Behavioral Neuroscience
2007, Vol. 121, No. 4, 742–750

0735-7044/07/$12.00 DOI: 10.1037/0735-7044.121.4.742

Dissociations of the Medial and Lateral Perforant Path Projections Into Dorsal DG, CA3, and CA1 for Spatial and Nonspatial (Visual Object) Information Processing

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Medial perforant path plasticity can be attenuated by 2-amino-5-phosphonovaleric acid (APV) infusions, whereas lateral perforant path plasticity can be attenuated by naloxone infusions. The present experiment was designed to evaluate the role of each entorhinal efferent pathway into the dorsal hippocampus for detection of spatial and nonspatial (visual object) changes in the overall configuration of environmental stimuli. Dorsal dentate gyrus infusions of either APV or naloxone attenuated detection of a spatial change, whereas only naloxone infusions disrupted novel object detection. Either APV or naloxone infusions into dorsal CA3 disrupted both spatial and novel object detection. APV infusions into dorsal CA1 attenuated detection of a spatial change, whereas naloxone infusions into dorsal CA1 disrupted novel object detection. These data suggest that each dorsal hippocampal subregion processes spatial and nonspatial (visual object) information from perforant path efferents in a unique manner that is consistent with the intrinsic properties of each subregion.

Keywords: APV, naloxone, spatial exploration, hippocampus, novelty detection

It has been shown both anatomically and neurophysiologically that the medial entorhinal cortex and lateral entorhinal cortex send projections to the dorsal hippocampus (here defined as the dorsal dentate gyrus [dDG], dorsal CA3 [dCA3], and dorsal CA1 [dCA1] subregions of the hippocampus proper). Martinez, Derrick, and colleagues (Breindl, Derrick, Rodriguez, & Martinez, 1994; Do, Martinez, Martinez, & Derrick, 2002; Kosub, Do, & Derrick, 2005; Martinez, Do, Martinez, & Derrick, 2002; Villarreal, Do, Haddad, & Derrick, 2002) have reported that the neurotransmitters used by these projections are distinct and exhibit associability in both dCA3 and dDG. No such experimental analysis has been carried out in dCA1, but anatomical data have suggested that the same dissociations exist (Steward, 1976; Witter, Wouterlood, Naber, & van Haeften, 2000). The medial perforant path (input to the dorsal hippocampus from the medial entorhinal cortex) uses glutamate receptors, and induced long-term potentiation (LTP) is attenuated by *N*-methyl-D-aspartate (NMDA) receptor antagonists like 2-amino-5-phosphonovaleric acid (APV), but μ opiate receptor antagonists have no effect. The lateral perforant path (input to the dorsal hippocampus from the lateral entorhinal cortex) uses μ opioid receptors, and lateral perforant path induced LTP is attenuated by μ opiate receptor antagonists such as naloxone, whereas NMDA receptor antagonists have no effect. It has been proposed (Naber, Lopes da Silva, & Witter, 2001; Naber, Witter, & Lopes da Silva, 1999; Sewards & Sewards 2003; Witter, 2003; Witter et al., 2000) that the medial entorhinal cortex contains spatial and idiothetic information, whereas the lateral entorhinal cortex contains nonspatial information made up of olfactory, auditory, and visual object information. It is still unclear what spatial and nonspatial information enters the dorsal hippocampus via the medial perforant path and lateral perforant path inputs and what the behavioral implications of these inputs may be.

There have been a number of studies exploring contributions of medial perforant path and lateral perforant path for learning and memory (Ferbinteanu, Holsinger, & McDonald, 1999; Kirby & Higgins, 1998; Myhrer, 1988; Vnek, Gleason, Kromer, & Rothblat, 1995; cf. Meilandt, Barea-Rodriguez, Harvey, & Martinez, 2004); however, it is still poorly understood how the lateral perforant path and medial perforant path interact with neurons in each dorsal hippocampal subregion. In the present study, an object exploration paradigm involving both spatial and nonspatial (visual object) changes to a configuration of objects in an environment (Lee, Hunsaker, & Kesner, 2005; Poucet, 1989; Save, Poucet, Foreman, & Buhot, 1992) was used to investigate the roles of medial perforant path and lateral perforant path inputs into dDG, dCA3, and dCA1. The role of the medial perforant path was assessed by infusion of APV, a selective NMDA receptor antagonist used to inhibit LTP at glutaminergic medial perforant path synapses. Naloxone, a μ-opioid antagonist with lower affinity for κ and δ opioids as well, was used to block LTP at opioidergic lateral perforant path synapses. Phosphate buffer solution (PBS) was infused as a vehicle control for the drugs mentioned above.

Method

Experimental Procedure

Subjects. Eighteen male Long–Evans rats served as subjects for this task. They weighed between 275 and 350 g when they

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This research was supported by National Science Foundation Grant IBN-0135273 and National Institutes of Health Grant R1MH065314. We have no conflicts of interest, financial or otherwise.

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arrived (Simonsen Laboratories, Inc., Gilroy, CA). They were acclimatized to the colony and handled by an experimenter for 7 days before receiving injection cannula implantation. During the course of the experiment, rats were free fed and had access to water ad libitum. The health of all rats was assessed weekly by a University of Utah Institutional Animal Care and Use Committee (IACUC) veterinarian. All experimental procedures and techniques conformed to University of Utah IACUC and Association for Assessment and Accreditation of Laboratory Animal Care International regulations and protocols.

