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The Role of the Direct Perforant Path Input to the CA1 Subregion of the Dorsal Hippocampus in Memory Retention and Retrieval

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Abstract

Subregional analyses of the hippocampus have suggested a selective role for the CA1 subregion in intermediate/long-term spatial memory and consolidation, but not short-term acquisition or encoding processes. It remains unclear how the direct cortical projection to CA1 via the perforant path (pp) contributes to these CA1-dependent processes. It has been suggested that dopamine selectively modulates the pp projection to CA1 while having little to no effect on the Schaffer collateral (SC) projection to CA1. This series of behavioral and electro-physiological experiments takes advantage of this pharmacological dissociation to demonstrate that the direct pp inputs to CA1 are critical in CA1-dependent intermediate-term retention and retrieval function. Here we demonstrate that local infusion of the nonselective dopamine agonist, apomorphine (10, 15 μ g), into the CA1 subregion of awake animals produces impairments in between-day retention and retrieval, sparing within-day encoding of a modified Hebb-Williams maze and contextual conditioning of fear. In contrast, apomorphine produces no deficits when infused into the CA3 subregion. To complement the behavioral analyses, electrophysiological data was collected. In anesthetized animals, local infusion of the same doses of apomorphine significantly modifies evoked responses in the distal dendrites of CA1 following angular bundle stimulation, but produces no significant effects in the more proximal dendritic layer following stimulation of the SC. These results support a modulatory role for dopamine in the EC-CA1, but not CA3-CA1 circuitry, and suggest the possibility of a more fundamental role for EC-CA1 synaptic transmission in terms of intermediate-term, but not short-term spatial memory.

Keywords

hippocampus; CA1; perforant; path; temporoammonic; apomorphine

INTRODUCTION

Contemporary analysis of hippocampal function has suggested that memory encoding and retrieval require unique computational processes that are independently supported by specific subregions and pathways projecting into this region (O'Reilly and McClelland, 1994; Treves and Rolls, 1994; Moser and Moser, 1998; Kesner et al., 2004; Daumas et al., 2005; Hasselmo, 2005; Rolls and Kesner, 2006). There is strong evidence suggesting the CA1 subregion plays a significant role in intermediate/long-term spatial memory and consolidation, but not short-term acquisition or encoding processes (O'Reilly and McClelland, 1994; Treves and Rolls, 1994; Maren et al., 1997; Bertaina-Anglade et al., 2000; Kesner et al., 2004; Lee and Kesner, 2004a; Remondes and Schuman, 2004; Daumas et al., 2005; Hasselmo, 2005; Sharifzadeh et al., 2006). Encoding of information acquired in

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a Hebb-Williams maze or in contextual fear conditioning, using a within-days analysis, is impaired by targeted lesions to the CA3 and dentate gyrus (DG) subregions, but not from targeted lesions to the CA1 subregion. In contrast, using a between-days analysis, retention and retrieval is disrupted following CA1, but not following CA3 or DG lesions (Lee and Kesner, 2004a; Lee and Kesner, 2004b; Jerman et al., 2006). Impairments have also been demonstrated in delay-dependent retrieval without impairing immediate recall or encoding of spatial information after infusing glutamatergic antagonists (Lee and Kesner, 2002), or cyclooxygenase-2 inhibitors (Sharifzadeh et al., 2006) into the CA1 subregion, but not when infused into the CA3 subregion. Similar retrieval deficits are apparent with selective lesions to the stratum lacuno-moleculare (s.l-m) dendritic layer, where the axons of the direct perforant path (pp) terminate (Remondes and Schuman, 2004).

Converging evidence suggests that the pp projection from the entorhinal cortex (EC) to the CA1 subregion of the hippocampus, also referred to as the temporoammonic pathway, may provide necessary environmental input to support specific computational resources underlying delay-dependent retention and retrieval of spatial information; whereas, the dentate/mossy fiber system within the trisynaptic circuitry provides the necessary framework for optimal storage or encoding of new information in the CA3 subregion (Colbert and Levy, 1992; Hasselmo et al., 1995; Kirkby and Higgins, 1998; Sybirska et al., 2000; Vinogradova, 2001; Brun et al., 2002; Remondes and Schuman, 2004; Hasselmo, 2005; Lisman and Grace, 2005). It is, therefore, of interest to selectively alter transmission of the direct pp with pharmacological methods to investigate the functional specificity of EC-CA1 activity, thus setting its contribution apart from indirect trisynaptic (i.e., CA3-CA1) connectivity. Similarly to previous data associated with targeted CA1 dysfunction, it is hypothesized that selective pharmacological disruption of EC-CA1 connectivity may in fact impair the rats' ability to integrate and update external environmental information with internal representations of space thereby producing errors in delay-dependent retrieval of relevant associative cues, while sparing encoding or short-term memory processes.

Recent studies suggest that the pp, but not the Schaffer collateral (SC) projection to CA1 is modulated by dopamine (DA) (Bruinink and Bischoff, 1993; Frey et al., 1993; Goldsmith and Joyce, 1994; Huang and Kandel, 1995; Gasbarri et al., 1997; Otmakhova and Lisman, 1999; Li et al., 2003). Thus, for the purpose of this series of experiments, we utilized pharmacological means of modifying the direct pp and driving the representation in CA1 towards processing trisynaptic inputs only. Our aim for the current series of experiments was threefold: (1) to elucidate the role of the direct pp in terms of intermediate-term spatial memory retention and retrieval, (2) to investigate differences in encoding (within-day) and retrieval (between-day) that may parallel previous CA1 dysfunction on a modified version of the Hebb-Williams maze and in contextual fear conditioning, and (3) to contribute to the existing data suggesting a neuromodulatory role for DA specific to EC-CA1 connectivity. We hypothesized that disruption of the pp projection to CA1 would specifically impair retention and retrieval and not encoding.

