, 6)} = 2.7$, $p = .15$). In addition, post-hoc comparisons showed that the performances of both injection groups were significantly different across post 3 and post 4 (all p's < .001). There was a trend toward an increase in performance in the SAL group ($F_{(1, 6)} = 5.2$, $p = .06$) but not in the MUS group ($F_{(1, 6)} < 1$, *n.s.*). The results indicate that when the dorsal hippocampus is inactivated rats not only fail to retrieve learned associations between standard contexts and rewarding locations but also are unable to learn new associations of ambiguous contexts with rewarding locations.

To eliminate any residual effects of muscimol, both groups were allowed 3 days of rest after post 4. From post 5 to post 6, rats were tested again with mixed contexts. This time, however, saline was administered to both groups. After three days of rest, the SAL group showed a high performance level across post 5 and post 6. The saline-injected MUS group showed an increase in performance between post 5 (68% correct) and post 6 (76% correct) (Fig. 6B). A repeated-measures ANOVA showed that there were significant effects of testing day ($F_{(1, 6)} = 6.9$, $p < .05$) and injection group ($\mathrm{F}_{(1, 6)} = 14.8$, $p < .001$), but no interaction of the two ($\mathrm{F}_{(1, 6)} < 1$, *n.s.*). Post-hoc comparisons showed that the MUS group's performance was below the SAL group's performance (all p's < .05). The MUS group exhibited enhanced performance when saline was injected ($F_{(1, 6)} = 5.7$, $p = .05$) but no further improvement was detected in the SAL group ($F_{(1, 6)} = 1.7$, $p = .24$). The results suggest that when the hippocampus functioned normally, rats were able to process the standard as well as ambiguous contexts.

Importantly, when standard contexts (Context A and B) and ambiguous contexts (Context A' and B') were plotted separately for more detailed analyses (see posts 3 and 4 in Fig. 6C), it is important to note that the SAL group not only improved performance further in the standard contexts but also showed some savings in performance when ambiguous contexts were presented (starting at approximately 70% for Contexts A' and B'). This contrasts with the chance performance in the ambiguous contexts exhibited by rats that received muscimol infusions in the hippocampus. When hippocampal inactivation was discontinued in the MUS group (post 5 and post 6 in Fig. 6D), however, performance returned to normal levels for standard contexts (paired samples t-test between post 2 and post 5, $t_{(6)} < 1$; *n.s.*). Furthermore, the slope of the learning curve associated with the SAL group for ambiguous context during post 3 and post 4 was also observed in the saline-injected MUS group in post 5 and post 6 as the rats tried to learn the ambiguous contexts. When the performance enhancement in ambiguous contexts (between post 3 and post 4 in the SAL group and between post 5 and post 6 in the MUS group) was compared between injection groups, there was no significant difference (all *p's =n.s.*).

As shown with hippocampal lesions in Experiment 1, hippocampal inactivation also resulted in perseverative behavior of choosing the food well on a particular side throughout the session (post 3 and post 4 in Fig. 6D). Specifically, in contrast to the SAL group, the MUS group showed a near-chance level of performance (51% for both post 3 and post 4, respectively) when muscimol was injected. During this time, the response bias was significantly higher in the MUS group than in the SAL group (Fig. 6D; $F_{(1, 6)} = 31.2$, $p < .01$ and all *p*'s from post-hoc comparisons < .05). This perseveration decreased during post 5 and post 6 as saline was injected, with a significant main effect of testing day (all *p's* from post-hoc pair-wise comparisons between post 4 and post 5, post 4 and post 6, and post 5 and post 6 were less than .05). The response bias overall appeared to remain higher in the MUS group during the saline-injection period, although this trend was not statistically significant (all *p's* > .05). This may indicate that the response strategy adopted during muscimol inactivation might have been carried over to post 5 and post 6 when saline was injected.

Probe task with partial cues

In partial cue-conditions in which only a single distal cue per cue-curtain remained (yielding two distal cues in the testing room), both SAL and MUS groups were tested with saline injections. Both groups were equally impaired in performing the task (Fig. 7). An ANOVA showed that the presence of entire cues made a significant difference in performance ($F_{(1, 6)}$ = 33.1, $p < .01$). Post-hoc comparisons using one-sample t-test revealed that the performances of both groups were not significantly different from the chance level (50%) when only partial cues were presented (all *p'*s > .05). The results suggest that rats were heavily dependent upon the distal cue-configurations created by cue-curtains in the current task.

Discussion

In our study, context is defined by the spatial configuration of two sets of distal cues and context was associated with a particular location in space. Our results showed that rats with dorsal hippocampal lesions were severely impaired in identifying a spatial location based on the configural relationships between distal cues, even when the two distal cue-sets were far apart as in Experiment 1. In Experiment 2, we have shown that the capability for associating similar contexts with a common spatial location was blocked by muscimol injection into the hippocampus, whereas saline-injected animals demonstrated significant savings in initial learning of the ambiguous contexts. The results suggest that manipulating the physical distance between different sets of distal cues is an effective way of varying the context while maintaining all other variables in the environment constant.

