Published in final edited form as:

Hippocampus. 2010 January ; 20(1): 208–217. doi:10.1002/hipo.20610.

The hippocampus contributes to memory expression during transitive inference in mice

Loren M. DeVito*, Ben Kanter*, and Howard Eichenbaum*

*Center for Memory & Brain, Boston University, Boston, MA, 02215

Abstract

There is substantial evidence that the hippocampus plays a role in transitive inference, the capacity to link overlapping memories and subsequently make novel judgments between elements of those memories that are only indirectly related. However, it is unclear whether the hippocampus is involved primarily during the original acquisition of the overlapping memories, or additionally during the flexible expression of those memories during transitive judgments. Here, we demonstrated that selective hippocampal damage produced after acquisition of the overlapping memories resulted in a severe impairment in subsequent transitive inference judgments, indicating that the hippocampus does play an important role beyond the initial learning phase. Furthermore, this study extends to mice a role for the hippocampus in transitive inference, as previously observed in other species.

Keywords

relational memory; odor learning; post-training; lesions; retrograde amnesia

Introduction

Transitive inference highlights a fundamental feature of relational memory, the ability to integrate experiences that share overlapping elements and then use this consequent relational network to guide novel judgments about elements that are related only indirectly within the network. Two variants of the transitive inference task have been used in neurobehavioral studies. One task involves the initial training on a series of “premises” posed as choices that form an orderly hierarchy, e.g., choose stimulus A over B, B over C, C over D, and D over E (Dusek & Eichenbaum, 1997; Heckers et al., 2004). Subsequently, the critical test for the hierarchical network involves assessment of the transitive choice of B over D. The other task involves learning multiple premises posed as associations that share common elements, e.g., A goes with B and B goes with C (Bunsey & Eichenbaum, 1996; Buckmaster et al., 2004); in this variant, subsequent testing for the relational network involves assessing the transitive association between indirectly related elements (A goes with C). In both versions of the task, training consists of rewarding the correct pairs or associations and then subsequently tests the ability to infer relationships between the indirectly related items by presenting unrewarded probe tests.

Rats and monkeys with damage to the hippocampus (Bunsey & Eichenbaum, 1996), fornix, or perirhinal and entorhinal cortex (Dusek & Eichenbaum, 1997; Buckmaster et al., 2004)

Corresponding author: Howard Eichenbaum, PhD, Center for Memory & Brain, Boston University, Boston, MA 02215, Phone: (617) 353-1426, Fax: (617) 353-1414, hbe@bu.edu.

*part of these results were presented previously at the Society for Neuroscience 2007 meeting.

can learn the overlapping premises but are severely impaired in subsequent transitive inference judgments about indirectly related elements in those memories. These findings suggest that the hippocampus is not required for the acquisition of overlapping memories, but is required for the ability to infer relations among them. On the other hand, it is possible that animals with hippocampal damage use an alternative representational strategy to learn the premise pairs during acquisition, but that strategy does not support the flexible use of this information in the probe tests. Consistent with this view, Frank et al. (2003) argued that the hippocampus contributes by altering the associative weights of individual stimulus elements during learning and does not participate during the expression of transitive inferences, and Greene et al., (2006) observed that hippocampal activation during premise learning predicted subsequent success in transitive inference expression in humans.

However, functional imaging studies have shown that the hippocampus is also activated during transitive inference judgments in humans (Heckers et al., 2004; Preston et al., 2004; Greene et al., 2006; Zalesak & Heckers, 2009). These observations suggest that the hippocampus may be directly involved during the inferential testing phase in at least in some circumstances, but imaging studies do not tell us whether the hippocampus is involved in the transitive judgment *per se* or instead is activated during retrieval of the original pairs that occurs during the transitive judgments. Therefore, these results leave open the question of whether the hippocampus is crucial only during initial premise learning or, additionally, during expression of transitive inference.

Here we tested whether selective damage to the hippocampus produced after training on the premises results in impairment on subsequent transitive inference judgments, employing the same hierarchical series variant of the task previously employed to assess the effects of hippocampal region damage produced prior to premise learning in rats. In addition, we examined this issue in mice in order to extend the range of species in which the role of the hippocampus in this form of flexible memory expression has been explored.

Materials and Methods

Subjects

Male C57 Bl6 mice were purchased from the Charles River Lab. All animals were maintained on a reverse 12-hour light/dark cycle [09:00 off; 21:00 on]. Animals were given *ad libitum* access to food and water, unless otherwise specified in the behavioral methods. Twenty animals were used in this study: ten animals received lesions of the hippocampus and ten served as sham-operated controls. The IACUC of Boston University approved the treatment and use of the animals in these experiments.

