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A Behavioral Analysis of the Role of CA3 and CA1 Subcortical Efferents During Classical Fear Conditioning

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It has been proposed that the hippocampus and subcortical structures interact during the processing of fear and anxiety-related information. It has been demonstrated that the subcortical efferents from CA3 and CA1 can be selectively disrupted without concomitant disruption to the afferents. The present experiment was designed to evaluate the role of CA3 efferents via the fimbria and the CA1 efferents via the dorsal fornix for encoding and consolidation/retrieval of classical fear conditioning. The present data suggest that the subcortical projections from CA3 and CA1 are differentially involved in the processing of classical fear conditioning, with CA3 subcortical efferents supporting acquisition of both cued and contextual fear but only supporting retention of contextual fear and CA1 subcortical efferents supporting the encoding and retrieval of both cued and contextual fear. These data further suggest that all hippocampal efferents are not homogeneous, and that the hippocampus and subcortex interact to process conditioned fear.

Keywords: dorsal fornix, fimbria, hippocampus, septum, classical fear conditioning

It has been proposed that septum and hippocampus interact to modulate anxiety and fear (Gray & McNaughton, 2000; Sheehan, Chambers, & Russell, 2004). The effects of lesions within the hippocampal-septal system are mixed—with some reports emphasizing an interaction between the hippocampus and septal nuclei, and others suggesting that the hippocampus and septal nuclei act independently (Bannerman, Matthews, Deacon, & Rawlins, 2001; Conejo, Lopez, Cantora, Gonzalez-Pardo, Lopez, & Begega, 2005; Gray & McNaughton, 1983; Maren & Fanselow, 1997; Phillips & LeDoux, 1995; Sparks & LeDoux, 1995; Thomas, Yadin, & Strickland, 1991; Vouimba, Garcia, & Jaffard, 1999). In addition, in studies of the direct interaction between the hippocampus and septum, full fimbria/fornix lesions are usually employed, which preclude analysis of the independent contributions of afferent and efferent pathways (cf. Maren & Fanselow, 1997; Phillips & LeDoux, 1995). Despite these difficulties, it has been clearly demonstrated that the medial and lateral septum process conditioned fear (Calandreau, Jaffard, & Desmendt, 2007), but they do so in qualitatively different ways from the contributions of the dorsal or ventral hippocampus (Bannerman et al., 2001; Hunsaker & Kesner, 2008). To date, the role of the hippocampal-septal efferents via the fimbria and dorsal fornix has not been specifically evaluated for fear-related information processing.

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CA3 subcortical efferents project via the fimbria to the lateral septum, medial septum, and diagonal band of Broca. CA1 subcortical efferents project via the dorsal fornix to the lateral septum, medial septum, and diagonal band of Broca, as well as to the mammillary bodies and other structures along the Papez circuit (Raisman, Cowan, & Powell, 1966; Swanson & Cowan, 1977; Wyss, Swanson, & Cowan, 1980). More specifically, CA3 projects to the septofimbrial nucleus, throughout the rostral lateral septum, the dorsal portion of the medial septum, as well as the dorsal-most portions of the vertical limb of the diagonal band of Broca. CA1 projects to the septofimbrial nucleus, the ventral portions of the medial septum, and the horizontal limb of the diagonal band of Broca. There are only sparse projections to the ventral-most portion of the caudal lateral septum. These differential targets of CA3 and CA1 subcortical efferents suggest that there may be functional heterogeneity between these two sets of efferents (cf. Hunsaker, Tran, & Kesner, 2008; Sheehan et al., 2004).

To investigate the roles of these two subcortical efferent pathways for the encoding and retrieval of classical fear conditioning, we have developed a transection paradigm whereby we selectively disrupt hippocampal efferents to the septum while leaving the afferents from the septum intact. In this experiment, one group of animals had CA3 subcortical efferents in the fimbria transected, but afferents left intact. A second group of animals had CA1 subcortical efferents in the dorsal fornix similarly transected (cf. Hunsaker, Allan, & Kesner, 2007; Hunsaker, Rogers, & Kesner, 2007; Hunsaker et al., 2008). This experiment was designed to better characterize the interaction between hippocampal efferent pathways and their septal targets during tests of learning and memory. After transecting these pathways, animals were run on a classical fear-conditioning paradigm to evaluate the effects of the transections for the encoding and consolidation/retrieval of tone-cued and contextual fear.