MATERIALS AND METHODS

All protocols conformed to the NAS Guide for the Care and Use of Laboratory Animals. The treatment of animals complied with all animal care guidelines approved by the Institutional Animal Care and Use Committee and the University Animal Resource Center.

CA1 Lesions

Fifteen rats were given lesions in the CA1 subregion of the dorsal hippocampal subregion. Each rat was given an intraperitoneal injection of atropine sulfate (0.2 mg/kg, i.p.) to control for respiratory difficulties. Rats were then deeply anesthetized with isoflurane gas and

placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) on an isothermal heating pad, to maintain body temperature constant at 37°C throughout the surgical procedure. The scalp was incised and retracted exposing bregma and lambda in the same horizontal plane. Small burr holes were drilled into the skull at the following coordinates: −3.6 mm posterior to bregma and 2.0 mm lateral to midline. An injection needle (28 gauge) was lowered into the CA1 subregion (1.9 mm ventral from dura) and an injection of ibotenic acid (6 mg/ml; 0.1–0.15 µl/site; 0.1 µl/min) was slowly infused bilaterally into the pyramidal cell layers of the CA1 region to have both longitudinal and mediolateral spread in the dorsal CA1 subregion. Injection was made using a 10-µl Hamilton syringe (Hamilton, Reno, NV) and microinjection pump (Cole-Parmer, Vernon Hills, IL). The injection needle was left in place for 1 min after the injection to allow for proper diffusion. Previous research has shown that similar lesion methods have been well suited for selective lesions of CA subregions of the hippocampus (Kesner et al., 2004; Lee and Kesner, 2004a; Jerman et al., 2006). Following surgery, the rats were allowed to recover on a heating pad before returning to their home cage. Rats were given acetaminophen (Children's Tylenol) (~150–200 mg/kg) dissolved in drinking water for 2–3 days post-surgery as an analgesic. They were given 1-week recovery before training on the Hebb-Williams maze, or being placed in the fear conditioning chamber. Rats were divided into groups for either the Hebb-Williams maze or the fear conditioning task.

Implantation of Guide Cannula

The same surgical procedures were followed in preparation to implant guide cannula and postsurgical recovery. Guide cannulae (22 G) were bilaterally implanted into either the CA1 ($n = 60$; 3.6 mm posterior to bregma, 3.6 mm lateral, and 2.9 mm ventral) or CA3 ($n = 13$; 3.6 mm posterior to bregma, 3.6 mm lateral, and 2.8 mm ventral) subregion and coupled with stylets (28 G) to prevent occlusion (Fig. 1). After cannulae implantation was performed, rats were given 1 week for recovery. Rats were divided into groups for either the Hebb-Williams maze or the fear conditioning task.

Intrahippocampal Microinjection

Apomorphine or control vehicle was injected immediately preceding behavioral conditioning (or testing). The doses of apomorphine chosen for this series of experiments were based on previous studies showing behavioral effects and injected intracerebrally (Jork et al., 1980; Costall et al., 1985; Morgenstern and Fink, 1985; Smialowski and Maj, 1985; LaHoste and Marshall, 1990; Kim and Levin, 1996). This series of behavioral experiments chose a moderately high dose of apomorphine as the lowest concentration (i.e., 10 µg), and a relatively higher concentration (i.e., 15 µg), in which apomorphine-HCL remained soluble in vehicle (i.e., <1% DMSO in bacteriostatic water).

The drug injection protocol was as follows: apomorphine was prepared the day of conditioning, using either a final concentration of 10, or 15 µg in 0.4 µl of <1% DMSO (Dimethyl sulfoxide) and bacteriostatic water. Control rats were injected with the vehicle only (<1% DMSO). Immediately prior to conditioning (or testing), injection of drug/vehicle was made bilaterally via an injection needle (28 gauge) at a rate of 0.1 µl/min, using a 10-µl Hamilton syringe and microinjection pump. The injection needle was left in place for 1 min after the injection to allow diffusion down the cannula. The rat was then placed in the fear-conditioning chamber for conditioning or in the start box to begin training on the Hebb-Williams maze.

Hebb-Williams Maze

The original Hebb-Williams maze (Hebb, 1946; Rabinovitch and Rosvold, 1951) consisted of a series of 12 problems, in which a unique route with varying difficulty was required to

reach the goal. The present version was modified to facilitate spatial and/or place learning with a moderate level of difficulty using a fixed configuration of inner barriers (i.e., Problem 4, described previously) (Rabinovitch and Rosvold, 1951; Lee and Kesner, 2004b; Rogers and Kesner, 2004). Two boxes, diagonally positioned on opposite sides of the maze, were designated as the start and goal boxes. During training, the rat had to learn to eliminate inappropriate navigational movements to derive the shortest path to the goal box, in which there was a food reward (Froot Loops cereal, Kellogg, Battle Creek Michigan). As soon as the rat entered the goal box, the door was closed and the trial ended. An intertrial interval (30–45 s) was used to clean the maze and allow the rat to finish eating the food reward. There were 10 trials each day per rat. Duration of training varied across days depending on the animals' latency to run; however, daily training sessions lasted ~30–45 min for each rat. All rats were handled for at least 5 days (>10 min per day for each rat) before they were shaped on a pretraining runway. Handling continued during shaping and postoperatively during training. All rats were kept at 85–90% of their original body weight during shaping and training.