To perform well in the current study, two components in the task need to be processed correctly. Specifically, rats first need to identify the configuration of distal cues (i.e., contextual component) and then must retrieve its associated food-well location (i.e., context-place paired associative component). Computational processes for pattern completion and pattern separation (Lee et al., 2004; Leutgeb et al., 2004; O'Reilly and McClelland, 1994; Rolls and Kesner, 2006; Vazdarjanova and Guzowski, 2004) may have relevance to the task components mentioned above. In a previous study (Lee et al., 2004), Lee and his colleagues introduced different amounts of mismatch to the configural relationships between proximal and distal cues around the maze and recorded ensembles of CA1 and CA3 neurons. In response to altered environments, CA3 neurons exhibited firing patterns similar to the ones associated with the original environment, whereas no similar firing pattern was observed in CA1 neurons. This so-called pattern completion or generalization may also be an underlying computational process in the current task. That is, Contexts A and A' were physically more similar to each other than to Contexts B and B'. If the hippocampal network generalized (or pattern-completed) the representation for Context A' into the representation for Context A when Context A' was first introduced, the rat's performance should not start from chance level (50%) and there should be some enhancement in performance. The same prediction can be made for Context B'. Our study demonstrated that control rats exhibited 75% performance level when the ambiguous contexts (Contexts A' and B') were presented from the start, whereas muscimol-injected animals showed exhibited chance-like performance. The results strongly suggest that the hippocampus is crucial for generalizing and disambiguating similar and dissimilar contexts, respectively.

The response bias score for the HP-LES group was found approximately at .5 (Fig. 3B) and this was smaller than the typical response bias such as the one observed in Experiment 2 (approximately at .8 as shown in Fig. 6D). This was due to the fact that lesioned rats tended to choose the food well on a particular side (e.g., left food well) during some part of trials and then switched their response bias to the opposite food well (e.g., right food well). This raised overall performance slightly above the chancel level when performances for all trials were averaged at the end of the session. In contrast, rats with muscimol inactivation in Experiment 2 rarely changed the direction of response bias throughout the session, which resulted in performance nearly at chance level. This qualitative difference in behavior between the lesioned rats and the muscimol-inactivated rats could be measured (i.e., percent shift) by calculating the proportion of the number of shifts in response (between selections for left and right food wells) in a given behavioral session (Fig. 8). When percent shifts for four groups (CTRL, HP-LES, SAL, and MUS) were compared with one-way ANOVA, there was a significant effect of group ($F_{(3,21)} = 26.3$, $p < .001$). Bonferroni *post-hoc* comparisons revealed that two control groups (CTRL and SAL) did not significantly differ from each other. However, both HP-LES and MUS groups showed significantly different proportional shifts as compared to control groups (*p'*s for both CTRL and SAL groups < .01). Importantly, the HP-LES group made significantly more frequent shifts between left and right food wells than did the MUS group ($p < .05$). It is unclear, however, why this difference in perseverative behavior was observed between the HP-LES group and the MUS group. One possibility is that lesions of the hippocampus made the animals less perseverative than muscimol-inactivated rats because other brain regions (e.g., prefrontal cortex, dorsal striatum) might try to learn the task in the absence of the hippocampus. The existence of the hippocampus in the MUS group, however, might have prevented other regions from performing the similar function.

Compared to the savings in performance observed in controls when learning ambiguous contexts, however, it is interesting that in the MUS-injected group learning those contexts started nearly at chance level when muscimol was discontinued (post 5) and saline was injected instead. This may be due to the development of strong response strategy during the MUS-injection period (post 3 and post 4) as shown in Figure 6D. Upon discontinuation of muscimol, the animals had to unlearn the irrelevant strategy adopted during the muscimol-injected period and switch to the relevant, context-dependent strategy. Careful examination of their learning curves in Figure 6C and 6D suggests that the animals in the MUS group injected with saline from post 5 unlearned the response strategy from post 5 and showed normal learning rate for the ambiguous contexts between post 5 and post 6 (as observed for control rats between post 3 and post 4).

The significantly reduced performance in the partial-cue conditions after saline injections in both SAL and MUS groups suggests that the rats used all cues during the task. Performance was approximately 10% greater than chance-level. It is unclear why the performance did not drop to 50% in the probe conditions. One possibility is that the rats may have been able to utilize the spatial relationship between the two distal cues remaining on the curtains. If that was the case, one may imagine it would be extremely hard to discern the angular distance between two single cues as compared to performing the same task with more cue sets. Another possibility is that the rats might have used the relationships between the single cues left on the curtains and fixed cues in the environment such as the middle arm of the maze. It is also possible that the ventral hippocampus, which was left intact in our study, was responsible for performance (Hobin et al., 2006; Rudy and Matus-Amat, 2005). Although our probe test could not pinpoint the source of the residual performance, it is nonetheless important to note that distal cues were used in their entirety in the current task, presumably for extracting contextual information from configural relationships.