Materials and Method

Subjects

Sixteen male Long–Evans rats, approximately 4 months of age and weighing 280 to 350 g at the start of the experiment served as subjects. The colony room was maintained on a 12-hr light/dark cycle. All rats had ad libitum access to water and maintained at 90% to 95% of their free feeding weight. All experimental protocols conformed to Institutional Animal Care and Use Committee (IACUC) and Assessment and Accreditation of Laboratory Animal Care (AAALAC) regulations. Before participating in the present experiment, all animals had participated in a study involving a modified Hebb Williams maze (Hunsaker et al., 2008).

Surgery

Male Long–Evans rats received a transection of CA3 subcortical efferents in the fimbria ($n = 5$), a transection of CA1 subcortical efferents in the dorsal fornix ($n = 5$), or a control surgery ($n = 6$; fimbria control $n = 3$; dorsal fornix control $n = 3$). The transection protocols used for these same animals have been detailed elsewhere (Hunsaker et al., 2007a,b, 2008). Briefly, animals were anesthetized with a mixture of ketamine and xylazine (65 mg/kg; 10 mg/kg) and for dorsal fornix transections twisted bipolar stimulating/recording electrodes were lowered into the medial septum (AP + 0.7, ML 0.1, DV ~4–5—all at a slight angle away from the midline (~5 degrees) to avoid rupturing any vasculature along bregma) and CA1 (AP −3.0, ML 1, DV ~1.5–2). A ground screw was secured in the skull above the rostral cortex in lowered to come in contact with dura. After dura was carefully punctured to allow easy penetration of electrodes, potentials were alternately evoked in the medial septum from CA1 and in CA1 from the medial septum. The placements of the electrodes were adjusted to maximize this signal in both directions, and then remained untouched throughout the remainder of the surgical procedure. A retractable wire knife was lowered stereotactically until it was situated adjacent to the dorsal fornix (AP −2.3, ML 0.5, DV ~2), after which the blade was protracted such that it was between CA1 and the dorsal fornix in the alveus. The knife was slowly raised until the medial septal responses evoked by CA1 stimulation were attenuated. It was then verified that stimulation of the medial septum was able to evoke responses in CA1. This was then performed on the other hemisphere, resulting in a bilateral transection of the dorsal fornix. The choice of the location of the dorsal fornix transection was such that there was never damage to CA1 or to the fimbria, also such that the transection was as rostral as possible without risking disruption of fibers from CA3 to the fimbria. Control surgery involved everything except the actual transection (i.e., the knife was lowered into place, protracted, and then immediately retracted again). For partial fimbria transections electrodes were lowered into the lateral septum (AP + 0.6, ML 0.6, DV ~3–4) and CA3 (AP −3.0, ML 3.0, DV ~3.0). After dura was carefully punctured to allow easy penetration of electrodes, potentials were alternately evoked in the lateral septum from CA3 and in CA3 from the lateral septum. The placements of the electrodes were adjusted to maximize this signal in both directions, and then remained untouched throughout the remainder of the surgical procedure. The retractable wire knife was lowered to be just ventral to the fimbria (AP 2.3, ML 3.2, DV ~4), after which the blade was protracted such that potentials in the lateral septum from CA3 stimulation were attenuated but the potentials in CA3 from lateral septal stimulation were unchanged. It was then verified that stimulation of the lateral septum was able to evoke responses in CA3. This was then performed on the other hemisphere, resulting in a bilateral transection of the fimbria. The location of the partial fimbria transaction was chosen such that CA3, the lateral septum, thalamic nuclei, and the dorsal fornix remained undisturbed.