Errors were recorded when a rat crossed one of the 11 error zones (Fig. 2A). Errors were then averaged across blocks of the first and last 3 trials for each day to produce a general learning index. It was assumed that encoding and retrieval interact on a trial-by-trial basis (one reason it makes it difficult to functionally differentiate them behaviorally) and thus indices were developed (Lee and Kesner, 2004b; Rogers and Kesner, 2004) to dissociate the two processes. A within-day (W/D) encoding score was calculated by subtracting B (the average number of errors across the last three trials of Day 1) from A (the average number of errors across the first three trials of Day 1), and D (the average number of errors across the last three trials on Day 2) from C (the average number of errors across the first three trials on Day 2). The encoding index was then derived from the average of the W/D encoding scores calculated for the first 2 days. A between-day (B/D) retrieval score was calculated by subtracting C from B and E (the average number of errors across the first three trials of Day 3) from D. The retrieval index was then derived from the average of the B/D retrieval scores calculated between Days 1 and 2 and between Days 2 and 3. A positive encoding index indicates that errors are decreasing between the first three trials of 1 day and the last three trials of the same day suggesting successful encoding whereas, a negative index indicates that errors are increasing between the first three trials of 1 day and the last three trials of the same day suggesting a disruption in encoding. A positive retrieval index indicates that errors are decreasing between the last three trials of 1 day and the first three trials of the next day suggesting successful retrieval, whereas, a negative index indicates that errors are increasing between the last three trials of 1 day and the first three trials of the next day suggesting a disruption in retrieval.

Rats were either selectively lesioned in the dorsal CA1 subregion (CA1-les; $n = 11$) or implanted with cannulae and divided into 3 groups (control, $n = 13$; Apo-10, $n = 13$; Apo-15, $n = 13$) to receive infusions of vehicle or apomorphine (10 or 15 μg , respectively) directly into the CA1 subregion prior to daily training (i.e., 3 consecutive days). Rats implanted with cannula in the CA3 subregion (CA3-control, $n = 9$) acted as an anatomical control to investigate the potential action of apomorphine outside but near to the intended injection site (i.e., CA1). Following a 7–10 day recovery period, approximately half of the rats that were tested on the Hebb-Williams maze were also used for the following fear conditioning task. No significant differences were later found between these two groups of rats.

Fear Conditioning

Two observation chambers were used during 3 consecutive days of testing for fear conditioning. All stimuli were controlled by a computer software package (Graphic State,

Coulbourn Instruments, Allentown, PA). All rats were placed into the fear-conditioning chamber without a tone or shock stimulus for a baseline period of 2 min (preconditioning baseline). During this 2-min period, the baseline level of locomotor activity of each rat was measured and recorded by counting the number of times the rat's two rear feet crossed the midline of the chamber (crossover score; Maren et al., 1997). After the 2-min exploratory baseline period, 10 trials of tone-shock pairs were administered for fear-conditioning. Each trial was initiated by a tone (10 s, 2 kHz, 85-dB A-scale) presented through the speaker. The tone coterminated with an electric foot-shock (2 s, 1 mA). After the tone-shock pair, a 64-s intertrial interval was given before the next tone-shock trial began. After the 10th tone-shock pairing, the rat was removed from the chamber. Freezing response (i.e., complete absence of movement except for respiratory movement) was measured during the preconditioning baseline and during each intertrial interval (64 s $\times$ 9) of conditioning. Freezing behavior was scored by monitoring the rat every 8 s by an observer blind to the experimental design, resulting in 15 observations for the preconditioning baseline periods and eight observations for each intertrial interval between tone-shock pair trials.

To test the effect of apomorphine on acquisition processes, rats with cannula were divided into two groups receiving infusions of apomorphine (Apo-10, $n = 9$; Apo-15, $n = 11$) (10 or 15 μ g, respectively) directly into the CA1 subregion before conditioning on Day 1. Another group of rats (CA1-les, $n = 9$) were selectively lesioned in the dorsal CA1 subregion prior to conditioning. Two control (control, $n = 15$; CA3-control, $n = 9$) groups were used. The first control group (control) received vehicle injections in the CA1 subregion immediately prior to conditioning on Day 1. The second control group (CA3-control) received 15 μ g of apomorphine into cannula implanted in the CA3 subregion immediately prior to conditioning on Day 1. The CA3-control group acted as an anatomical control (see Fig. 1 for cannula placements).

All groups of rats were tested for retrieval of contextual fear 24 hr after conditioning in the same context as Day 1. Each rat was placed in the fear-conditioning chamber for 8 min with the same visual and olfactory cues present on Day 1; however, the original cue (i.e., tone) was not present. A final group of rats (Apo-15₂, $n = 9$) received infusions of apomorphine (0.4 μ l; 15 μ g) immediately prior to testing only. This group of rats was used to assess retrieval of the context or cue-shock association without the potential of disrupting acquisition processes. The other groups of rats were tested drug-free. Freezing behavior was analyzed, resulting in 60 observations.

The rats were tested for retrieval of cued fear 48 hr after conditioning in a context that was novel when compared with the context from Day 1. The Apo-15₂ group of rats was injected with apomorphine (0.4 μ l; 15 μ g) immediately prior to testing. The other groups of rats were tested drug-free. All rats were placed in the novel chamber for 2 min (precued-retrieval baseline), while freezing behavior was recorded by a blind observer. After the 2-min baseline period, the same tone used for Day 1 of fear-conditioning was presented for 8 min continuously to test cued retrieval of tone-shock association. Freezing behavior was scored for both precued-retrieval baseline (15 observations) and cued-retrieval (60 observations) periods.