Behavioral apparatus. The behavioral apparatus was an open, circular platform (a dry land version of the Morris water maze—i.e., a cheeseboard; cf. Gilbert & Kesner, 2003; and Lee et al., 2005). The platform was painted white and elevated 70 cm from the floor. The surface of the apparatus was 119.0 cm in diameter and 3.5 cm thick. One hundred seventy-seven food wells (2.5 cm × 1.5 cm) were drilled into the surface of the platform in evenly spaced rows and columns (7.5-cm separation). The holes were covered by a thick white vinyl shower curtain draped over the platform. The platform was kept in a well-lit room with one door, an air tank, and posters of various sizes and colors placed on the walls. Rats were kept in a cage outside the testing room during intersession intervals (ISIs). A video camera attached to the ceiling of the room was adjusted to capture the entire platform and connected to a VCR and monitor in an adjacent room. Three groups of six objects were chosen, and these combinations were the object sets used throughout this experiment. For each test that a rat received, a different object set was used, and no object set was ever repeated for any subject.

Surgical method. Prior to tests, experimentally naive rats received chronic dDG ($n = 6$), dCA3 ($n = 6$), or dCA1 ($n = 6$) cannulae. Subjects were anesthetized with isoflurane (1%–4% [vol/vol] at 1–2 L/min) and placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) on an isothermal pad. dDG coordinates were 3.6 mm posterior to bregma, 2.1 mm lateral to midline, and 4.0 mm ventral to the skull surface. dCA3 coordinates were 3.6 mm posterior, 3.6 mm lateral, and 3.6 mm ventral. dCA1 coordinates were 3.6 mm posterior, 2.1 mm lateral, and 2.0 mm ventral. Burr holes were drilled for each stylus as well as two holes for jeweler's screws that were used as anchors for the dental cement. Cannulae implanted were 22 gauge (GA) stylae through which 26-GA injection cannulae could be inserted to project 1.0 mm from the bottom of the stylus into the brain. All dorsoventral measurements were made from the tip of an injection cannula projecting through a stylus. Cannulae supplies and screws were purchased from Plastics One, Inc. (Roanoke, VA). Once the screws and the stylae were in place, dental cement (Orthojet Powder and Liquid, Lang Dental, Wheeling, IL) was mixed and placed on the skull to anchor the stylae. Once the cement was dry and the stylae were secure, the rat was removed from the earbars. As the rat recovered, injection cannulae were removed from the stylus, and dust caps with dummy cannulae were put in their place. After surgery, rats were allowed to recover in their home cage and given acetaminophen (2 mL/50 mL water) and 100 g of ground food mixed with 10–15 g of sucrose.

Behavioral method. After surgery, rats were given a 7-day recovery period prior to experimentation during which they were weighed daily and handled by an experimenter. During these handling periods, injection cannulae were inserted and removed from the stylae to habituate the rat to the experience prior to experimentation.

For Session 1, no objects were present on the board. Rats were placed on the board and allowed 6 min to explore. After 6 min of exploration, the rat was placed in a cage outside the testing room for a 3-min ISI. Before Session 2, six objects to which the rat had never been exposed were placed on the maze in a geometric pattern (cf. Figure 1). Rats were allowed 6 min to explore both the board and the objects, followed by an ISI. For Session 3, the rat was placed on the maze with all conditions identical to Session 2. After Session 3, the rat received an intracranial infusion of PBS (0.4 μL, 125 mM in distilled water), APV (0.4 μL mixed at 30 mM in 125 mM PBS), or naloxone (0.4 μL mixed at 30 mM in 125 mM PBS) at a flow rate of 6.0 μL/hr (0.07 μL/min). The rat was placed in a cage for an additional 10 min prior to Session 4. This resulted in a 15-min ISI after Session 3. Session 4 was identical to Sessions 2 and 3. During the 3-min ISI after Session 4, two of the objects were moved in space (cf. Figure 1). Session 5 began when the rat was placed on the board with the objects in the new spatial configuration. After 6 min of exploration, the rat was removed to the cage for a 3-min ISI. Session 6 was identical to Session 5. After Session 6, a novel object was substituted for the object occupying the upper left-hand corner of the geometric pattern. The rat was placed on the board after the ISI and allowed to explore for the 6-min Session 7. After Session 7, testing was complete. The test was repeated three times for each rat, once with a PBS infusion, once with naloxone, and once with APV. A Latin square design was used to control for order effects—in short, rats from each group were run in a precise, pseudorandom order to control for multiple exposures to task requirements affecting performance. No order effects were observed (see the Results section for analysis). To reduce any potential proactive and retroactive interference between testing sessions, we separated each test by 2 days.

Dependent measures. The dependent measure for object exploration was time of exploration of each object. Exploration was recorded when the rat sniffed, pawed at, or looked at an object from a distance of under 1 cm for greater than 0.5 s. To quantify habituation of object exploration, we subtracted the time spent exploring each object during Session 2 from the time spent exploring the same objects during Session 4 The habituation index was thus the arithmetic mean of the difference scores for all five objects (A, B, C, D, and E) between Sessions 2 and 4 (habituation). A spatial mismatch index was calculated to quantify the amount of exploration for the two objects moved from their original locations (displaced object exploration): The sum of the exploration time for both of the displaced objects (Objects D and E) during Sessions 3–4 was subtracted from the sum of the exploration time for the same objects during Sessions 5–6. The same index was calculated for the nondisplaced objects (nondisplaced object exploration: Objects A, B, and C) to assess whether a spatial reconfiguration of objects in an environment would result in generalized reexploration of all objects or specific exploration of only the spatially rearranged objects. We calculated the novel object mismatch index by subtracting the average exploration time for the four objects that were not changed (Objects B, C, D, and E) from the time spent exploring the newly introduced object during Session 7 (Object F: novel object exploration; calculation after Poucet, 1989, and Lee et al., 2005).

