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A Double Dissociation of Subcortical Hippocampal Efferents for Encoding and Consolidation/Retrieval of Spatial Information

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ABSTRACT: CA3 lesions impair encoding, whereas CA1 lesions impair retrieval during learning of a Hebb-Williams maze. CA3 efferents in the fimbria were transected, taking care to spare cholinergic and GABAergic afferents. CA1 efferents in the dorsal fornix were similarly transected. Fimbria transections, but not dorsal fornix transections, resulted in deficits for the encoding of spatial information during learning of a Hebb-Williams maze. Dorsal fornix, but not fimbria, transections resulted in deficits for retrieval of spatial memory during learning of a Hebb-Williams maze. These results reveal a double dissociation for the roles of CA3 and CA1 subcortical efferents in encoding and retrieval processes that mirror the double dissociation seen after excitotoxic lesions of CA1 and CA3. These data provide support for the theory that the cholinergic projections from the septal nuclei modulate the dynamics for encoding and consolidation/retrieval in the hippocampus.

KEY WORDS: dorsal fornix; fimbria; acetylcholine (ACh), encoding and retrieval; maze learning; animal cognition; computational model; learning and memory

INTRODUCTION

Research has shown that one can observe behavioral deficits following dysfunction of CA3 without concomitant deficits following dysfunction of CA1. Lesions of CA3, but not CA1, impair acquisition of object-place and odor-place paired-associate learning (Gilbert and Kesner, 2003; Kesner et al., 2005). CA3, but not CA1, lesions impair the encoding of spatial information during learning of a Hebb-Williams maze (Jerman et al., 2006; Vago et al., 2007). In contrast, one can observe deficits after dysfunction of CA1 without concomitant deficits following CA3 dysfunction. Lesions to CA1, but not CA3, produce deficits for temporal ordering of visual objects (Hoge and Kesner, 2007), consolidation or retrieval of contextual fear conditioning (Lee and Kesner, 2004), and consolidation or retrieval of spatial information during learning of a Hebb-Williams maze (Jerman et al., 2006; Vago et al., 2007). Also, when rats encounter a changed cue configuration, CA3 place fields shift only during the first day; whereas CA1 place fields do not shift until day 2 (Lee et al., 2004). These results suggest that CA3 and CA1 are dissociable across the temporal domain. CA3 is critical early in learning (i.e., for encoding and short term consolidation/retrieval) and CA1 is critical later on when information has to be remembered (i.e., for consolidation or intermediate to long-term retrieval).

To date, the specific mnemonic functions of CA1 and CA3 subcortical efferents have not been investigated. 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 (Papez, 1937; Raisman et al., 1966; Swanson and Cowan, 1977, 1979; Wyss et al., 1980; Gaykema et al., 1991; Vann and Aggleton, 2004). These two hippocampal projections synapse on different neuron populations within the medial and lateral septum (Raisman et al., 1966; Swanson and Cowan, 1977, 1979; present report). Hasselmo and colleagues have proposed a critical role for these pathways for regulating the dynamics of encoding and retrieval (Hasselmo and Schnell, 1994; Hasselmo et al., 1995; Hasselmo and McGaughy, 2004). They posited that these efferent pathways to the medial and lateral septum act upon the medial and lateral septum to modulate the encoding and retrieval dynamics in the hippocampus proper (i.e., feedback modulation). They have postulated that ACh is critical for this modulation. Previous work supports this assertion by demonstrating that a transection of dorsal CA3 efferents in the fimbria acts similarly to infusions of scopolamine (a cholinergic antagonist) into dorsal CA3 (Hunsaker et al., 2007b) (for a quantitative analysis of the models that were tested in this experiment the reader is referred to Hasselmo and Schnell, 1994; Hasselmo et al., 1995; Hasselmo and McGaughy, 2004; Hasselmo, 2005).

To investigate the roles of these subcortical efferent pathways, one group of animals had CA3 subcortical efferents in the fimbria transected, but afferents left intact (cf. Hunsaker et al., 2007a,b). Another group of animals had CA1 subcortical efferents in the dorsal fornix similarly transected. After transecting these pathways, animals were run on a Hebb-Williams maze to evaluate the effects of the transections for the encoding and consolidation/retrieval of spatial infor-

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mation (Rogers and Kesner, 2003; Jerman et al., 2006). The data reveal that subcortical efferents support encoding and consolidation/retrieval of spatial information. Transecting CA3 subcortical efferents in the fimbria causes encoding deficits, whereas transecting CA1 subcortical efferents in the dorsal fornix results in consolidation/retrieval deficits.

MATERIALS AND METHODS

Subjects

Twenty-three Long-Evans rats, ~4 months of age and weighing 280–340 g at the start of the experiment served as subjects. The colony room was maintained on a 12 h light/dark cycle. All rats had ad libitum access to water but were food deprived to 85–90% of free feeding weight. All surgical and experimental protocols conformed to University of Utah IACUC and AAALAC protocols and regulations. The health of the animals was assessed weekly by an IACUC veterinarian.