Electrophysiological Recording

Each of the rats ($n = 13$) was anesthetized with urethane (1.6 mg/kg, i.p.) and placed in a stereotaxic apparatus. Body temperature was maintained at 37 $\pm$ 1°C with a heating pad, and monitored continuously. An incision on the scalp was made to expose the skull and three unilateral (right side) burr holes (3 mm in diameter/hole) were drilled to accommodate recording and stimulating electrodes. Two additional holes for skull screws were also drilled for the ground and recording reference. One stimulating electrode (SNEX-200 bipolar

stainless steel, 0.5 mm vertical tip distance between the two poles, Rhodes Medical Instruments, Woodland Hills, CA) was directed toward the angular bundle (i.e., to stimulate the pp) or directed at the SCs, and a recording electrode (simultaneous bipolar electrode-cannula, 125 μ m, 1 mm vertical tip distance between the two poles, Plastics One, Roanoke, VA) was directed toward the CA1 region of the dorsal hippocampus using the following coordinates: the angular bundle/medial pp: 8.0 mm posterior to bregma, $\pm$ 4.4 mm lateral to midline, and 2.8 mm ventral from dura; the SC: 3.6 mm posterior to bregma, and 2.5–2.7 mm lateral to midline; dorsal CA1: 3.6 mm posterior to bregma, and 1.6–1.8 mm lateral to midline (see Fig. 5 for electrode placements). The exact vertical coordinates of the recording electrode was determined by optimizing the characteristic shape of the evoked potential.

An acute surgical preparation was used to acquire data in vivo. Rats were fixed with connector cables to both the recording and stimulating electrodes. Biphasic stimulation impulses (0.1–0.5 ms in duration, 0.1–1 mA in amplitude) were delivered using constant low frequencies (<0.5 Hz) and generated by a Neurodata PG4000 digital stimulator, and SIU90 stimulation isolation unit (Cygnus Technologies, Scottsdale, AZ). To optimize the magnitude of the evoked response, responses from the simultaneous electrode/cannula in the CA1 subregion were monitored, while final depths of recording and stimulating electrodes were adjusted at increments of 50 μ m. As previously demonstrated (Heinemann et al., 2000; Jones, 1993), trisynaptic responses were less evident at low frequency stimulation. Once short-latency (4–6 ms) monosynaptic responses from CA1 were recorded, stimulus intensity was reduced and the tissue was allowed to stabilize for 1–2 min. To address stability of the evoked population response, baseline field potentials were then monitored for 10 min prior to making the first baseline recording (–10 min). Ten minutes following the first baseline recording, a second baseline recording was made (0 min), and immediately followed by drug or vehicle infusion. Tissue was given another 1–2 min to stabilize. An additional seven (10, 20, 30, 40, 50, 60, and 70 min) 2 min recordings were made every 10 min following stabilization and all measurements were reported as averages ($\pm$ SEM). Electrical signals from the brain were amplified (gain 50–100; Neuralynx amplifier, Tucson, AZ), filtered (0.1–10 kHz), digitized (20 kHz), and monitored on a virtual oscilloscope and computer screen using a commercial software package (Sciworks; Datawave Technologies, Longmont, CO). Sciworks was also used for automatic control of data acquisition and for later off-line analysis. Basic physiological function of the animal was carefully monitored during the data collection, so as to avoid any confounds with the electrophysiological profile. Following data acquisition, the animals were perfused, and the brains were processed to examine histological placement of electrodes. The field EPSP amplitude was measured as an average of the difference scores between maximum and minimum evoked response values. Amplitude measurements were reported as a percentage of average baseline EPSP amplitude (see Fig. 5).

Histology

At the conclusion of all testing, each rat with cannulae was injected with a Chicago blue dye solution (0.4 μ l), deeply anesthetized with an intraperitoneal injection of sodium pentobarbital (100 mg/kg) and perfused intracardially with normal saline followed by a 10% formalin solution. Each brain was then stored in a 10% formalin/30% sucrose solution at 4°C for 72 hr, frozen (–18°C) and cut in 40- μ m coronal sections on a cryostat. Every third section was collected on a glass slide and stained with crystal violet. These sections were examined for histological verification of the lesion and cannulae (see Fig. 1).

RESULTS

The Effects of Apomorphine on Encoding and Retrieval of Maze Learning

As predicted, the results indicate that neither CA1 lesions nor apomorphine produce any deficits in encoding the necessary spatial information for navigation within-days but do produce significant deficits in retrieval of the necessary spatial information between-days (Fig. 3) when injected into the CA1, but not the CA3 subregion. The general learning index was calculated from the mean number of errors for each block of 3 trials (across days) and was analyzed between groups. An ANOVA with a repeated measures design was performed with the experimental groups (control-vehicle, CA3-control, Apo-10 μ g, Apo-15 μ g, and CA1-les) across blocks. There was no effect of group, but there was a significant effect for blocks, $F(4, 232) = 90.62$; $P < 0.001$ and a significant interaction, $F(16, 232) = 2.75$; $P < 0.001$. To explore the interaction between blocks (errors) and experimental groups, specific encoding and retrieval indices were developed and used in further analyses (see methods for a description of indices).

