The startled seahorse: is the hippocampus necessary for contextual fear conditioning?

Stephen Maren, Stephan G. Anagnostaras and Michael S. Fanselow

It is widely believed that the hippocampus plays an important role in learning and memory in several species including rats, monkeys and humans. The type of memory served by this structure has been described as explicit, configural, declarative, spatial, relational and episodic. In simpler terms, many would agree that the hippocampus assembles cognitive representations of stimuli and their relationships for storage in long-term memory.

Despite its various roles, the general wisdom is that the hippocampus is not required for simple forms of associative learning, such as Pavlovian (classical) conditioning. Nonetheless, several investigators have suggested that the hippocampus may have a role in mediating a special case of Pavlovian conditioning, that is, conditioning to context[1]. In the past few years, considerable support for this view has emerged[2–4].

In the learning laboratory, 'context' refers to the collection of stimuli associated with the training environment. Contexts are multimodal (including olfactory, visual, auditory, tactile and spatial stimuli) and tonic. Contexts can be contrasted with discrete conditional stimuli (CSs), such as tones or lights, which are unimodal and phasic. In conditioning experiments, contextual conditioning occurs when training consists of either 'signaled' delivery of an unconditional stimulus (US) by a CS or 'unsignaled' delivery of a US (that is, the context signals US delivery). In either case, it has been argued that in order for contextual conditioning to proceed, a configural representation of the many stimulus elements within the context must be formed[5]. It is this process of cognitive representation of context that is thought to require the hippocampus[1,6]. Notwithstanding, it is conceivable that contexts could also be acquired as individual elemental associations, and this process would not be expected to require the hippocampus. The rules governing the selection of configural versus elemental strategies for acquiring contextual representations are not known, although it is assumed that configural strategies normally supersede elemental strategies.

The lion's share of work implicating the hippocampus in contextual conditioning has been derived from studies of fear conditioning in rats. In fear conditioning tasks, discrete and/or contextual CSs that are paired with footshock come to evoke conditional fear responses (CRs) such as defecation, potentiated acoustic startle, elevated blood pressure and freezing. In many laboratories, contextual fear is assessed by returning the rats to the conditioning chamber and measuring freezing. That the hippocampus plays an important role in contextual fear conditioning is indicated by studies demonstrating that dorsal hippocampal lesions attenuate freezing to contextual CSs, but do not alter freezing behavior to discrete CSs (Refs 2,3). Hippocampal lesions also impair contextual fear conditioning following unsignaled training[7,8]. While these results suggest that the hippocampus is required for contextual fear conditioning, recently published data challenge this notion.

In a recent study, McNish *et al.*[9] re-examined the role of the hippocampus in contextual fear conditioning by simultaneously measuring two contextual fear responses: freezing and fear-potentiated startle. Fear-potentiated startle to context was characterized by an increase in the amplitude of the acoustic startle response in a shock-associated context compared to startle amplitude in that same context before fear conditioning. Consistent with earlier reports, these authors found that dorsal hippocampal lesions impaired freezing in the conditioning context. Surprisingly, however, they did not observe impairments in fear-potentiated startle in this same context. Thus, by one measure of fear, context conditioning was disrupted by hippocampal damage, but by another measure of fear it was intact. In the light of these results, McNish *et al.* suggested that deficits in contextual freezing are not the result of a failure in contextual learning, but rather are the result of 'response competition' between freezing and the motor hyperactivity that typically accompanies hippocampal damage[10,11]. Other groups, including our own, have reported increased motor activity and reduced freezing in rats with hippocampal lesions[7,12]. Based on these collective results, McNish *et al.* argue that the hippocampus is not essential for contextual learning. It should be noted, however, that McNish *et al.* did not measure hyperactivity in their report. At first glance, these data would appear to call for a revision of current thinking regarding the role of the hippocampus in contextual learning. However, when the full range of effects of hippocampal lesions on contextual fear is taken into account, it becomes apparent that a response competition explanation of contextual freezing deficits is indefensible.

Competing responses or symptoms of a common deficit?

As stated above, we and others have reported elevated motor activity in rats with dorsal hippocampal lesions that accompanies reduced contextual freezing in these animals[8,12]. In our study[8], rats with lesions in other regions of the hippocampal formation (the fornix or entorhinal cortex) displayed a similar pattern of results. Across these different lesion groups, pre-training motor activity correlated well with post-training freezing, but within each group this correlation was poor. On this basis, we argued that it is unlikely that motor activity and freezing deficits are causally related. To bolster this claim, we have compiled a correlation between freezing and motor activity from all of the experiments in which we measured these parameters. In these experiments, motor activity was measured during a 3 min pre-shock period in the same chambers where conditioning took place. As shown in Fig. 1, there was no significant correlation between motor activity and contextual freezing in rats with hippocampal lesions.