Surgery

In order to target the hippocampus, bilateral lesions were made using NMDA or sterile PBS for sham operations (Sigma, 10 mg/mL) delivered via a microinfusion pump connected to a 5- μ L Hamilton syringe. Fiber-sparing neurotoxic lesions were performed in order to selectively destroy cells within the hippocampus. Animals were anesthetized with a ketamine/xylazine cocktail (0.01 mL/g), and diazepam (0.02 mL) was administered pre-operatively in order to prevent seizures. After the animal was placed into a stereotaxic head frame, the skull was exposed, and the coordinates of bregma were measured. The skull overlying the four coordinates was drilled and dura was removed. Before infusions were made, the syringe was lowered 0.2 mm for the first two coordinates (dorsal hippocampus) and 0.5 mm for the last two coordinates (ventral hippocampus) past the injection site and kept a lower depth for one minute in order to increase spread of drug diffusion. The syringe was then raised to the injection site and the drug was infused over a 2-minute period (3-

minute infusion for the last coordinate). The needle was left in place for another 5 minutes before being slowly withdrawn. The complete dorsal and ventral hippocampus was targeted (including the CA fields, dentate gyrus, and subiculum) at four stereotaxic coordinates: AP + 1.7, ML +/- 1.2, DV - 1.5; AP + 2.3, ML +/- 1.75, DV - 1.75; AP + 2.8, ML +/- 3, DV - 3; AP + 3.1, ML +/- 2.85, DV - 3.75 (Franklin & Paxinos, 1997); 50 nL was infused into the first three sites and 75 nL was infused into the fourth site. In sham-operated controls, infusions were made in the same four coordinates in the same manner as the NMDA infusions, except that sterile PBS was instead delivered through the Hamilton syringe. After all infusions, the animal was sutured and given 0.4 mL of Lactated Ringer's solution and a hot water bottle in order to hydrate and return body temperature to normal. After surgery, the animal received Children's Tylenol in its water and was provided with soft food and Nutrical. Each animal was allowed two weeks to recover before returning to behavioral testing.

Behavioral Methods

Pre-operative premise pair training

The hierarchical series variant of the transitive inference task was adapted for mice from Dusek and Eichenbaum (1997). Animals were placed on food restriction and maintained at 85% of free feeding weight. They were shaped over a three-day period to dig for chocolate sprinkle rewards buried in sand that filled small plastic cups. Once they were reliably digging, subjects were given a simple olfactory discrimination of ten trials across two days in order to teach them to dig in a cup guided by the odor of the sand. All stimuli were composed at 1% concentration of odorant by weight in sand.

Following the preliminary discrimination problem, animals were trained on a series of overlapping premise pairs (A+ vs B-, B+ vs C-, C+ vs D-, and D+ vs E-; where A = paprika [CVS, Woonsocket, RI]; B = coffee [Folger's, Cincinnati, OH]; C = basil [McCormick, Hunt Valley, MD]; D = cumin [McCormick, Hunt Valley, MD]; and E = cocoa [Hershey's, Hershey, PA]; + and - refer to rewarded and non-rewarded odors, respectively). A choice was defined as a significant displacement of the sand by the mouse's paw. After the first training stage, a reward was no longer buried in the sand but instead a sprinkle was dropped onto the cup if the animal chose the correct cup. This helped prevent extinguishing during probe trials in which the cups were not baited. Training involved two 8-trial sessions per day across four training stages that began with large blocks of trials on the same premise and then involved progressively greater intermixing of premise pair presentations as illustrated in Table 1. Animals were trained to reach a criterion of 75% accuracy on each premise pair across two consecutive days (i.e., 6 out of 8 trials on each of the 4 premise pairs) at each stage of training.

Probe tests

The day after reaching criterion on the last training stage, animals were given probe tests for transitive (B vs D) and non-transitive (A vs E) pairs, novel choices between items that had not previously been presented together. Four BD and four AE probe tests were intermixed within presentations of the training premises over a two-day period such that the probe tests were presented on trials 3, 6, 11, and 14. The appropriate inferential judgment (choosing B over D) on the BD probe required that animals had linked the odor premises so that they could make the inference across the missing overlapping element C. In contrast, the appropriate choice of A over E could be made without reference to the structure of the odor premises because odor A was always rewarded and odor E was never rewarded; thus, the AE pair served as a control for the presentation of novel pairs. Neither cup was baited during probe trials. We compared the amount of time the animals spent digging in each cup as the

measure of preferential choice, using a preference index (PI) that normalized total digging time in both test cups (Bunsey and Eichenbaum, 1996). For the B vs D test, $PI = (B-D)/(B+D)$; for A vs E, $PI = (A-E)/(A+E)$, where each letter corresponds to the digging time in the cup with that odor.