The results generally indicate an overall decrease in the number of errors for all groups across blocks suggesting that apomorphine does not disrupt general learning ability for spatial types of information. Additionally, the CA1-lesion group and both drug-treated groups (Apo-10, Apo-15) demonstrated retention and retrieval deficits with similar increases in the number of errors (compared with control) between days and decreases in number of errors within and across days (Fig. 2). The CA3 anatomical control group did not demonstrate any deficits in general learning, but did show some facilitation with faster decreases in number of errors within and across days. These data suggest that apomorphine acts specifically in the CA1 subregion to disrupt retrieval processes, but has little to no effect when localized to the CA3 subregion.

A one-way ANOVA was performed separately for the encoding and retrieval indices with the experimental groups (control-vehicle, CA3-control, Apo-10 μ g, Apo-15 μ g, and CA1-les). There were no significant differences in terms of the within-day (W/D) encoding index indicating that all experimental groups were similar in reducing their errors within days. There was a significant effect of apomorphine and CA1-lesions in terms of the between-day (B/D) retrieval index, $F(4, 63) = 12.86$, $P < 0.001$. A post hoc analysis (Tukey HSD) showed a significant difference ($P < 0.001$) between control groups (control, CA3-control) and the CA1-lesion group as well as between control groups and both drug-treated groups (Apo-10, Apo-15) indicating a significant increase in the number of errors between days after CA1 lesions or infusion of apomorphine into CA1. There was no significant difference between the control and CA3-control group, indicating that apomorphine failed to produce any deficits in retrieval when infused into the CA3 region, specifically.

The Effects of Apomorphine on Acquisition of Contextual Fear

Given the observed selective deficits in retrieval of spatial cues necessary for navigation of a maze and previous evidence implicating CA1 in retrieval (Hasselmo, 1995; Hasselmo et al., 1995; Sybirska et al., 2000; Kesner et al., 2004; Lee and Kesner, 2004b; Jerman et al., 2006; Rolls and Kesner, 2006), we reasoned that similar selective deficits may be apparent in additional CA1-dependent tasks. It has been shown previously that CA3-lesioned rats are significantly impaired on the acquisition, but not retention of contextual fear-conditioning whereas, CA1 lesioned rats are significantly impaired on retention but not acquisition (Lee and Kesner, 2004a). We predicted that apomorphine would produce similar selective retention deficits when infused into the CA1, but not CA3 subregion.

A one-way ANOVA was performed with different experimental groups (control, control-CA3, Apo-10, Apo-15, Apo-15, and CA1-les) for the preconditioning baseline. All groups

displayed minimal amounts of freezing. No significant differences in the amount of freezing were found among any of the groups. No significant changes in locomotor activity in comparison to control animals were observed due to either dose of apomorphine. Thus, behavioral deficits could not be attributed to locomotor changes.

An ANOVA with a repeated measures design was performed with different experimental groups across blocks of conditioning to investigate any deficits in encoding processes within the 10 trials. For the purpose of analysis, average freezing during the ITI was averaged and split into three blocks for each rat (Block 1 = avg. freezing during first three ITI periods; Block 2 = avg. freezing during second three ITI periods; Block 3 = avg. freezing during last four ITI periods). A significant effect was found for blocks, $F(2, 114) = 18.62$, $P < 0.001$. There was no significant effect of group across blocks, nor was there a significant interaction effect. Although there were some initial impairments by the CA1 lesion and Apo-15 group in Block 1, these impairments disappeared by Block 2. All experimental groups displayed significantly higher levels ($P < 0.05$) of freezing during every ITI compared with baseline levels, suggesting that acquisition processes were not impaired. These results indicate that neither CA1 lesions nor infusion of apomorphine into the dorsal hippocampus impair encoding of contextual fear (Fig. 4).

The Effects of Apomorphine on Retention and Retrieval of Contextual Fear

To test intermediate-term retention and retrieval (24 hr postconditioning), the same groups of rats were then tested (i.e., freezing response was measured) on retrieval of contextual fear in the same context on Day 2 without the presence of the original cue (i.e., tone) and on retrieval of cued fear in a novel context with the presence of the cue on Day 3. A one-way ANOVA was performed for the retrieval of contextual fear among the different experimental groups. There was a significant effect of group, $F(5, 63) = 20.96$, $P < 0.001$ (Figs. 4B,D). A post hoc analysis (Tukey HSD) showed that all drug groups (Apo-10, Apo-15, and Apo-15), as well as the lesion group (CA1-les), significantly differed ($P < 0.01$) from the control-vehicle group (Fig. 4B). There was no significant difference between the CA3-control and vehicle control group. These results suggest that similarly to CA1-selective lesions, apomorphine disrupts retention and retrieval of context-associated fear when infused into the CA1, but not CA3 subregion.

On Day 3, all rats were introduced to a novel observation chamber, in which they all showed minimal freezing behavior (Fig. 4C). A one-way ANOVA was performed for the amount of freezing at baseline among all groups. No significant locomotor effects were observed, nor were there any differences among groups. After the 2-min baseline period and in response to the 8-min continuous presentation of the cue, all rats showed increased levels of freezing compared with the baseline indicating cue-associated fear was preserved. A one-way ANOVA was performed for the retrieval of cued fear between different drug groups. There were no significant differences among the groups (Fig. 4C,D). The results indicate that neither apomorphine nor targeted CA1 lesions produce deficits in cue-associated fear, a behavior that has not previously been shown to be sensitive to CA1 dysfunction.