The concept of context has been used by many researchers who ascribe different meanings and connotations to the term. In addition to the frequently used definition of multisensory cues in the environment (Dellu et al., 1997; Good and Bannerman, 1997; Good and

Honey, 1991; Kim and Fanselow, 1992; Moses et al., 2007; Mumby et al., 2002; Piterkin et al., 2008; Rajji et al., 2006; Winocur and Olds, 1978), the term has been used to refer to the configuration of distal and/or local cues (Anderson et al., 2006; Hayman et al., 2003; Lee et al., 2004; Rudy and Sutherland, 1995; Shapiro et al., 1997) as well as internal, motivational state or task demand (Hirsh, 1974; Kennedy and Shapiro, 2004; Smith and Mizumori, 2006). Although it is possible that the hippocampus may be responsible for processing all these different types of stimuli and their relationships, it would be helpful to develop more sophisticated subdivisions or terms to bring this area closer to more rigorous electrophysiological investigation. Therefore the current study operationally defined the term context as the spatial configuration of distal, visual cue-sets. This definition also reflects the long-held view that the place cell in the hippocampus uses spatial relationships between distal stimuli in the environment to represent a certain location in space (Muller and Kubie, 1987; O'Keefe and Nadel, 1978). Compared to the traditional idea that context means static background behind a target event (Hirsh, 1974), the role of context in our study was not tested through its modulatory effect on the performance of a target behavior. Instead, the context formed by distal cue-sets determined where the animal needed to go in space to find reward. It would be interesting to investigate in future studies whether the hippocampus is differentially recruited when context in the environment plays a modulatory role for a target event as compared to situations when, as in the current study, context itself is directly used in connection with the target event.

Figure Legends

Figure 1. Contextual manipulations and behavioral testing environments. A. Schematic overview of the behavioral testing environment. Only the center arm of the maze was used throughout the study. Two movable cue-curtains were overlaid in front of the circular curtained background. Three visually distinctive cues were attached to each cue-curtain. By adjusting the angular distance between the cue-curtains, the configuration of distal cues was parametrically adjusted. The plus sign denotes the food-well in which reward was given and the minus sign indicates the absence of reward. Two metal discs covered both left and right food wells. *Left*: Context A – Cue-curtains were aligned along the midline of the middle arm (Angular distance = 0°). Reward was provided upon selection of the left food well. *Right*: Context B – Cue-curtains were separated by 80°, and the reward was placed in the right food well. Unused arms were depicted in gray. B. Configuration of six distal cues (Context A) attached to the cue-curtains against the background curtains.

Figure 2. Histological verifications of the dorsal hippocampal lesions. Three sections along the anterior-posterior axis are shown from both control (CTRL) and lesion (HP-LES) groups. Principal cell layers in the dorsal hippocampus were removed in the HP-LES group, whereas no damage was observed in the CTRL group.

Figure 3. Impaired performance in retrieving contextual memories. **A.** The HP-LES group was significantly impaired in performing the task after surgery, as compared to the CTRL group. **B.** The HP-LES group showed stronger response bias after surgery than the CTRL group. **C.** Simple

object discrimination task. Reward was always given under the toy figure (Barney). Both groups performed equally well in this task. Error bars indicate standard error.

Figure 4. Schematic illustration of the behavioral paradigm used in Experiment 2. The spatial configuration of distal cues was manipulated similarly as in Experiment 1. Standard contexts (Contexts A and B) were the same as in Experiment 1. Intermixed with the standard contexts were two ambiguous contexts (Contexts A' and Context B') in Experiment 2. Context A' was constructed by separating the cue-curtains 14° apart from Context A, and Context B' was made by separating the cue-curtains 14° from Context B (i.e., 66°). The arrow with gray gradients indicates the parametric changes in angular distance between distal-cue sets (context A correlated with Context A' more closely than Context B' or B, and *vice versa*). Black and white words used for labeling context groups (black for Context A and A' and white for Context B and B') indicate discrete responses associated with the two categories of context. Reward was given in the left food well for Contexts A and A' and in the right food well for Contexts B and B'.

Figure 5. Verification of cannula tips and the visual estimation of muscimol diffusion. **A.** Positions of cannula tips in the dorsal hippocampus in all animals used in the study. **B.** *Left*: A representative photomicrograph showing bilateral cannula penetration in the dorsal hippocampi. *Right*: Spread of fluorescent muscimol in the dorsal hippocampus.

Figure 6. Performance in standard and ambiguous contexts. **A.** Overall experimental schedule for drug injection and behavioral testing in the SAL group and MUS group. STD represents the task involving standard contexts (Contexts A and B) and MIX stands for the session in which the

standard contexts were intermixed with ambiguous contexts (Contexts A' and B'). **B.** Performance of SAL and MUS groups in different testing phases: *post 1 and post 2* (testing with standard contexts only as in Experiment 1), *post 3 to post 6* (testing with standard and ambiguous contexts intermixed). Two adjacent arrowheads associated with each testing day indicate drug injection conditions with the first arrowhead representing the SAL group and the second arrowhead representing the MUS group (open arrowhead – saline injection, filled arrowhead – muscimol injection). **C.** Performance graphs (shown in B) were separately plotted for standard and ambiguous contexts in SAL and MUS groups. STD – standard contexts (Contexts A and B). AMB – Ambiguous contexts (Contexts A' and B'). Error bars indicate standard error. **D.** Response bias in different testing phases.

Figure 7. Performance in the probe task with partial cues. Trials in which only partial distal cues were presented were provided in some of the trials during the saline-injected sessions for both SAL and MUS groups. Partial cue-based performance was compared with performance in normal conditions with all cues. Both groups showed impaired performance when the distal cue-configurations were disrupted by the removal of distal cues. Error bars indicate standard error.