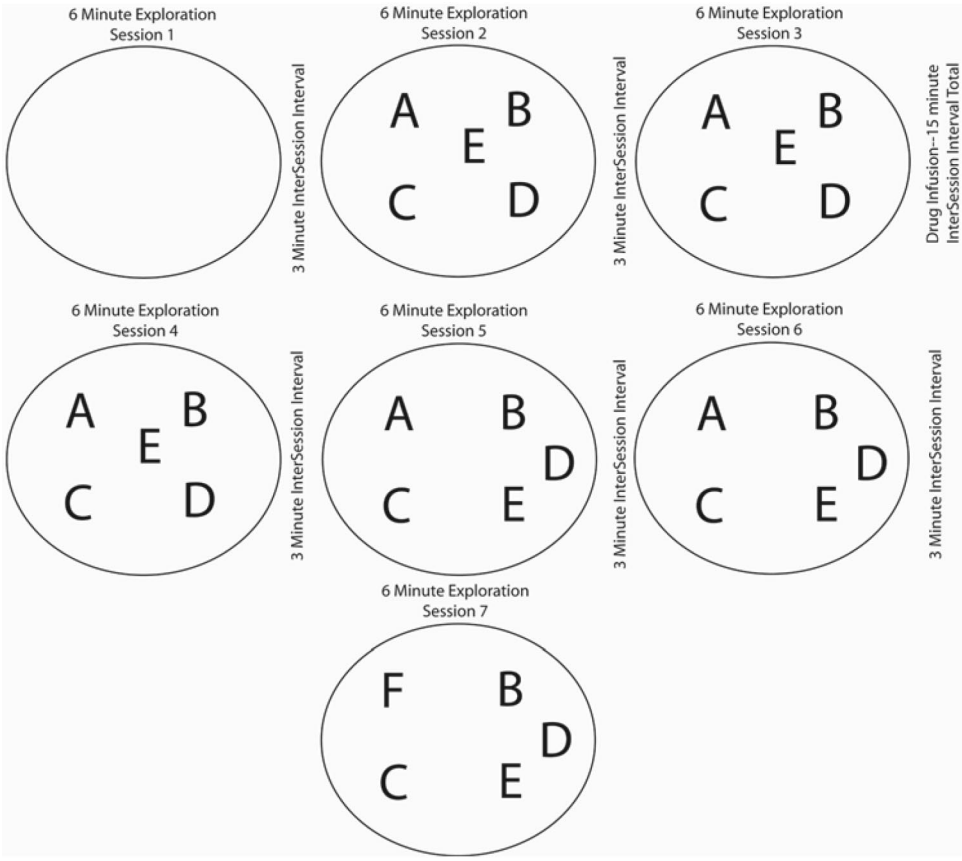

Figure 1. Diagram of the behavioral paradigm.

To measure general locomotor activity, we drew nine equal-sized grids on the television monitor covering the platform. The number of these grids crossed was used as a measure of general locomotor activity. A rat was scored as having crossed a grid if all four limbs entered an adjacent grid.

Histological methods. After all experimentation had been completed, rats were given an infusion of Chicago Blue Dye (Sigma-Aldrich, St. Louis, MO) to aid in identification of the cannulae tip. The rat was given 5 min to recover prior to receiving an overdose of sodium pentobarbital or chloral hydrate (both 100 mg/kg ip) and perfused with PBS and 10% (wt/vol) formalin. The rat was perfused with the cannulae intact, and the cannulae were removed during the brain extraction process. The brain was removed and placed in 30% sucrose/10% formalin (wt/vol) at 4 °C for at least 72 hr prior to sectioning. A tissue block containing the hippocampus was cut at 40 μm and every third section was placed on a slide and cover-slipped for microscopic analysis. The cannula track was reconstructed on the basis of stereotaxic data taken during surgery and microscopic visualization of cannula tracks and tip of injection cannulae. The sections were not counterstained.

Data Analysis

A Latin square analysis was performed on the grouped data to verify that there were no order effects. Two-way repeated measures analysis of variance (ANOVA) with subregions (dDG, dCA3, and dCA1; $n = 6$ for each group) as the between factor and drug infused (APV, naloxone, or PBS) as the within factor was used for analysis of all object exploration data. Tukey's honestly significant difference (HSD) post hoc paired comparison tests were conducted on effects of drug within subregion, because all other post hoc analyses were not particularly informative for our investigations.

Three-way repeated measures ANOVA with subregions as the between factor and drug infused and session as repeated within factors was conducted on the grid crossing–general activity data. This allowed for evaluation of any possible effects of interactions between session, drug infused, and subregion for general locomotor behavior.

All statistical analyses were performed using either SYSTAT 11 or SigmaSTAT 3.11 statistical analysis software packages (SYSTAT Software, Inc., Redmond, CA). To control for Type I error, we set alpha at $p < .05$ for all analyses, and statistical power was above .80 for all analyses.

