HIPPOCAMPUS 17:333–337 (2007)

RAPID COMMUNICATION

Environmental Complexity Affects Contextual Fear Conditioning Following Hippocampal Lesions in Rats

Sandra N. Moses,[1] Gordon Winocur,[1–3]* Jennifer D. Ryan,[1,3,4] and Morris Moscovitch[1,3,4,5]

ABSTRACT: Contextual fear conditioning has become a benchmark measure for hippocampal function, even though several studies report successful acquisition in hippocampal-damaged rodents. The current study examined whether environmental complexity may account for these discrepancies. We directly compared single-session contextual fear conditioning in rats in a simple vs. complex environment. Hippocampal lesions led to reduced fear conditioning in both contexts, as measured by freezing, but the effect was significantly greater in the complex context. As well, lesions led to generalized fear when the complex context was paired with shock, but not when the simple context was paired. We suggest that the representation of the simple context formed by rats with hippocampal lesions was adequate to support associative learning, but the representation of the complex context, which depended to a greater extent on relational learning, was not. The results were interpreted as consistent with theories of hippocampal function that emphasize its role in integrating multiple stimulus elements in a memory trace. © **2007 Wiley-Liss, Inc.**

KEY WORDS: hippocampus; rats; relational learning; memory; generalization

When an animal is administered an aversive foot-shock (US), unconditioned fear responses become associated with discrete stimuli (CSs) that are paired with the shock, as well as with the general context in which the experience occurred. Attempts to understand the mechanisms of such conditioning have focused on several brain regions, particularly the hippocampus. Following extensive investigation, there is broad consensus that the hippocampus is not directly implicated in classical CS-US conditioning (Kim and Fanselow, 1992; Phillips and LeDoux, 1992; but see Maren et al., 1997), but the evidence with respect to contextual fear-conditioning (CFC) is mixed. Several investigators have reported that damage to the hippocampus severely impairs CFC (Kim and Fanselow, 1992; Phillips and LeDoux, 1992; Maren et al., 1997; Rudy et al., 2002), others have reported no effect (McNish et al., 1997; Frankland et al., 1998; Good and Honey, 1991; Winocur, 1997), and one group even reported enhanced CFC under some conditions (Winocur et al., 1987).

Variations in fear conditioning tasks, type of lesion, and measures of conditioned fear undoubtedly account for some of the discrepancy among the reports (see reviews by Phillips and LeDoux, 1994; Holland and Bouton, 1999; Gewirtz et al., 2000), but one variable that has received scant attention is the complexity of the environment in which conditioning occurred. Frequently, when HPC lesions were found to impair CFC, animals were trained in a chamber with transparent walls that allowed visual access to a wide range of background cues (e.g., Kim and Fanselow, 1992; Phillips and LeDoux, 1992, 1994; Maren et al., 1997). In contrast, in those studies where HPC lesions did not impair CFC, training typically was conducted in a more confined environment where fear responses could be conditioned to a limited number of salient contextual stimuli (e.g., Winocur et al., 1987; Good and Honey, 1991; Winocur, 1997). Interestingly, parallel results of intact contextual learning in a relatively simple environment, following HPC lesions, have been obtained in nonaversive tasks. (Hirsh, 1974; Winocur and Olds, 1978; Skinner et al., 1994).

It is widely held that the hippocampus plays a crucial role in integrating and forming relationships among multiple stimulus elements for purposes of guiding appropriate behavior (e.g., Cohen and Eichenbaum, 1993; Rudy and Sutherland, 1995; Nadel and Moscovitch, 1997; Fanselow, 1999; Rosenbaum et al., 2001; Moses and Ryan, 2006). By this view, the hippocampus is not considered to be involved in forming direct associations between specific stimuli and unconditioned responses, and it follows that HPC lesions should have limited effects on CFC in a relatively uniform environment where discrete background cues can become directly associated with the fear response. By contrast, in a complex environment in which an array of contextual cues is associated with the unconditioned fear response, HPC lesions are likely to impair the representation of the context and, hence, have a detrimental effect on CFC.