Figure 8. Percent shift in response direction during behavioral session. Graphs were based on the behavioral data from the CTRL group, the HP-LES group (Experiment 1), the SAL group, and the MUS group (Experiment 2). Percent shift was calculated by counting the number of directional shifts divided by the total number of trials within a session. Error bars indicate standard error.

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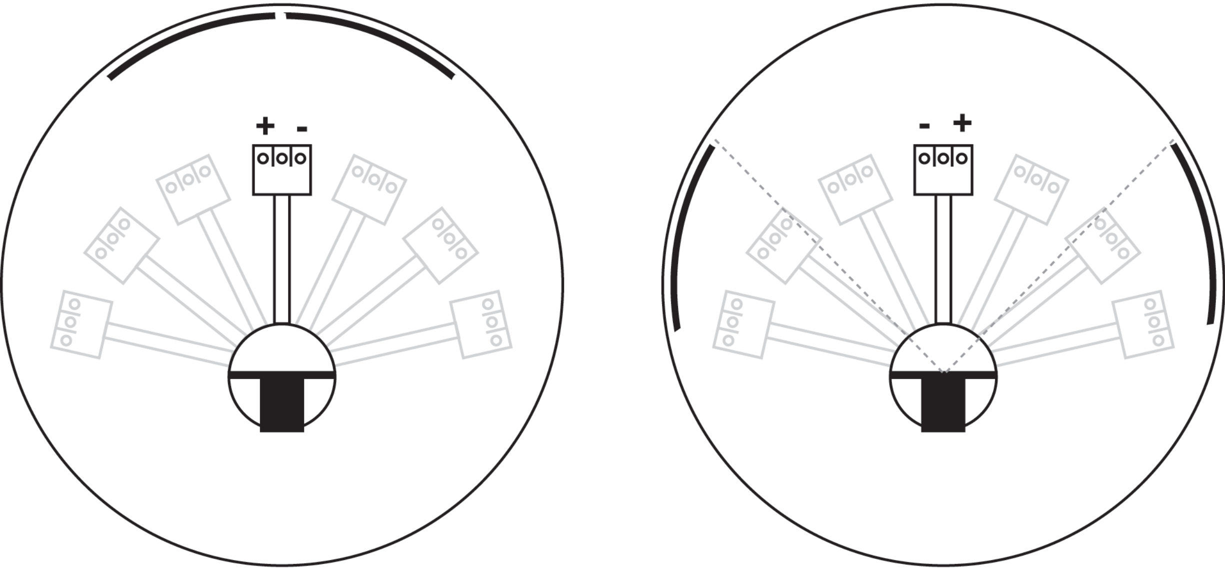
Figure 1

A.



Context A (angular distance = 0°)

Context B (angular distance = 80°)

B.