Stephen Maren is at the Department of Psychology and Neuroscience Program, University of Michigan, Ann Arbor, MI 48109-1109, USA.

tel: +1 313 936 6532
fax: +1 313 763 7480
e-mail: maren@umich.edu

Stephan G. Anagnostaras and Michael S. Fanselow are at the Department of Psychology, University of California, Los Angeles, CA 90095-1563, USA.

 1364-6613/98/$19.00 PII: S1364-6613(98)01123-1

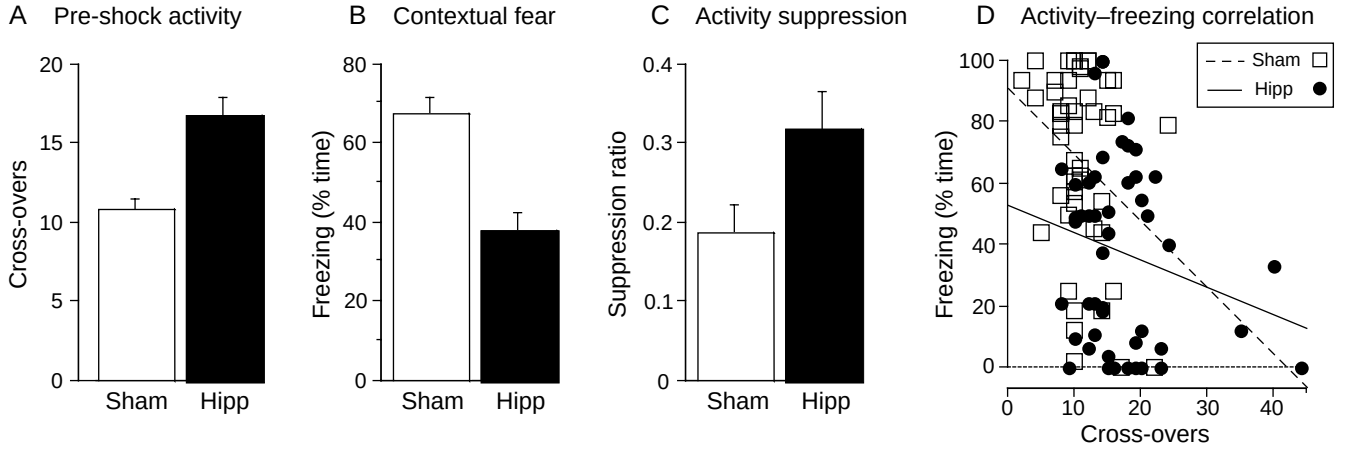

Fig. 1 Motor activity and freezing following hippocampal lesions. The data shown are pooled from five separate experiments conducted under similar and balanced conditions. In all cases, hippocampal lesions were made before training so that an assessment of both hyperactivity and contextual freezing could be made in the same rats. **(A)** Pre-shock activity. On the conditioning day, horizontal cage cross-overs were assessed during the 3 min period before the first shock. Rats that received electrolytic lesions of the dorsal hippocampus (n = 48) made more cross-overs than sham animals (n = 49), which had lesions of other hippocampal regions, during this period [$F(1,95) = 24.3$; $p < 0.0001$]. **(B)** Contextual fear conditioning. Following fear conditioning, hippocampal rats showed about half as much contextual learning as sham animals [$F(1,95) = 23.4$; $p < 0.0001$]. **(C)** Activity suppression. A prediction implicit in the argument of McNish *et al.* is that hippocampal rats should show normal suppression of motor activity following fear conditioning. Higher baseline activity in hippocampal rats might mask this effect. To correct for baseline activity differences, we have characterized contextual fear learning with an activity suppression ratio using the 3 min pre-shock cross-overs, and cross-overs assessed during the first 3 min period of a contextual fear test [SR = (post) / (pre + post)]. A representative subset of animals was examined using this measure. Hippocampal rats (n = 21) exhibited about half as much suppression as sham rats [n = 23; $F(1,42) = 5.4$; $p < 0.05$]. This suppression ratio indicates that there is a context-conditioning deficit in hippocampal rats that cannot be accounted for by changes in baseline activity. **(D)** Activity–freezing correlation. The critical piece of data missing in the analysis of McNish *et al.* is a correlative analysis of motor activity and freezing – a strong negative correlation between activity and contextual freezing is a necessary condition for the response competition hypothesis to be correct. By combining data from several experiments, we are able to rule out the response competition hypothesis definitively. For sham animals (n = 49), there was a small, but marginally significant negative correlation between pre-shock cross-overs and contextual freezing ($r = -0.30$; $p < 0.05$). For animals with hippocampal lesions (n = 48), this correlation was weaker and not significant ($r = -0.22$; $p > 0.10$). Hippocampal lesions produce hyperactivity, but this is not the source of deficits in contextual freezing.