Surgery

Experimentally naïve 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 ($n = 13$; fimbria control $n = 6$; dorsal fornix control $n = 7$). All rats were anesthetized with ketamine and xylazine (55 mg/kg and 5–10 mg/kg i.p.) and placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) on an isothermal pad. We chose ketamine/xylazine as the anesthetic so we could record neurophysiologically from the animals during the transections as well as test them behaviorally, and it was easier to obtain clean signals under ketamine/xylazine than under barbiturate anesthesia (unpublished observations). For partial fimbria transections, burr holes were drilled above the lateral septum (anterior 0.6 mm from bregma and lateral 0.6–0.8 mm relative to midline), dorsal fimbria (posterior 2.3 mm and lateral 3.2 mm), and CA3 (posterior 3.0 mm and lateral 3.0 mm). Coordinates were based on studies of the hippocampo-septal system (McLennan and Miller, 1974; Rawlins et al., 1979; McNaughton and Miller, 1986; Hunsaker et al., 2007a,b) and two stereotaxic atlases (Paxinos and Watson, 1997; Kruger et al., 2005). We chose to evoke responses in the lateral septum instead of the medial septum because the responses evoked by CA3 stimulation were more reliable in the lateral septum. Bipolar recording electrodes from Plastics One (Roanoke, VA) were lowered into the lateral septum and CA3 and adjusted dorsoventrally to obtain maximal signal of resting neural activity. The signal was amplified 2,000 times (Grass-Telefactor P511, West Warwick, RI) and visualized on a virtual oscilloscope (SciWorks 3.0; Datawave Technologies, Berthoud, CO) on a PC computer running Windows XP (Microsoft, CO; Redmond, WA). Once all electrodes were in place and resting activity visualized, CA3 was stimulated with 0.4 μs square pulses at 0.5 Hz and 0.4 mA using an analog stimulator (Ortec 4710 Dual Channel Stimulator, Oak Ridge, TN), and stimulus isolation unit (Grass-Telefator PSIU-6D) and responses were recorded in the lateral septum. Once the evoked potentials were recorded, the lateral septum was stimulated and responses from CA3 evoked without manipulating any electrodes. Once both CA3 and lateral septal potentials could be reliably evoked, electrodes remained untouched. Pretransection evoked responses ($n = 6$ per rat; $n = 3$ per hemisphere) and theta after a firm tail pinch were collected for offline analysis. Stimulation of CA3 continued at a rate of 0.33–0.5 Hz throughout surgery. A fine wire knife retracted into a small diameter handle was lowered to the ventro-lateral region of the fimbria (ventral from the dura mater ~5.0 mm). The blade was protracted and CA3 subcortical efferents in the fimbria were slowly transected until the lateral septal evoked responses were reduced in amplitude. The lateral septum was stimulated to again evoke responses in CA3, verifying that afferent fibers in the fimbria from the medial septum and diagonal band of Broca were not damaged. The knife was retracted to avoid further damage to the fimbria and removed from the brain. Posttransection evoked responses ($n = 6$ per rat; $n = 3$ per hemisphere) and theta after a firm tail pinch were collected for offline analysis. The transection was repeated in the other hemisphere for a bilateral partial fimbria transection. This protocol has been proven effective for selectively transecting CA3 efferent axons via the fimbria (Hunsaker et al., 2007a,b).

For dorsal fornix transections, the transection was performed similarly. Burr holes were drilled above CA1 (posterior 3.0 mm and 2.0 mm), the medial septum (anterior 1.0 mm, and lateral 0.1 mm), and the dorsal fornix (2.1 mm posterior and 1.0 mm lateral). Responses in the medial septum to CA1 stimulation were evoked. The recording site in the medial septum was chosen over a site in the lateral septum because the responses evoked by CA1 stimulation were more reliable in the medial septum. Care was taken to place the CA1 electrode dorsal to the pyramidal cell soma (e.g., in or near the alveus) to prevent backfiring the Schaffer collaterals and the fimbria. Responses in CA1 evoked by medium septal stimulation were recorded. Stimulation and recording parameters were exactly the same as for the fimbria transection surgery. The dorsal fornix was transected until the response in the medial septum evoked by CA1 stimulation was eliminated or dramatically reduced, after which the knife was retracted, taking care to not damage the corpus collosum and overlying cortex. Pretransection evoked responses ($n = 6$ per rat; $n = 3$ per hemisphere) and theta after a firm tail pinch and posttransection evoked responses ($n = 6$ per rat; $n = 3$ per hemisphere) and theta after a firm tail pinch were collected for offline analysis. Control animals had the neurophysiological processes performed, but no transections were made. After surgery, all animals received acetominophen (200 mg/100 ml water) in their drinking water and were provided with crushed food for 3 days postsurgery and monitored for abnormal activity (e.g., epileptiform activity). No behavioral seizures were observed.

Neuroanatomical Characterization of the Dorsal Fornix Transection

Two additional animals received unilateral dorsal fornix transections followed by biotinylated dextran-amine (BDA–an anterograde tracer) infusions to evaluate the efficacy of the dorsal fornix transection (cf. Hunsaker et al., 2007a). After 7 days, both rats were anesthetized with isoflurane (2–4% at 1–2 l/min). Animals were secured to a stereotaxic frame and their incisions reopened. One of the animals that had undergone a dorsal fornix transection received bilateral 1.0 μl injections of a 10% (w/v) aqueous solution of BDA into CA1 at a flow rate of 0.05 μl/min. The injection site used was the CA1 stimulation site for each animal. The needle was left in place for 5 min after BDA infusion. The other animal that had undergone a unilateral dorsal fornix transection received a bilateral injection of BDA (1.0 μl) into the lateral septum/medial septum area (on the border between the two regions and corresponding with the recording/stimulating locations). In both the CA1 and lateral septum/medial septum injection groups, BDA staining in the target region on the side of the transection was compared to the nontransected hemisphere.

Behavioral Apparatus and Procedure

The Hebb-Williams maze was modified to emphasize spatial learning (Rogers and Kesner, 2003; Jerman et al., 2006) The base was painted gray and measured 76.2 × 76.2 cm^2. The walls were 30.5-cm high, made of 0.6-cm-thick transparent Plexiglas. Four identical boxes, one of which served as a start box and another as a goal box, were placed at the four corners of the maze. The inner barriers were made of 1.3-cm-thick Plexiglas, measuring 25.4 cm in height. A 7.6-cm-wide black strip was placed on the bottom of the Plexiglas. This spatial arrangement of the maze was meant to emphasize the use of extra-maze visual cues, which included easily visible posters and three-dimensional objects attached to the walls of the room surrounding the maze.

Rats were handled for 5 days, until they freely ate a food reward (Froot Loops cereal; Kellogg's, Battle Creek, MI), after which they were trained. To prevent the rats from learning spatial cues in the testing room prior to task acquisition, training was conducted in a different room and on a different behavioral apparatus (a wooden linear runway; i.e., shuttle box). The shuttle box was 50.8 cm long with walls 10.2 cm high, and had an open top. On each end of the runway, there were two small wooden boxes connected to the shuttle box-a start box and a goal box. Each rat was placed in the start box and the door was opened to provide access to a food reward in the goal box. Once rats learned to run to the goal box to obtain a food reward in response to the opening of the start box door, acquisition began on the Hebb-Williams maze.

Rats learned the Hebb-Williams maze over three consecutive days with a static configuration of inner barriers. Doors for all the boxes were open except the start box, which was opened at the beginning of each trial. The goal box contained a food reward (i.e., one full Froot Loop). The objective of each trial was to find the shortest path from the start box to the rewarded goal box. There were 11 error zones, and when a rat crossed into an error zone in a given direction an error was recorded (Fig. 1). As the rat learned the task, it eliminated navigational errors that deviated from the shortest path to the goal box. When the rat entered the goal box, the door was closed. A 30-s intertrial interval was given before the next trial, during which the goal box and the start box were switched. Hence, the goal box of the previous trial became the start box for the next trial. Ten trials were conducted per day for each rat. The total number of errors per trial was recorded as the dependent measure.