Figure 2

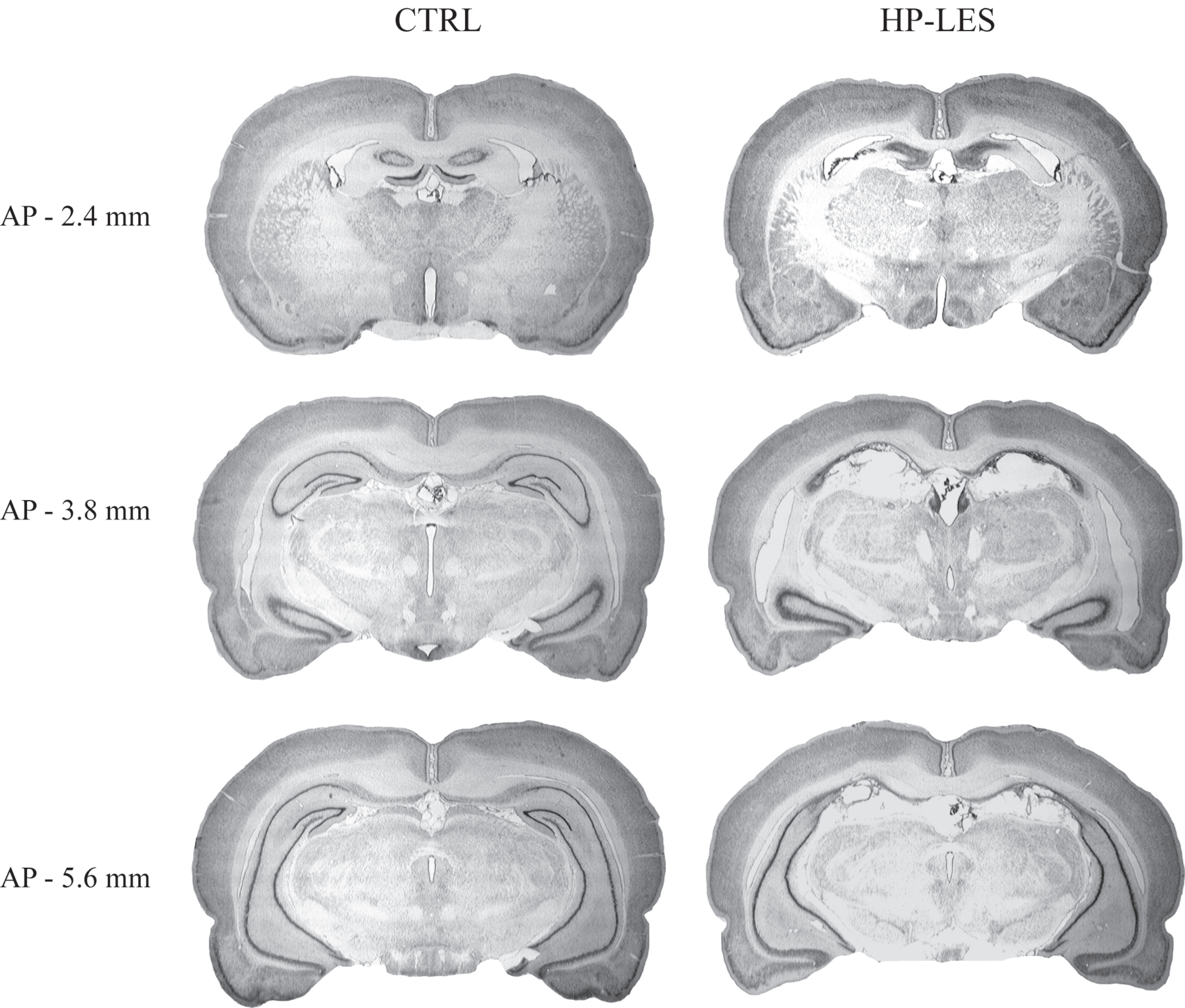
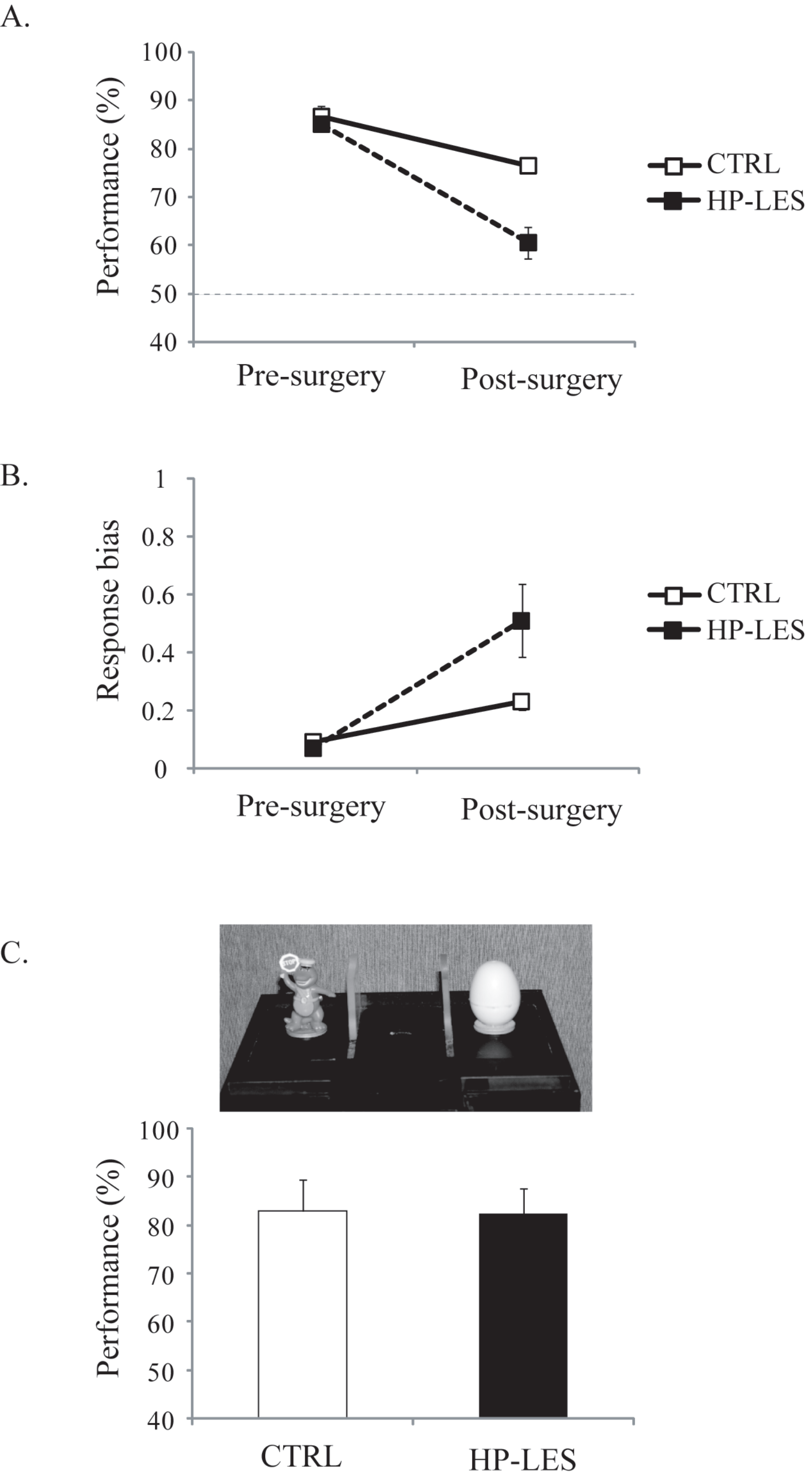