The Effect of Apomorphine on EC-CA1 Synaptic Transmission

With dense dopaminergic projections and the majority of DA receptors localized in the dendritic layers of CA1 associated with the pp input from layer III of EC (Goldsmith and Joyce, 1994; Gasbarri et al., 1997), it was reasoned that apomorphine may be acting on those receptors specifically. To substantiate that the observed behavioral impairments were due to the selective infusion of apomorphine into the CA1 subregion, in vivo recordings were performed. Baseline evoked responses as recorded from the distal dendrites of the CA1 subregion after pp stimulation consisted of a positive population excitatory postsynaptic

field potential. Baseline evoked responses as recorded from the proximal dendritic layer of the CA1 subregion after SC stimulation consisted of a negative population excitatory postsynaptic field potential (Fig. 5).

As predicted, this set of in vivo experiments revealed that apomorphine produced a modification of evoked population responses recorded in CA1 from pp stimulation and no significant changes in evoked responses after SC stimulation. Specifically, apomorphine produced increases in amplitude of evoked responses (mean increase, 33.48 $\pm$ 5.26%) for at least 1 hr and up to 2.5 hr from angular bundle stimulation following infusion of apomorphine (10 μ g, Apo-10 and 15 μ g, Apo-15; 0.4 μ l) directly into the dorsal CA1 region. There were however, no reliable changes to the fEPSP induced by SC stimulation after apomorphine infusion into the CA1 region of a separate group of animals (apo-15, n = 4; apo-10, n = 4) (Fig. 5). No significant differences were found between apomorphine and vehicle infusion in this group of animals. These data support the prediction that the direct pp projection to CA1 is critical for a selective role of CA1 in intermediate-term memory retrieval. These data also suggest that DA can selectively modulate the EC-CA1, but not the SC-CA1 projection.

A repeated measures ANOVA was performed to assess dose-dependent differences of apomorphine and vehicle infusion (Apo-15 μ g, n = 5; Apo-10 μ g, n = 4; Vehicle, n = 4) across time (−10, 0, 10, 20, 30, 40, 50, 60, and 70 min) revealing a significant effect for apomorphine in animals receiving stimulation to the pp, $F(2,10) = 17.578$, $P < 0.001$. The analysis also revealed a significant effect for time, $F(8,80) = 18.31$, $P < 0.001$, signifying the dramatic change in amplitude from baseline recordings. A post hoc analysis (Tukey HSD) demonstrated that both doses of apomorphine significantly differed from control vehicle ($P < 0.01$), but not from each other, indicating that there was a significant amplitude change due to apomorphine infusion and no apparent dose-dependent differences. There was also a significant interaction between time and apomorphine, $F(16,80) = 3.66$, $P < 0.001$, indicating the effect of apomorphine significantly increased the magnitude of the evoked response within 10 min after infusion and continued to increase until about 30–40 min, in which the response began to plateau. To further investigate the differences at each time point, an independent samples t -test was performed revealing significant differences ($P < 0.05$) between the drug and vehicle groups at all time points, beginning at 10 min post-drug infusion and reaching maximum differences at 40 min. Even 60 min after apomorphine infusion, there was little indication for the facilitation response to return to baseline. In fact, in some cases, the facilitation was found to plateau around 60 min and show no decrease even after 2.5 hr. No significant differences were found between apomorphine and vehicle control when the SC was stimulated. These data support the observed behavioral impairments in that both Hebb Williams maze and contextual fear testing were well within the time constraints of the observed electrophysiological changes. The observed effects of apomorphine provide further evidence for selective dopaminergic modulation at the level of the direct pp projection to CA1.

Histology

Histological analyses revealed accurate placement of all cannula and electrodes. Further analyses revealed that the volume used for injection did not diffuse much further than the CA1 region, but was apparently confined to the area above the granule cell layer of DG and lateral to the injection sites throughout the CA1 stratum (Fig. 1). However, it must be noted that diffusion of Chicago blue is only an approximation of the diffusion of apomorphine with the same volume. Serial coronal sections (40 μ m) of the dorsal hippocampus (N = 15 for each animal) from the lesion group were carefully examined under high magnification microscope for the existence of intact pyramidal cells in CA1 and compared with those from a control brain. The imaging software (ImageJ, NIH, Washington, D.C.) used for analyses

showed the extent of CA1 lesions in all animals to have $\sim 72 \pm 10\%$ reduction in pyramidal cell distribution (Fig. 1D).

DISCUSSION

This series of experiments utilized a model of dopaminergic modulation in an attempt to selectively modify the direct pp and assess its contribution to the dorsal CA1 subregion in terms of (a) general maze learning, (b) short-term encoding of spatial and contextual information, and (c) intermediate-term retrieval of spatial, contextual, and cue-associated information. The electrophysiological data support the behavioral observations to the extent that apomorphine is able to selectively modify the functioning of the direct pp, generally indicating that the pp plays an important modulatory role in CA1-dependent memory retention and recall, but not short-term encoding, and has raised the possibility of a more fundamental role for EC-CA1 synaptic transmission in intermediate-term, but not short-term spatial memory.

The present study demonstrates that infusion of a nonselective DA agonist, apomorphine, prior to learning produces deficits in intermediate-term (i.e., 24 hr delay) recall of spatial and contextual types of memory, sparing short-term or immediate recall, encoding, and thus acquisition of spatial information necessary for goal-directed navigation, and cue/context-associated fear. When injected outside the CA1 subregion (i.e., CA3), between-day retrieval on a Hebb-Williams maze or in contextual fear conditioning is not affected, indicating the anatomical specificity of disruption to CA1. In support of a role for EC-CA1, but not CA3-CA1 connectivity, local infusion of the same doses of apomorphine in an anesthetized rat selectively modifies evoked CA1 responses by pp stimulation, but has no effect on responses elicited by SC stimulation.