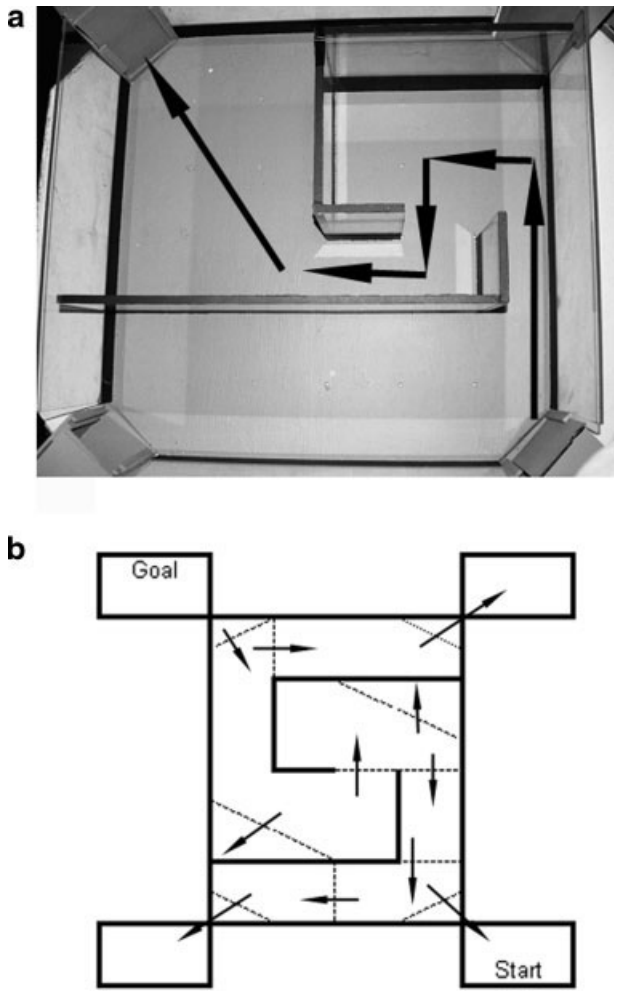

FIGURE 1. (a) Modified Hebb-Williams Maze paradigm with optimal pathway marked. (b) Schematic with error zones and direction of entry into error zones marked.

Histology

At the conclusion of testing, each rat was anesthetized with an intraperitoneal injection of sodium pentobarbital (70 mg/kg i.p.). Rats were perfused intracardially with phosphate buffered saline (PBS) and 10% (w/v) formalin. The brain was removed from the skull and stored at 4°C in a solution of 30% sucrose/

10% formalin (w/v) for 72 h prior to further processing. The brain was frozen and 40 μm sections were cut, and every other section from the medial septum through the hippocampus was collected and stained for AChsterase after the procedure of Karnovsky and Roots (1964).

Animals that had received infusions of BDA were sacrificed after a 7-day survival period and perfused in preparation for counter-staining with streptavidin bound fluorescein and processed after the protocol described by Vinkenoog et al. (2005). Eighty-micrometer sections were cut on a vibrating microtome. Every other section was taken from lateral septum/medial septum (∼AP +1.5 mm) to the dorsal hippocampus (∼AP −4.0 mm; both measurements based on Paxinos and Watson (1997)). Sections were counterstained by incubating the sections for 24 h at 4°C in streptavidin bound fluorescein. After staining, the sections were rinsed, mounted on slides, and dehydrated. After dehydration, slides were stored at 0°C.

The levels of BDA staining in the target region (bilateral lateral septum/medial septum in the case of CA1 injection and bilateral CA1 in the case of lateral septum/medial septum injection) were verified using a fluorescence microscope and compared to nontransected hemispheres. The presence of the bright green fluorescent tag was used as a criterion for labeling. ImageJ (v1.34j; National Institute of Health; Bethesda, MD) was used to assess labeling in hemispheres with transections (after either a CA1 injection or a lateral septum/medial septum injection of BDA) compared to the nontransected hemisphere. Labeling was analyzed by separating the two hemispheres into separate files using ImageJ. The files were thresholded to the same luminance to reduce background optical artifacts. Once the staining in the regions was clearly visible and artifacts reduced, labeled pixels were counted and compared to the total number of pixels in the file (The total number of pixels was identical for both files).

Dependent Measures and Statistical Analysis

The average number of errors made per trial was calculated and blocked into five-trial blocks to produce a general learning index for that day (e.g., first five trials of day 1 = block 1, second five-trial block during day 1 = block 2, etc). For a detailed analysis of encoding and consolidation/retrieval processes, separate indices were developed. It is assumed that encoding and consolidation/retrieval processes interact on a trial-by-trial basis in any behavioral task. Thus, it is difficult to discriminate between the contributions of encoding and consolidation/retrieval during individual trials using a behavioral analysis. For this experiment, encoding and consolidation/retrieval were given operational definitions based on calculations of trial errors. An encoding index was calculated by subtracting the mean number of errors made per trial during the last five trials of day 1 from the mean number of errors made during the first five trials of day 1. A consolidation/retrieval index was calculated by subtracting the mean number of errors made per trial during the first five trials of day 2 from the mean of the last five trials of day 1.

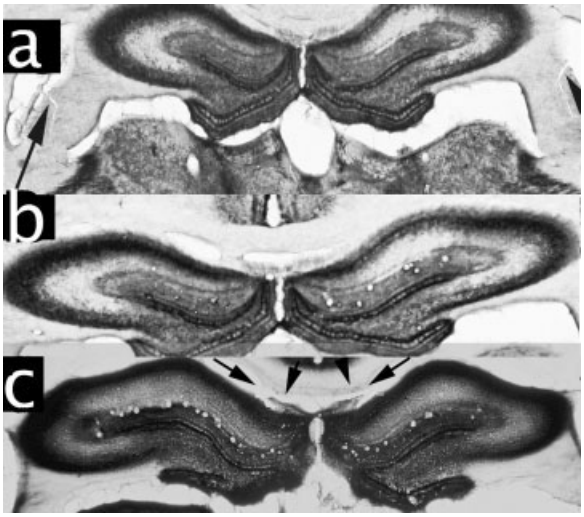

FIGURE 2. (a) Photomicrograph of a typical partial fimbria cut stained for AChsterase. Arrows point to site of partial fimbria transection. (b) Photomicrograph of a typical control brain stained for AChsterase. (c) Photomicrograph of a representative dorsal fornix transection stained for AChsterase. Black arrows point to site of dorsal fornix transection.

