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Role of dCA3 Efferents via the Fimbria in the Acquisition of a Delay Nonmatch to Place Task

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ABSTRACT: Dorsal CA3, but not dorsal CA1, lesioned rats are impaired in the acquisition of a delay nonmatch to place task. In this study, dorsal CA3 efferent fibers in the fimbria were transected; while taking care to spare afferent fibers from the medial septum. Neurophysiological, anatomical tracing, and histochemical data suggest that the transection was selective to dorsal CA3 efferent fibers and spared afferents from the medial septum. Rats showed a deficit for acquisition, but not for performance once learned. One possible explanation is that a small change to the cholinergic inputs to dCA3 caused by a decrease in dorsal CA3 efferent signals reaching the medial septum may impair new learning but not performance of a task once learned. © 2007 Wiley-Liss, Inc.

KEY WORDS: fimbria; lateral septum; medial septum; dorsal hippocampus; acetylcholine (ACh); modulation

INTRODUCTION

One can observe behavioral deficits following dorsal CA3 (dCA3) dysfunction without concomitant deficits following dysfunction of dorsal CA1 (dCA1). Lesions of dCA3, but not dCA1, impair acquisition of object-place and odor-place paired-associate learning (Gilbert and Kesner, 2003). Dorsal CA3, but not dCA1, lesions impair within-day learning (or encoding) of a Hebb–Williams maze paradigm (Vago et al., 2003; Jerman et al., 2006). Dorsal CA3, but not dCA1, lesions also impair acquisition of a delayed nonmatching to place (DNMP) task on an eight-arm radial maze with ten-second delays between the sample and choice phase. It appears that dCA3 mediates encoding or acquisition processes, whereas dCA1 appears to mediate retrieval or consolidation processes (cf. Kesner et al., 2004; Rolls and Kesner, 2006). There is accumulating evidence that suggests differences between dCA3 and dCA1 place cell firing dynamics. When rats encounter a changed cue configuration, the dCA3 place field center of mass shifts location during the first day, but not subsequent days. The dCA1 place field center of mass starts to shift from Day 2 onward, but not during Day 1 (Lee et al., 2004). In the DNMP task, a lesion to either dCA3 or dCA1 produces a deficit at 5-min delays, suggesting that dCA3 and dCA1 interact via the Shaffer collaterals to process spatial information within an intermediate-term memory system (Lee and Kesner, 2003).

Subcortical efferents from dCA3 via the fimbria project onto cholinergic neurons in the medial septum and diagonal band of Broca, or to the lateral septum (Gaykema et al., 1991). The medial septum and diagonal band of Broca, in turn, provide cholinergic and GABAergic inputs to the dorsal hippocampus. The lateral septum may process the hippocampal inputs, then transfer the information to the medial septum or other subcortical structures. The role of this output in modulating acquisition and retention of behavioral tasks has not been evaluated, but computational models suggest that any disruption would cause acquisition deficits without concomitant retrieval deficits (Hasselmo et al., 1995; Hasselmo and McGaughy, 2004; cf. Hunsaker et al., in press).

Hippocampo-septal connections have been characterized via degeneration, immuno-fluorescence, and anatomical tract tracing (Raisman et al., 1966; Swanson and Cowan, 1977; Wyss et al., 1980; Gaykema et al., 1991). Efferent projections from dCA3 pyramidal cells via the fimbria onto cholinergic neurons in the medial septum and diagonal band of Broca may provide an anatomical locus for modulating cholinergic input to the dorsal hippocampus. Computational models suggest that this pathway may be important for acquisition and recall of information during learning. High levels of ACh favor acquisition over recall, whereas low levels of ACh favor recall over acquisition (Hasselmo and Schnell, 1994; Hasselmo et al., 1995; Hasslemo and McGaughy, 2004; cf. Hunsaker et al., in press).

To investigate the roles of dCA3 subcortical efferents, efferents in the fimbria were transected, but afferents were left intact. Dorsal CA1 was lesioned using ibotenic acid to remove the dCA1 targets of the Shaffer collaterals. A dCA1 lesion was chosen over a Shaffer collateral transection, since dCA1 lesions do not cause deficits for acquisition of a DNMP task at short (10-s) delays (Lee and Kesner, 2003). Furthermore, inactivations of the perforant path input into dCA1 do not result in deficits in this task at short delays (Vago et al., 2003). Also, a Shaffer collateral transection would potentially damage afferents from the mossy fiber or perforant pathways, whereas ibo-

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tenic acid lesions of dCA1 do not damage these fibers (Gilbert and Kesner, 2003; Lee and Kesner, 2003; Jerman et al., 2006).

The purpose of the present experiment is to use the DNMP task to determine the behavioral effects of transecting dorsal CA3 subcortical efferents via the fimbria (FIMB) on the acquisition and performance of a DNMP task. For comparison, these data were compared to data from rats having excitotoxic dCA1 lesions, or a partial fimbria transection paired with an excitotoxic dCA1 lesion (FIMB + dCA1) for the acquisition and performance of the same DNMP task.

METHODS

Subjects

Twenty-three Long Evans rats (260–400 g) served as subjects for this study. They were housed individually in the colony in standard plastic rodent cages and maintained on a 12-h light/dark cycle. They had access to food and water ad libitum prior to surgery. After recovery from surgery, they were deprived to 85% of free feeding weight and given ad libitum access to water. All animal care and experimental procedures conformed to the University of Utah Institution for Animal Care and Use Committee (IACUC) guidelines for care and use of experimental animals. The health of the animals was assessed weekly by an IACUC veterinarian.