After surgery, animals were placed in their cages on a heating pad for 1 to 2 hours to recover and given sweetened, crushed food, as well as given acetaminophen in their water (Children's Tylenol; 50 ml/100 ml water) for 3 days after surgery as an analgesic. Animals were given 2 weeks to recover from surgery before starting any experimentation.

Behavioral Apparatus and Procedure

Experimental apparatus. Two chambers were used during 3 days of testing. The first chamber was used for encoding and for the contextual fear retention test. One chamber (28 × 21 × 22 cm; Coulbourn Instruments; Allentown, PA) consisted of two transparent Plexiglas walls (rear wall and front door) and two aluminum sidewalls. The chamber floor was made up of 18 steel rods connected to a shock delivery apparatus used to deliver the shock. The chamber was surrounded by five small dolls and there were posters on the walls to provide a clear context. Before and after conditioning, the chamber was cleaned with an unscented cleaning solution to remove any olfactory cues an animal may have left during conditioning.

The second observation chamber was used during tests of auditory-cued fear retention. This chamber (32 cm^2) was constructed entirely of transparent Plexiglas. No cues surrounded this chamber, only a green curtain and the table upon which the chamber was set. The floor of the chamber was covered with a single paper towel before placing the rat inside. Before and after conditioning, the chamber was cleaned with the same unscented cleaning solution used in the other chamber to remove olfactory cues.

Behavioral methods

Encoding—Day 1. Rats were placed in the conditioning chamber for 2 minutes before the first auditory stimulus, after which they received 10 auditory-shock pairings separated by 74 seconds. An auditory stimulus (10-s duration, 2 kHz, 85-dB) was presented through a speaker to initiate each trial. An electric foot shock (2-s duration, 0.75 mA) was presented coterminal with the auditory stimulus. A 74-s intertrial interval (ITI) separated each successive tone + shock pairing. After 10 tone + shock pairings and subsequent ITIs, rats remained in the chamber for an additional 2 minutes. A freezing response was measured by an observer who scored freezing behavior every 8 seconds during the preexposure period and ITI (the 2 seconds after the shock were discarded and freezing during the subsequent 72 seconds was recorded) and every 4 seconds during the tone stimulus; resulting in two auditory observations and nine ITI observations for each trial. The first nine trials of acquisition were blocked into 3-trial blocks for analysis of acquisition. Tone and contextual (ITI) freezing were also blocked

into a single 10-trial block for further characterization of any specific tone or contextual fear acquisition deficits. It is understood that the contextual measure is not completely dissociable from tone-related freezing, but previous research has shown that separating tone and contextual freezing as we have here is a fair approximation of separate processes (Hunsaker & Kesner, 2008).

Contextually fear retention test—Day 2 or 3. Each rat was tested for retrieval of contextually cued fear either 24 or 48 hours after acquisition (half on Day 2 and half on Day 3, counterbalanced with auditory-cued fear retention tests). The rat was placed in the encoding chamber for 8 minutes in the absence of the auditory cue and shock for eight minutes (blocked for analysis into eight, 1-min trials). Freezing behavior was measured every 8 seconds. Because of extinction in the control group, only the first 4 minutes of testing were blocked and used for statistical analysis.

Auditory-cued fear retention test—Day 2 or 3. Each rat was tested for retrieval of auditory-cued fear either 24 or 48 hours after acquisition (half on Day 2 and half on Day 3, counterbalanced with contextual fear retention tests). The rat was placed in the encoding chamber for 8 minutes in the continuous presence of the auditory cue for 8 minutes (blocked for analysis into eight, 1-min trials). Freezing behavior was measured in 8-s intervals. Because of extinction in control animals, only the first 4 minutes of testing were blocked and used for statistical analysis.

Histology

At the conclusion of testing, each rat was deeply anesthetized with an injection of sodium pentobarbital (70 mg/kg i.p.), perfused intracardially with PBS and 10% (wt/vol) formalin, and the brain was removed from the skull and stored at 4 °C in a solution of 30% sucrose in 10% formalin (wt/vol) for 72 hours before further processing. The brain was frozen and 40 μm sections collected through the medial septum and hippocampus for Nissl and acetylcholinesterase staining.