Post-operative premise pair training and probe tests

Following a two-week recovery period after surgery, the animals were again placed on food restriction and maintained at 85% of free feeding weight. In order to rule out the possibility that performance on the task was affected by loss of memory of the individual premises, all animals were re-trained on Stage 4 of training until they reached pre-operative criterion levels of 75% across all premises. After reaching Stage 4, the animals were again tested on the BD and AE probes intermixed among premises across two consecutive days. In addition to comparing performance between the two groups, a within-subjects analysis between pre- and post-operative scores for each animal was also assessed to determine the extent to which each animal's level of performance changed due to assignment of surgery group.

Repeat post-operative probe tests

After completion of the probe tests, animals were given one week of rest without behavioral training. Then, they were re-tested on probe trials and premise pairs over a two-day period. These additional tests were performed in order to determine the time course of hippocampal involvement in the task, as well as to assess the ability to perform transitive inference with a delay between training and test independently of strengthening the memory of the premise pairs. In addition to comparing performance between the two groups, a within-subjects analysis between the first post-operative and second test scores for each animal was used to determine the extent to which each animal's performance changed.

Histology

After behavioral testing, all animals were given an overdose of sodium pentobarbital and perfused transcardially with 4% formalin. The brains were removed and post-fixed for an hour in formalin, and then cryoprotected in 30% sucrose solution (in 7.4 pH PBS). Coronal sections were cut (40 μ m) using a freezing microtome. Every section was mounted on gelatin-coated slides and dried overnight. Slides were soaked in xylenes and then run through a series of ethanol dehydrations, stained with cresyl violet, and then rehydrated. The extent of the lesion was determined using a light microscope to study the stained sections.

Results

Histology

NMDA infusions resulted in a substantial loss of cells within the hippocampal formation, including Ammon's horn, the dentate gyrus, and the subiculum (Figure 1A, B). An average of 57% of the hippocampus was damaged across animals, ranging from 8%-92% total ablation (Figure 1C). The extent of damage in the hippocampus was not correlated with performance on the initial BD probe test ($r = 0.04$, $p = 0.907$). Four animals also had partial unilateral damage to the medial entorhinal cortex, and some had damage to the dorsal and medial thalamus as well. Only one animal had slight amygdala damage. Both the sham injection procedure and the neurotoxic lesion resulted in some damage to parietal cortex overlying the infusions; the size of the damaged area did not differ between groups ($t_{(1,15)}=1.07$, $p = 0.318$).

Pre-operative acquisition of the premise pairs and performance on the probe tests

All twenty animals successfully acquired the four premise pair problems over an average of 26.15 $\pm$ 1.76 days (Figure 2A). As compared to Stages 1 and 2 (trend for Stage 3), a significantly greater number of trials were required to reach criterion on Stage 4 (One-Way ANOVA: $F_{(3,79)} = 2.25$, $p = 0.089$; Stage 1 v Stage 4 ($p = 0.023$); Stage 2 v Stage 4 ($p = 0.043$); Stage 3 v Stage 4 ($p = 0.058$)).

On the days in which the probe tests were administered, performance differed across the premise pairs, with the highest levels of accuracy on the DE pair (One-Way ANOVA: $F_{(3,79)} = 6.17$, $p = 0.001$; AB v BC ($p = 0.001$); BC v CD ($p = 0.006$); BC v DE ($p < 0.001$); Figure 2B). In addition, all animals performed successfully on the BD and AE probes at levels that differed significantly from chance (BD: $t_{(1,39)} = 62.41$, $p < 0.001$; AE: $t_{(1,39)} = 267.33$, $p < 0.001$; Figure 2C).

After pre-operative probe testing, the sham and hippocampal lesion groups were matched on performance, such that they were statistically indistinguishable in the number of days to reach criterion performance on all four stages of training ($t_{(1,19)} = 0.98$, $p = 0.334$), on the individual premise pairs (2-Way ANOVA: group $F_{(1,79)} = 0.63$, $p = 0.429$, premise pair $F_{(3,79)} = 6.04$, $p = 0.001$, group X pair interaction $F_{(3,79)} = 0.57$, $p = 0.636$), on BD performance ($t_{(1,19)} = 0.24$, $p = 0.627$), and on AE performance ($t_{(1,19)} = 0.88$, $p = 0.360$).