Partial Fimbria Transection

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 to regulate body temperature at 37°C throughout surgery. Burr holes were drilled bilaterally above the lateral septum [anterior 0.6 mm from bregma and lateral 0.6–0.8 mm from midline—cf. Fig. 1A for histologically verified final electrode positions on a plate modified from Paxinos and Watson (1997)], fimbria (posterior 2.3 mm and lateral 3.2 mm), and dCA3 (posterior 3.0 mm and lateral 3.0 mm). Ventral coordinates were based upon studies of the hippocamposeptal system (McLennan and Miller, 1974; McNaughton and Miller, 1986). All surgical coordinates were obtained by comparing two stereotaxic atlases (Kruger et al., 1995; Paxinos and Watson, 1997) and previous literature. Bipolar recording electrodes from Plastics One (Roanoke, VA) were lowered into the lateral septum and dCA3, and adjusted dorsoventrally to obtain maximal signal of resting neural activity. The signal was amplified 2,000 times and visualized using an analog oscilloscope (Hameg HM-2053 20 MHz Storage Scope; Mainhausen, Germany) and a virtual oscilloscope (Scope, Data Translation; Marlboro, MA) on a PC computer running Windows 2000 (Microsoft, CO; Redmond, WA). Once all electrodes were in place and resting activity visualized, evoked potentials were recorded in the lateral septum via stimulation of dCA3 using 0.4 μs duration monophasic 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; West Warwick, RI). Once the maximal evoked potential was visualized, the lateral septum was stimulated and evoked responses from dCA3 obtained without moving any electrodes. We assume the lateral septum to dCA3 evoked potentials result via the medial septum either trans-synaptically via the lateral septum or as a direct result of lateral septum stimulation in such close proximity to the medial septum (i.e., lateral septum stimulation directly stimulated the medial septum). Once both dCA3 and lateral septum potentials could be reliably evoked, pretransection evoked responses and theta were collected for offline analysis. Stimulation of dCA3 continued at a rate of 0.33–0.5 Hz throughout surgery. A fine wire knife retracted into a small diameter handle (<1/2 mm diameter) connected to a stereotaxic arm was lowered until it was located in the ventro-lateral region of the fimbria (ventral from dura ~5.0 mm). The blade was protracted 0.5 mm and the fimbria was slowly transected while monitoring evoked responses in the lateral septum until they were eliminated or dramatically attenuated (Fig. 1B—left hand traces are pre- and right hand traces are post-transection). It was then verified that the evoked response in dCA3 to septal stimulation remained intact. The knife was retracted to avoid further damage to the fimbria and removed. Post-transection evoked responses and theta were collected for offline analysis. The transection was repeated on the other side.

For the FIMB + dCA1 group, the fimbria cut followed the above protocol after which six burr holes were drilled in the skull above dCA1 and a 28 gauge injection cannula mounted on a stereotaxic arm was lowered into dCA1 and 0.1–0.15 μl ibotenic acid [8 mg/ml in phosphate-buffered saline (PBS) vehicle] was injected at a rate of 6.0 μl/h using a 10 μl syringe connected to microinjection pump directly into the pyramidal cell layer of dCA1. The dCA1 lesion coordinates were as follows: All sites were located posterior 3.6 mm. The lateral coordinates were 1, 2, and 3 mm lateral from the midline suture. The injection cannula was lowered into the pyramidal cell layer of dCA1 located 2.1, 2.2, and 2.4 mm ventral (cf. Lee and Kesner, 2003). The injection cannula was left in place for 1 min after injection to allow the drug to efficiently diffuse before the injection cannula was removed from the brain. Control animals had the electrophysiological process performed without the fimbria transection and received PBS injections into dCA1. Dorsal CA1-lesioned rats received ibotenic acid-induced lesions of dCA1 but no electrophysiology.

Two additional animals received unilateral partial fimbria transections followed by biotinylated dextran-amine (BDA—an anterograde tracer) infusions to evaluate the efficacy of the partial fimbria transection. 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 partial fimbria transection received 1.0 μl injections of a 10% aqueous solution of BDA bilaterally into dCA3 at a flow rate of 0.05 μl/min (3.0 μl/h). The injection site used was the dCA3 stimulation site for each animal. The needle was left in place for 5 min after BDA infusion to allow for diffusion of BDA. The other