What then is one to make of the between-group correlation in these measures? Rather than citing motor hyperactivity as the cause for contextual freezing deficits, we have argued that these two consequences of hippocampal damage reflect a common syndrome[8]. As others have argued[13], increased motor activity in rats with hippocampal damage may reflect a failure to habituate exploratory activity. We imagine that this failure to habituate exploration results from an inability of the hippocampal rat to encode a contextual representation of the environment[12,14]. Thus, we argue that deficits in contextual freezing and motor hyperactivity in novel environments in rats with hippocampal damage reflect a deficit in contextual representation.

Recent versus remote memories

As in humans[15] and monkeys[16], hippocampal damage in rats is associated with a temporally graded retrograde amnesia. In other words, recent memories are much more sensitive to hippocampal damage than are remote memories. In fear conditioning, for instance, dorsal hippocampal lesions impair contextual freezing when made one day following training, but not when made 28 days following conditioning[2]. Hippocampal lesions disrupted context fear selectively, because fear conditioning to a tone CS was not affected by hippocampal lesions at any time. Importantly, neither context fear nor tone fear varied over the retention intervals in control animals. In a related study, it was found that pre-exposing rats to the conditioning context 28 days before training eliminated lesion-induced deficits in contextual freezing[17].

Consider the implications for a response competition view of these data. If deficits in contextual freezing were the result of motor hyperactivity, then hyperactivity should have interfered with contextual freezing at both the one day and 28-day training-to-lesion intervals. Similarly, pre-exposed animals should not have been protected from the hippocampal deficit. These were not the observed results. Freezing was disrupted when lesions were made shortly, but not a long time, after training, and context pre-exposure protected against this deficit. Even though the lesion-to-testing interval was held constant in these studies, it is possible that one could argue, in each case, that the 28-day retention interval somehow protects against hippocampal lesion-induced increases in motor activity. However, this argument is not tenable in view of recent data showing that temporally graded context-freezing deficits can be demonstrated in the same animal. Anagnostaras *et al.* (1996 *Soc. Neurosci. Abstr.* 22, 1380) trained individual rats on both a recent and a remote context memory. These rats exhibited impaired freezing in the recently trained context, but normal freezing in the remotely trained context. The response competition argument requires that the same rat is both hyper- and hypoactive!

Of course, a response competition view also predicts that freezing to tone CSs should be disrupted by motor hyperactivity. However, tone fear is typically immune to the effects of hippocampal lesions[2,3]. Nevertheless, the argument has been made that tone conditioning may be less susceptible to hippocampal damage than is context conditioning because it is a stronger memory. In the Kim and Fanselow[2] study, however, context-conditioning deficits in hippocampal rats were found at levels of context fear that either equaled or exceeded levels of tone fear. A response competition view cannot possibly explain this pattern of results.

Neurons or axons?

The work discussed thus far has made use of electrolytic hippocampal lesions. These lesions destroy not only neurons in the dorsal hippocampus, but also axonal projections that merely pass through the hippocampus. Because axonal projections running through the dorsal hippocampus have been implicated in hippocampal lesion-induced increases in motor activity[18], we recently made neurotoxic lesions of the dorsal hippocampus that selectively destroyed neurons in the hippocampus, leaving the fibers intact[6]. As expected, only the electrolytic lesions increased motor activity.