The assumption made in these calculations and subsequent analyses was that the encoding and very short-term retrieval of spatial information predominated during day 1, and intermediate to long-term retrieval of consolidated spatial information predominated during the first five trials during day 2 (Rogers and Kesner, 2003; Jerman et al., 2006). Acquisition was analyzed by performing two-way repeated measures analysis of variance (ANOVA) with groups as the between factor and blocks of five trials as the within factor. All analyses were performed using the statistics toolbox on MATLAB (v6.5 R13; The MathWorks, Natick, MA). Encoding and consolidation/retrieval indices were analyzed by performing one-way ANOVA with groups as the between factor. Tukey's HSD post hoc paired comparisons were performed on all significant effects. Alpha was set at $P < 0.05$ for all analyses.

RESULTS

Histology

Figure 2a shows an AChsterase-stained section from a rat that had received a transection of CA3 subcortical efferents in the fimbria with the transection pointed to by arrows. The presence of AChsterase in the hippocampus suggests that cholinergic inputs were spared (Hunsaker et al., 2007a,b). Figure 2b shows a representative control brain stained for AChsterase. Figure 2c shows an AChsterase-stained section from a rat that had received a transection of CA1 subcortical efferents in the dorsal fornix with the transection pointed to by arrows. The continued presence of AChsterase in the hippocampus suggests

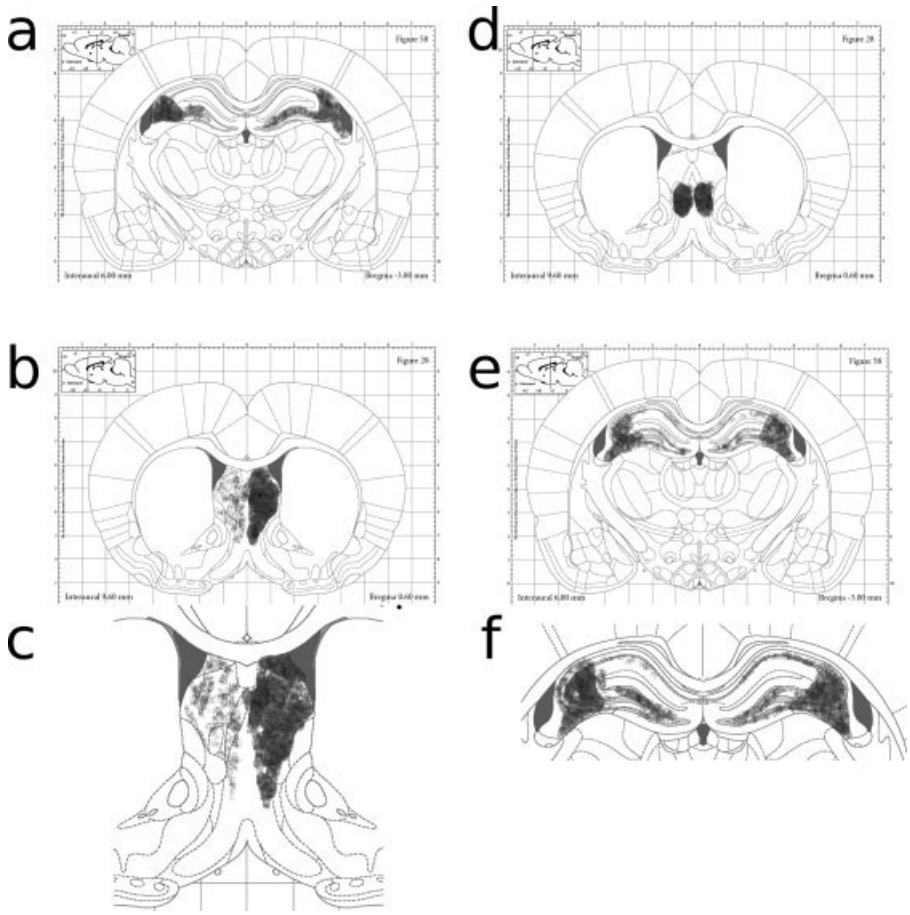

FIGURE 3. Partial fimbria transection. (a) BDA injection into CA3. Note that BDA is pooled at the site of the partial fimbria transection. (b) Septal staining after infusion of BDA injection into CA3. The transected hemisphere is to the left of the figure. Notice the right hemisphere shows greater staining. (c) Closer view of the septal staining after CA3 infusions of BDA. (d) BDA injection into the septum. (e) Hippocampal staining after BDA infusion into the septum. (f) Close-up view of the staining in the hippocampus after septal BDA infusions. This figure was reproduced from Hunsaker et al., Hippocampus, 2007a, 17, 494–502 © John Wiley and Sons, reproduced by permission. See text for further descriptions.

that cholinergic inputs from the medial septum and diagonal band of Broca were spared.

In a previous report (Hunsaker et al., 2007a), two animals received BDA injections 7 days postunilateral partial fimbria transection and the results are shown in Figures 3a–f. These data are replots of the data reported in Hunsaker et al. (2007) and are reproduced here for comparison with the dorsal fornix transaction. Figure 3a shows the infusion site into the dorsal CA3. What can be seen is that CA3 stains densely, and the superior portion of the fimbria stains darkly (these are BDA carrying axon tracts). Notice that on the left side (the transected side in Fig. 3a) the BDA staining stops at the site of the partial fimbria transaction, whereas on the right side the BDA continues along its path toward the septum. Figure 3b shows the resultant BDA staining in the lateral septum after infusions of BDA into CA3. The left side of Figure 3b is the transected hemisphere. Relative to the nontransected hemisphere, the transected hemisphere contains ~63% fewer labeled pixels, and thus a 63% reduction in BDA staining. Figure 3c shows a higher magnification of the septal staining after a BDA infusion into dorsal CA3, emphasizing the differences between the two hemispheres.

Figure 3d shows a typical BDA infusion into the lateral/medial septum. Figure 3e shows the resultant BDA staining in the hippocampus after a septal infusion of BDA. Notice that the BDA stained axon tracts are not disrupted by the partial fimbria transaction and CA3 is densely stained, with the dentate gyrus (DG) and CA1 only lightly stained. Figure 3f is a magnified view of Figure 3e to further emphasize that there were no differences between the two sides. These data suggest that hippocamposeptal efferents were selectively disrupted, whereas hippocamposeptal afferents were left intact.