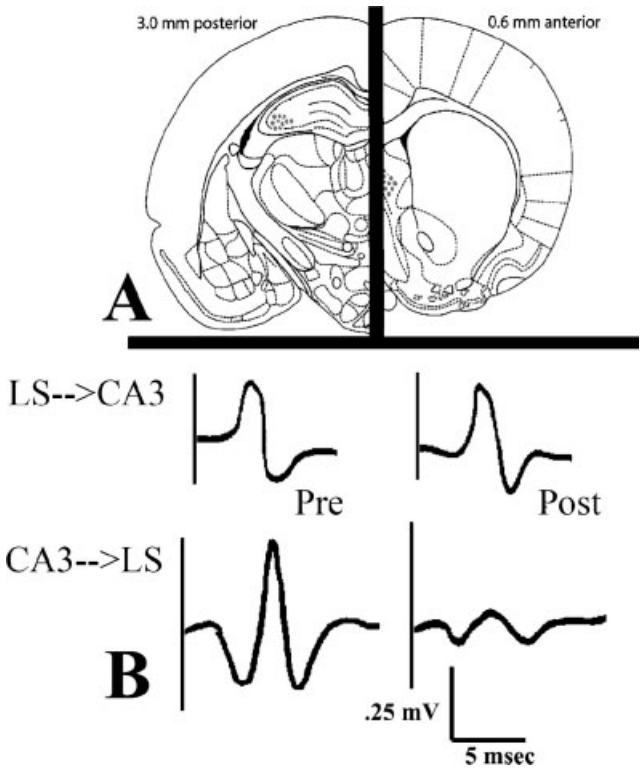

FIGURE 1. (A) Histologically verified electrode tip locations. Bilateral locations have been imposed over a single hemisphere of a plate modified from Paxinos and Watson (1997). (B) Tracings of CA3 (LS→CA3. CA3 response evoked by stimulation of the lateral septum) and LS (CA3→LS. Lateral septum response evoked by CA3 stimulation) both pre- (left) and post (right) partial fimbria transection. Notice that the LS→CA3 evoked response did not show a significant amplitude change, whereas the CA3→LS response changed dramatically. LS = lateral septum.

animal that had undergone a partial fimbria transection received an injection of BDA (1.0 μl) into the lateral septum/medial septum area (on the border between the two regions and corresponding to the recording/stimulating locations). In both the dCA3 and lateral septum/medial septum injection groups, presence of BDA in the target region on the side of the transection was compared to the nontransected hemisphere.

Histology

After completing behavioral experimentation, each animal was sacrificed by an injection of 1 ml pentobarbital (70 mg/ml PBS) and intracardially perfused with PBS and 10% formalin. This was followed by 5% sucrose in PBS for 5 min. The brain was stored at 4°C for 24 h in a 10% sucrose/formalin solution followed by 24 h in a 25% sucrose/formalin solution. A tissue block containing the lateral septum, medial septum, and hippocampus was frozen (−18°C) and cut into 40-μm sections. Alternate sections were stained with cresyl violet and AChesterase 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 staining with streptavidin bound fluorescein and processed after the protocol of 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 were after Paxinos and Watson (1997)]. Sections were counterstained by incubating the sections in 5% normal goat serum followed by 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 presence of BDA in the target region (bilateral lateral septum/medial septum in the case of dCA3 injection and bilateral dCA3 in the case of lateral septum/medial septum injection) was verified using a fluorescence microscope and compared to control hemispheres. The presence of the bright green fluorescent tag was used as a criterion for labeling. ImageJ 1.35j (NIH; Bethesda, MD) was used to assess labeling in hemispheres with transections (dCA3 injection and lateral septum/medial septum injection groups) 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 eliminate background staining and optical noise from the microscope. Once only the labeling in the regions of interest was present, pixels were counted and compared to the total number of pixels in the file (The total number of pixels was identical for both files).

Behavioral Method

Apparatus

A wooden eight arm radial maze, painted white with 5.7-cm clear plexiglass walls down each arm, was used in this experiment. Each arm had a well drilled into the distal end (1.5 cm × 2.5 cm) into which Froot Loops cereal reward (Kellogg's; Grand Rapids, MI) could be placed but not seen from the platform. The maze had a white bucket suspended over the platform that could be lowered from outside the room to block the rats' view of the arms during delay intervals. Surrounding the maze were ten distinct cues suspended from the ceiling of the room housing the maze. Transparent Plexiglass guillotine doors to each arm could be raised and lowered from outside the room to allow or restrict access to each arm.

Behavioral procedure

After surgery, animals received a 7-day recovery period, after which they were habituated to the maze. During habituation, animals were placed in the center of the maze with all the arms accessible and five pieces of Froot Loops cereal on the distal third of each arm. The number of Froot Loops was reduced each day until there was only 1/3 of one in the food well at the end of each arm (7 days of habituation). During habituation, doors were raised after the rat had visited each arm to bar entry to previously visited arms. When rats consistently ran to the ends of the arms and consumed Froot Loops, training