Results

Table 1 contains arithmetic means (and standard error of the means) for all object exploration data analyzed. All significant effects are marked in Table 1. To test for overall order effects, we performed a Latin square analysis. There was no effect for test order within subjects (row), $F(2, 37) = 1.25$, $p = .30$, and no effect for test order across subjects (column), $F(17, 37) = 0.94$, $p = .54$,

Table 1
Mean Difference Scores for Reaction to Spatial and Nonspatial (Visual Object) Novelty

	Habituation		Nondisplaced		Displaced		Novel object	
Drug	*M*	*SEM*	*M*	*SEM*	*M*	*SEM*	*M*	*SEM*
				DG				
APV	−1.95	0.16	−0.25	0.66	−0.50***	0.40	2.56	0.19
NLX	−1.71	0.31	−0.33	1.36	−0.60***	0.30	−0.10*	0.13
PBS	−2.20	0.25	−0.32	0.11	2.33	0.40	3.85	0.70
				CA3				
APV	−1.39	0.16	−01.3	0.18	−0.50***	0.38	0.63*	0.45
NLX	−1.70	0.26	−0.60	0.32	1.04*	0.38	−0.12***	0.45
PBS	−2.29	0.24	−0.10	0.14	2.32	0.46	3.50	0.43
				CA1				
APV	−1.77	0.38	−0.53	0.24	0.00***	0.17	5.17	0.75
NLX	−1.65	0.28	−0.64	0.21	2.33	0.50	0.50***	0.58
PBS	−2.10	0.31	−0.58	0.15	2.33	0.57	4.25	1.50

Note. Significant differences from Tukey's honestly significant difference post hoc comparisons are marked and reported. Notice that there were no significant differences between drug infusion groups for habituation and nondisplaced object exploration, whereas there were significant differences for both displaced object exploration and novel object detection. APV = 2-amino-5-phosphonovaleric acid; NLX = naloxone; PBS = phosphate buffer solution. * $p < .05$. ** $p < .01$. *** $p < .001$. (all relative to the PBS infusion group)

but there was an effect for drug treatment, $F(2, 37) = 8.22$, $p = .01$. Because there were no test order effects, ANOVA analyses were performed.

Habituation

All rats in the study reduced their object exploration over time as measured during Sessions 4 and 2 (data not shown). A two-way repeated measures ANOVA revealed that there were no significant group differences for subregion, $F(2, 53) = 1.53$, $p = .25$, or drug infused, $F(2, 53) = 1.91$, $p = .17$. Also, there was no significant interaction between subregion and drug infused, $F(4, 35) = 0.42$, $p = .80$.

Displaced Object Exploration

Figure 2 shows the data for exploration of displaced objects (i.e., Sessions 5 and 6 subtracted from Sessions 3 and 4). A two-way repeated measures ANOVA revealed that there was no significant effect of subregion, $F(2, 53) = 2.13$, $p = .15$, but there was a significant effect of drug infused, $F(2, 53) = 33.00$, $p < .001$, and a significant interaction between subregion and drug infused, $F(4, 53) = 3.95$, $p = .01$. On the basis of a Tukey's HSD post hoc analysis, rats with infusions of APV or naloxone into dDG explored the displaced objects significantly less than those receiving PBS ($ps < .001$), but no significant differences in exploration for rats receiving APV infusions compared with those receiving naloxone into the dDG were observed ($p = .93$). Thus, it appears that within dDG, the medial perforant path and lateral perforant path inputs interact to process spatial information. Within dCA3, rats that received APV infusions explored the displaced objects less than those receiving PBS ($p < .001$), rats that received naloxone also explored the displaced objects less than those receiving PBS ($p = .05$), and rats receiving infusions of APV explored the displaced objects less than those receiving naloxone ($p = .004$). Thus, it appears that within dCA3, medial perforant path and lateral perforant path inputs showed a graded effect, suggesting cooperation or associability between pathways. Within dCA1, rats that received APV infusions explored the displaced objects less than those receiving PBS and naloxone ($p < .001$), whereas rats receiving infusions of PBS did not differ from those receiving naloxone ($p = 1.000$). Thus, in dCA1, it appears that the medial perforant path inputs are involved for processing spatial information, whereas the lateral perforant path inputs are not involved.

Nondisplaced Object Exploration

All rats behaved similarly and explored the nondisplaced objects less during Sessions 5 and 6 relative to Sessions 3 and 4 (data not shown). A two-way repeated measures ANOVA revealed that there were no significant effects for subregion, drug infused, and the interaction between subregion and drug infused: $F(2, 53) = 3.47$, $p = .054$; $F(2, 53) = 3.00$, $p = .07$; and $F(4, 53) = 0.17$, $p = .95$, respectively.

Novel Object Exploration

Figure 2 shows novel object exploration data from Session 7. A two-way repeated measures ANOVA shows that there was a significant effect for subregion, $F(2, 53) = 5.17$, $p = .02$, and drug infused, $F(2, 53) = 21.40$, $p < .001$, and an interaction between subregion and drug infused, $F(4, 53) = 3.11$, $p = .03$. Tukey's HSD post hoc comparisons revealed that within dDG, rats receiving naloxone infusions explored the novel object less than those receiving PBS ($p = .02$), but there were no significant differences between rats that received infusions of PBS and APV ($p = .75$)