We then examined contextual fear conditioning in these rats. Consistent with earlier results, we found that

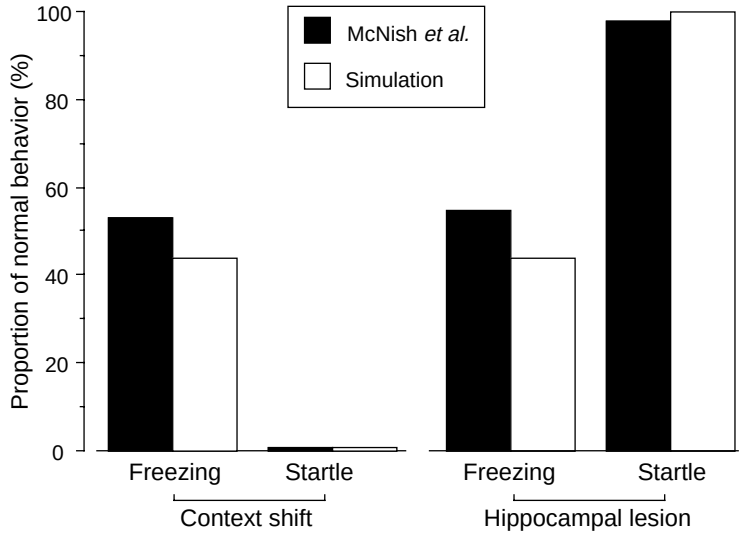

Fig. 2 The actual data of McNish *et al.* and the data from our simulation presented as a proportion of normal behavior displayed. Our model assumes that there are 100 shock-associated units, half of which are configural; the other half are elemental. Context shifts eliminate half of each type of unit. Hippocampal lesions eliminate all of the configural units but none of the elemental units. A subtractive-response elicitation threshold of 25 units is set for startle, and a threshold of 10 is set for freezing. Freezing is determined by both the available configural and elemental units, while startle is determined only by the elemental units. Therefore, freezing = (elemental + configural) –10 and startle = elemental –25. In the figure, context shift represents the measure of fear (either freezing or potentiation of startle) in the novel test chamber divided by the measure of fear obtained in the training chamber. For hippocampal lesions, the hippocampal data are divided by the sham data. The model represents the first simulation that was conducted and no data-fitting techniques were used.

hippocampal lesions made shortly after training severely disrupted contextual freezing, and lesions made a long time after training yielded small deficits in freezing. Interestingly, we also found that neurotoxic hippocampal lesions made before training did not affect the acquisition of contextual fear conditioning. In other words, rats with pre-training neurotoxic lesions conditioned to context normally. To explain these results, we argued that intact rats use a configural strategy to acquire contextual fear (which is sensitive to post-training hippocampal lesions), but that rats with neurotoxic hippocampal lesions use an elemental strategy to acquire context fear (without a hippocampus a configural strategy is not available). In either case, it cannot be argued that motor hyperactivity is responsible for the pattern of deficits that we observed, because neurotoxic hippocampal lesions did not yield enhanced motor activity. And even if motor hyperactivity were recruited as an explanation, it cannot explain temporally graded retrograde amnesia or the selectivity of the deficit for post-training as compared to pre-training lesions. Again, it seems that a response competition argument fails to explain the existing data.

It should also be pointed out that reversible pharmacological manipulations of the hippocampus prevent contextual conditioning[17]. In these experiments, intra-hippocampal infusion of an *N*-methyl-D-aspartate receptor antagonist during training attenuated contextual freezing measured during a drug-free test. A response competition account is unable to account for these data.

Startle versus freezing

The foregoing arguments make a strong case that deficits in contextual freezing in hippocampal rats are not merely the outcome of motor hyperactivity. But what accounts for the pattern of results reported by McNish *et al.*? Why do hippocampal lesions affect contextual fear as measured by freezing but not by potentiated startle? One hint at an explanation can be found by a close inspection of their freezing data. Typically, in studies of contextual conditioning, freezing is eliminated completely by changes in context cues and by post-training lesions of the hippocampus[2,19]. By comparison, McNish *et al.* reported rather small deficits with both of these manipulations. Perhaps the residual freezing following either context shift or hippocampal lesions was mediated by conditioning to some salient element of the context. This element could have been provided by McNish and colleagues' use of small, confining chambers. If this is true, then a few simple assumptions would allow us to simulate the McNish *et al.* data, namely: (1) hippocampal lesions selectively eliminate the use of configural cues (an assumption that is consistent with most views of hippocampal function), (2) 'freezing has a lower response threshold than fear-potentiated startle' (this assumption is quoted from the conclusions of McNish *et al.*), and (3) potentiated startle is more sensitive to elemental than to configural cues. This last assumption may be the most controversial of the three, but there is certainly substantial evidence in the Pavlovian conditioning literature that different aspects of the CS control different CRs, even though all are related to a single CS–US association[20]. These three assumptions yield a model that predicts the entire pattern of results reported by McNish *et al.*, that is: (1) hippocampal lesions cause a partial reduction in freezing, (2) hippocampal lesions do not affect startle, and (3) context shifts partially reduce freezing and completely eliminate startle. Figure 2 illustrates the results of this simulation. Obviously, additional research must test the veracity of this model, but we feel confident that whatever the eventual solution is it will not be an explanation that relies on response competition.