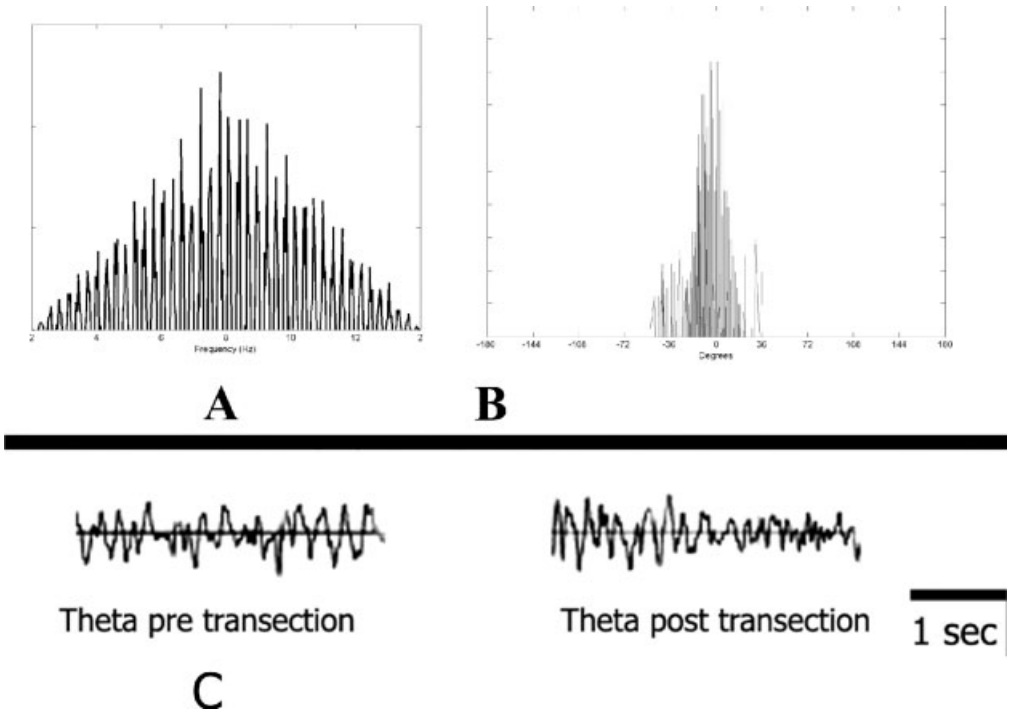

FIGURE 2. (A) Frequency correlogram with pretransection theta rhythm compared to post-transection theta. Frequency of theta did not change pre- to post-transection. (B) Power correlogram with pretransection theta rhythm compared to post-transection theta. Note that power of theta did not change pre- to post-transection. (C) Raw trace of theta rhythm pre- and postfimbria transection.

began. Training consisted of a forced choice paradigm. The rat was placed in the center platform with all doors closed. One door was opened and the rat ran down the open arm for a reward. This continued until the rats ran directly to the ends of the arms to obtain Froot Loops reward and finished all eight forced choice trials in under 4 min.

Acquisition consisted of eight trials per day of study and choice phases, replicating the delay nonmatch to place (DNMP) protocol used by Lee and Kesner (2003). The rat was given a door to enter and obtain a reward during the sample phase. When the rat returned to the center of the maze, the door was raised and the bucket was lowered for a 10-s delay. At the end of the delay, the rat was presented with the arm it had previously visited (now unrewarded) and an adjacent, rewarded arm for the choice phase (randomized right/left). If the rat entered the unrewarded arm, the entrance to the rewarded arm was blocked and the trial was recorded as an error. Once animals reached criterion (>90% correct over three consecutive days), they were given a 5-min delay test wherein 5-min delays were randomly intermixed with 10-s delays (four of each delay per eight trial block). Delay tests were conducted for 4 days, after which the animals were prepared for histological analysis.

Dependent measures and statistical analysis

When the rat made a correct choice during the choice phase, the experimenter recorded the latency from the moment the bucket was raised until the rat obtained the reward. If the rat went down the unrewarded arm an "X" was recorded and the door to the rewarded arm was immediately closed to prevent the animal from receiving reward. Entry to an arm was defined as the rat's hind legs completely crossing into arm. The ratio of correct/total trials was converted to a percentile score as a measure of performance. Interactions among transectioned, lesioned, and control animals were examined by performing two-way repeated measures (RM) analysis of variance (ANOVA) with groups (FIMB, CTRL, dCA1, and FIMB + dCA1) as the between subjects factor and blocks of trials as the repeated within subjects factor. All analyses were performed using SigmaStat 3.11 commercial statistical analysis software package (SYSTAT Software; Redmond, WA). For evoked response data acquired during surgery, the statistics toolbox on MATLAB (v6.5 R13; The MathWorks; Natick, MA) was used to run one-tailed *t*-tests (reduction of amplitude post- relative to pretransection) on evoked response amplitudes collected before and after partial fimbria transection.

RESULTS

Partial Fimbria Transection

Figure 1A shows the locations of histologically verified electrode tips. Bilateral locations have been projected onto a single hemisphere. Figure 1B shows the average results of pre- and

postfimbria transection-evoked potentials from stimulation of dCA3 and recording in the lateral septum (Fig. 1B) and from stimulation in the lateral septum and recording in dCA3 (Fig. 1B). The responses shown are traces of responses printed out from the virtual oscilloscope. The dCA3-evoked potential elicited by stimulation of the lateral septum did not change pre- to postfimbria transection, whereas the evoked response in the lateral septum elicited by stimulation in dCA3 was reduced in amplitude. Amplitude change of the evoked reponses pre- and postfimbria transection was analyzed using Data Translation SCOPE software and MATLAB. The overall change in amplitude of the evoked response was analyzed. There was an overall reduction in the evoked response post-transection as compared to pre-transection, from [mean ± standard error of mean (SEM)] 0.20 ± 0.04 mV to 0.05 ± 0.02 mV [$t(54) = 14.19$, $P < 0.0001$]. Evoked responses in dCA3 elicited by lateral septum stimulation were analyzed the same way. Overall, there was no significant change in the evoked response, from 0.28 ± 0.07 mV to 0.30 ± 0.06 mV [$t(54) = -1.78$, $P = 0.81$]. These results provide evidence that the partial fimbria transection did not significantly affect hippocampal afferent fibers, only efferents. Theta was measured to assess whether a fimbria transection dramatically changed afferent input from the medial septum and diagonal band of Broca and is shown in Figure 2. Theta was measured pre- and post-transection in five animals. There was no significant change in theta frequency [$t(18) = -0.09$, $P = 0.46$; cf. Fig. 2A for pre–post correlogram] or power [$t(18) = -0.013$, $P = 0.23$; cf. Fig. 2B for pre–post correlogram]. The data for theta power and frequency are presented as correllograms comparing pre and post-transection measurements to emphasize that there were no significant changes after the transection. Raw traces of theta are provided in Figure 2C. These results suggest theta was not significantly disrupted, which was expected since transections were selective to hippocampal efferents and spared the cholinergic fibers from the medial septum and diagonal band of Broca.

