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Septal Infusions of the H-Channel Blocker ZD7288 Impair Spontaneous Alternation but not Inhibitory Avoidance

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by

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Under the Direction of Marise B. Parent

ABTRACT

It is well established that the septo-hippocampal system is involved in memory. The medial septum is connected to the hippocampus via the fimbria fornix, which consists mostly of acetylcholine and γ-aminobutyric acid (GABA) projection neurons. The contributions of the cholinergic projection to memory have been studied extensively; whereas, the role of the GABAergic projection is not well characterized. The present experiment tested whether septal infusions of the selective H-channel blocker ZD7288 would impair spontaneous alternation (SA) and continuous multiple inhibitory avoidance (CMIA). Fifteen minutes prior to assessing SA or CMIA, different groups of male Sprague-Dawley rats were given septal infusions of saline or ZD7288 (0.2, 0.6 or 1.5 nmol / 0.5µl). Our results indicate that septal infusions of ZD7288 impaired SA in a dose-dependent manner; the same infusions did not affect CMIA. This is the first demonstration that H-channels on septo-hippocampal GABAergic projection neurons are involved in memory.

INDEX WORDS: Learning, memory, GABA, SA, CMIA, ZD7288.

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Introduction

Converging lines of evidence suggest that there is a close interaction between the septum and the hippocampus during learning and memory. For example, lesions of the septum (Hepler et al. 1985; Feasey-Truger et al. 1992; M'Harzi and Jarrard 1992; Kelsey and Vargas 1993; Bannerman et al. 2004) or fimbria-fornix (Mahut 1972; van der Staay 1989; Guillazo-Blanch et al. 2002; Winters and Dunnett 2004) impair hippocampal-dependent learning and memory. Furthermore, septal manipulations influence hippocampal function such as the theta rhythm, which is a prominent 4-10 hertz field potential oscillation that is important for memory (Allen and Crawford 1984; Mizumori et al. 1989; Givens and Olton 1990; Givens et al. 1992; Lawson and Bland 1993; Oddie et al. 1996; Partlo and Sainsbury 1996; Vertes and Kocsis 1997; Asaka et al. 2000; Vertes et al. 2004; Yoder and Pang 2005). Moreover, hippocampal manipulations prevent memory deficits produced by septal treatments. For example, hippocampal infusions of glucose, pyruvate, or acetylcholinesterase (AChE) inhibitors reverse the memory deficits produced by the septal infusions of the γ-aminobutyric acid (GABA) agonist muscimol (Parent et al. 1997; Degroot and Parent 2000, 2001; Krebs and Parent 2005a,b).

Septo-hippocampal cholinergic projections are involved in the interaction between the medial septum and the hippocampus during learning and memory (Walsh et al. 1996; Cassel et al. 2002; Pizzo et al. 2002; Janisiewicz and Baxter 2003; Parent and Baxter 2004). For instance, septal treatments that impair learning and memory also decrease hippocampal acetylcholine (ACh) levels (Walsh et al. 1996; Mishima et al. 2000; Lehmann et al. 2002). Moreover, in some cases only those doses of a drug that decrease hippocampal ACh impair memory (Brioni et al. 1990). In contrast, septal treatments that enhance memory generally increase hippocampal ACh (Tarricone et al. 1991, 1993; Darnaudery et al. 2002). In addition, hippocampal grafts containing ACh-producing cells reverse the memory deficits produced by septal lesions, fimbria fornix

lesions or selective lesions of the septo-hippocampal cholinergic projections (Tarricone et al. 1996; Cassel et al. 1997, 2002; Dickinson-Anson et al. 1998). Similarly, pharmacological manipulations that enhance hippocampal ACh reverse memory deficits produced by septal drug treatments (Parent et al. 1997; Ragozzino et al. 1998; Degroot and Parent, 2000, 2001; Krebs and Parent 2005a).