If HPC lesions lead to impaired fear conditioning to a complex context because representation of that context in memory is impoverished, the shock-induced fear response should generalize to other envi-

[1] **Rotman Research Institute, Baycrest Centre, Toronto, Canada;**
[2] **Department of Psychology, Trent University, Peterborough, Canada;**
[3] **Department of Psychology, University of Toronto, Toronto, Canada;**
[4] **Department of Psychiatry, University of Toronto, Toronto, Canada;**
[5] **Department of Psychology, Baycrest Centre, Toronto, Canada**
Grant sponsor: Natural Sciences and Engineering Research Council of Canada; Grant number: AP8181.
*Correspondence to: Gordon Winocur, Ph.D., Rotman Research Institute, Baycrest Centre, 3560 Bathurst Street, Toronto, Ontario, Canada M6A 2E1. E-mail: gwinocur@rotman-baycrest.on.ca
Accepted for publication 26 January 2007
DOI 10.1002/hipo.20275
Published online 5 April 2007 in Wiley InterScience (www.interscience.wiley.com).

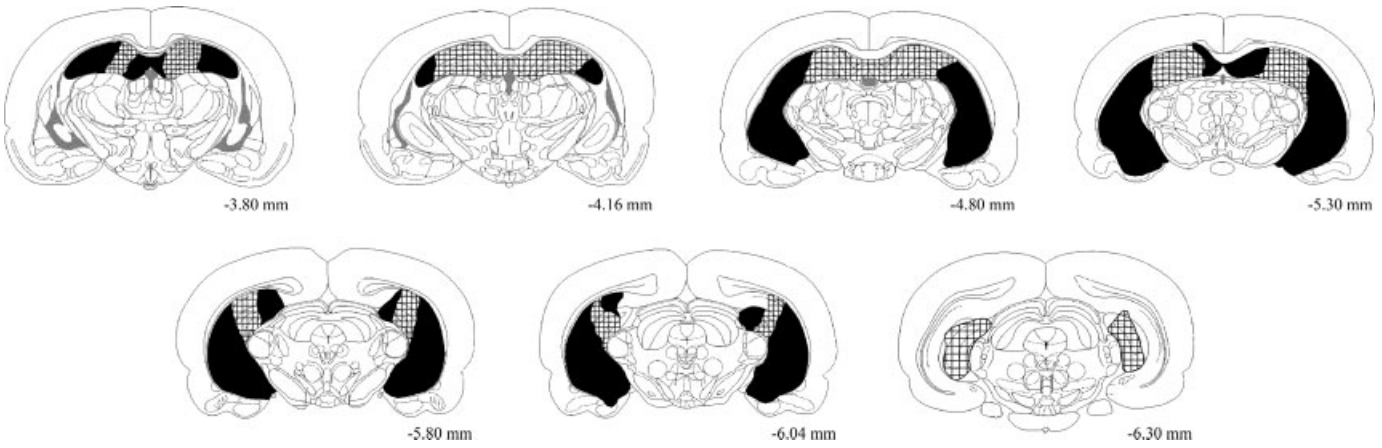

FIGURE 1. Coronal reconstructions of minimal (solid) and maximal (cross-thatched) extents of hippocampal lesions. Numbers represent distance in millimeters from bregma.

ronments. On the other hand, if, following HPC lesions, a simple context were represented effectively in memory, thus enabling the formation of a strong conditioned fear response, less generalization of that response would be expected. These predictions are tested in the present study as part of an examination of the interactive effects of HPC lesions and environmental complexity on CFC.

Twenty-eight male Long Evans rats, obtained from the Charles River Laboratories in St. Constant, Quebec, were maintained on a 12:12 light/dark cycle. Experimentation was carried out during the dark phase of the cycle. Fourteen rats received bilateral NMDA neurotoxic lesions of the hippocampus, and 14 received control surgery (Winocur et al., 2005). The simple context was a rectangular chamber ($50 \times 40 \times 18$ cm^3) with white walls and a floor that consisted of metal rods, spaced 1.3 cm apart. The top was open for observation. The chamber was placed on a table, 1.3 m above the floor, against a wall. The complex context was a chamber ($40 \times 40 \times 25$ cm^3), with clear walls, a hinged Plexiglas ceiling that contained several holes for ventilation, and a grid floor. This chamber was placed on a table in the center of a different room. This room contained visually accessible standard furniture (e.g., desk, table, bookshelf along one wall, etc.), as well as pictures, light fixtures, etc. on the walls. In both rooms, illumination was provided by overhead fluorescent lights under rheostatic control. Thus, the simple context consisted essentially of a homogeneous white surround. By comparison, the complex context consisted of multiple stimulus elements with the potential of forming multiple relations (associations) between them.