Dependent Measures and Statistical Analysis

Freezing scores during encoding and retrieval tests were transformed to percentages and were blocked for statistical analysis. A two-way repeated measures ANOVA with groups as the between factor and blocks of three trials as the within factor was employed for testing group differences during the encoding of delay fear conditioning (e.g., the first nine trials of acquisition were blocked into three, 3-trial blocks). The last trial was excluded arbitrarily to keep all blocks the same size and to keep the number of blocks at three, as has been presented previously (Hunsaker & Kesner, 2008). One-way ANOVA with groups as the between factor were used to analyze encoding (single 10-trial acquisition block during acquisition) and retention of contextual and auditory-cued fear (single 4-min block during the tests) between groups. All main effects were considered significant at $p < .05$. Fisher's least significant difference (LSD) post hoc paired comparisons tests were performed upon all significant effects. Overall acquisition of fear conditioning for each group is presented in three blocks of trials, plotted as means $\pm SE$. Contextual and auditory-cued fear encoding were collapsed into a single 10-trial block for analysis. Retention tests are presented as mean percent freezing $\pm SE$ for single 4-min blocks.

Results

Histology

The results of the partial fimbria and dorsal fornix transections are depicted in Figure 1. More thorough anatomical, neurophysiological, and neurochemical analyses of the lesions of these same animals have been reported elsewhere (Hunsaker et al., 2007, 2008). In short, for animals with dorsal fornix transections, the responses in the medial septum from CA1 stimulation were reduced by the transection, whereas the CA1 responses from medial septal stimulation were unchanged. For animals with partial fimbria transections, the responses in the lateral septum from CA3 stimulation were reduced by the transection, whereas the CA3 responses from the lateral septum were unchanged. Anatomical tract tracing experiments using biotynilated dextran amine previously reported by Hunsaker et al. (2008) revealed that the dorsal fornix transection disrupted ~50% of CA1 subcortical efferents, without significantly disrupting projections from the medial septum into the hippocampus. It should be noted that since only 50% of these efferents were transected, the dorsal fornix transection was not complete. Partial fimbria transections disrupted ~63% of CA3 subcortical efferents, without significantly disrupting projections from the septum into the hippocampus. Again, it must be emphasized that this manipulation was incomplete. In the case of either transection, there was no disruption in acetylcholinesterase staining in the hippocampus after the transection, additionally suggesting that there was no large-scale disruption to the projections from the medial septum into the hippocampus (Figure 1A–C). Detailed anatomical, neurophysiological, and histochemical data that demonstrate the specificity and efficacy of these manipulations have been reported previously (cf. Hunsaker et al., 2007a,b, 2008). The reader is referred to those reports since all the animals in the present study were those used by Hunsaker et al. (2008).

Behavior

Figure 2A shows the overall acquisition of classical fear conditioning. A two-way repeated measures ANOVA with groups (fimbria transection, dorsal fornix transection, control) as the between factor and blocks of 3-trials as the within repeated factor were

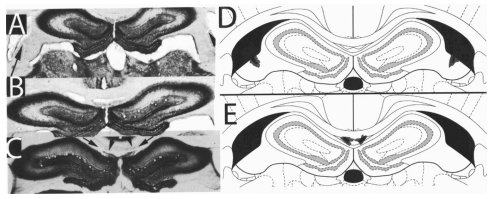

Figure 1. Histology. (A) Acetylcholinesterase (AChE) stained section from a partial fimbria transection. (B) AChE stained section from a control animal. (C) AChE stained section from a dorsal fornix transection. There were no differences in AChE reactivity between control animals and animals receiving either of the transections. In both (A) and (C) the transections are signaled by arrows. (D) Diagram of a partial fimbria transection after (A). (E) Diagram of a dorsal fornix transection. In both (D) and (E) the largest (gray) and smallest (black) lesions are depicted. The reader is referred to Hunsaker et al. (2008) for more detailed neurophysiological, anatomical, and histochemical analyses of these lesions.