Some evidence indicates that septo-hippocampal ACh may be involved in but not necessary for learning and memory (Pang and Nocera 1999; Parent and Baxter 2004). For instance, selective lesions of the septo-hippocampal cholinergic projections often produce little or no deficits in learning and memory or hippocampal theta rhythm (Berger-Sweeney et al. 1994; Lee et al. 1994; Torres et al. 1994; Bassant et al. 1995; Baxter et al. 1995, Baxter and Gallagher 1996; Dornan and al. 1996; McMahan et al. 1997; Chappell et al. 1998; Pang and Nocera 1999; Frick et al. 2004; Kirby and Rawlins 2004; Frielingsdorf et al. 2006). Furthermore, the effects of some septal drug treatments on hippocampal ACh do not parallel the effects of these treatments on memory. Specifically, septal injections of carbachol or scopolamine impair hippocampal-dependent spatial learning despite increasing hippocampal ACh levels (Elvander et al. 2004). Furthermore, septal infusions of cholinergic agonists, which usually enhance memory or reverse memory deficits (Izquierdo et al. 1992; Givens and Olton 1995; Pang and Nocera 1999), decrease extracellular hippocampal ACh levels (Gorman et al. 1994). Similarly, although multiple doses of the benzodiazepine agonist zolpidem produce comparable memory deficits, only one of the memory-impairing doses decreases hippocampal ACh levels (Herzog et al. 2000). In addition, septal infusions of adenosine tri-phosphate (ATP) sensitive potassium (K+) modulators increase hippocampal ACh levels despite impairing memory (Stefani and Gold 2001).

Limited evidence suggests that septo-hippocampal GABAergic projections participate in learning and memory (Alreja et al. 2000; Wu et al. 2000; Pang et al. 2001). For instance, combined lesions of cholinergic and GABAergic projections produce larger deficits in memory and hippocampal theta than do lesions of either projection alone (Pang et al. 2001; Yoder and

Pang 2005). Importantly, electrophysiological evidence indicates that many compounds that affect memory when infused into the septum influence the functioning of septo-hippocampal GABA projections, but not the cholinergic ones (Alreja et al. 2000; Wu et al. 2000).

Hyperpolarization-activated cyclic nucleotide gated channels (H-channels) are located on the cell bodies and dendrites of septo-hippocampal GABAergic projection neurons, but not on septo-hippocampal cholinergic projections (Morris et al. 2004; Kocsis and Li 2004; Sotty et al. 2003; Xu et al. 2004). Furthermore, there are no known H-channels on the newly discovered septo-hippocampal glutamatergic projections (Sotty et al. 2003). Therefore, one way to selectively disrupt the function of the septo-hippocampal GABAergic neurons may be to block the H-channel. H-channels contribute to the rhythmicity observed in a variety of neurons, including septo-hippocampal GABAergic projection neurons (Luthi and McCormick 1998; Robinson and Siegelbaum 2003; Morris et al. 2004; Xu et al. 2004). Blocking H-channels on the septo-hippocampal GABAergic projections disrupts hippocampal theta rhythm (Xu et al. 2004; Kocsis and Li 2004) which would be expected to impair memory (Pan and McNaughton 1997). As a result, the purpose of the present study was to test whether blocking septal H-channels would impair septo-hippocampal-dependent memory. Specifically we determined whether septal injections of the H-channel blocker ZD7288 would impair spontaneous alternation (SA), a measure of spatial working memory and/or continuous multiple trial inhibitory avoidance (CMIA), a measure of emotional long term memory.

Results

Fig.1 shows the approximate location of the septal infusions for those rats whose data were included in the statistical analysis. Septal drug infusions significantly impaired SA performance in a dose-dependent manner [$F(3,56) = 5.63$; $p < .05$; see Fig. 2A]. Specifically, the percent alternation scores of rats given the 0.6 or 1.5 nmol doses of ZD7288 were significantly lower than those of rats given vehicle ($p < .05$). The percent alternation scores of rats given the

0.2 nmol dose of ZD7288 were not significantly different from those of vehicle rats ($p > .05$). Drug infusions into the septum did not significantly affect the number of arms the rats entered in the maze [$F(3,56) = 1.074$; $p > .05$; see Fig. 2B].