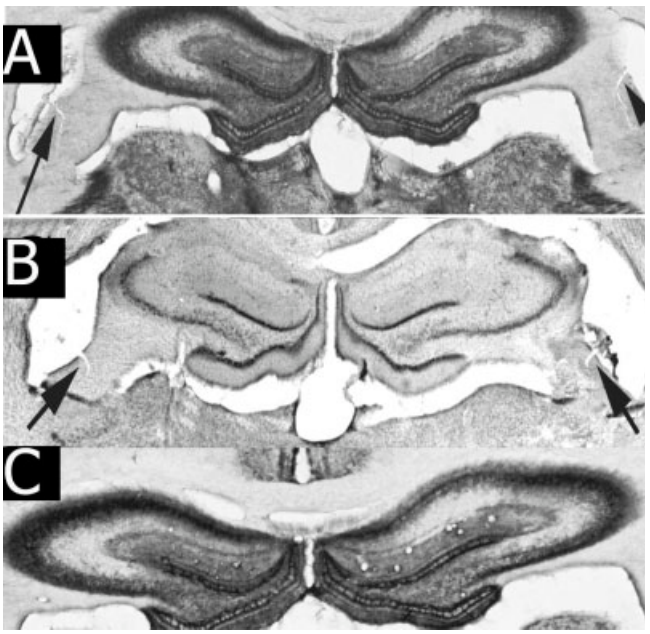

FIGURE 3. (A) Acetylcholinesterase stain of rodent dorsal hippocampus showing continued presence of AChesterase as well as fimbria transection (approximately AP-2.5 mm from bregma). The photomicrographs were converted to greyscale to emphasize overall contrast and allow easier visualization of the fimbria transection. (B) Typical fimbria transection plus CA1 lesion. To show the fimbria cut the section shown was taken approximately AP-2.3–2.5 mm posterior to bregma. (C) Control brain processed for AChesterase.

Histology

Figure 3A shows an AChesterase-stained section from a rat that had a partial fimbria transection. The continued presence of AChesterase banding in the dorsal hippocampus and overlying cortices reflects a continued cholinergic presence. Normal AChesterase banding was also observed in the ventral hippocampus (data not shown). Figure 3B shows a typical fimbria transection paired with a dCA1 lesion in a cresyl violet-stained dorsal hippocampus. A control dorsal hippocampus stained for acetylchoinesterase is presented (Fig. 3C—note that there is no difference between control and FIMB). Lesions to dCA1 and the fimbria transection were verified using a Nissl stain (cresyl violet) and visual inspection under a light microscope. Lesions and cuts were analyzed in the dorsal hippocampus [defined as the septal or dorsal 50% of the overall hippocampus (Moser and Moser, 1998; Bannerman et al., 1999)]. Lesions in the dCA1 lesion group were similar to lesions obtained previously in our lab and were quantified using a reconstruction procedure on ImageJ Software [volumetric analysis method after Gilbert et al. (2001)]. In short, 85% of dCA1 pyramidal cells were ablated. The 15% sparing was in the most medial portion of dCA1. Dorsal CA3 and the dorsal dentate gyrus (DG) were not damaged. The sparing was also more caudal than rostral. For FIMB + CA1 animals, the transections were identical to FIMB animals, and the dCA1 lesion to the dCA1 lesion group.

Two animals had BDA injections 7 days postunilateral fimbria transection and the results are shown in Figures 4A–D. Figures 4A,B show the results for BDA injection into dCA3 after a fimbria transection. Note that in Figure 4A there is a pooling of BDA at the cut site (signaled by a black arrowhead). Figure 4B shows the resultant BDA staining in the lateral septum. The left side of Figures 4A,B is the transected hemisphere. Relative to the nontransected hemisphere, the transected hemisphere contains ~63% fewer labeled pixels, and thus a 63% reduction in BDA labeling. Figures 4C,D show the results after BDA injection into lateral septum and medial septum post-transection. There were no differences between transected and nontransected hemispheres. Figures 4C,D show the input into the dorsal hippocampus after septal infusions of BDA. Notice that the pathway followed by the afferent fibers into the dorsal hippocampus was not disrupted by the fimbria transection. This region of interest was blown up in Figure 4D and the medial septum and diagonal band of Broca to dCA3 pathways is labeled by black arrows. This is important since the