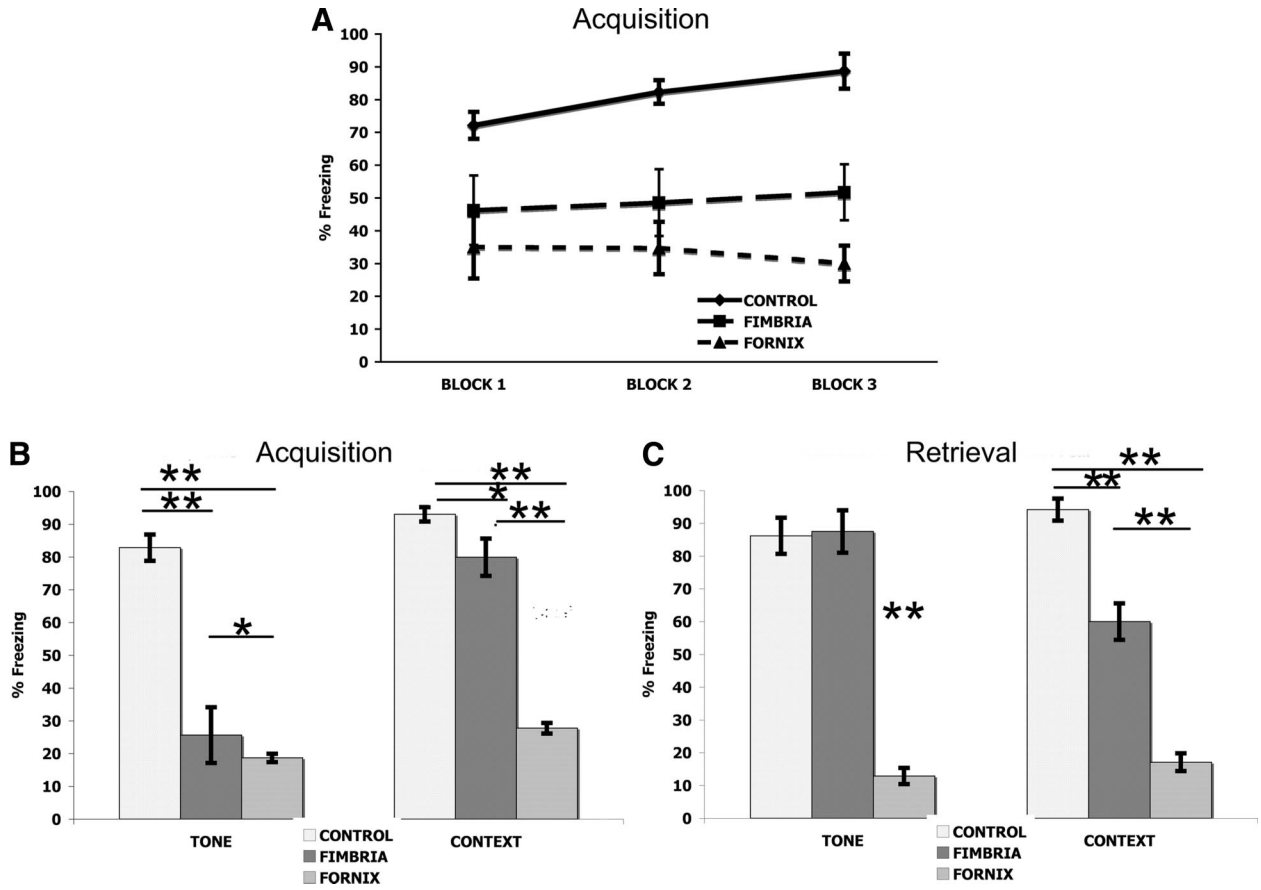

Figure 2. Encoding and Consolidation/Retrieval of delay fear conditioning. (A) Both the partial fimbria transection group and the dorsal fornix transection groups showed overall acquisition deficits relative to control animals. (B) The partial fimbria transection and dorsal fornix transected animals showed deficits for the encoding of tone and contextual fear during acquisition. $^{*}p < .05$. $^{**}p < .01$. (C) The partial fimbria transection group showed no differences from controls for retention of tone-cued fear, whereas the dorsal fornix transected animals showed large deficits. Both the partial fimbria and dorsal fornix transected animals showed deficits relative to controls for the retention of contextual fear. $^{*}p < .05$. $^{**}p < .01$.