To characterize the fornix transections performed in the present experiment, two animals had BDA injections 7 days postunilateral dorsal fornix transection and the results are shown in Figures 4a–f. Figure 4a shows the infusion site into the dorsal CA1. What can be seen is that CA1 stains densely. Figure 4b shows the resultant BDA staining in the medial septum after infusions of BDA into CA1. The left side of Figure 4b is the transected hemisphere. Relative to the nontransected hemi-

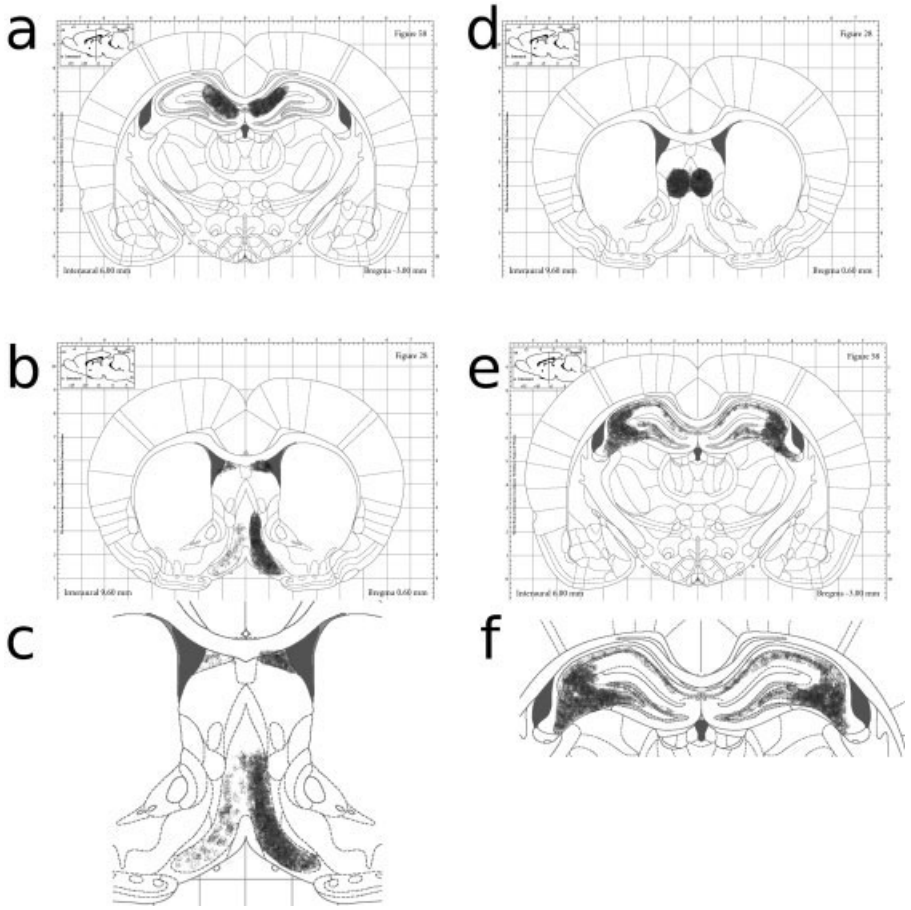

FIGURE 4. Dorsal Fornix Transection. (a) BDA injection into CA1. Note that BDA is pooled at the site of the dorsal fornix transection. (b) Septal staining after infusion of BDA injection into CA1. The transected hemisphere is to the left of the figure. Notice the right hemisphere shows greater staining. (c) Closer view of the septal staining after CA1 infusions of BDA. (d) BDA injection into the septum. (e) Hippocampal staining after BDA infusion into the septum. (f) Close-up view of the staining in the hippocampus after septal BDA infusions. See text for further descriptions.

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Figure 4d shows a typical BDA infusion into the lateral/medial septum. Figure 4e shows the resultant BDA staining in the hippocampus after a septal infusion of BDA. Notice that the BDA stained axon tracts are not disrupted by the dorsal fornix transaction and CA3 is again densely stained, with the DG and CA1 only lightly stained. Figure 4f is a magnified view of Figure 4e to further emphasize that there were no differences between the two sides. These data suggest that hippocamposeptal efferents were selectively disrupted, whereas hippocamposeptal afferents were left intact.

The main difference encountered between the results of these two transections was that there was a slight difference in localization of BDA in the medial septum and diagonal band of Broca after infusions into CA1 instead of CA3. Also, there was slightly more BDA in the hippocampus after medial septum/lateral septum infusion in the dorsal fornix transected animals, but we hypothesize this was the result of subtle differences in the location of BDA infusion, and not due to any anatomical differences or blocked pathways, since the overall pattern of BDA staining in the hippocampus was similar between the two studies.

Neurophysiology

Neurophysiological results for animals receiving transections of the fimbria have been reported previously (Hunsaker et al., 2007a,b). For all reports, three potentials were recorded from each hemisphere of each rat for each condition. Since there were five rats in each group, this resulted in 30 potentials per group per analysis ($n = 30$). In short, CA3 responses evoked by stimulation of lateral septum did not change pre- to post-transection, whereas the response in the lateral septum evoked by stimulation of CA3 was reduced in amplitude (Fig 5a). Amplitude changes of the responses pre- and post-transection were analyzed by performing one-tailed *t*-tests. There was a signifi-

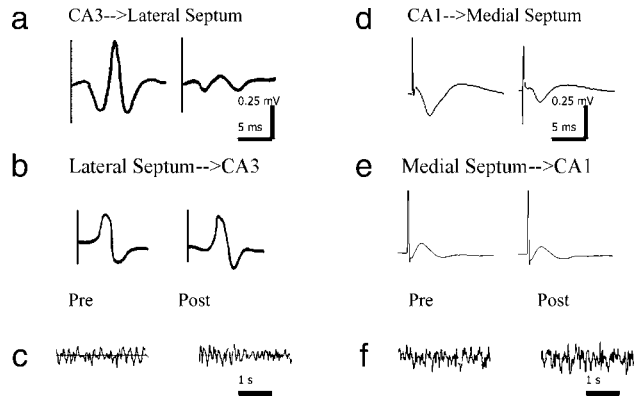

FIGURE 5. Partial fimbria transection. (a) Evoked responses in the lateral septum to CA3 stimulation. Notice the response was attenuated after the transection. (b) Evoked response in CA3 to lateral septum stimulation. Notice there was no change in the response after the transection. (c) Theta rhythm pre and postfimbria transection. Dorsal fornix transection. (d) Evoked response in the medial septum to CA1 stimulation. Notice the response was attenuated after the transection. (e) Evoked response in CA1 to medial septum transection. Notice there was no attenuation in the response after the transection. (f) Theta rhythm pre and postdorsal fornix transection. Plates a–c reproduced from Hunsaker et al., Hippocampus, 2007a, 17, 494–502 © John Wiley and Sons, reproduced by permission.