Post-operative premise pair and probe test performance

The number of days to regain pre-surgical criterion performance on Stage 4 of training did not differ significantly between the two groups ($t_{(1,19)} = 1.15$, $p = 0.234$; Figure 3A). However, on the days in which probe tests were administered, the animals with hippocampal lesions outperformed sham animals on the premise pairs (2-way ANOVA: group $F_{(1,79)} = 6.28$, $p = 0.014$, pair type $F_{(3,79)} = 11.27$, $p < 0.001$, and group X pair interaction $F_{(3,79)} = 2.88$, $p = 0.041$; Figure 3B). *Post hoc* analyses revealed significant group differences only on the AB and DE pairs (AB: $t_{(1,19)} = 4.44$, $p = 0.049$, BC: $t_{(1,19)} = 1.44$, $p = 0.245$, CD: $t_{(1,19)} = 2.48$, $p = 0.132$, DE: $t_{(1,19)} = 8.39$, $p = 0.01$). Consistent with these findings, within the sham group, performance did not significantly differ across premises (1-Way ANOVA: $F_{(3,39)} = 1.6$, $p = 0.206$). By contrast, within the group of animals with hippocampal lesions, performance did significantly differ across premise pairs (1-Way ANOVA: $F_{(3,39)} = 13.38$, $p < 0.001$) such that performance on the BC pair was significantly lower than all other pairs (AB v BC: $p < 0.001$; BC v CD: $p < 0.001$; BC v DE: $p < 0.001$).

Animals with hippocampal damage were severely impaired compared to the sham group on the transitive BD probe test ($t_{(1,19)} = 11.72$, $p = 0.003$; Figure 3C). Furthermore, the sham group performed at levels that were significantly different from chance ($t_{(1,19)} = 35.63$, $p < 0.001$), whereas animals with hippocampal lesions did not ($t_{(1,19)} = 0.00$, $p = 0.954$). A within-subjects analysis comparing performance before and after surgery revealed that sham-operated animals performed equivalently on both tests ($t_{(1,9)} = 1.40$, $p = 0.195$), whereas animals with hippocampal lesions performed less well following surgery ($t_{(1,9)} = 4.40$, $p = 0.002$). Indeed, in each animal with hippocampal damage, the PI was lower in the post-operative test than in the pre-operative test. Consequently, the pre- to post-operative difference in PI was much larger in the hippocampal lesion group than the sham group ($t_{(1,19)} = 7.85$, $p = 0.012$; Figure 3D).

Animals with hippocampal lesions outperformed sham operated animals on the non-transitive AE probe ($t_{(1,19)} = 11.59$, $p = 0.003$; Figure 3E). Both groups performed at levels that were significantly better than chance (sham: $t_{(1,19)} = 139.67$, $p < 0.001$; lesion: $t_{(1,19)} = 252.83$, $p < 0.001$). A within-subjects analysis comparing PI before and after surgery revealed that sham operated animals performed less well after surgery ($t_{(1,9)} = 2.63$, $p =$

0.027), whereas animals with hippocampal lesions performed similarly on both tests ($t_{(1,9)} = -0.03$, $p = 0.974$). The pre- to post-operative difference in PI did not differ between the two groups ($t_{(1,19)} = 2.14$, $p = 0.161$; Figure 3F). In addition, the two groups spent an equivalent amount of time digging in the probe cups, indicating similar levels of activity and motivation ($t_{(1,131)} = 2.31$, $p = 0.129$).

Repeat post-operative probe tests

On the second post-operative tests, group performances did not differ on the premise pairs (2-Way ANOVA: group $F_{(1,75)} = 0.30$, $p = 0.585$, pair $F_{(3,75)} = 15.79$, $p < 0.001$, and group X pair interaction $F_{(3,75)} = 0.65$, $p = 0.584$; Figure 4A). Within both groups, performance levels differed across premise pairs, with both groups performing lowest on the BC pair (sham: $F_{(3,39)} = 6.80$, $p = 0.001$; AB v BC: $p = 0.001$, CD v BC: $p < 0.001$, DE v BC: $p = 0.002$; lesion: $F_{(3,35)} = 9.16$, $p < 0.001$; AB v BC: $p < 0.001$, CD v BC: $p < 0.001$, DE v BC: $p < 0.001$).

Group performances on the BD pair did not significantly differ ($t_{(1,18)} = 0.00$, $p = 0.924$; Figure 4B), although the sham group performed at a level that was significantly different from chance ($t_{(1,18)} = 13.56$, $p = 0.002$), whereas performance of the hippocampal lesion group was only marginally above chance ($t_{(1,18)} = 4.24$, $p = 0.055$). Thus, animals with hippocampal lesions were less impaired on retest than in the initial post-operative testing on transitive inference. Within-subjects comparisons revealed that the PI scores of neither the sham group ($t_{(1,9)} = 0.16$, $p = 0.869$) nor the hippocampal lesion group ($t_{(1,9)} = -1.73$, $p = 0.121$) differed significantly between the test and re-test after surgery. However, the test to re-test difference in PI was significantly different between the two groups ($t_{(1,18)} = 12.83$, $p = 0.002$; Figure 4C).