One day prior to training rats were preexposed in a counter balanced order to both the simple and complex contexts for 15 min each to reduce effects of novelty, (Phillips and Ledoux, 1992; Rudy and O'Reilly, 1999). Training followed over two days. On day 1 rats were placed in either the simple or complex context and allowed to explore freely for 5 min. Half the rats in each context then received a series of 10 foot shocks (1.5 mA; 1 s) with a variable interval (10–120 s) between each shock. After the last shock, the rat remained in the chamber for 60 s.

On training day 2 rats were placed in the context which they had not experienced on day 1. The rats that were shocked on day 1 received no shock on day 2, and the rats that were not shocked on day 1 received shock on day 2 in an identical manner as aforementioned.

Testing began 24 h after the second training day and occurred over two days. On Test day 1, half of the rats were placed in the simple context and half were placed in the complex context for 8 min. On Test day 2, rats were placed in the context which they had not experienced on the previous day for 8 min. The design allowed for each animal to be tested in the context in which it had been shocked (memory test) and, in a test of generalization, in the context in which it had never been shocked.

Freezing was defined, after Anagnostaras et al. (2001), as an immobilized crouching response in which the only detectable movement was the rat's breathing. The amount of time spent freezing was recorded throughout prexposure, training, and test sessions.

HPC lesions varied in size with most affecting 50–95% of the hippocampus proper. Six of the 14 lesioned rats sustained damage to 75–95% of the hippocampus, including extensive damage to all the subfields [CA1-CA3, dentate gyrus (DG)]. Two rats had relatively small lesions that affected 20–30% of the hippocampus but were included as their performance fell within the range of their group. Overall, the median value for HPC destruction was 68%, with the extent and pattern of damage similar in all conditions. In all cases, extra-HPC damage was minor or nonexistent (Fig. 1).

Table 1 provides the minimum, median, and maximum times spent freezing by HPC and operated control (OC) groups in the simple and complex contexts during the 15 min preexposure sessions, the 60 s period following fear conditioning, and the 60 s control period before which no shock was delivered. The preexposure data, which represent freezing times averaged over 15, 60-s periods, clearly show that there was no effect of lesion or context on freezing prior to the administration of shock. Not shown in the table, but consistent with this

TABLE 1.

Time Spent Freezing by HPC and OC Groups in Simple and Complex Contexts During Pre-exposure, Post-shock, No-shock Control Conditions

		Group	
Context	Condition	HPC	OC
Simple	Pre-exposure	(0, 0, 2)	(0, 1, 3)
	No-shock	(0, 0, 0)	(0, 0, 0)
	Post-shock	(50, 60, 60)	(45, 60, 60)
Complex	Pre-exposure	(0, 0, 3)	(0, 1, 2)
	No-shock	(0, 0, 0)	(0, 0, 12)
	Post-shock	(48, 60, 60)	(57, 60, 60)

Freezing in the post-shock and no-shock control conditions is measured over a 60 s period; in the pre-exposure condition, freezing was measured over 15 min and is presented as an average over 15, 60-s intervals. The numbers in each cell are the minimum, median, and maximum freezing times (in seconds) for the respective groups.

finding, is that in the 5-min pre-shock period on the training days, HPC and OC groups showed virtually no freezing in either context. Similarly, as can be seen in Table 1, the immediate response to shock by HPC and OC groups was identical in both contexts. Most rats in both groups froze for the entire 60-s period, in contrast to the corresponding period in the no-shock condition, where there was virtually no freezing.

Figure 2 shows time spent freezing by each rat in the HPC and OC groups when tested in the context that was paired with shock (Fig. 2A) and when tested for generalization of the fear response to the context that was not paired with shock (Fig. 2B). As can be seen, the groups exhibited variable amounts of freezing in the various conditions and this was reflected in a significant 3-way, group x context-same x context-different, interaction $F_{1,24} = 18.40$, $P < 0.001$.

Subsequent analyses of freezing in the context that was paired with shock (Fig. 2A) confirmed that both groups exhibited more freezing in the simple context than the complex context, $F_{1,24} = 13.35$, $P = 0.001$ and that, overall, rats with HPC lesions exhibited less freezing than controls, $F_{1,24} = 25.83$, $P < 0.001$. Although the group x context interaction was not statistically significant, $F_{1,24} = 0.52$, $P = 0.48$, several lines of evidence indicate that rats with HPC lesions were more impaired in acquiring a conditioned fear response in the complex context than in the simple context. First, as can be seen in Figure 2A, whereas there was no significant difference in freezing behavior between OC groups tested in the simple and complex contexts, $t_{12} = 1.77$, $P = 0.11$, the corresponding difference between HPC groups was highly significant, $t_{12} = 4.48$, $P = 0.002$, with the HPC group in the simple context exhibiting considerably more freezing at test. Second, a measure of overlap between two groups (U' ranges from 0 to 24 for $n = 7$) identified a complete separation of observed freezing times between HPC and OC groups in the complex context - that is, no OC rat exhibited shorter freezing times than any of the HPC rats ($U' = 0$) as shown in the left hand panel of Figure 2. By contrast, there was overlap in the simple context as reflected by the fact that seven instances of overlap occurred between OC and HPC rats ($U' = 7$). This indicates that the dis-

