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Article *in* Hippocampus · January 2002

DOI: 10.1002/hipo.10071 · Source: PubMed

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HIPPOCAMPUS 12:561–565 (2002)

The Hippocampus and Pavlovian Fear Conditioning: Reply to Bast et al.

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In a recent article that appeared in *Hippocampus*, we reviewed findings supporting a mnemonic role for the dorsal hippocampus (DH) in Pavlovian (contextual and tone) fear conditioning (Anagnostaras et al., 2001). We also detailed a view that has emerged over the years from this work that suggests that the hippocampus plays a highly selective role in the acquisition and temporary storage of contextual representations, as opposed to a role in conditional stimulus–unconditional stimulus (CS–US) associations or in permanent storage for which the amygdala has been heavily implicated (Kim and Fanselow, 1992; Phillips and LeDoux, 1992; Young et al., 1994; Maren and Fanselow, 1996; Maren et al., 1996,1997, 1998; Anagnostaras et al., 1999). Because the evidence that DH lesions produce a temporally graded retrograde amnesia selective for contextual fear that accords well with declarative memory deficits in amnesic humans, we have further argued this may be a good model system with which to study the transformation of memory from a hippocampus-dependent to a hippocampus-independent (cortical) state (i.e., consolidation) (Scoville and Milner, 1957; Squire, 1992; Squire and Alvarez, 1995; Hodges and Graham, 1998; Squire et al., 2001; Murre et al., 2001; Frankland et al., 2001).

In a letter to *Hippocampus* regarding our recent article, Bast et al. (2001b) expand on our review to discuss their recent data (and the data of others), focusing in particular on findings from lesions of the ventral hippocampus (VH) and discussing how these are problematic for the view we presented. Although a specific hypothesis on the role of the VH in fear conditioning has not yet been formulated, several interesting findings were reviewed, emphasizing, in particular, the effects of complete or VH lesions on both tone fear conditioning and on remotely acquired fear (e.g.,Mumby et al., 1999; Sutherland et al., 2001). These findings are in contrast to DH lesions, in which a severe and selective deficit for recently acquired contextual fear, but not for tone or remotely acquired fear, is typically found (Kim and Fanselow, 1992; Phillips and LeDoux, 1992; Maren et al., 1997; Anagnostaras et al., 1999). In this reply, we address and expand on some of the issues raised by Bast et al. (2001b). Although this can help clarify where agreements lie in some of the empirical findings, we feel there is not yet enough experimental evidence to offer a specific role for the VH in fear conditioning, and it is not yet clear whether these findings challenge the views offered in our review. However, we agree that, as the interface between the DH and amygdala, future work in the area of VH will be essential to our understanding of the neural circuits involved in contextual fear conditioning:

1. Complete, excitotoxic hippocampal lesions abolish Pavlovian conditioned freezing. It is evident from several studies that complete hippocampal lesions, which in rat memory studies have only been done with excitotoxins, produce a nonselective and nonspecific deficit in contextual and tone fear conditioning, for both recent and remote fear. In several studies using kainite-colchicine lesions of the hippocampus in Sutherland's laboratory, the findings indicate substantial impairments in remote and recent fear, as well as similar deficits in other tasks such as the hidden-platform Morris water maze (Weisend et al., 1996; Sutherland et al., 2001). Indeed, in our own work, we have recently found that complete hippocampus lesions produced by ibotenic acid in the manner described by Jarrard (1989) produce a severe temporally ungraded retrograde amnesia of both contextual and tone fear (Anagnostaras et al., 1998). Considering this kind of evidence, Nadel and Moscovitch (1997, 2001) have offered a view in which the hippocampus is permanently involved in memory storage, challenging considerable work on human amnesics, including that collected by Squire, which indicates a temporary role for the hippocampus in memory storage (Knowlton and Fanselow, 1998; Squire et al., 2001; Murre et al., 2001). In the Nadel and Moscovitch account, multiple memory traces come to be formed over time as the result of rehearsal or retrieval, which allows this view to accommodate the immunity to partial hippocampal lesions that memory gains over time. Although there is little evidence to support directly the idea that multiple memory traces are formed within the hippocampus, the view is consistent with the empirical data from both rats and humans that retrograde amnesia is temporally graded after partial damage to the hippocampus. In addition at first glance, the finding that complete hippocampus lesions appear to produce a temporally ungraded amnesia is problematic for the Squire consolidation view (Nadel and Bohbot,