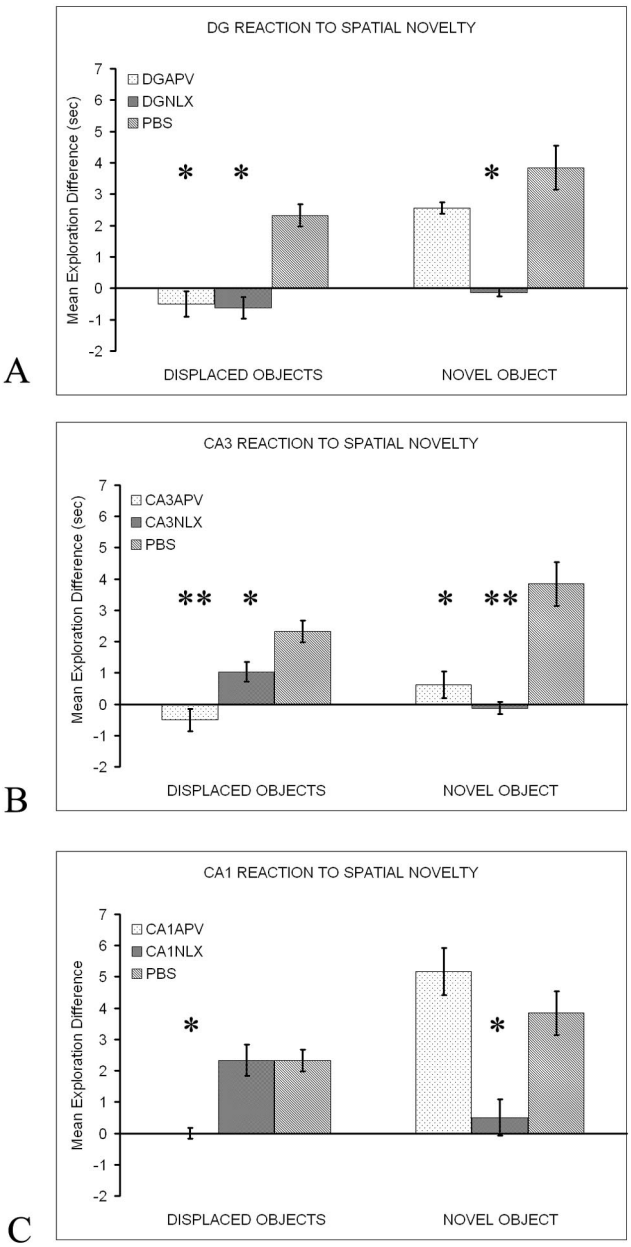

Figure 2. The effects of naloxone (NLX) and 2-amino-5-phosphonovaleric acid (APV) infusions for spatial (bars on the left) and nonspatial (visual object; bars on the right) novelty detection organized by drug infused within subregion. A: Displaced object and novel object exploration for the dorsal dentate gyrus (DG). B: Displaced object and novel object exploration for dorsal CA3. C: Displaced object and novel object exploration for dorsal CA1. Error bars represent standard error of the mean. PBS = phosphate buffer solution. $^{*}p < .05$. $^{**}p < .01$.

and APV and naloxone ($p = .11$). Thus, in dDG, it appears that the inputs from the lateral perforant path are sufficient to process nonspatial (visual object) information and that inputs from the medial perforant path are not involved. Within dCA3, rats that received APV infusions explored the novel object less than those receiving PBS ($p = .02$) but not less than those receiving naloxone ($p = .63$), and rats receiving naloxone infusions explored the novel object less than those receiving PBS ($p = .001$). Thus, it appears that within dCA3, medial perforant path and lateral perforant path inputs interact for processing nonspatial (visual object) information. Within dCA1, rats that received APV did not differ significantly from those receiving PBS ($p = .57$), but rats receiving infusions of naloxone explored the novel object less than rats receiving either PBS or APV ($p < .001$). This suggests that the medial perforant path and lateral perforant path inputs remain functionally segregated in dCA1.

General Activity–Grid Crossings

Figure 3 shows grid crossing data for all groups (plotted as arithmetic means, plus or minus standard error of the means). A three-way repeated measures ANOVA showed there was no significant effect of subregion, $F(1, 53) = 1.01$, $p = .38$, or drug infused, $F(2, 53) = 0.38$, $p = .69$, but there was an effect of session, $F(6, 318) = 119.0$, $p < .0001$. There were no significant interactions between drug infused and subregion, $F(2, 53) = 1.53$, $p = .23$; session and drug infused, $F(12, 318) = 1.02$, $p = .44$; session and subregion, $F(6, 318) = 0.56$, $p = .76$; or session, drug infused, and subregion, $F(12, 318) = 0.87$, $p = .58$.

Histology

All sections were analyzed under a light microscope for cannula placement, and no rats were excluded as a result of misplaced cannulae. Figure 4 shows a diagram of reconstructed cannula placements for all rats on plates adapted from Paxinos and Watson (1997). Note that all cannula are in the intended hippocampal subregion.