performed on the data as plotted. There was a significant group effect, $F(1, 15) = 12.00$, $p < .002$, an effect for blocks, $F(2, 30) = 6.55$, $p < .006$, and a group $\times$ block interaction, $F(2, 30) = 7.87$, $p < .001$. A Fisher's LSD post hoc paired comparisons test revealed that both the fimbria transection and dorsal fornix transection groups showed an overall acquisition deficit relative to control animals (all ps < 0.01). Fimbria transected animals and dorsal fornix transected animals did not differ ($p > .1$). Despite the deficit in acquisition, the transection groups did show some acquisition and were not at floor levels. It also should be noted that no animals appeared either hyperactive or showed freezing behaviors during the 2-min baseline period before acquisition, though quantitative data were not collected.

To evaluate whether it was a specific deficit in tone or contextual fear encoding, the acquisition data were separated into tone encoding and contextual encoding (Figure 2B), and the separated data were collapsed into a single 10-trial block for analysis. One-way ANOVA with group as the between factor were used to analyze tone and contextual fear encoding. For tone acquisition, there was an effect for group, $F(2, 15) = 45.98$, $p < .001$. A Fisher's LSD post hoc paired comparison revealed that both the fimbria transection and dorsal fornix transection group froze less than the control group (ps < 0.01). The fimbria transection and dorsal fornix transection groups did not differ ($p > .1$). For context acquisition, there was an effect for groups, $F(2, 15) = 96.17$, $p < .001$. A Fisher's LSD post hoc paired comparisons test revealed that the dorsal fornix transection groups froze less than the control group ($p < .01$). The dorsal fornix transection group froze less than the fimbria transection group ($p < .05$). The fimbria transection group also froze less than the control group ($p < .05$). This suggests the fimbria transection group showed a lesser impairment relative to the dorsal fornix transection group. Additionally, though the dorsal fornix group showed impairments in acquisition relative to the other groups, they were not at baseline freezing levels. Their learning was impaired relative to the other groups, but not absent.

One-way ANOVA with group as the between factor were used to evaluate the tone and contextual fear retention data. The tone and contextual fear data were blocked into single 4-min blocks for analysis (Figure 2C). For the retention of tone-cued fear, there was

an effect for groups, $F(2, 15) = 64.97$, $p < .001$. A Fisher's LSD post hoc paired comparisons test revealed that the dorsal fornix transection group froze less than both the fimbria transection group and controls (ps < 0.01). Fimbria transected animals and control animals froze similarly ($p > .1$). For the retention of contextual fear, there was an effect for groups, $F(2, 15) = 96.25$, $p < .001$. A Fisher's LSD post hoc paired comparisons test revealed that fimbria transected animals froze less than control animals ($p < .01$), but froze more than dorsal fornix transected animals ($p < .01$). Dorsal fornix transected animals froze less then control animals, as well ($p < .01$). This pattern of results suggests that the fimbria transection group showed a lesser impairment relative to the dorsal fornix transection group.

Discussion

The present data suggest that although the CA3 and CA1 efferents into the lateral and medial septum (and CA1 efferents to the subcortex) both participate in the acquisition of conditioned fear, they do so in a slightly different manner. This is reflected in the fact that animals without CA3 efferents to the lateral septum have some consolidation/retrieval of contextual fear and efficient consolidation/retrieval auditory-cued fear, whereas animals without CA1 efferents appear to have little to no memory whatsoever of the conditioned fear. Although the animals without CA1 subcortical efferents (the dorsal fornix transection group) as well as the animals without CA3 subcortical efferents (the partial fimbria transection group) showed deficits for acquisition, they were not at floor and did appear to acquire some cued fear, just less efficiently than the control group. This is reflected in the fact that the animals froze more during conditioning than they did during the 2-min baseline condition and the first trial of conditioning.