Animals with hippocampal lesions again performed at higher levels than the sham group on AE ($t_{(1,18)} = 5.89$, $p = 0.027$; Figure 4D). Both groups performed at levels that were significantly different from chance (sham: $t_{(1,18)} = 142.93$, $p < 0.001$; lesion: $t_{(1,18)} = 398.30$, $p < 0.001$). Within subjects comparisons showed that the PI scores of neither the sham group ($t_{(1,9)} = -0.69$, $p = 0.507$) nor the hippocampal lesion group ($t_{(1,9)} = -0.29$, $p = 0.777$) differed significantly between the post-operative test and re-test. The test to retest difference in PI was not significantly different between the two groups ($t_{(1,18)} = 0.51$, $p = 0.484$; Figure 4E).

Discussion

Although previous studies have indicated that the hippocampus plays an important role in transitive inference, whether its role was in the initial learning of premises or in the expression of the inferences remained unclear. Here, we show for the first time that, even following the completion of premise training and demonstration of the existence of a relational representation in successful transitive inference, damage to the hippocampus produced a significant deficit in subsequent transitive inference judgments. Previous studies have shown that surrounding cortical structures are important for relational memory. The present results indicate that the hippocampus itself is essential in this circuit but that damage to the hippocampus can be rescued by recruitment of these surrounding regions, as demonstrated by partial recovery of function after one week. These results show for the first time that, regardless of prior training, the hippocampus is needed to guide flexible memory processes.

Animals with hippocampal damage showed enhanced performance on end-anchored premises and AE probes

Interestingly, animals with hippocampal lesions outperformed sham-operated animals on the AB and DE premise pairs, and performed better on all other premise pairs than on the BC pair, a pattern not seen in the sham-operated group. One possible explanation is that animals without a hippocampus are more strongly guided by the consistent reinforcement associations of the end anchor stimuli A (always rewarded) and E (never rewarded), whereas intact animals are also guided by the internal stimuli (B, C, and D) that have reinforcement contingencies dependent upon the other stimulus with which they are paired. This explanation is supported by the additional observation of superior performance of animals with hippocampal lesions on the AE (non-transitive) probe, wherein both reliably reinforced and unreinforced stimuli are presented. These findings are consistent with the view that, whereas relations among memories of stimulus elements guide normal animals, animals without an intact hippocampus rely primarily upon hippocampal-independent reinforcement associations for individual stimulus elements or configurations (Eichenbaum et al., 1992). Since the learning of the premise pairs occurred prior to hippocampal damage, the present findings suggest that animals initially learned both the reinforcement associations of individual stimuli and the relations among those stimuli, and that the hippocampal lesions eliminated the relational representations as observed in subsequent testing. Enhanced performance on end-anchored premises and probes was not observed in previous studies (Dusek & Eichenbaum, 1997; Buckmaster et al., 2004). This could be either a consequence of the time at which the lesion was made, such that post-training lesions exacerbate the differences between individual and relational representations, or a species-selective effect.

Partial recovery of BD performance in animals with hippocampal damage at the repeat test

The data from the probe tests administered one week after the original test demonstrates the importance of the hippocampus even after a delay in exposure to the probe pairs. Since the probe tests are never rewarded, improvement in performance indicates that there was a partial recovery in relational memory abilities. The recovery was only partial because the hippocampal group did not perform at levels that were significantly different from chance. Also, abnormally elevated scores on AE persisted during the repeat test, indicating that damage to the hippocampus results in a lasting change in representation of the item associations, which may reflect a different strategy used to direct behavior in novel situations. Since surrounding cortical areas, such as the entorhinal cortex, have been implicated in relational memory, it is possible that, over time and repeated testing, these cortical regions may compensate for the loss of hippocampal function.

This study is the first to test transitive inference abilities in animals after a significant delay between training and test. Ellenbogen et al. (2007) reported that delay between training and testing, as well as sleep, resulted in relational memory ability that correlated significantly with the amount of delay. These results suggest that a time delay between training and testing is required for developing a relational memory organization. However, Titone et al. (2004), as well as Greene et al. (2006), found that subjects trained and tested on transitive inference in one session were able to exhibit significant relational memory. In the present study the delay between training and inference testing was much longer than the delays examined in Ellenbogen et al. (2007), making it impossible to examine whether organizational changes like those reported in that study occurred in our mice. Nevertheless, our findings from the second post-operative test suggest that, in the absence of hippocampal function, strengthening of the associations between related items may gradually occur within cortical systems in support of hierarchical binding.

How does the hippocampus contribute to transitive judgments?