Discussion

Medial Perforant Path and Lateral Perforant Path

The experimental results provide compelling evidence for distinct pathways into the dorsal hippocampus from the medial ento-

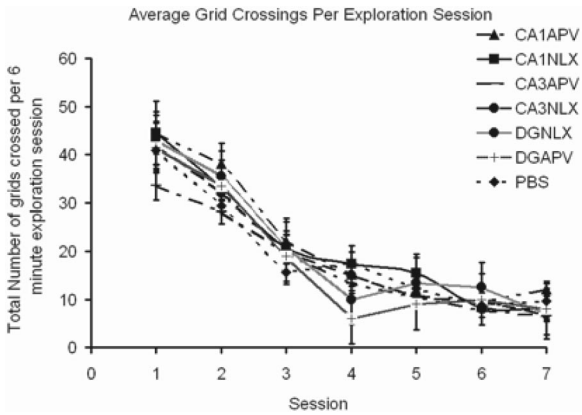

Figure 3. Data for general locomotor activity measured by grid crossings. Notice that for all groups grid crossing activity went down as a function of session. Error bars represent standard error of the mean. APV = 2-amino-5-phosphonovaleric acid; NLX = naloxone; DG = dentate gyrus; PBS = phosphate buffer solution.

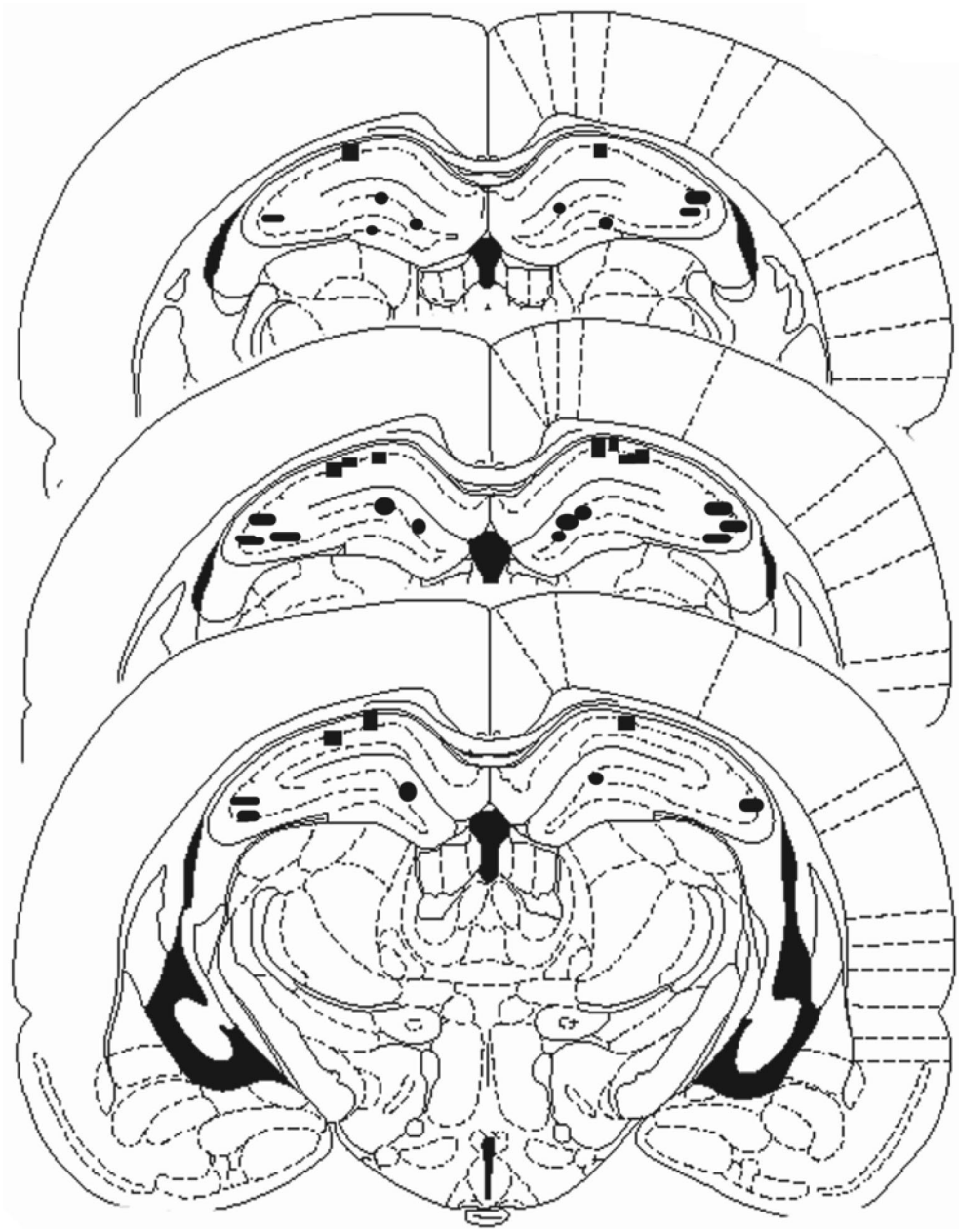

Figure 4. Schematic of cannula placements for all rats, displayed on a plate from Paxinos and Watson (1997). Note that all cannulae were placed in the intended subregion. Squares represent dorsal CA1 cannula placements, rounded rectangles represent dorsal CA3 cannula placements, and circles represent dorsal dentate gyrus cannula placements. From *The Rat Brain in Stereotaxic Coordinates* (composite of Figures 20, 21, and 22), by G. Paxinos and C. Watson, 1997, New York: New York Academy of Sciences. Copyright 1997 by the New York Academy of Sciences. Adapted with permission.