Although counterintuitive, it has been demonstrated that encoding (or acquisition) and consolidation/retrieval processes are partially dissociable (cf. Aggleton & Saunders, 1997; Vann & Aggelton, 2004). In fact, recent studies have shown similar phenomena in primates and rodents with lesions to the fornix (Aggleton & Saunders, 1997; Buckley, Eilson, & Gaffan, 2008; Eacott & Gaffan, 2005; Mitchell, Browning, Wilson, Baxter, & Gaffan, 2008) and in studies with lesions along the Papez circuit (targets of the projections in the fornix; cf. Vann & Aggleton, 2004). In fact, the same fimbria transection animals that participated in the present experiment previously caused acquisition or encoding deficits on a modified Hebb-Willliams maze, without concomitant deficits for retrieval (i.e., they had difficulty learning the task, but remembered what they had learned as well as the control group; Hunsaker et al., 2008). That is not to say that encoding and retrieval are completely dissociable, but it means that there can be intact retrieval after impaired acquisition, and vice versa (cf. Hunsaker et al., 2008).

It has been proposed that the hippocampus and subcortical structures form a neural circuit that modulates anxiety in the animal (Gray & McNaughton, 2000). It has also been determined that there are unique patterns of behavioral and cognitive deficits after selective lesions at different locations within the septal-hippocampal circuit (Gray & McNaughton, 1983). The model proposed by Gray and McNaughton (2000) posits dynamic, real-time interactions between the hippocampus and septum during some learning and memory paradigms. There are reports of medial septal lesions resulting in deficits for classical fear conditioning (Bannerman et al., 2001; Maren & Fanselow, 1997; Sparks & LeDoux, 1995; cf. Calandreau et al., 2007 for an inactivation experiment), but the present data do not match these previous findings. It has also been demonstrated that fornix lesions result in deficits for fear conditioning (Phillips & LeDoux, 1995). The present deficit pattern does not mimic those seen after dorsal and ventral CA1 and CA3 excitotoxic lesions (Hunsaker & Kesner, 2008), nor do they mimic the effects of full dorsal or ventral hippocampal lesions (Bannerman et al., 2001). These data suggest that the current transections disrupted an interaction between the hippocampus and the subcortex, and did not result in specific septal or hippocampal deficits per se. Further lesion and inactivation studies are necessary to better characterize the hippocampal-subcortical interactions disrupted by these selective transections.

In previous reports (Hunsaker et al., 2007a, 2008), two animals received biotinylated dextran amine (BDA; an anterograde tracer) infusions 7 days postunilateral partial fimbria transection and two animals had BDA injections 7 days postdorsal fornix transection. The results suggested that the CA3 and CA1 subcortical efferents project to separate populations within the septal nuclei (the study did not investigate other subcortical structures aside from the septal nuclei). After infusions of BDA into CA3, there was staining present in the septofimbrial nucleus, throughout the rostral lateral septum, the dorsal portion of the medial septum, as well as the dorsal-most portions of the vertical limb of the diagonal band of Broca. After infusions of BDA into CA1, there was staining in the septofimbrial nucleus, the ventral portions of the medial septum, and the horizontal limb of the diagonal band of Broca. There was only sparse staining in the ventral-most portion of the caudal lateral septum. The differential targets of CA3 and CA1 subcortical efferents suggest that there may be functional heterogeneity between these two sets of efferents (cf. Hunsaker et al., 2008; Sheehan et al., 2004). Additionally, during this anatomical tract tracing experiment, it was demonstrated that the transections of the fimbria disrupted roughly 63% of CA3 efferent fibers the dorsal fornix transections disrupted slightly more than 50% of the CA1 efferent fibers.