Figure 3



A.
100
90
80
70
60
50
40
Performance (%)
Pre-surgery
Post-surgery
CTRL
HP-LES
B.
1
0.8
0.6
0.4
0.2
0
Response bias
Pre-surgery
Post-surgery
CTRL
HP-LES
C.
STOP
100
90
80
70
60
50
40
Performance (%)
CTRL
HP-LES

Figure 4

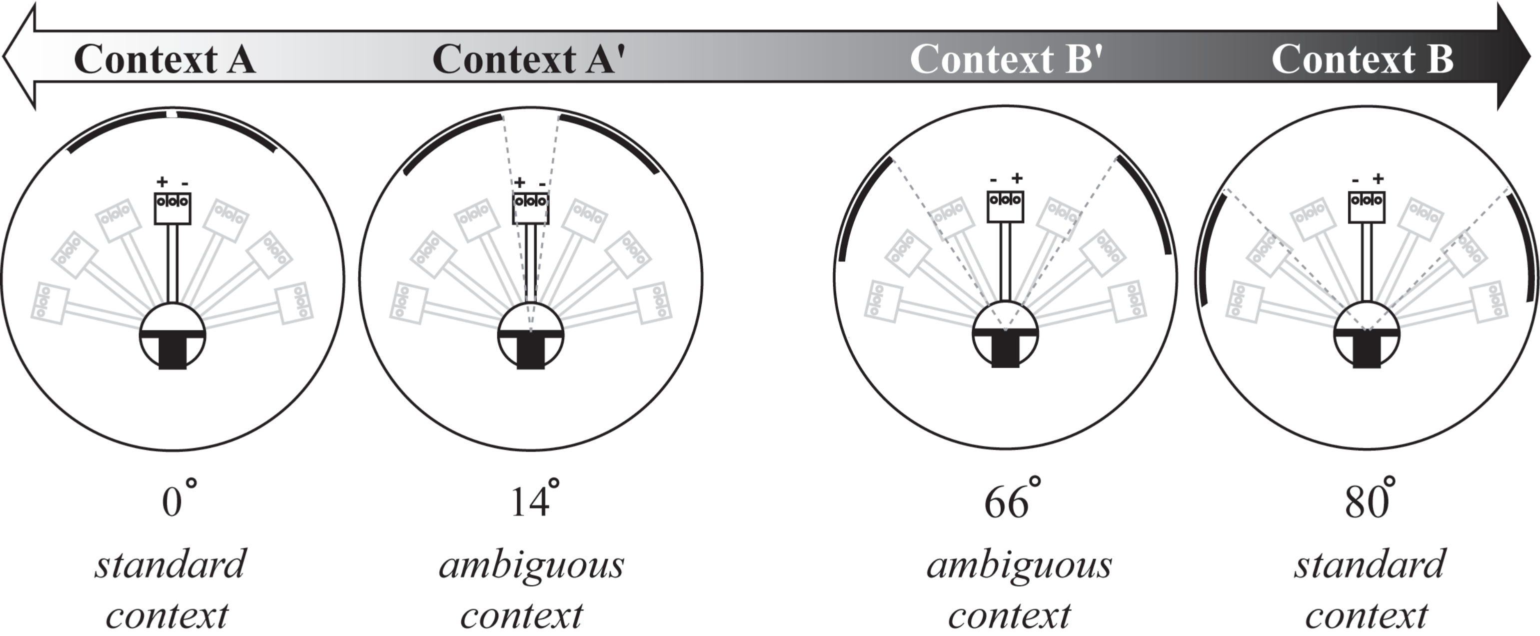

Context A
Context A'
Context B'
Context B
+ -
+ -
- +
- +
0°
standard context
14°
ambiguous context
66°
ambiguous context
80°
standard context