The current data from this series of behavioral and electro-physiological experiments is supported by previous evidence for specific dopaminergic modulation of the direct pp (Empson and Heinemann, 1995; Huang and Kandel, 1995; Swanson-Park et al., 1999; Otmakhova and Lisman, 1999, 2000; Li et al., 2003), and findings that have localized dense mesencephalic projections and high levels of DA receptors in dendritic layers of CA1 that receive afferent input via the pp (Frey et al., 1991; Bruinink and Bischoff, 1993; Goldsmith and Joyce, 1994; Carr and Sesack, 1996; Gasbarri et al., 1997). Thus, the observed deficits are assumed to be attributed to apomorphine acting on those receptors specifically. Furthermore, these data parallel impairments demonstrated in previous studies involving DA (Murphy et al., 1996; Zahrt et al., 1997) and CA1 dysfunction (Sybirska et al., 2000; Lee and Kesner, 2002; Kesner et al., 2004; Lee and Kesner, 2004a; Remondes and Schuman, 2004; Dumas et al., 2005).

Models of cellular and systems-level consolidation (Treves and Rolls, 1994; Otmakhova and Lisman, 1999; Vinogradova, 2001; Kesner et al., 2004; Hasselmo, 1995, 2005; Lisman and Grace, 2005; Rolls and Kesner, 2006) propose that dynamic interactions between hippocampal networks and neocortex are necessary for supporting a role for CA1 in terms of intermediate/long-term retention, retrieval and consolidation of spatial memory. The current data support such models, such that modification of EC-CA1 synaptic connectivity by a DA agonist is assumed to alter the operating characteristics in terms of storage, retention, or retrieval capacity within a 24 hr intermediate-term time frame. Within the context of this framework, there is evidence that parallels cellular and molecular consolidation processes necessary for gene transcription and protein synthesis after induction of LTP in this region. For example, increased expression of LTP-related immediate early genes (i.e., BDNF, zif268, c-FOS, and JUNB) in dorsal CA1 has been observed within 24 hr after the exposure to learning (Frey et al., 1991; Bertaina-Anglade et al., 2000; Gusev et al., 2005).

Consolidation processes during the intermediate-term, 24-hr time frame that support later retrieval may depend on an active dialogue between EC and CA1, and DA may be modulating proper EC-CA1 interactions. The extent to which DA interferes with the memory trace during the 24-hr window of vulnerability remains unclear; however, the data associated with the Apo₂-15 group support an extensive role for EC-CA1 interactions in retrieval alone by showing deficits when apomorphine is injected into CA1 prior to testing.

One interpretation of the current data is dependent upon previous data that have implicated the CA1 region in match-mismatch operations, and detection of novelty (Hasselmo et al., 1995; Vinogradova, 2001; Remondes and Schuman, 2004; Lisman and Grace, 2005; Rolls and Kesner, 2006). The CA1 subregion may function to compare information from the two afferent inputs, in which case, the CA3 input contains the contents of processed forms of memory, while the pp supplies unprocessed sensory information. The comparison of converging inputs may lead to the detection of novelty (Vinogradova, 2001), mismatch from expectations (Hasselmo and Schnell, 1994), and/or a facilitation of retrieval mechanisms (Lee and Kesner, 2002). Increased availability of DA to a certain threshold within the hippocampal-VTA circuitry may provide a means to disrupt or “switch” ongoing informational processes to allow for the initiation of, or detection of novel information processes (Swerdlow and Koob, 1987; Lisman and Grace, 2005). Depending on the level of DA, the “comparator” role of CA1 may essentially fluctuate between the computational algorithm necessary for either encoding or recall. Under the constraints of Hasselmo’s model of encoding/retrieval dynamics (Hasselmo and Schnell, 1994; Hasselmo et al., 1995), a mismatch between SC- and EC-CA1 inputs would result in high levels of cholinergic modulation. This modulatory change supports the appropriate dynamics for learning, such that the autoassociative network in CA3 is strengthened and CA3-CA1 synaptic connectivity is suppressed preventing the recall of previously stored associations from interfering with new learning. The current data supports Hasselmo’s model such that increases in DA availability and receptor function in the CA1 region appear to interfere with the comparator function by preventing the matching of information arriving via the SC input with the direct cortical input and reception of current sensory information. Thus, if the direct pp input is selectively disrupted, the encoding/retrieval algorithm would be driven towards the resulting mismatch (novelty) signal. A match however, appears necessary for optimal retrieval in the Hebb-Williams maze and fear conditioning, and accordingly a behavioral deficit is observed.

The present electrophysiological data indicate that apomorphine produces facilitation of field potentials in EC-CA1 synaptic connectivity. Although the current data differ from inhibitory responses found in vitro (Otmakhova and Lisman, 1999), the observations are supported by previous data indicating that facilitation of LTP, specifically, the late protein-synthesis dependent component of LTP in CA1 is dependent upon DA receptor activation (Frey et al., 1993; Huang and Kandel, 1995; Otmakhova and Lisman, 1996; Swanson-Park et al., 1999; Li et al., 2003). Differences may be due to the isolation of the hippocampal slice in vitro, in which the origins of the pathways are lost. Together with the behavioral data, the selective facilitation in synaptic transmission through the direct pp by apomorphine raises several computational questions, such as the extent to which these processes following learning are affected by hyperexcitability or noise; however, it remains clear that modification of the direct pp disrupts CA1-dependent behavior. Future studies may develop more specific lesion techniques that may allow one to selectively destroy the pp fibers coming from EC Layer III to CA1 or Layer III pyramidal neurons without producing seizure-like activity (Wu and Schwarcz, 1998). Such studies will further contribute to the understanding of EC-CA1 connectivity and function.