Conclusions

The work by McNish *et al.* has provided new insights into the neural mechanisms of contextual fear conditioning. Clearly, models that invoke a role for the hippocampus in the mediation of contextual learning are impelled to explain the selective deficits in freezing that McNish *et al.* have described. From our perspective, it is insufficient to invoke response competition as an explanation for this pattern of results. Although the exact cause of this pattern of deficits is not yet known, we feel that it is premature to conclude that the hippocampus is not essential for contextual fear conditioning. Further parametric examination of fear-potentiated startle in rats with hippocampal lesions is required.

References

1 Nadel, L. and Willner, J. (1980) Context and conditioning: a place for space *Physiol. Psychol.* 8, 218–228

2 Kim, J.J. and Fanselow, M.S. (1992) Modality-specific retrograde amnesia of fear *Science* 256, 675–677

3 Phillips, R.G. and LeDoux, J.E. (1992) Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning *Behav. Neurosci.* 106, 274–285

4 Selden, N.R. *et al.* (1991) Complementary roles for the amygdala and hippocampus in aversive conditioning to explicit and contextual cues *Neuroscience* 42, 335–350

5 Fanselow, M.S. (1986) Associative vs topographical accounts of the immediate shock freezing deficit in rats: implications for the response selection rules governing species specific defensive reactions *Learn. Motiv.* 17, 16–39

6 Maren, S., Aharonov, G. and Fanselow, M.S. (1997) Neurotoxic lesions of the dorsal hippocampus and Pavlovian fear conditioning in rats *Behav. Brain Res.* 88, 261–274

7 Kim, J.J., Rison, R.A. and Fanselow, M.S.

(1993) Effects of amygdala, hippocampus, and periaqueductal gray lesions on short- and long-term contextual fear *Behav. Neurosci.* 107, 1093–1098

8 Maren, S. and Fanselow, M.S. (1997) Electrolytic lesions of the dorsal hippocampus, fimbria/fornix, or entorhinal cortex produce anterograde deficits in contextual fear conditioning in rats *Neurobiol. Learn. Mem.* 67, 142–149

9 McNish, K.A., Gewiritz, J.C. and 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

10 Douglas, R.J. and Isaacson, R.L. (1964) Hippocampal lesions and activity *Psychonomic Sci.* 1, 187–188

11 Blanchard, D.C. *et al.* (1977) Movement arrest and the hippocampus *Physiol. Psychol.* 5, 331–335

12 Good, M. and Honey, R.C. (1997) Dissociable effects of selective lesions to the hippocampal subsystems on exploratory behavior, contextual learning, and spatial learning *Behav. Neurosci.* 111, 487–493

13 O'Keefe, J. and Nadel, L. (1978) *The Hippocampus as a Cognitive Map*, Clarendon Press

14 Fanselow, M.S. (1996) Modality-specific memory of fear: differential involvement of the amygdala and hippocampal formation in Pavlovian fear conditioning, in *Perception, Memory and Emotion: Frontiers in Neuroscience* (Ono, T. *et al.*, eds), pp. 499–512, Pergamon Press

15 Squire, L.R. and Alvarez, P. (1995) Retrograde amnesia and memory consolidation: a neurobiological perspective *Curr. Opin. Neurobiol.* 5, 169–177

16 Zola-Morgan, S. and Squire, L.R. (1990) The primate hippocampal formation: evidence for a time-limited role in memory storage *Science* 250, 288–290

17 Young, S.L., Bohenek, D. and Fanselow, M.S. (1994) NMDA processes mediate anterograde amnesia of contextual fear conditioning induced by hippocampal damage: immunization against amnesia by context preexposure *Behav. Neurosci.* 108, 19–29

18 Whishaw, I.Q. and Jarrard, L.E. (1995) Similarities vs differences in place learning and circadian activity in rats after fimbria-fornix section or ibotenate removal of hippocampal cells *Hippocampus* 5, 595–604

19 Fanselow, M.S. (1990) Factors governing one trial contextual conditioning *Anim. Learn. Behav.* 18, 264–270

20 Holland, P.C. (1984) Origins of behavior in Pavlovian conditioning, in *The Psychology of Learning and Motivation* (Bower, G., ed.), pp. 129–174, Prentice–Hall

Response from McNish, Gewirtz and Davis

In our recent article examining the role of the hippocampus in contextual fear conditioning, we developed a paradigm which produced contextual freezing and fear-potentiated startle that was specific to a context previously paired with shock[1]. Lesions of the central nucleus of the amygdala blocked both freezing and fear-potentiated startle, consistent with the notion that this structure is critically involved in mediating conditioned fear responses. In contrast, lesions of the dorsal hippocampus disrupted contextual freezing, but had no effect on fear-potentiated startle. Based on these results, we concluded that despite a disruption of freezing, fear to the context was preserved in animals with hippocampal lesions.