Figure 5

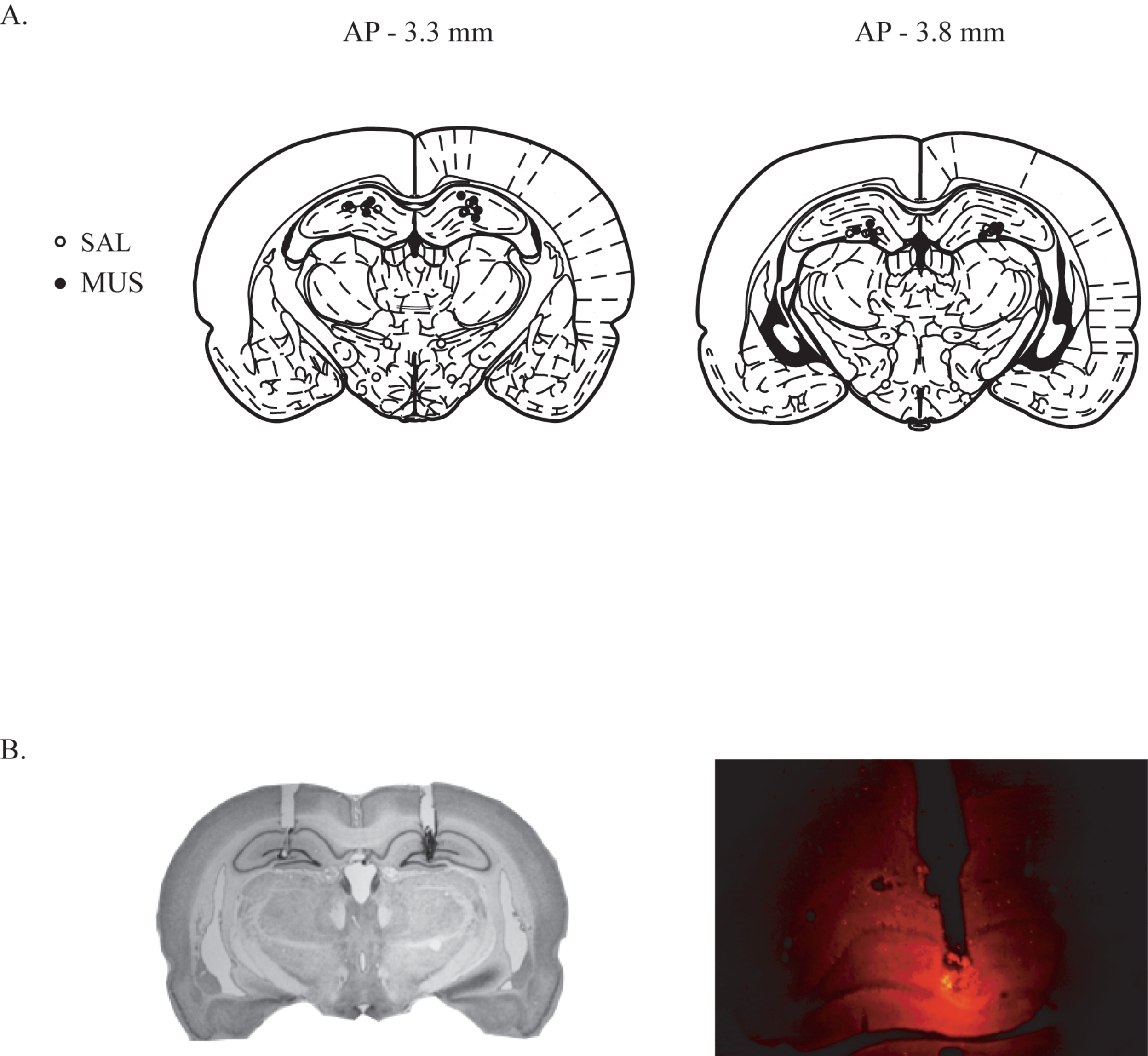


Figure 6

A.

Block		Pre-surgery	Post1	Post2	Post3	Post4	Post5	Post6
Context		STD	STD	STD	MIX	MIX	MIX	MIX
Drug Injection	SAL-group	N/A	N/A	SAL	SAL	SAL	SAL	SAL
	MUS-group	N/A	N/A	SAL	MUS	MUS	SAL	SAL