Unlike previous reports by Hunsaker et al. (2007a,b, 2008) that posited the subcortical outputs of the hippocampus were primarily modulatory on the hippocampus via a feed forward inhibition system, the present data suggest that information processing within the medial and lateral septum may be directly affected by efferents from the hippocampus during the conditioning of auditory-cued and contextual fear (cf. Gray & McNaughton, 2000; Sheehan et al., 2004). Any differences between the effects of transecting CA1 or CA3 subcortical efferents may be attributed to the differences in the specific targets of the projections. It has previously been demonstrated that there are dissociations between the medial and lateral septum for auditory-cued and contextual fear processing (Calandreau et al., 2007). The present data suggest that the CA3 efferents (primarily) to the lateral septum are involved for the acquisition of contextual fear, involved in consolidation/retrieval of contextual fear, but only involved for the acquisition of auditory-cued fear (i.e., the transection left auditory-cued fear consolidation/retrieval intact). Also of note is that the retrieval deficits were never as severe as those displayed by the dorsal fornix transection group. The present data also suggest that the CA1 efferents (primarily) to the medial septum (and structures

along the Papez circuit) are involved during acquisition and (potentially) for the consolidation/retrieval of auditory-cued and contextual fear, though the present data are unclear on the nature of the retrieval deficits, though these data are congruent with previous findings (Phillips & LeDoux, 1995). The present data do not lend themselves to a specific analysis of encoding as fully separable from consolidation or retrieval processing as well as previous studies (i.e., there needs to be some sort of measurable consolidation/retrieval to determine whether some learning had taken place—such as that seen in the animals with partial fimbria transections; cf. Hunsaker & Kesner, 2008; Hunsaker et al., 2008).

An alternative explanation for the deficits seen after dorsal fornix transactions may be that subcortical efferents from the subiculum were also disrupted by the dorsal fornix transactions—not just those from CA1. These subcortical efferents from the subiculum also have been shown to project not only to the medial septum, but also to structures along the Papez circuit (Gray & McNaughton, 2000; Swanson & Cowan, 1977; Vann & Aggleton, 2004), which may also have been involved.

The present data add to the understanding of the neural circuitry underlying affective and fear related learning, as well as provide further insight into the subcortical projections of CA1. What the present data suggest is that the accepted view of the hippocampus supporting contextual and the amygdala mediating both contextual and elemental (e.g., auditory) fear conditioning is at best incomplete (cf. Quinn, Wied, Ma, Tinsley, & Fanselow, 2008). These findings also suggest that using fear conditioning as a test of amygdala function may be confounded by the presence of hippocampal-septal (and hippocampal-subcortical) interactions (cf. Conejo et al., 2005). Additionally, these data suggest that hippocampal and septal interactions are involved during fear-related learning and not just during anxiety-related processing (Gray & McNaughton, 2000). Of particular interest is the finding that disrupting subcortical efferents from either CA1 or CA3 was sufficient to disrupt acquisition of classically conditioned fear. Retrieval of conditioned contextual fear was dependent upon subcortical projections from CA3 (the data do not allow any meaningful interpretations for CA1 subcortical efferents other than that they are critical for acquisition). The present study reveals a role for subcortical structures in both hippocampus dependent and (presumably) hippocampus independent processing of conditioned fear.

In the case of the present experiment, it appears that the hippocampus and subcortical structures interact to efficiently process the auditory and contextual cues during the acquisition and retrieval of classical fear conditioning. This suggests that the medial septum, in addition to sending cholinergic and GABAergic projection fibers into the hippocampus may potentially play an important role in the formation of a representation of the tone and context that are associated with an aversive shock. The lateral septum, it appears, also may have a role in the formation and recall of these representations. Additional subcortical effects of the transections may potentially contribute to deficits seen after the dorsal fornix transections. The medial septum has strong, reciprocal connectivity with the entorhinal cortex and the amygdala, and any alteration in the outputs from the septum to these areas may also potentially underlie the present effects (Gray & McNaughton, 2000; Sheehan et al., 2004; Swanson & Cowan, 1977; Vann & Aggleton, 2004). Overall, these data suggest that the role of the hippocampal subcortical efferents and their role in learning and memory processing should be reevaluated.

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