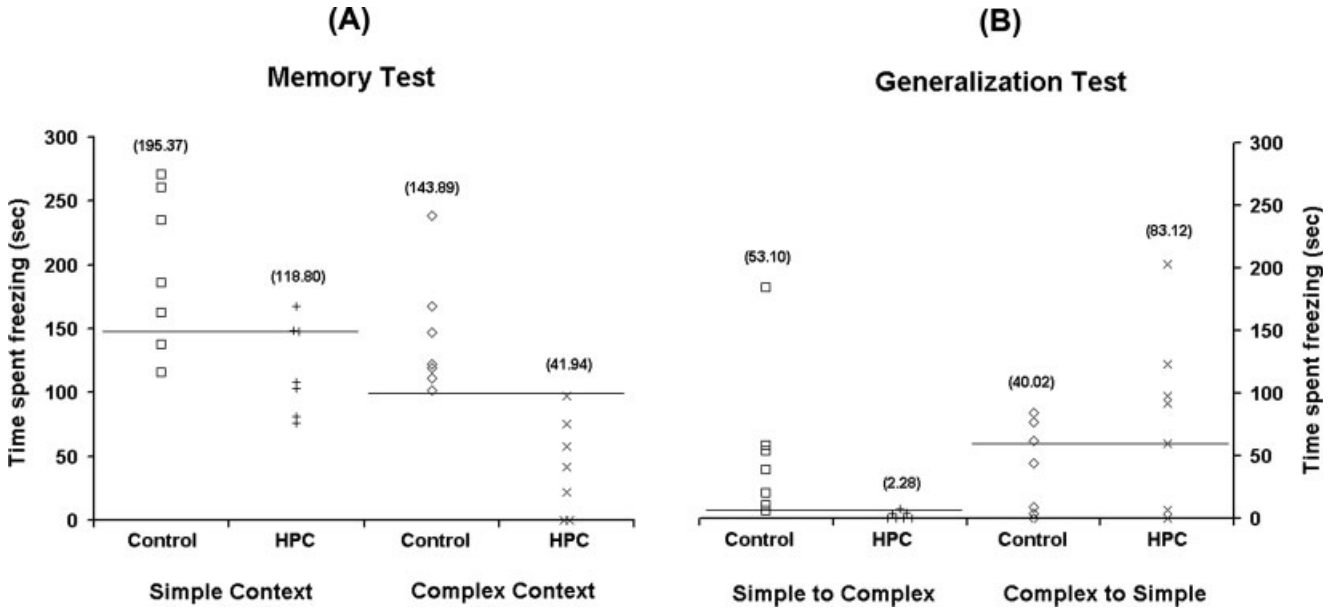

FIGURE 2. (A) Dot plot showing the time spent freezing by each rat in the simple and complex contexts following fear conditioning in which shock was delivered in the same context. Hippocampal lesions impaired contextual fear conditioning in both contexts but hippocampal and control groups showed more freezing in the simple than the complex context. (B) Dot plot showing the time spent freezing by each rat in tests of generalization. Rats with hippocampal lesions exhibited more generalization when shocked in the complex context than in the simple context. Control rats showed similar generalization in both conditions. The horizontal lines represent, the median value across HPC and OC rats in each contextual condition and provide a reference for between-group comparisons. The numbers in brackets represent group means.

tributions of freezing times in the HPC and OC groups are more similar in the simple than in the complex context.