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FIGURE 1.

Histological verification of cannulae placement and lesion for behavioral analyses. (A) Photomicrograph of control brain. (B) Photomicrograph illustrating extent of CA1-specific excitotoxic lesion in the dorsal portion of the CA1 subregion. (C) Photomicrograph of coronal section of dorsal hippocampus with cannula track terminating in CA1 subregion. (D) Photomicrograph of cannula track terminating in the CA3 subregion. (E) Representative schematic of cannula placement for all rats (indicated by each dot/open square, $n = 73$) and approximate spread of drug assessed by Chicago blue dye infusion (indicated by circular shaded area) in anterior and posterior directions.

FIGURE 2.

Modified Hebb-Williams maze—general learning index. (A) Schematic illustrating configuration of inner barriers and error zones. Error zones and associated direction are indicated by dashed lines with numbers and arrows, while inner barriers are indicated by solid lines. Errors were operationalized by a crossing of defined zones by both hind paws in the assigned direction. Numbers associated with each error zone were used in the behavioral analysis (i.e., recording sequences and number of errors in each trial). (B) A general learning index was formulated from mean number of errors during each 3-trial block for 3 days of testing. Note that the strongest deficits came between days. Mean number of errors for each group are indicated for each 3-trial block. Compared with control (white diamonds) and CA3-control (black diamonds), the CA1 lesion group (white triangles), and both CA1-drug-treated groups (Apo-10, black squares; Apo-15, black triangles) showed increased number of errors on Blocks 3 and 5, indicating a deficit in retrieval between days. It should be noted that there were no significant differences between any of the groups across blocks, suggesting that general learning was spared.

FIGURE 3.

Encoding and retrieval differences during 3 days of exposure to the modified Hebb-Williams maze. (A) Within-day (W/D) encoding index scores are indicated. Higher scores indicate better encoding. (B) Between-day (B/D) retrieval index scores are indicated. Higher scores indicate better retrieval. See methods for description of indices. Both CA1 lesions and apomorphine produced deficits in between-day retrieval, but not within-day encoding ($P < 0.001$). CT, control; CA3-CT, CA3 control, Apo-10, apomorphine 10 μg ; apo-15, apomorphine 15 μg ; CA1-les, CA1 lesions.

FIGURE 4.

Freezing behavior on 3 days of acquisition and retrieval testing in contextual fear conditioning. (A) Freezing behavior during intertrial intervals (ITI) of the fear-conditioning paradigm on Day 1. [Block 1 = avg. freezing during first three ITI periods; Block 2 = avg. freezing during second three ITI periods; Block 3 = avg. freezing during last four ITI periods]. Average % freezing $\pm$ SEM for each group during baseline and during each block of conditioning is indicated. There were no significant differences among groups during the baseline preacquisition period. All groups appeared to acquire the tone–shock association as measured by the amount of freezing and difference from baseline. Control group (white diamonds), CA3-control group (open squares), Apo-10 drug-treated group (black squares), Apo-15 drug treated group (black triangles), Apo-15₂ (black circles), CA1-lesion group (open triangles). (B) Context test. Average % freezing $\pm$ SEM for all groups was measured in same context used for conditioning on Day 1 with no shock or cue (i.e., tone) present during the 8-min period. Both apomorphine (10, 15 μ g) and CA1 lesions produced significant deficits in retrieval for context–shock association compared with the control groups (CT, CA3-CT). The Apo-15 drug-treated group of rats showed significantly less freezing than the Apo-10 drug-treated group of rats. The CA3-control group (apomorphine, 15 μ g) showed no deficits. (C) Baseline—novel context. Average % freezing $\pm$ SEM for all groups was measured in novel context on Day 3 during a 2-min precued retrieval baseline, during which no cue (i.e., tone) was present. There were no significant differences between groups (D) Cue (Tone) test. Average % freezing $\pm$ SEM on Day 3 in a novel context during the presentation of the cue (i.e., tone) for all groups is indicated. There were no significant differences among groups.

FIGURE 5.

Selective increase in evoked postsynaptic potential (EPSP) amplitude in CA1 following apomorphine injections into the CA1 subregion and stimulation of angular bundle. (A) Electrode arrangements. A bipolar stimulating electrode was positioned in either the angular bundle to stimulate the pp (A/P = -8.1, lateral = 4.4, ventral = 2.8) or the SC (A/P = -3.6, lateral = 3.6, ventral = 2.9). A simultaneous bipolar electrode/22 G cannula was positioned in the CA1 region to record from the respective afferent input (A/P = -3.6, lateral = 1.6–1.8, ventral = 2.2–2.8) and inject drug/vehicle. (B) Between subjects comparison of evoked responses recorded in CA1 after pp stimulation (biphasic pulses < 0.5 Hz). Vehicle or apomorphine (10 or 15 µg) was injected after 2nd baseline measure (0 min) into CA1 cannula. Amplitude differences between max and min of average evoked responses were measured. Both 10 µg (black squares) and 15 µg (black triangles) of apomorphine significantly increased the max and min of evoked responses when compared with vehicle injections (white diamonds). (C) Between subjects comparison of evoked responses recorded in CA1 after SC stimulation. Max amplitude was measured. Neither dose of apomorphine produced any noticeable change to evoked responses. Insets show representative evoked responses from the different dendritic layers after respective stimulation before (gray) and after (black) apomorphine injection. Scale: 0.5 mV by 5 ms.