Previous experimental and theoretical studies have concluded that the hippocampus should play a role in the integration of information from premise pair training into a relational network of memories (Eichenbaum, 1997, 2004; Wallenstein et al., 1998; Eichenbaum et al., 1999; Greene et al., 2006; Siekmeier et al., 2007). Other empirical and theoretical efforts have suggested instead that the hippocampus adjusts weights for individual items during learning and this information is integrated into cortical representations (Frank et al., 2003; Van Elzaker et al., 2003). These views differ on how the hippocampus serves its role in integrating representations of the ambiguous stimulus elements, but they agree that its role occurs during the development of representations and that the ability for transitive inference is an emergent property of that integrated representation.

These considerations suggest that the hippocampus should not be essential to the transitive judgments *per se*. Nevertheless, functional imaging studies have shown hippocampal activation during transitive inference judgments (Heckers et al., 2004; Preston et al., 2004; Greene et al., 2006; Zalesak & Heckers, 2009). One possible explanation for these findings is that the hippocampus is engaged in the processing of the novel experience with transitive pairings, much as it is engaged with processing any novel experience (Stern et al., 1996) or reflects the retrieval of memories of the previously learned items and pairings. However, Heckers et al. (2004) observed that the amount of activation was greater when subjects were exposed to novel judgments about transitively related pairs compared to novel stimulus pairings in which a choice could be made without transitive inference. Furthermore, Zalesak & Heckers, (2009) found that the magnitude of hippocampal activation scaled with the degree of relational processing required in a larger (A-F) hierarchical network. These findings suggest that the hippocampus plays a role during the transitive judgment itself. The present observations support that view and show that the role of the hippocampus is significant for novel transitive judgments, while not important for novel judgments that can be supported without transitive inference.

What role might the hippocampus play during transitive judgments? Potentially important clues may come from the observations on enhanced performance on end-anchored judgments, on post-operative training, and on comparison between initial and repeat post-operative testing. The enhancement of performance on end-anchored pairs, as discussed above, likely reflects a shift in the representations guiding performance on premise pairs and the end-anchored novel probe AE, and this change could have also eliminated the representation guiding novel transitive judgments. It is notable that 5-8 days of retraining were required for both groups to post-operatively re-reach criterion performance on the premise pairs. The distinct representational formats may have been enhanced in this period, such that intact animals re-established a relational representation whereas animals with hippocampal damage exacerbated their representations of the individual pairings. Another possibility is suggested by the observation that animals with hippocampal damage significantly improved on re-testing in the post-operative period, such that there was no longer a significant impairment on the second test. It is possible that animals with hippocampal damage did not fully re-integrate the premise pairs during post-operative retraining; this would explain why they failed in the transitive probe BD. However, the cortical network might have incorporated that further experience with the premise and probe pairs during the one-week inter-test period, thereby mediating a recovery of transitive ability.

On the evolution of transitive inference and hippocampal function

The capacity for transitive inference has been previously shown in humans (Piaget, 1928; Bryant & Trabasso, 1971; Greene et al., 2001; Smith & Squire, 2005; Titone et al., 2004),

monkeys (McGonigle & Chalmers, 1977; Buckmaster et al., 2004), rats (Dusek & Eichenbaum, 1997), birds (Paz-y-Mino et al., 2004), and fish (Grosenick et al., 2007). Previous studies on mice have reported mixed results on whether this species is capable of associative inference (Ohta et al., 2002; Van Dijk et al., 2008) and one study has reported that mice show transitive inference in the hierarchical series variant of the task (DeVito et al., in press), but none of the studies on mice examined the effects of hippocampal damage. Here we confirm that mice are capable of learning a hierarchical series of premises and demonstrate transitive inference between indirectly related elements of the series. Furthermore, the present findings show that, as in monkeys and rats, the hippocampus plays an important role in transitive inference in mice. Also, the present findings extend to transitive inference evidence that the hippocampus plays a critical role in other tasks that demand relational memory in mice (Rondi-Reig et al., 2001; Etchamendy et al., 2003; Rajji et al., 2006; Mingaud et al., 2007). Since both the evolution and function of the hippocampus is highly conserved across species and the ecological relevance of this particular aspect of episodic memory has been demonstrated, it is very likely that this type of flexible modulation and integration of associated items is an essential feature of information processing that is crucial to understanding the overall mnemonic function of the hippocampus (Suzuki & Clayton, 2000; Manns & Eichenbaum, 2006). Furthermore, the demonstration of robust transitive inference in mice presents an important opportunity to explore the putative contributions of different genes and receptors to relational memory using transgenic animals.

Acknowledgments

Thanks to Christine Lykken for assistance with processing histology.