DOI 10.1002/hipo.10071

2001). However, as we stated in our review, the lack of data on several fronts is problematic for the Nadel view. In the standard consolidation model, it is argued that memory is transferred from the hippocampus to the cortex. Indeed, there is an abundance of evidence that hippocampus damage produces impairments in recent memory while sparing remote memory, and recent evidence indicates that cortical impairments or damage can produce a reverse temporal gradient, impairing remote memory while sparing recent memory (Hodges and Graham, 1998; Graham, 1999; Frankland et al., 2001; Murre et al., 2001). Moreover, as we stated in our review (Anagnostaras et al., 2001), several problems with studies using complete hippocampus lesions in rats suggest that the effects may in fact be expected from the Squire consolidation model. Specifically, we suggested that kainite-colchicine lesions produce distal damage to the cortex. A study of rats lesioned from the Sutherland laboratory indicated a 10% reduction in cortical volume (Day et al., 1999) and damage to the amygdala has also been shown after kainate injection into the hippocampus (Jarrard and Meldrum, 1990). Indeed, we have recently completed an extensive study in rats with complete and partial ibotenic acid lesions of the hippocampus in a within-subject design very similar to that used by Anagnostaras et al. (1999; for preliminary data, see Anagnostaras et al., 1998). Although the full details of this study will be published elsewhere, we found (1) a severe loss of remote and recent context and tone fear after complete hippocampus lesions, similar to partial amygdala lesions; (2) substantial cortical damage after partial (dorsal) or complete ibotenic acid lesions directed at the hippocampus; (3) damage to the cortex as a better predictor of remote context and tone memory deficits than hippocampus damage; and (4) severe hyperactivity in rats with complete hippocampus lesions, with a limited ability to produce the freezing response. Indeed, the mnemonic impairments after complete hippocampus lesions very much resembled the effects produced by partial amygdala lesions.

2. Evidence of distal damage and disruption of the cortex and amygdala is consistent with both the Squire consolidation model and the Nadel multiple memory trace model. It is important to note that any lesions that produce distal damage in the cortex and amygdala are predicted to produce a remote and permanent memory impairment by the consolidation model. This is because that model assumes permanent memory is stored in cortex (Squire and Alvarez, 1995). Indeed, the effects of those lesions would probably be predicted by almost any model, because the amygdala has been shown to be permanently involved in fear associations in rats (Gale et al., 1999; Lee et al., 1996; Maren et al., 1996). Moreover, even small partial lesions of the amygdala produce a severe retrograde amnesia for both context and tone fear (Maren, 1999a). Therefore, because the amygdala and cortex are believed to be essential to permanent memory in fear conditioning, the effects of complete but selective hippocampus lesions on fear conditioning remain unanswered until lesions can be produced that are clearly shown not to produce damage or disruption in the cortex or amygdala. A study of trace eyeblink conditioning by Kim et al. (1995) may by informative in this regard. Those researchers made complete aspirations of the hippocampus after eyeblink conditioning. Hippocampectomy performed 1 day, but not 1 month, after training eliminated expression of eyeblink conditioning. Although aspiration lesions will certainly interrupt fibers of passage, perhaps they do not cause as much distal damage or disruption as excitotoxic lesions. Alternatively, this may be because the primary site of eyeblink conditioning is in the cerebellum, far away from the site of the lesion, rather than in the amygdala, as in fear conditioning. One additional problem with conclusions drawn from studies using excitotoxins relates to the evidence that these toxins produce cell death through oxidative stress and mitochondrial pathology (e.g., Liang et al., 2000). Cells in the hippocampus probably exhibit massive discharge for a sustained period of time, perhaps producing catastrophic interference in other structures that may try to encode this persistent noisy output (e.g., McClelland et al., 1995). This could interfere with previously established memories. Moreover, this massive glutamate output could also produce oxidative stress in distal cortical cells, making them more susceptible to cell death later, or reducing the efficacy of learning and memory mechanisms, which considerably overlap with the mechanisms involved in mitochondrial stress. Indeed, in unpublished work examining ibotenic acid lesions of the hippocampus, we have found considerably more cortical damage when histology was done 6 months, as compared with 4 days after the lesion. Therefore, we suggest that excitoxins may be less than ideal for the study of retrograde amnesia. Indeed, when we compared ibotenic acid lesions of the DH with electrolytic lesions of roughly the same (small) size, electrolytic lesions were found to produce a highly selective deficit for recent context fear (with $<$50-day gradient), but excitoxic lesions produced an extended gradient ($>$100 days) and modest tone deficits (Anagnostaras et al., 1998; compare Anagnostaras et al., 1999 with Maren et al., 1997). These findings will be published in detail elsewhere. Therefore, we suggest that considerable additional work is necessary to develop effective ways of producing large lesions of the hippocampus without producing damage or functional disturbance elsewhere. This problem appears to be particularly great in rodents, where, in contrast to monkeys or humans, the hippocampus makes up a very significant portion of the overall brain volume. In rats, the surface area of the hippocampal formation is $\sim$1.2 cm^2, whereas the entire isocortex is only slightly larger at 1.5 cm^2 (Amaral and Witter, 1995). It may very difficult to disrupt such a large proportion of the brain without severely disturbing other brain structures. Perhaps molecular genetic techniques will offer a way to induce selective cell death in the hippocampus without producing it elsewhere.