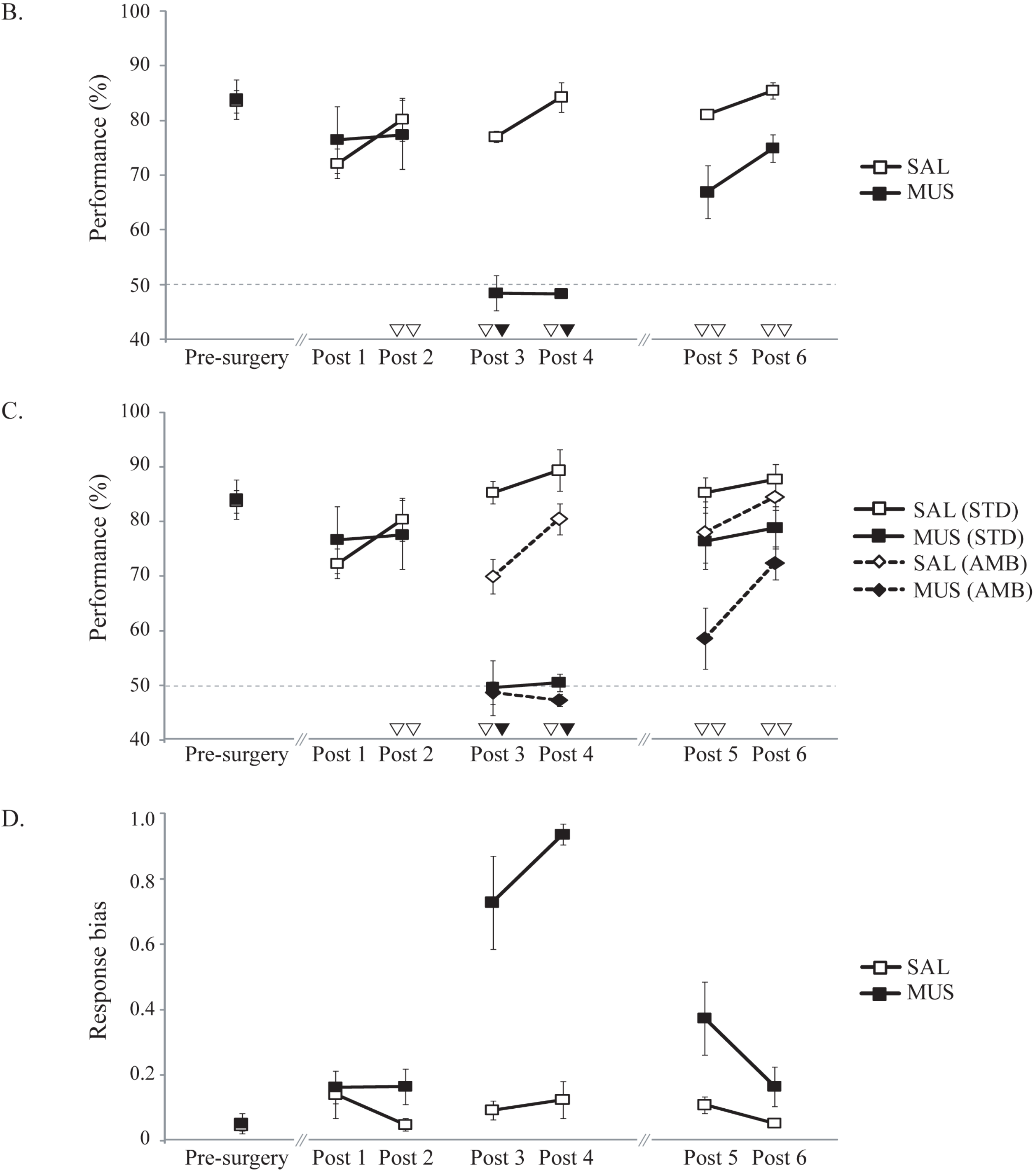


Figure 7

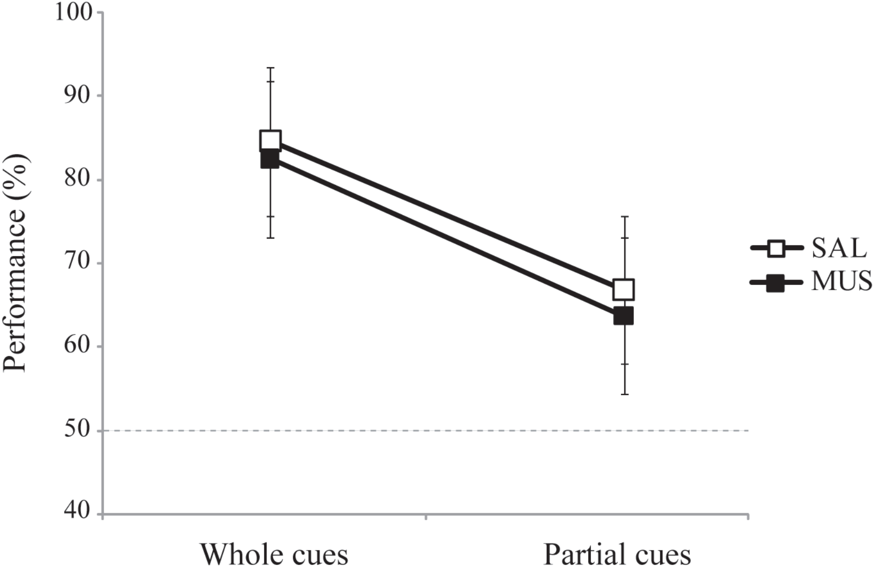
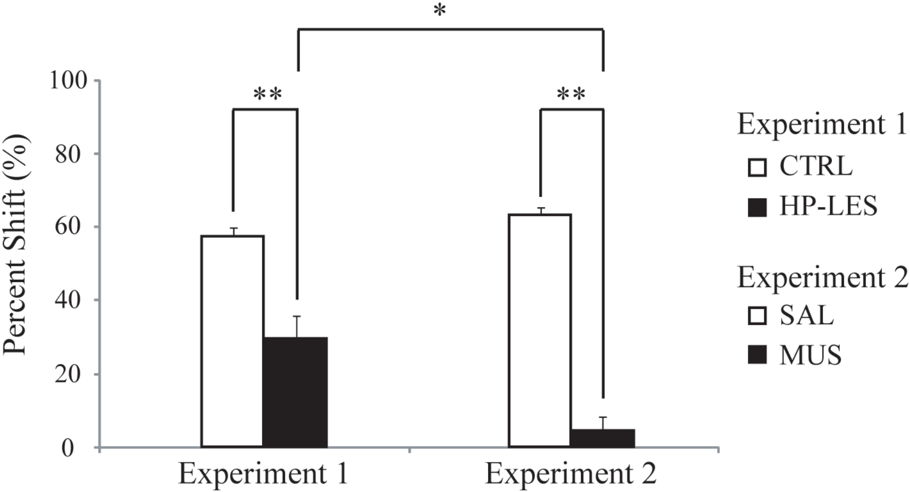

Performance (%)
100
90
80
70
60
50
40
Whole cues
Partial cues
SAL
MUS

Figure 8


*
**
**
Percent Shift (%)
100
80
60
40
20
0
Experiment 1
Experiment 2
Experiment 1
□ CTRL
■ HP-LES
Experiment 2
□ SAL
■ MUS