cant overall reduction in the amplitude of the evoked responses posttransection ($n = 30$) as compared to pretransection ($n = 30$), from (mean ± standard error of mean (SEM)) 0.20 ± 0.04 to 0.05 ± 0.02 mV [$t(58) = 14.19$, $P < 0.0{,}001$]. There was no significant change in the response amplitude in CA3 to lateral septal stimulation: from 0.28 ± 0.07 to 0.30 ± 0.06 mV [$t(58) = -1.78$, $P = 0.81$] (Fig 5b). These results provide evidence that the partial fimbria transection did not significantly affect the hippocampal afferent fibers from the medial septum and diagonal band of Broca, only CA3 efferents. Theta after a firm tail pinch was recorded to assess whether the transection of subcortical CA3 efferents altered baseline input from the medial septum and diagonal band of Broca and results are shown in Figure 5c. There was no significant change in theta frequency [$t(18) = 0.21$, $P = 0.42$] or power [$t(18) = 0.33$, $P = 0.38$]. These results suggest that theta was not significantly affected, which was expected since transections were selective to CA3 efferents and spared the afferent cholinergic and GABAergic fibers from the medial septum and diagonal band of Broca. This result is similar to that reported previously by Rawlins et al. (1979) who showed that cutting hippocampal efferents (esp. the fornix) disrupted theta less effectively than cutting septal efferents that entered the hippocampus. It must be noted that potentials were recorded in the lateral septum as opposed to the medial septum or diagonal band of Broca because the evoked responses were more reliable in the lateral septum than in the medial septum or diagonal band of Broca.

CA1 responses evoked by stimulation of medial septum did not change pre- to post-transection, whereas the response in medial septum evoked by stimulation of CA1 was reduced in amplitude (Fig 5d). For all reports, for each condition three potentials were recorded from each hemisphere of each rat. Since there were five rats in each group, this resulted in 30 potentials per group per analysis ($n = 30$). Amplitude changes of the responses pre- and post-transection were analyzed by performing one-tailed t-tests. There was a significant overall reduction in the amplitude of the evoked responses posttransection ($n = 30$) as compared to pretransection ($n = 30$), from (mean ± SEM) 0.24 ± 0.02 mV to 0.04 ± 0.01 mV [$t(58) = 9.8{,}203$, $P < 0.0{,}001$]. There was no significant change in the response amplitude in CA1 to medial septal stimulation; from 0.11 ± 0.02 mV to 0.12 ± 0.06 mV [$t(58) = -1.3{,}364$, $P = 0.19$] (Fig 5e). These results provide evidence that the dorsal fornix transection did not significantly affect the hippocampal afferent fibers from the medial septum and diagonal band of Broca, only CA1 efferents. Theta after a firm tail pinch was recorded to assess whether the transection of subcortical CA1 efferents altered baseline input from the medial septum and diagonal band of Broca and results are shown in Figure 5f. There was no significant change in theta frequency [$t(18) = 0.20$, $P = 0.46$] or power [$t(18) = 0.33$, $P = 0.23$]. These results suggest that theta was not significantly affected, which was expected since transections were selective to CA1 efferents and spared the cholinergic and GABAergic fibers from the medial septum and diagonal band of Broca. It must be noted that potentials were recorded in the medial septum as opposed to the lateral septum or diagonal band of Broca because the evoked responses were more reliable in the medial septum than in the lateral septum or diagonal band of Broca.

Behavior

Figure 6a shows acquisition trends for all groups in the present study. Note that control animals and animals with partial fimbria transections (for clarity during the results, partial fimbria transactions will have (CA3) written after them to remind the reader that CA3 fibers are disrupted) show a similar learning trend and make a similar number of errors per trial. Animals with dorsal fornix transactions (for clarity during the results, fornix transections will have (CA1) written after them to remind the reader that CA1 fibers are disrupted) learn quite rapidly within each day, but do not appear to be able to consolidate and/or retrieve the information between days, resulting in the observed saw-tooth pattern. Acquisition of the Hebb-Williams maze across 3 days was assessed by performing a two-way repeated measures ANOVA with transection groups as the between factor and blocks of five trials as the within factor. There was a significant effect for transection groups ($F(2,149) = 19.09$, $P < 0.0{,}001$), an effect for trial blocks ($F(5,149) = 5.07$, $P = 0.0{,}003$), but no interaction between transection groups and trial blocks ($F(10,149) = 1.16$, $P = 0.32$). Tukey's HSD post hoc paired comparisons were run on the main effect for groups and showed that animals with dorsal fornix transactions (CA1) made an overall greater number of errors per trial than control and fimbria transection (CA3) groups ($P < 0.01$), which did not differ from each other ($P > 0.05$).