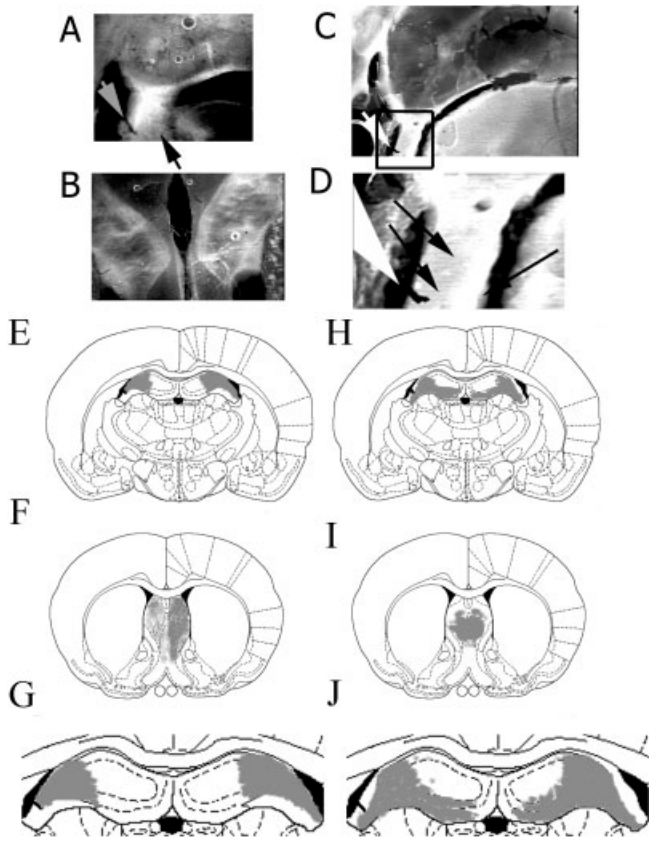

FIGURE 4. (A) BDA injection into CA3. Note BDA is pooled at the site of the partial fimbria transection (Black arrowhead). (B) Results of BDA injection into CA3. The transected hemisphere is to the left of the figure. Notice the right hemisphere is dramatically more illuminated. (C) Fimbria and dorsal hippocampus in the transected hemisphere after medial septum/lateral septum infusion of BDA (arrowhead). (D) Blow-up of boxed area in (C). (E,F) Reconstruction of A–D on plates modified from Paxinos and Watson (1997).

transection did not effect medial septum/diagonal band of Broca efferent fibers entering the dorsal hippocampus, they traveled in a more medial portion of the fimbria relative to where the transection was made. Figures 4E,F are reconstructions of the data presented in Figures 4A–D on plates from Paxinos and Watson (1997).

Behavioral Results

Acquisition

Two-way repeated measures ANOVA was performed on the blocked acquisition data with lesion group as the between variable and blocks of 16 trials as the repeated within variable. Figure 5A shows the acquisition trends for the groups. Notice that dCA1 and CTRL groups show a similar trend, whereas FIMB and FIMB + CA1 groups show an impairment, from which FIMB recovered but FIMB + CA1 did not. In all graphs, dCA3 data shown are from Lee and Kesner (2003) and were not included in any analysis. There was a significant effect for groups [$F(3,19) = 31.64$; $P < 0.001$], blocks of trials [$F(5,137) = 25.2$; $P < 0.001$], and an interaction between groups and blocks of trials [$F(15,137) = 2.08$; $P = 0.03$]. To further characterize these effects, Tukey's HSD post hoc paired comparison tests were performed. Animals with lesions to dCA1 did not differ from CTRL for any blocks of trials ($P = 0.24$). FIMB animals displayed lower levels of acquisition for Blocks 1 and 2 ($P < 0.001$), but did not differ from CTRL of CA1 for the final four blocks ($P = 0.13$). FIMB + dCA1 animals showed significantly attenuated levels of acquisition relative to all groups for all blocks of trials ($P < 0.001$).