Additional evidence that HPC lesions had a greater effect on CFC in the complex context comes from analysis of the generalization findings. These data yielded a significant group x context-different interaction, $F_{1,24} = 6.17$, $P = 0.02$, indicating that differences between groups in this test depended on the direction of the context shift. As can be seen from a comparison of Figures 2A,B both OC groups exhibited the same generalization pattern. That is, relative to their performance in the context that had been paired with shock, controls in both generalization conditions exhibited similar decreases in freezing behavior. As can be seen in Figure 2B, there was no difference in amount of time spent freezing between controls in the two generalization test conditions $t < 1$. Essentially the same pattern was seen in the HPC group that was shocked in the simple context and tested for generalization in the complex context. Their ability to discriminate between the simple context and the complex context in the generalization test (Fig. 2B) can be interpreted as further evidence that considerable CFC occurred in these animals in the simple context. In contrast, the HPC group that was shocked in the complex context exhibited essentially the same amount of freezing when tested for generalization to the simple context as did the HPC group that was tested in the complex context after having been shocked in that context. This indicates that rats with HPC lesions shocked in the complex context generalized whatever fear response they had acquired and that the learning was not strongly associated with the specific context in which the shock was delivered.

We interpret the substantial fear conditioning that occurred in HPC rats in the simple context, where the environment consisted essentially of a single explicit stimulus, as being similar to classical fear conditioning in which a shock-mediated UCR becomes associated with a discrete CS. As such, this finding is consistent with numerous demonstrations of normal classical fear conditioning in rats with HPC lesions (e.g., Kim and Fanselow, 1992; Phillips and LeDoux, 1994) as well as with other evidence that HPC lesions have little or no effect on fear conditioning in relatively simple contexts (Winocur et al., 1987; Winocur, 1997). At the same time, the results showed that rats with HPC lesions did exhibit less CFC in the simple context than controls, indicating that, even in relatively uncomplicated contexts, CFC is not necessarily fully spared following such lesions (for similar results Chen et al., 1996; Rudy et al., 2002).

The finding that HPC lesions severely impaired CFC in the complex context confirms numerous reports of this effect (e.g., Kim and Fanselow, 1992; Phillips and LeDoux, 1992; Maren et al., 1997). It is noteworthy that rats with HPC lesions exhibited some modest fear conditioning in the complex context, which generalized to the simple context; there was no generalization, however, from simple to complex context. The asymmetry between the two generalization conditions likely occurred because rats were able to create a functional representation of the simple context, but the representation of the complex context was extremely impoverished. In this situation the simple context elicited fear probably because it was remembered more accurately than the complex context. Importantly, this generalization effect was absent in control rats who could represent the simple and complex context in memory equally well.

The ability of rats with HPC lesions to learn a new response without using contextual cues and the resultant tendency to generalize that response to another environment that shares some features with the original environment has been observed recently in other tasks (e.g., socially-acquired food preference; Winocur et al., in press).

Overall, the results are consistent with theories of HPC function that emphasize HPC involvement in learning about relationships among multiple elements (Cohen and Eichenbaum, 1993; Rudy and Sutherland, 1995; Nadel and Moscovitch, 1997; Fanselow, 1999; Rosenbaum et al., 2001; Moses and Ryan, 2006). They also underscore the importance of distinguishing between different kinds of contextual representations and the memories they support (Rosenbaum et al., 2001). Contextual cues can be linked relationally to each other to form detailed spatial (O'Keefe and Nadel, 1978) or nonspatial (Eichenbaum, 1999) representations of an event. Such representations, when reinstated, support detailed and flexible episodic memories of the experience. Alternatively, each contextual cue or combination of cues can be linked independently and directly to the response that is part of the task at hand. These associations give rise to less complex, rigid memories that are dependent on the presence of specific background cues or landmarks. Following the distinction between relational and associative learning, the two types of contextual representations have been characterized as expressions of *relational and associative contextual learning* (Rosenbaum et al., 2001), with the hippocampus implicated in the former but not the latter. Efficient fear conditioning in the complex context of the present study required that rats form representations of the environment in relational terms, thereby placing rats with HPC lesions at a disadvantage, and promoting generalization to other contexts. By contrast, in the simple context, the highly salient background cue could be associated readily with the UCR through extra-HPC structures, and little generalization to other contexts would be evident. While normally less efficient than relational learning, under such conditions, associative contextual learning in rats with HPC lesions can be expected to approximate that of normal rats.

Acknowledgments

The authors thank Andrea Goode and Marie-Ève Couture for their excellent technical support, and Malcolm Binns for his help in analyzing the data. The research was funded by grants to G.W. and M.M. from The Natural Sciences and Engineering Research Council of Canada.

REFERENCES

Anagnostaras SG, Gale GD, Fanselow MS. 2001. Hippocampus and contextual fear conditioning: Recent controversies and advances. Hippocampus 11:8–17.