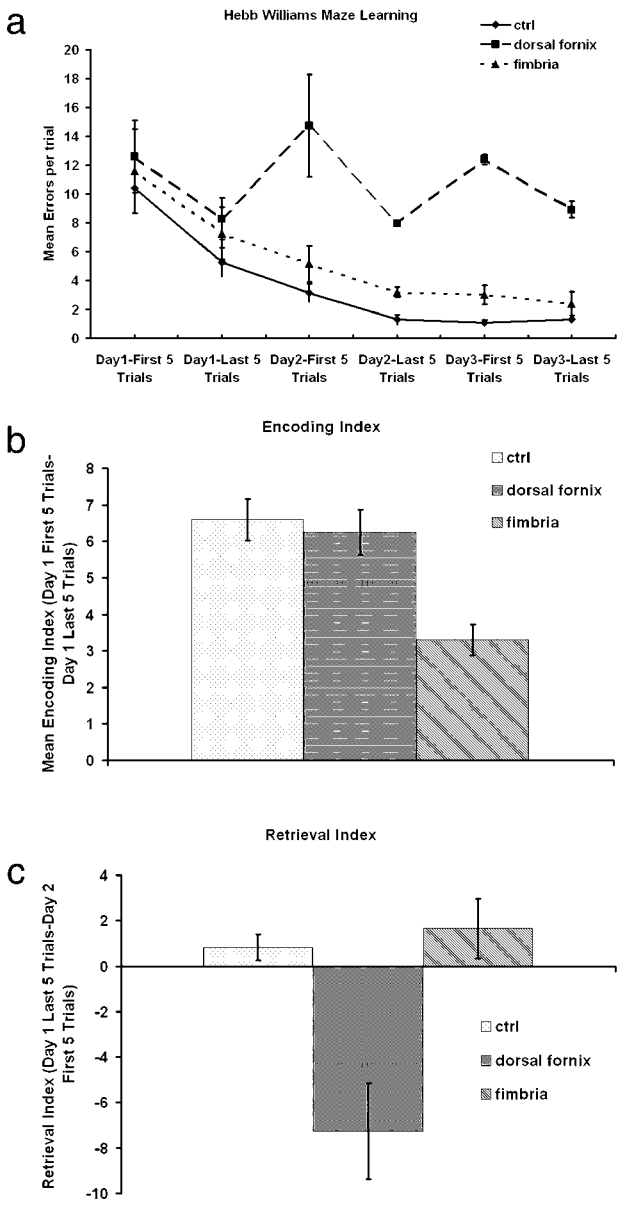

FIGURE 6. (a) Overall acquisition of the Hebb-Williams maze. Note that dorsal fornix animals encode within each day but are unable to recall information between days. (b) Encoding Index. Note that fimbria transected animals were impaired relative to controls and dorsal fornix transected animals. (c) Retrieval Index. Note that dorsal fornix transected animals were impaired relative to controls and fimbria transected animals.

To further examine learning within a given day, an encoding index was calculated by subtracting the average number of errors made per trial during the final five trials during day 1 from the first five trials of day 1 for each individual animal (Fig. 6b). This measure takes into account each animal's baseline performance so it is a measure of learning during the first day-reflective of encoding and short-term retrieval processes. A one-way ANOVA was performed to test between-group differences for encoding ($F(2,24) = 4.61$, $P = 0.021$). Tukey's HSD post hoc paired comparisons verified there were group differences. The fimbria transection (CA3) group displayed less efficient encoding relative to control and dorsal fornix transection (CA1) groups ($P < 0.05$). Control and dorsal fornix transection (CA1) groups did not differ ($P > 0.05$).

To evaluate how well animals could remember learned information after a 24 h delay, a consolidation/retrieval index was calculated by subtracting the average number of errors made per trial during the first five trials of day 2 from the last five trials of day 1 (Fig. 6c). This compares each animal's retention of information during day 2 compared to how well that animal had learned during day 1-reflective of intermediate and long-term retrieval processes. A one-way ANOVA was performed to assess differences between transection groups. There was a significant effect for transection groups ($F(2,24) = 9.02$, $P = 0.001$). Tukey's HSD post hoc analysis verified that control and fimbria transection (CA3) groups did not differ ($P > 0.05$), but the dorsal fornix transection (CA1) group showed less efficient consolidation/retrieval relative to the control and fimbria transection (CA3) groups ($P < 0.01$).

DISCUSSION

Hippocampo-Septal Anatomy

A circuit between the hippocampus and medial septum/diagonal band of Broca has been characterized, as well as an efferent pathway from the hippocampus to the lateral septum (Papez, 1937; Raisman et al., 1966; McLennan and Miller, 1972; Swanson and Cowan, 1977, 1979; Rawlins et al., 1979; Wyss et al., 1980; McNaughton and Miller, 1986; Gaykema et al., 1991; Hasselmo and Schnell, 1994; Hasselmo et al., 1995; Hasselmo and McGaughy, 2004; Vann and Aggelton, 2004). Briefly, CA3 subcortical efferents in the fimbria terminate in the lateral septum, medial septum, and diagonal band of Broca. CA1 subcortical efferents in the dorsal fornix also terminate in the lateral septum, medial septum, and diagonal band of Broca, but they synapse on distinct neuron populations. The medial septum and diagonal band of Broca send cholinergic efferents via the fimbria into the hippocampus that have been implicated in the hippocampal theta rhythm and modulation of learning and memory (McLennan and Miller, 1972; Rawlins et al., 1979; McNaughton and Miller, 1986; Hasselmo and Schnell, 1994; Hasselmo et al., 1995; Hasselmo and McGaughy, 2004; Hasselmo, 2005). GABAergic efferents may be involved as well, but they potentially act too rapidly to modulate encoding and consolidation/retrieval as operationally defined in this report (e.g., as measured in seconds as opposed to the millisecond time course of the GABAergic modulation within theta phase precession and similar processes; cf. Wallenstein and Hasselmo, 1997; Hasselmo and Fehlau, 2001). ACh has a more robust effect in stratum radiatum than stratum lacunosum-moleculare and stratum lucidum in slice preparations. The recurrent collaterals and Schaffer collaterals terminate in the stratum radiatum, whereas the perforant path termi-

nates in stratum lacunosum-moleculare and the mossy fibers terminate in stratum lucidum. This connectivity suggests that the mossy fiber pathway and perforant pathway inputs are not as dramatically affected by ACh influx as the recurrent collateral and Schaffer collateral inputs. These data suggest that ACh modulates the hippocampus by selectively affecting the signal-to-noise (S/N) ratio within the recurrent collaterals and Schaffer collaterals in the stratum radiatum (Hasselmo and Schnell, 1994; Hasselmo et al., 1995).

Proposed Circuitry

Based on the models proposed by Hasselmo and colleagues, it is suggested that CA3 and CA1 work in parallel to provide a feedback control over the cholinergic modulation of the hippocampus. It is proposed that CA3 sends a "mismatch" signal to the lateral septum, medial septum, and diagonal band of Broca in response to information that needs to be encoded. This means that if information from the perforant path and mossy fiber pathways do not match the information in the recurrent collateral network, then CA3 signals the septum to raise levels of ACh in the hippocampus to facilitate encoding and attenuate retrieval processes. Conversely, CA1 sends a "match" signal to the medial septum and diagonal band of Broca in response to information that needs to be retrieved. This means that if information from the perforant path matches the information in the Schaffer collaterals, then CA1 signals the septum to attenuate levels of ACh in the hippocampus to facilitate retrieval and attenuate encoding processes (for a quantitative analysis of the computational model being tested by the present experiment the reader is referred to Hasselmo and Schnell, 1994; Hasselmo et al., 1995).