Conte Center for Schizophrenia Research NIH MH60450

References

- Bryant P, Trabasso T. Transitive inferences and memory in young children. *Nature*. 1971; 232:255–257. [PubMed: 4937078]
- Buckmaster C, Eichenbaum H, Amaral D, Suzuki W, Rapp P. Entorhinal cortex lesions disrupt the relational organization of memory in monkeys. *The Journal of Neuroscience*. 2004; 24:9811–9825. [PubMed: 15525766]
- Bunsey M, Eichenbaum H. Conservation of the hippocampal memory function in rats and humans. *Nature*. 1996; 379:255–257. [PubMed: 8538790]
- DeVito LM, Konigsberg R, Lykken C, Sauvage M, Young WS 3rd, Eichenbaum H. Vasopressin 1b receptor knockout impairs memory for temporal order. *The Journal of Neuroscience*. In press.
- Dusek J, Eichenbaum H. The hippocampus and memory for orderly stimulus relations. *Proceedings of the National Academy of Sciences USA*. 1997; 94:7109–7114.
- Eichenbaum H. Hippocampus: Cognitive processes and neural representations that underlie declarative memory. *Neuron*. 2004; 44:109–120. [PubMed: 15450164]
- Eichenbaum H. Declarative memory: Insights from cognitive neurobiology. *Annual Review of Psychology*. 1997; 48:547–572.
- Eichenbaum, H.; Cohen, N.; Otto, T.; Wible, C. Memory representation in the hippocampus: Functional domain and functional organization. In: Squire, L.; Lynch, G.; Weinberger, N.; McGaugh, J., editors. *Memory: Organization and Locus of Change*. New York: Oxford University Press; 1992. p. 163-204.
- Eichenbaum H, Dudchenko P, Wood E, Shapiro M, Tanila H. The hippocampus, memory, and place cells: Is it spatial memory or a memory space? *Neuron*. 1999; 23:209–226. [PubMed: 10399928]
- Ellenbogen J, Hu P, Payne J, Titone D, Walker M. Human relational memory requires time and sleep. *Proceedings of the National Academy of the Sciences USA*. 2007; 104(18):7723–7728.

- Etchamendy N, Desmedt A, Cortes-Torrea C, Marighetto A, Jaffard R. Hippocampal lesions and discrimination performance of mice in the radial maze: sparing or impairment of performance depending on the representational demands of the task. *Hippocampus*. 2003; 13:197–211. [PubMed: 12699328]
- Frank M, Rudy J, O'Reilly R. Transitivity, flexibility, conjunctive representations, and the hippocampus II. A computational analysis. *Hippocampus*. 2003; 13:341–354. [PubMed: 12722975]
- Franklin, K.; Paxinos, G. The mouse brain in stereotaxic coordinates. San Diego: Academic Press; 1997. p. 45-56.
- Greene A, Spellman B, Dusek J, Eichenbaum H, Levy W. Relational learning with and without awareness: transitive inference using nonverbal stimuli in humans. *Memory and Cognition*. 2001; 29:893–902.
- Greene A, Gross W, Elsinger C, Rao S. An FMRI analysis of the human hippocampus: inference, context, and task awareness. *Journal of Cognitive Neuroscience*. 2006; 18:1156–1173. [PubMed: 16839289]
- Grosenick L, Clement T, Fernald R. Fish can infer social rank by observation alone. *Nature*. 2007; 445:429–432. [PubMed: 17251980]
- Heckers S, Zalesak M, Weiss A, Ditman T, Titone D. Hippocampal activation during transitive inference in humans. *Hippocampus*. 2004; 14:153–162. [PubMed: 15098721]
- Manns J, Eichenbaum H. Evolution of declarative memory. *Hippocampus*. 2006; 16:795–808. [PubMed: 16881079]
- McGonigle B, Chalmers M. Are monkeys logical? *Nature*. 1977; 267:694–696. [PubMed: 406574]
- Mingaud F, Le Moine C, Etchamendy N, Mormède C, Jaffard R, Marighetto A. The hippocampus plays a critical role in encoding discontinuous events for subsequent declarative memory expression in mice. *Hippocampus*. 2007; 17:264–70. [PubMed: 17265459]
- Ohta A, Akiguchi I, Seriu N, Ohnishi N, Yagi H, Higuchi K, Hosokawa M. Deterioration in learning and memory of inferential tasks for evaluation of transitivity and symmetry in aged SAMP8 mice. *Hippocampus*. 2002; 12:803–810. [PubMed: 12542231]
- Paz-y-Mino C, Bond A, Kamil A, Balda R. Pinyon jays use transitive inference to predict social dominance. *Nature*. 2004; 430:778–781. [PubMed: 15306809]
- Piaget, J. Judgment and Reasoning in the Child. Kegan; Paul; Trench; Trubner, editors. London: 1928.
- Preston A, Shrager Y, Dudukovic N, Gabrieli J. Hippocampal contribution to the novel use of relational information in declarative memory. *Hippocampus*. 2004; 14:148–152. [PubMed: 15098720]
- Rajji T, Chapman D, Eichenbaum H, Greene R. The role of CA3 hippocampal NMDA receptors in paired associate learning. *Journal of Neuroscience*. 2006; 26:908–915. [PubMed: 16421310]
- Rondi-Reig L, Libbey M, Eichenbaum H, Tonegawa S. CA1-specific N-methyl-D-aspartate receptor knockout mice are deficient in solving a nonspatial transverse patterning task. *Proceedings of the National Academy of Sciences*. 2001; 98:3543–3548.
- Siekmeier P, Hasselmo M, Howard M, Coyle J. Modeling of context-dependent retrieval in hippocampal region CA1: Implication for cognitive function in schizophrenia. *Schizophrenia Research*. 2007; 89:177–190. [PubMed: 17055702]
- Smith C, Squire L. Declarative memory, awareness, and transitive inference. *The Journal of Neuroscience*. 2005; 25:10,138–10,146. [PubMed: 15634762]
- Stern C, Corkin S, Gonzalez R, Guimaraes A, Baker J, Jennings P, Carr C, Sugiura R, Vedantham V, Rosen B. The hippocampal formation participates in novel picture encoding: evidence from functional magnetic resonance imaging. *Proceedings of the National Academy of Sciences USA*. 1996; 93:8660–8665.
- Suzuki W, Clayton N. The hippocampus and memory: a comparative and ethological perspective. *Current Opinions in Neurobiology*. 2000; 10:768–773.
- Titone D, Ditman T, Holzman P, Eichenbaum H, Levy D. Transitive inference in schizophrenia: impairments in relational memory organization. *Schizophrenia Research*. 2004; 68:235–47. [PubMed: 15099606]