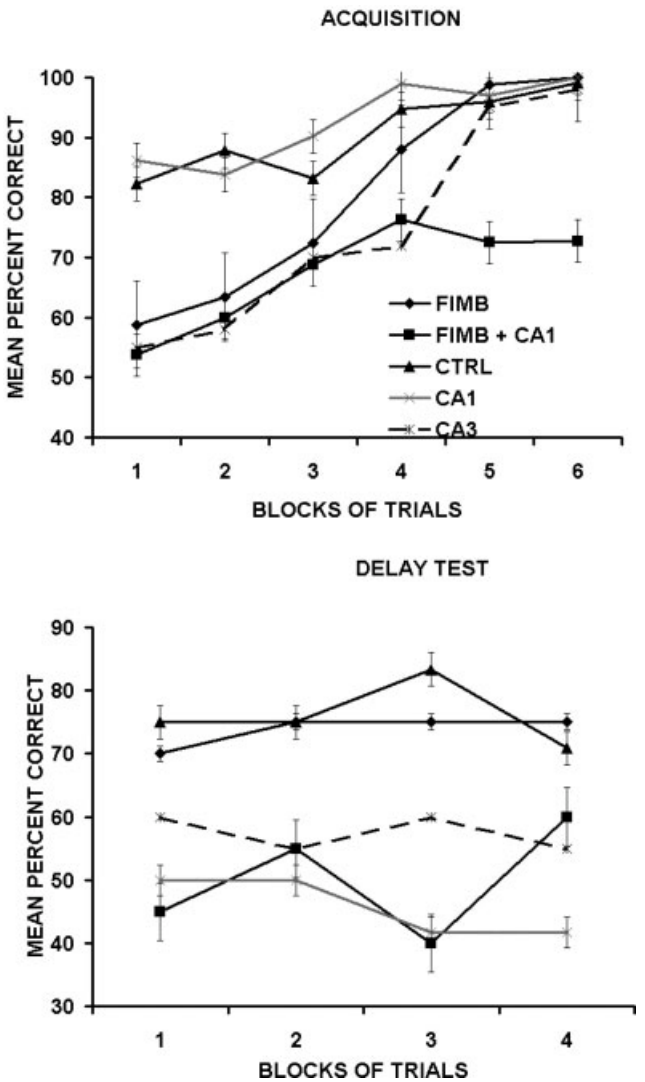

FIGURE 5. (A) Acquisition. Note that CTRL and CA1 groups have almost identical acquisition trends. The FIMB + CA1 group shows a deficit on all blocks, and the FIMB group shows an initial impairment followed by significant learning. On the final two blocks, there are no significant deficits for FIMB versus CTRL, but there is for FIMB + CA1 versus CTRL and FIMB. See acquisition results for further discussion. (B) Delay Test. CA1 and FIMB + CA1 animals show a deficit relative to CTRL. See delay test results. All CA3 data presented are the average values for the dCA3 group are from Lee and Kesner (2003).

Variable delay test

For 10-s delay data taken during the 5-min delay test, all animal's performance was indistinguishable: all data points were between 97.5 and 100% correct for 10-s delays (data not shown). Figure 5B shows the results of the 5-min delay tests analyzed in the same manner as acquisition. There was a significant effect for groups [$F(3,19) = 13.38$; $P < 0.001$], but not for blocks of trials [$F(3,91) = 0.37$; $P = 0.78$] or for the interaction between group and blocks of trials [$F(9,91) = 0.92$; $P = 0.51$]. Tukey's HSD test verified that FIMB did not differ from CTRL ($P = 0.45$). dCA1 and FIMB + dCA1 performed more poorly than both CTRL and FIMB ($P < 0.001$) and performed at chance levels. FIMB + dCA1 did not differ from dCA1 ($P = 0.50$). These results suggest that dCA1 lesions, not the fimbria transections, mediated the deficits present at 5-min delays.

Latency

A two-way repeated measures ANOVA was performed on the blocked latency data with lesion group as the between variable and blocks of 16 trials as the repeated within variable. There was no significant effect for groups [$F(3,19) = 0.22$; $P = 0.88$], but there was a significant effect for blocks of trials [$F(5,137) = 18.78$; $P < 0.001$]. There was no significant interaction between lesion group and blocks of trials [$F(15,137) = 0.57$; $P = 0.89$]. All animals' latency decreased as blocks of trials progressed—all animals completed each trial more quickly and efficiently on later blocks compared to earlier blocks of trials.

DISCUSSION

The acquisition data suggest that a selective transection of dCA3 efferents in the fimbria disrupts DNMP acquisition, but rats were able to reach criterion by the sixth block of trials. These data are similar to what has been reported for dCA3 lesions (Lee and Kesner, 2003). This result, in combination with the observation that dCA1 lesioned rats acquired the task as readily as controls, suggests that dCA3 efferents via the fimbria are involved in the acquisition of new spatial information. The continued presence of AChesterase in the hippocampus indicates there was no large-scale disruption of the medial septum/diagonal band of Broca cholinergic innervation of the hippocampus.

To anatomically characterize the partial fimbria transection, an anterograde tracing experiment using biotinylated dextranamine (BDA—an anterograde tracer; Vinkenoog et al., 2005) was performed on two animals. The data suggest that dCA3 to septum efferents were selectively transected, whereas medial septum/diagonal band of Broca to dCA3 afferent fibers were not damaged. This, in addition to the neurophysiological evidence presented, supports the conclusion that the partial fimbria transection was selective to dCA3 subcortical efferent fibers.

It is clear that a selective transection of dCA3 efferents via the fimbria disrupts acquisition of a DNMP task, whereas performance once learned (even at a long delay) is not significantly affected. Jerman et al. (2006) dissociated acquisition (encoding) from retrieval (performance) using a Hebb–Williams maze. We assume encoding and retrieval interact during every trial. Jerman et al. (2006) showed that even though these processes interact, they can be dissociated behaviorally. In the present experiment, it is assumed that early in acquisition encoding was more prominent than retrieval, whereas during later phases of acquisition, retrieval was more prominent than encoding processes. However, during acquisition of a DMNP task, there are many different processes that mediate learning and performance which, in turn interact with each other. The animals have to be able to make a coherent representation of the layout of the environment, remember the arm just visited, as well as learn the nonmatch rule. In this experiment, since there is a trial-by-trial interaction between encoding and retrieval, it is difficult to characterize the precise nature of the learning deficit in this task. The possible cause of the present deficit can be elucidated, but discovering the precise nature of the deficit requires further research.