rhinal cortex and lateral entorhinal cortex. The role of the medial perforant path inputs was assessed by infusion of APV, a selective NMDA receptor antagonist that was used to inhibit LTP at medial perforant path synapses, which are glutaminergic. Naloxone, a μ-opioid antagonist with lower affinity for κ and δ opioids as well, was used to block LTP at lateral perforant path synapses, which are primarily μ-opioidergic. The data support the hypothesis that nonspatial (visual object) information enters the dorsal hippocampus via lateral perforant path and spatial information via medial perforant path and are consistent with data that have demonstrated a role of the medial entorhinal cortex for spatial information processing. Hargreaves, Rao, Lee, and Knierim (2005) found that neurons in the medial entorhinal cortex fire with a higher degree of spatial selectivity than neurons in the lateral entorhinal cortex; they also found that neurons in the lateral entorhinal cortex show limited firing on the basis of nonspatial (visual object) factors

(unpublished observations mentioned in Knierim, Lee, & Hargreaves, 2006). These studies, along with others (Naber et al., 1999, 2001; Sewards & Sewards, 2003; Steward, 1976; Witter, 2003; Witter et al., 2000), provide evidence for a spatial loop between the dorsal hippocampus and the medial entorhinal cortex, suggesting that medial perforant path inputs to the dorsal hippocampus from the medial entorhinal cortex carry spatial information reflective of dorsal hippocampal or postrhinal activity, whereas the lateral perforant path inputs carry nonspatial (visual object) information that reflects activity in the perirhinal cortex (Burwell, Bucci, Sanborn, & Jutras, 2004; Eacott & Gaffan, 2005; Hargreaves et al., 2005; Norman & Eacott, 2005; cf. Canning, Wu, Peloquin, Kloosterman, & Leung, 2000; Liu & Bilkey, 1996).

dDG

Medial perforant path and lateral perforant path project onto similar populations of dDG cells. There are laminar differences based on pathway termination, but the information carried by the pathways could potentially interact at the dDG (Abraham, Bliss, & Goddard, 1985). Attenuating either medial perforant path or lateral perforant path input into dDG is sufficient to disrupt reexploration of a spatial reconfiguration of familiar objects, whereas disruption of the lateral perforant path inputs, but not medial perforant path inputs, attenuates novel object detection. These data suggest that dDG is attuned to small changes in combinations of spatial and nonspatial (visual object) information. At the dDG, spatial and nonspatial (visual object) information may be combined into a coherent representation of space with very high levels of spatial resolution. This means that a constellation of nonspatial (visual object) landmarks from the lateral entorhinal cortex may combine with idiothetic and vestibular information from the medial entorhinal cortex to form a conjunctive representation of context (O'Reilly & Rudy, 2001). Conjunctive coding would provide for economical storage and processing of spatial information (Rudy & Sutherland, 1995). It has been shown that medial perforant path and lateral perforant path inputs show associability within dDG (Abraham et al., 1985). This suggests that medial perforant path spatial inputs are combined with lateral perforant path nonspatial (visual object) inputs into a single conjunctive representation in dDG before being sent to dCA3 via the mossy fibers.

It is interesting to note that blocking the medial perforant path was not sufficient to disrupt novel object detection. This suggests that at dDG granule cells, visual object identification is mediated by lateral perforant path inputs, whereas both lateral perforant path and medial perforant path inputs are involved in spatial information processing. These data suggest that the individual object information (e.g., the objects on the board that were moved) comes from the lateral entorhinal cortex and enters the hippocampus via the lateral perforant path. The spatial or contextual information (e.g., the arrangement of cues on the boards as well as the distal contextual information) comes from the medial entorhinal cortex and enters the hippocampus via the medial perforant path.

The present data support models that propose orthogonalization (i.e., spatial pattern separation) as the primary role for dDG. Rolls (1996) and Rolls and Kesner (2006) proposed that dDG mediates fine-scale orthogonalization (e.g., when two spatial locations have a high degree of similarity or spatial overlap). The critical role for dDG in pattern separation is, in part, due to projections from the medial and lateral entorhinal cortex to dDG, which emphasize differences in patterns because the probability of separate input patterns activating the same granule cells is minimal (Rolls, 1996). Gilbert, Kesner, and Lee (2001) reported previously that pattern separation (or metric information processing; cf. Goodrich-Hunsaker, Hunsaker, & Kesner, 2005, 2007) depends on dDG, not dCA3 or dCA1 (Gilbert & Kesner, 2006; Gilbert et al., 2001).

dCA3

Medial perforant path and lateral perforant path projections into dCA3 show the same distribution as seen in dDG, with medial perforant path terminating in a layer more proximal to the pyramidal cell soma (Do et al., 2002). The present results support the model proposed by Marr (1971) and extended by Rolls (1996) and Rolls and Kesner (2006) that dCA3 is an auto-associative network. The present data indicate that nonspatial (visual object) information enters dCA3 via lateral perforant path and is associated with spatial information entering either via medial perforant path or mossy fibers. Blockade of either lateral perforant path or medial perforant path into dCA3, but not dDG or dCA1, causes concomitant disruptions for spatial and object novelty detection. It appears that within dCA3, spatial and nonspatial (visual object) information is combined into a conjunctive representation. This being the case, dCA3 could mediate associative learning as long as information introduced by the medial perforant path and the lateral perforant path contained elements of the association (cf. Gilbert & Kesner 2003; Kesner, Hunsaker, & Gilbert, 2005).