3. Damage in the area of ventral hippocampus and ventral subiculum impairs freezing. As Bast et al. (2001b) are careful to point out, the amygdala receives its hippocampal afferents from the VH, not the DH. Indeed, this projection has been implicated in context-shock associations (Maren and Fanselow, 1996). This has been particularly problematic for studies of lesions in this area because it is difficult to argue that lesions of this region would not affect the amygdala, producing either cell death or functional disruption by deafferentation. For example, after lesions of the ventral subiculum, Maren (1999b) found a severe reduction of tone and context fear conditioning. Although the disruption of freezing did not appear to be due to hyperactivity, it remains unclear whether it is due to amygdala disruption. Likewise, as re-

viewed above complete hippocampus lesions seem to produce a similar disruption of conditioned freezing in general. Therefore, in contrast to the DH, lesions that include the VH are complicated by behavioral results that could reflect a generalized loss of fear (or of conditioned fear) requiring extensive controls for performance of fear and for distal damage. Considerable further study, particularly employing techniques such as those used by Bast et al. (2001a) will help elucidate whether a mnemonic role for the ventral hippocampal area, independent of effects on the amygdala, is indicated in fear conditioning.

4. Temporary inactivation studies can complement lesion studies, particularly for memory acquisition, but are not more decisive in the role of a structure in memory storage. Bast et al. (2001b) review a number of interesting and recent studies completed in their laboratory and others, in particular those using tetrodotoxin (TTX) or muscimol inactivation to investigate role of the hippocampus in fear conditioning. In one study, it was found that pre-training TTX infusion induced deficits to both context and tone conditioning, whereas muscimol infusion induced context deficits with milder (not significant but numerically present) tone conditioning deficits (Bast et al., 2001a). However, although we agree that pharmacological inactivation methods are quite useful in the study of memory acquisition (anterograde), we consider them less useful for the study of memory storage (retrograde) for several reasons. Most pharmacological agents do not last very long, while the time course for consolidation and transformation of memory is believed to be at least several days if not much longer (e.g., Squire, 1992; Kim and Fanselow, 1992). Most cannula preparations cannot be kept for adequate periods of time; even when chronic microinfusions have been performed, substantial neuroadaptation may occur (Bannerman et al., 1995). Moreover, molecular studies suggest several waves of consolidation, which may involve a repetition of molecular steps (Bourtchuladze et al., 1998). In theory, one then needs to identify these phases specifically and infuse the agent at the various times, in order to determine realistically the role of each structure. Lastly, the time course of memory phases revealed by inactivation appear to be shorter but may mirror the pattern revealed by lesions. In one particularly illuminating study, TTX inactivation in between the time of training and testing of the DH, basolateral amygdala (BLA), and perirhinal cortex revealed a wave of consolidation requiring sodium channel activation for progressively longer periods in these respective brain structures (Sachetti et al., 1999). TTX inactivation selectively affected context conditioning in the DH only up to 1.5 h after training, BLA inactivation affected both tone and context conditioning only up to 48 h after training, with perirhinal cortex inactivation affecting both up to 96 h. In contrast lesions of the DH affect context conditioning for at least 1–2 weeks after training, and BLA lesions affect both context and tone conditioning for the lifetime of the rat (Kim and Fanselow, 1992; Anagnostaras et al., 2001; Gale et al., 1999; Maren et al., 1996; Lee et al., 1996). Nonetheless, the general sequence of structures involved in consolidation is that which could be expected from lesion studies. Therefore, considerable further study, particularly examining the effects of inactivation on recall during testing after long periods of consolidation is required. Even then, it is not known if structural memory circuits may still be available for recall during inactivation by various agents (e.g., Corcoran and Maren, 2001). Therefore, while inactivation studies will add to our growing knowledge of the roles of memory structures in memory, and offer some advantages over lesions, we do not believe they offer more decisive evidence over lesions when investigating memory storage.