One possible explanation for the fimbria cut induced acquisition deficit may be in the prominent cholinergic medial septum/diagonal band of Broca to dCA3 projection (Raisman et al., 1966; Swanson and Cowan, 1977; Wyss et al., 1980; Gaykema et al., 1991), since even a small disruption of modulatory cholinergic or GABAergic projections could potentially disrupt the ability of dCA3 neurons to encode new information (Hasselmo et al., 1995; Hasselmo and McGaughy, 2004). The theoretical consequence of a small reduction of the cholinergic innervation of the dHIP would be an increase in the effective strength of the reciprocal, recurrent dCA3 connections, which would impair new learning because existing associations would dominate the dCA3 cell firing. In this sense, low ACh is appropriate for consolidation or recall but disruptive to new learning (Hasselmo and Schnell, 1994; Hasselmo et al., 1995; Hasselmo and McGaughy, 2004). With low ACh, memory traces in dCA3 would interfere with processing new information. In addition, lowered ACh levels after a fimbria cut would impair new learning because long-term potentiation (LTP) in the dCA3 to dCA3 synapses (recurrent collaterals) would be reduced. This means the recurrent collaterals would be unable to encode but would preferentially complete patterns of activity previously encoded by the system (i.e., retrieve). These acquisition deficits may emerge when the task is difficult to learn and requires multiple trials over various days because interference between consolidation or recall of past spatial information and encoding of new spatial information becomes pronounced.

To test these hypothesis more directly, Hunsaker et al. (2007) transected CA3 subcortical efferents in the fimbria. They ran the animals on a spatial and nonspatial exploration paradigm. They compared the deficits shown by the fimbria transection groups to effects caused by scopolamine and physostigmine infusions. They found that a partial fimbria transection causes deficits very similar to those caused by infusions of

scopolamine directly into dCA3. These results suggest that a reduction in cholinergic efficacy produces the same results as observed following a transection of CA3 subcortical efferents in the fimbria.

Consistent with these hypotheses are observations of Rogers and Kesner, who examined the effects of scopolamine (a cholinergic antagonist) and physostigmine (a cholinergic agonist) injections into dCA3 for the acquisition of a modified Hebb–Williams maze (2003) and delay fear conditioning (2004). Scopolamine acted to inhibit or slow acquisition (referred to by the authors as encoding), but had no effect on consolidation or retrieval. Physostigmine did not cause an encoding deficit, but did cause a consolidation/retrieval deficit. The same effects were observed for delay contextual fear conditioning (Rogers and Kesner, 2004). Pereira et al. (2005) showed that pretraining injections of 192 IgG-saporin in the dorsal hippocampus was sufficient to disrupt acquisition of the standard Hebb–Williams maze. These data are also consistent with data that suggest the medial septum and diagonal band of Broca project to dCA3, and that CA3 projects to the medial septum and diagonal band of Broca (Raisman et al., 1966; Swanson and Cowan, 1977; Wyss et al., 1980; Gaykema et al., 1991; Rolls and Kesner, 2001), which emphasizes the importance of dCA3 for encoding.

To put the 5-min delay test results into the context of cholinergic modulation, the working memory nature of the test must be addressed. During acquisition, ACh from the medial septum and diagonal band of Broca sets the dynamics for learning by reducing interference between the recurrent collateral system and the mossy fiber/perforant path inputs by reducing the relative strength of the recurrent collaterals relative to the mossy fiber/perforant pathways (Hasselmo et al., 1995; Hasselmo and McGaughy, 2004). For working memory tasks, all that is required of the animal is to remember the last arm visited through the delay until the test phase; interference does not necessarily become a factor after acquisition when only single-trial encoding and retrieval is necessary. If the fimbria transection reduces ACh by reducing excitation of the medial septum and diagonal band of Broca cholinergic neurons, information in the recurrent collaterals would be favored over the incoming mossy fiber of perforant path inputs, emphasizing recall of past information over encoding. This pushes dCA3 into the role of a working memory buffer (Rolls and Kesner, 2001, 2006; Kesner et al., 2004) or recall mechanism (Hasselmo and Schnell, 1994; Hasselmo et al., 1995; Hasselmo and McGaughy, 2004), and away from the role of pattern associator/separator.

Consistent with this hypothesis are studies of encoding and retrieval involving scopolomine and physostigmine infusions into dCA3 (Rogers and Kesner, 2003, 2004). The pattern of results for scopolomine (which reduced ACh efficacy) and physostigmine (which increased ACh efficacy) is intriguing. Scopolomine injections did not effect consolidation or recall, whereas physostigmine did. Scopolomine injections disrupted encoding (learning), whereas physostigmine did not disrupt encoding. This was observed during Hebb–Williams maze learning (Rogers and Kesner, 2003) and delay fear conditioning (Rogers and Kesner, 2004). Also, post-training injections of 192 IgG-saporin that drastically reduced the MS cholinergic projection into dCA3 were insufficient to cause a deficit at long delays on a previously learned DMP task (Winters and Dunnett, 2004).

These results suggest that a reduction in ACh entering dCA3 via the fimbria would not have disruptive effects for performance of a learned task, since interference between encoding and retrieval is not as important during performance of a task as during acquisition—in other words, any reduction in ACh would have little effect on recall, but a large effect on acquisition (Hasselmo and Schnell, 1994; Hasselmo et al., 1995; Hasselmo and McGaughy, 2004). The models predict that there would be an initial impairment of acquisition from which the animals would eventually recover, without a concomitant deficit in recall during subsequent tests of performance; the present data support this assertion.

In summary, dCA3 and its subcortical efferents are involved in acquiring spatial tasks that require gradual learning over multiple trials to reach asymptotic performance. The dCA3 to dCA1 Shaffer collateral efferents appear to be important for intermediate-term memory, but the efferent output from dCA3 via the fimbria appears not to be involved in working memory, consolidation, or recall; only gradual learning over multiple trials.

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