It appears that dCA3 is attuned to detect a mismatch in a combination of spatial and nonspatial (visual object) information, as blockade of either pathway caused the rat to not react to spatial or nonspatial (visual object) novelty (cf. Hasselmo, 2005). An intact dCA3 network reacts to subtle changes in the conjunctive firing patterns of the medial perforant path and the lateral perforant path, as well as subsequent interactions with the mossy fiber and recurrent collateral inputs (Rolls & Kesner, 2006). This suggests that any small environmental change (reflected as a change in medial perforant path and/or lateral perforant path firing) would be encoded by dCA3 as novel. This is supported by the current data because spatial novelty (familiar objects moved to novel spatial locations) and visual object novelty (familiar spatial location occupied by a novel object) both induced reexploration in PBS-treated rats but not after treatment with APV or naloxone. The lack of reexploration after APV or naloxone infusions suggests that a combination of activity patterns from the medial perforant path and the lateral perforant path is processed by dCA3 pyramidal cells, not just the spatial or nonspatial (visual object) information per se. In this case, dCA3 would act as a match–mismatch comparator of the conjunctive or cumulative firing patterns of the afferent pathways—a match (e.g., sufficient similarity) would correspond to a lack of reexploration and a mismatch (e.g., insufficient similarity) would induce the rat to recode the information and reexplore (cf. Hasselmo, 2005).

dCA1

Steward (1976) showed that the lateral perforant path inputs from the lateral entorhinal cortex project to the subicular third of dCA1; medial perforant path inputs from the medial entorhinal

cortex project to the third of dCA1 proximal to dCA2; and there is overlap in the intermediate third of dCA1—where the stylae were placed in the current experiment. Additional data have suggested that the medial perforant path and lateral perforant path input to dCA1 is distinct (Witter et al., 2000). These data support models that demand a robust perforant path to subserve retrieval (cf. Rolls & Treves, 1992) and suggest that the models are incomplete because they do not differentiate between medial perforant path and lateral perforant path inputs.

The medial perforant path and lateral perforant path inputs appear to affect distinct populations of dCA1 neurons. This allows for Schaffer collateral efferents to interact with neurons receiving medial perforant path inputs separately from neurons receiving lateral perforant path inputs in dCA1. This provides dCA1 with the ability to maintain spatial (via an interaction between medial perforant path inputs and Schaffer collateral inputs in a given neuron) and nonspatial (visual object) information (via an interaction between the lateral perforant path inputs and Schaffer collateral inputs in a different neuron) for longer temporal intervals than dCA3 or dDG. In dCA1, Schaffer collateral interactions with lateral perforant path inputs would remain separate from Schaffer collateral interactions with medial perforant path inputs until dCA1 efferents reach the medial entorhinal cortex, at which point these two outputs would be integrated to form strong spatial representations with strong idiothetic tuning and maintain fidelity of information within dCA1 (Fyhn, Molden, Witter, Moser, & Moser, 2004; Hafting, Fyhn, Molden, Moser, & Moser, 2005; Sargolini et al., 2006). This hypothesis is supported by data indicating that dorsal hippocampus lesions decouple place-specific firing in the medial entorhinal cortex while not affecting idiothetic correlates of the same units (Fyhn et al., 2004).

Alternatively, the independence of medial perforant path and lateral perforant path input patterns could provide for higher fidelity of match–mismatch processing and temporal sequencing or ordering of information. If lateral perforant path and medial perforant path independently enter dCA1 and their firing patterns are conserved, the ability of dCA1 to accurately determine whether these patterns are similar or dissimilar to incoming Schaffer collateral inputs would be increased. In fact, for efficient match–mismatch processing for either spatial or nonspatial (visual object) information, it is imperative that medial perforant path and/or lateral perforant path inputs be relatively free from interference—for both spatial and nonspatial (visual object) information (cf. Hasselmo, 2005; Hasselmo & McGaughy, 2004; Hasselmo & Schnell, 1994). If medial perforant path and lateral perforant path firing patterns in dCA1 are conserved, then the ability of rats to preserve a temporal code would be facilitated. In fact, temporal ordering and retention of information over a trace interval appears to be a function unique to CA1 within the hippocampus (Fortin, Agster, & Eichenbaum, 2002; Kesner, Gilbert, & Barua, 2002; McEchron, Tseng, & Disterhoft, 2003; Rogers, Hunsaker, & Kesner, 2006; Weible, O'Reilly, Weiss, & Disterhoft, 2006).

General Remarks

The present experimental results suggest that the dentate gyrus combined the medial and lateral perforant path inputs to generate a spatial representation, but only the lateral perforant path input is used to identify the visual objects occupying the spatial locations. In CA3, the data suggest that the medial and lateral perforant path inputs are combined to generate a spatial representation that contains within it a representation of the visual objects occupying the spatial locations. In CA1, it appears that the medial and lateral perforant path inputs do not mix much because only the medial perforant path appears to be involved in generating a representation of space, whereas the lateral perforant path is used to identify visual objects. These data support previous data that suggest that the dentate gyrus, area CA3, and area CA1 all process information on the basis of their proposed mnemonic functions (Rolls, 1996; Rolls & Kesner, 2006). The data also suggest that the dentate gyrus, CA3, and CA1 receive information from both the medial and lateral entorhinal cortex that provides information necessary for proper hippocampal function (Witter, 2003; Witter et al., 2000). These data also support previous data that have shown that the dentate gyrus, CA3, and CA1 can be dissociated from each other using behavioral tasks (Gilbert & Kesner, 2003, 2006; Gilbert et al., 2001; Kesner, Lee, & Gilbert, 2004; Rolls & Kesner, 2006).

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Received November 29, 2006
Revision received February 14, 2007
Accepted March 6, 2007 ■