CA3

It is clear that transecting CA3 subcortical efferents in the fimbria disrupts encoding, but not retrieval, of spatial information during learning of a Hebb-Williams maze task. One explanation is the prominent cholinergic medial septum/diagonal band of Broca to CA3 projection (Raisman et al., 1966; Swanson and Cowan, 1977, 1979; Gaykema et al., 1991), since a disruption of these cholinergic projections would theoretically disrupt the ability of CA3 to encode spatial information (Hasselmo and Schnell, 1994; Hasselmo and McGaughy, 2004; Hasselmo, 2005; Hasselmo et al., 2005; Hunsaker et al., 2007a,b). CA3 sends subcortical efferents via the fimbria that terminate on cholinergic neurons in the medial septum and diagonal band of Broca, so a transection would result in reduced activation of cholinergic neurons that send projections to CA3 via the fimbria (Gaykema et al., 1991), and also indirectly affect the medial septum via the lateral septum. The consequence of reducing the cholinergic projection to CA3 would be a decrease in the signal to noise ratio within the recurrent collaterals. In addition, reducing levels of ACh in CA3 would impair encoding since long-term potentiation (LTP) in the recurrent collaterals would be attenuated (Hasselmo et al., 1995; Hasselmo, 2005).

Hasselmo and colleagues have proposed that ACh subserves the encoding and consolidation/retrieval dynamics observed in CA3. They suggest that high levels of ACh would facilitate encoding, but would disrupt recall. They also proposed that medial septum/diagonal band of Broca activity would be low when CA3 activity was high, suggesting an interaction. When CA3 subcortical efferents in the fimbria are transected, the lateral septum, medial septum, and diagonal band of Broca would not have information concerning the CA3 activity state, but would continue to receive the signals from CA1. This means the ACh levels in the hippocampus would be at baseline levels or lower to facilitate consolidation or retrieval, and not at the higher than baseline levels that favor encoding processes.

ACh sets the dynamics for encoding in CA3 by increasing the signal to noise ratio in the recurrent collaterals (Hasselmo et al., 1995; Hasselmo, 2005). Transecting CA3 subcortical efferents reduces ACh by attenuating excitation of the medial septum/diagonal band of Broca cholinergic neurons. This would favor consolidation/recall over encoding and push CA3 into the role of a working memory buffer (Kesner and Rolls, 2001; Kesner et al., 2004; Rolls and Kesner, 2006), and thus produce an encoding deficit during learning of a Hebb-Williams maze.

The pattern of results for scopolamine (a cholinergic antagonist) and physostigmine (a cholinergic agonist) infusions into CA3 is intriguing. Scopolamine infusions into CA3, but not physostigmine infusions, disrupt encoding. In contrast, physostigmine infusions into CA3, but not scopolamine infusions, disrupt recall. This was observed during a spatial exploration paradigm (Hunsaker et al., 2007b), during Hebb-Williams maze learning (Rogers and Kesner, 2003), and during delay fear conditioning (Rogers and Kesner, 2004). Pretraining injections of 192-IgG saporin into the hippocampus, which attenuate cholinergic input into the hippocampus by eliminating the cholinergic projection cells in the medial septum/diagonal band of Broca, disrupted encoding on a Hebb-Williams Maze (Pereira et al., 2005), whereas posttraining injections were insufficient to cause a retrieval deficit at long delays on a delay match-to-sample task (cf. Winters and Dunnett, 2004).

CA1

Transecting CA1 subcortical efferents in the dorsal fornix attenuated retrieval, but not encoding, of spatial information during learning of a Hebb-Williams maze. Hasselmo and colleagues have proposed that ACh modulates the encoding and consolidation/retrieval dynamics observed in CA1 (Hasselmo and Schnell, 1994; Hasselmo, 2005). They stated that low ACh favors consolidation/retrieval of information. They also proposed that medial septum/diagonal band of Broca activity would be low when CA1 activity is high, suggesting an interaction. After transecting CA1 subcortical efferents in the dorsal fornix, the medial septum/diagonal band of Broca would not receive information concerning the CA1 activity state, but would continue to receive signals from CA3. This suggests that

ACh levels in the hippocampus would be at baseline levels or higher to facilitate encoding, and not at lower than baseline levels that facilitate consolidation or retrieval. This would favor encoding over consolidation/recall and push CA1 into the role of a mismatch processor (Kesner and Rolls, 2001; Kesner et al., 2004; Hasselmo, 2005; Rolls and Kesner, 2006), and thus produce a retrieval deficit during learning of a Hebb-Williams maze.

Pretraining infusions of 192-IgG saporin disrupt encoding during learning of a Hebb-Williams maze (Rogers and Kesner, 2003) but posttraining infusions do not affect recall of a delay match-to-sample task (Rogers and Kesner, 2004). The principal finding of these manipulations is that consolidation and recall are favored over encoding when the cholinergic input is attenuated. Infusions of physostigmine into CA3 support the present assertion since increasing efficacy of ACh in the hippocampus disrupts consolidation/retrieval, but has no effect for encoding during fear conditioning and the Hebb-Williams maze (cf. Winters and Dunnett, 2004; Pereira et al., 2005).

General Remarks

Neither CA1 nor CA3 subcortical efferent projections were able to compensate for a disruption of the other. CA3 subcortical efferents in the fimbria appear to be involved in encoding of spatial information, whereas CA1 subcortical efferents in the dorsal fornix appear to be important in consolidation/retrieval of spatial information. These data are the first direct support for both the CA1 and CA3 cholinergic modulation models postulated by Hasselmo and colleagues in the 1990s that the hippocampus and the septum/diagonal band of Broca interact to control encoding and consolidation/retrieval dynamics in the hippocampus (Hasselmo and Schnell, 1994; Hasselmo et al., 1995; Hasselmo and McGaughy, 2004; cf. Hasselmo, 2005). These results support reports showing that CA3 lesions attenuate encoding, whereas CA1 lesions attenuate retrieval during the same tasks (Lee and Kesner, 2003, 2004; Jerman et al., 2006; Rolls and Kesner, 2006; Vago et al., 2007). Furthermore, these results clearly demonstrate a double dissociation between CA3 and CA1 subcortical efferents, with CA3 subcortical efferents (via the fimbria) involved in encoding and CA1 subcortical efferents (via the dorsal fornix) involved in retrieval of spatial information during learning of the same task. These results parallel the double dissociations previously shown following CA3 and CA1 lesions (Gilbert and Kesner, 2003; Kesner et al., 2004, 2005; Jerman et al., 2006; Rolls and Kesner, 2006; Vago et al., 2007).

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