Our interpretation of the effects of hippocampal lesions on contextual freezing challenges the notion that context conditioning, like spatial learning, is a hippocampal-dependent task. This notion was encouraged by demonstrations that lesions of the hippocampus disrupted freezing to contextual cues, but had no effect on freezing to explicit cues[2,3]. One interpretation of these findings is that the hippocampus is critically involved in forming complex, polymodal associations, as would be required in forming a representation of context but not in unimodal or 'elemental' associations[4]. An alternative interpretation is that hippocampal lesions enhance motor activity, which preferentially disrupts weak conditioned freezing responses[5]. Given that contextual fear is likely to be less strong than fear to explicit cues[6], one might expect the lesions to have a greater impact on freezing to contextual cues. The commentary by Maren *et al.* attempted to rule out the response competition hypothesis. Furthermore, they propose a model that appears to simulate our data while preserving the central role of the hippocampus in contextual fear conditioning. Below, we will outline why response competition, coupled with a strength of conditioning argument, is a reasonable alternative explanation for the effects of hippocampal lesions on freezing. We will also highlight several problems inherent in the model proposed by Maren *et al*.

K.A. McNish, J.C. Gewirtz and M. Davis are at the Departments of Psychiatry and Psychology, Yale University School of Medicine, Connecticut Mental Health Center, New Haven, CT 06508, USA.

tel: +1 203 789 7448
fax: +1 203 562 7079
e-mail: michael.davis@yale.edu

Response competition

Hippocampal lesions increase motor activity

It has frequently been reported that hippocampal lesions increase motor activity. Recently, Maren and Fanselow[7] have reported that across-groups increases in motor activity produced by lesions of the dorsal hippocampus, entorhinal cortex and fimbria-fornix were highly correlated with the disruption of freezing. They have argued that these effects are not causal but reflect a common underlying syndrome, because within a given group the correlations between activity and freezing deficits are poor. However, the lack of significant within-group correlations does not discount a causal relationship. Because the lesions significantly enhanced motor activity, there is a narrower distribution of activity levels within a group than across groups, decreasing the likelihood of finding a significant correlation within a group. The important point is that because their experimental manipulation was at the group level, it is the significant between-groups correlation that is relevant, not the non-significant within-group correlations.

Interestingly, it has recently been reported that excitotoxic dorsal hippocampal lesions produced increases in activity, deficits in freezing and impairments in spatial learning[5]. In contrast, entorhinal cortex lesions also disrupted spatial learning, but had no effect on either activity or freezing. This suggests that there is a closer relationship between motor activity and freezing than between freezing and spatial learning. If the freezing deficits truly reflected a disruption of contextual fear conditioning, one would have expected them to go hand-in-hand with deficits in spatial learning.

In an attempt to rule out a response competition account, Maren *et al*. cite a study showing that local infusion of the *N*-methyl-D-aspartate (NMDA) antagonist DL-2-amino-5-phosphonovalerate (APV) into the dorsal hippocampus during contextual fear conditioning disrupted freezing measured the next day[8]. However, the dose of APV infused into the hippocampus (10 μg) was twice the maximal dose given intraventricularly (5 μg) to block contextual fear conditioning[9]. Because lower, rather than higher, doses of APV given locally would be expected to block conditioning, these data do not rule out the possibility of spread to extra-hippocampal structures or the ventricles. Hence, further studies are required to demonstrate the importance of NMDA receptors in the hippocampus in contextual fear conditioning.

Hippocampal lesions disrupt freezing to explicit cues

An important foundation of Maren *et al.*'s thesis is that: 'dorsal hippocampal lesions attenuate freezing to contextual conditioned stimuli (CSs) but do not alter freezing behavior to discrete CSs'. However, they have recently reported that chemical lesions of the dorsal hippocampus disrupted freezing

 1364-6613/98/$19.00 PII: S1364-6613(98)01124-3