- Van Dijck A, Vloeberghs E, Van Dam D, Staufenbiel M, De Deyn PP. Evaluation of the APP23-model for Alzheimer's disease in the odour paired-associate test for hippocampus-dependent memory. *Behavioral Brain Research*. 2008; 190(1):147–51.
- Van Elzakker M, O'Reilly R, Rudy J. Transitivity, flexibility, conjunctive representations, and the hippocampus I. An empirical analysis. *Hippocampus*. 2003; 13:334–340. [PubMed: 12722974]
- Wallenstein G, Eichenbaum H, Hasselmo M. The hippocampus as an associator of discontiguous events. *Trends in Neuroscience*. 1998; 21:317–323.
- Zalesak M, Heckers S. The role of the hippocampus in transitive inference. *Psychiatry Research: Neuroimaging*. 2009 in press.

Figure 1. Histological verification of the extent of hippocampal damage. **A.** Representative sections from four targeted coordinates along the anterior-posterior axis of the mouse hippocampus in a sham-operated animal. **B.** Corresponding sections from an animal given NMDA infusions into the hippocampus. **C.** A diagram shows the extent of the largest (light gray) and smallest (black) lesion across the ten animals.

Figure 2. Pre-operative performance of all mice. **A.** Performance on each training phase. **B.** Performance on premise pairs presented during probe testing. **C.** Preference index for non-transitive (AE) and transitive (BD) probes. * $p < 0.05$

Figure 3. Post-operative performance. **A.** Performance ($\pm$ s.e.m.) in re-training on the final stage of premise pair learning. **B.** Performance on premise pairs presented during probe testing. **C.** Preference index for the transitive probe B versus D. **D.** Pre- to post-operative differences in PI for BD. **E.** Preference index for the non-transitive probe A versus E. **F.** Pre- to post-operative differences in PI for AE. * $p < 0.05$. SHAM: sham-operated group; HPX: hippocampal lesion group.

Figure 4.

Performance on the repeat post-operative testing. **A.** Performance ($\pm$ s.e.m.) on premise pairs presented during probe testing. **B.** Preference index for the transitive probe B versus D. **C.** Test to re-test differences in PI for BD. **D.** Preference index for the non-transitive probe A versus E. **E.** Test to re-test differences in PI on AE. * $p < 0.05$. SHAM: sham-operated group; HPX: hippocampal lesion group.

Table 1

Stages of training in learning the premise pairs.

Stage	Training Schedule
1	Day One: Session 1: AB (8 trials) Session 2: BC (8 trials) Day Two: Session 1: CD (8 trials) Session 2: DE (8 trials)
2	Session 1: AB & BC (4 trials each) Session 2: BC & DE (4 trials each)
3	Session 1: AB, BC, CD & DE (2 trials each) Session 2: Repeat
4	Session 1: Pseudorandom presentation of all pairs; 2 trials of each pair Session 2: Repeat