Chen C, Kim JJ, Thompson RF, Tonegawa S. 1996. Hippocampal lesions impair contextual fear conditioning in two strains of mice. Behav Neurosci 110:1177–1180.

Cohen NJ, Eichenbaum H. 1993. Memory, amnesia, and the hippocampal system. Massachusetts: The MIT Press.

Eichenbaum H. 1999. The hippocampus and mechanisms of declarative memory. Behav Brain Res 103:123–133.

Fanselow MS. 1999. Learning theory and neuropsychology: Configuring their disparate elements in the hippocampus. J Exp Psychol: Anim Behav Proc 3:275–283.

Frankland PW, Cestari V, Filipkowski RK, McDonald RJ, Silva AJ. 1998. The dorsal hippocampus in essential for context discrimination but not for contextual conditioning. Behav Neurosci 70:44–61.

Gewirtz JC, McNish KA, Davis M. 2000. Is the hippocampus necessary for contextual fear conditioning? Behav Brain Res 110: 83–95.

Good M, Honey RC. 1991. Conditioning and contextual retrieval in hippocampal rats. Behav Neurosci 105:499–509.

Hirsh R. 1974. The hippocampus and contextual retrieval of information from memory: a theory. Behav Biol 12:421–444.

Holland PC, Bouton ME. 1999. Hippocampus and context in classical conditioning. Curr Opin Neurobiol 9:195–202.

Kim JJ, Fanselow MS. 1992. Modality-specific retrograde amnesia of fear. Science 256:675–677.

Maren S, Aharonov G, Fanselow MS. 1997. Neurotoxic lesions of the dorsal hippocampus and Pavlovian fear conditioning in rats. Behav Brain Res 88:261–274.

McNish KA, Gewirtz JC, Davis M. 1997. Evidence of contextual fear after lesions of the hippocampus: a disruption of freezing but not fear-potentiated startle. J Neurosci 17:9353–9360.

Moses SN, Ryan JD. 2006. A comparison and evaluation of the predictions of relational and conjunctive accounts of hippocampal function. Hippocampus 16:43–65.

Nadel L, Moscovitch M. 1997. Memory consolidation, retrograde amnesia and the hippocampal complex. Curr Opin Neurobiol 7:217–227.

O'Keefe J, Nadel L. 1978. The Hippocampus as a Cognitive Map. Oxford, UK: Oxford University Press.

Phillips RG, LeDoux JE. 1992. Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. Behav Neurosci 106:274–285.

Phillips RG, LeDoux JE. 1994. Lesions of the dorsal hippocampal formation interfere with background but not foreground contextual fear conditioning. Learn Mem 1:34–44.

Rosenbaum RS, Winocur G, Moscovitch M. 2001. New views on old memories: Re-evaluating the role of the hippocampal complex. Behav Brain Res 127:183–197.

Rudy JW, O'Reilly RC. 1999. Contextual fear conditioning, conjunctive representations, pattern completion, and the hippocampus. Behav Neurosci 113:867–880.

Rudy JW, Sutherland RJ. 1995. Configural association theory and the hippocampal formation: An appraisal and reconfiguration. Hippocampus 5:375–389.

Rudy JW, Barrientos RM, O'Reilly RC. 2002. Hippocampal formation supports conditioning to memory of a context. Behav Neurosci 116:530–538.

Skinner DM, Martin GM, Harley C, Kolb B, Pridgar A, Bechara A, van der Kooy D. 1994. Acquisition of conditional discriminations in hippocampal lesioned and decorticated rats: Evidence for learning that is separate from both simple and classical conditioning and configural learning. Behav Neurosci 108:911–926.

Winocur G. 1997. Hippocampal lesions alter conditioning to conditional and contextual stimuli. Behav Brain Res 88:219–229.

Winocur G, Olds J. 1978. Effects of context manipulation on memory and reversal learning in rats with hippocampal lesions. J Comp Physiol Psychol 92:312–321.

Winocur G, Rawlins JNP, Gray JA. 1987. The hippocampus and conditioning to contextual cues. Behav Neurosci 101:617–625.

Winocur G, Moscovitch M, Caruana DA, Binns M. 2005. Retrograde amnesia in rats with lesions to the hippocampus on a test of spatial memory. Neuropsychologia 43:1580–1590.

Winocur G, Moscovitch M, Sekeres M. (in press). Memory consolidation or transformation: Context manipulation and hippocampal representations of memory. Nature Neuroscience.