5. In humans and monkeys, damage to the area of hippocampus corresponding to rat ventral hippocampus produces selective mnemonic recent memory deficits. A noteworthy piece of data regarding this recent controversy over ventral hippocampus damage in rats comes from studies of humans and nonhuman primates. The rat or mouse 'ventral hippocampus' anatomically corresponds to the human or monkey "anterior" or "rostral" hippocampus (e.g., Amaral and Witter, 1995; Rempel-Clower et al., 1996). In most rodent studies, the DH is explored, because it is conveniently oriented for stereotactic placement studies toward the top of the skull; in contrast, much of the ventral hippocampus lies below the thalamus and is very posterior, making placements that do not produce extrahippocampal damage quite difficult (Paxinos and Watson, 1998). In contrast, most studies in humans and monkeys most often depict the anterior (i.e., rodent ventral) hippocampus, perhaps because it is the portion most accessible from the inferior view of the medial temporal lobe. Therefore, it is illuminating that studies that include significant or complete anterior hippocampal damage in humans or monkeys still find a highly selective and temporally graded retrograde amnesia for explicit memory (Rempel-Clower et al., 1996; Zola-Morgan and Squire, 1990). These findings suggest that the primate anterior/rodent ventral hippocampus does not play a more general role in memory than has been explicitly proposed for the rat dorsal (i.e., primate posterior/caudal) hippocampus. Alternatively, the primate anterior hippocampus may play a more general role in fear conditioning than it does in declarative memory, or the rat ventral hippocampus may be more generally involved in memory than the primate anterior hippocampus perhaps because the cortex is more evolved. However, we think a more likely explanation is that very significant damage or impairment has been made to extrahippocampal structures in rat studies of ventral hippocampus, both because of its placement close to the amygdala and because of its relative size compared with the cortex.

CONCLUSIONS

The commentary by Bast et al. (2001b) is indeed timely, and it is obvious that the study of the rodent ventral hippocampus may tell us a great deal about the role of the hippocampus in memory, particularly because of its connectivity. However, considerable more data remains to be collected, given that many issues remain unresolved regarding the effects of VH manipulations (especially after training) and whether these effects are independent of effects on the amygdala. Therefore, we do not believe that the existing data warrant a reformulation of the specific role in the acquisition and temporary maintenance of contextual representations we have offered for the dorsal hippocampus in Pavlovian fear conditioning,

which accords well with data from studies in humans and monkeys (Anagnostaras et al., 2001; Maren et al., 1998). Indeed, Bast et al. (2001b) do not offer a specific hypothesis for the role of the ventral hippocampus in fear conditioning. Their data suggest that its role is probably mnemonic but may differ from that of the dorsal hippocampus. Therefore, future studies should be fruitful in shedding light on this role. However, we recommend some vigilance in considering how these data will accord with existing data from humans and monkeys.

Acknowledgments

This work was supported by NSF grant IBN0091487 (to M.S.F.). S.A. was supported by NIH grant F32 NS10932.

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