[Insert Figure 1 and 2 about here]

Pretraining septal drug infusions did not affect the number of trials to criterion for CMIA training [$\chi^2(3,56) = 1.27$ $p > .05$; see Fig. 3A]. Fig. 3B demonstrates that the drug infusions into the septum also did not significantly affect subsequent CMIA retention performance [$\chi^2(3,56) = 0.819$; $p > .05$].

[Insert Figure 3 about here]

Discussion

The present findings demonstrate that septal infusions of the H-channel blocker ZD7288 impair spontaneous alternation performance in a dose-dependent manner. In addition, the infusions did not affect the number of arms the rats entered in the maze, indicating that the impairing effects of the drug were not due to changes in activity levels. Furthermore, the impairing effects of ZD7288 are task-dependent. The same doses of ZD7288 that impaired SA did not affect CMIA acquisition or retention. To the best of our knowledge, these results are the first to show that septal H-currents conducted by H-channels participate in memory. More specifically, the present findings indicate that septal H-channels play a facilitatory role in spatial working memory, but do not appear to be involved in emotional long-term memory. Given that H-channels contribute to the rhythmic oscillatory activity of cells, these task-dependent effects of ZD7288 may be due to the possibility that reverberating neural activity is necessary for short-term memory but not long-term memory. Indeed, evidence indicates that rhythmic activity in the theta band is involved in active maintenance and recall of working memory processes (Rizzuto et al. 2003; Huhn et al. 2005; Raghavachari et al. 2006; Jensen 2006).

The present findings are consistent with previous research showing that septal infusions of ZD7288 impair hippocampal theta (Xu et al. 2004; Kocsis and Li 2004). Theta synchronization is important for short-term memory processes (Vertes 2005; Sakata 2006; Jensen 2006), and even a slight reduction in theta frequency impairs spatial learning (Winson 1978; Pan and McNaughton 1997). The present data are in contrast with previous results showing that deletion of the HCN1 gene from mice forebrain enhances learning and memory, increases the power of theta oscillation, and enhances long-term potentiation (Nolan et al. 2004). These latter data suggest that H-channels play an inhibitory role in memory processes in the forebrain. These brain region differences in the facilitatory versus inhibitory role of H-channels in memory could be due to variation in the type, distribution and kinetics of H-channels in the septum versus the forebrain (Monteggia et al. 2000; Santoro et al. 2000; Morris et al. 2004).

Given that H-channels are located on septo-hippocampal GABAergic projection neurons, but not on cholinergic projections, (Sotty et al. 2003; Morris et al. 2004; Xu et al. 2004; Kocsis and Li 2006), the present findings also suggest that the septo-hippocampal GABAergic projection plays a role in spatial working memory. These data are consistent with electrophysiological data showing that ACh agonists in the medial septum, which enhance memory (Tarricone et al. 1991, 1993; Givens and Olton 1994, 1995; Bland and Oddie 1998; Carey et al. 2001), likely do so via the GABAergic rather than the cholinergic projection (Wu et al. 2003). These results are also congruent with previous findings indicating that lesions of septo-hippocampal GABAergic projection neurons eliminate hippocampal theta rhythm in rats under urethane anesthesia (Yoder and Pang 2005). The present results are in contrast, though, with evidence indicating that kainic acid-induced lesions of the septo-hippocampal GABAergic projection do not impair memory (Pang et al. 2001) or locomotion-induced theta (Yoder and Pang 2005). The reasons for this discrepancy are unclear, but may be related to differences in methodologies used or to the extent of the lesions. Septal infusions of ZD7288 do not likely influence memory by acting on the

recently discovered septal glutamate projection neurons (Sotty et al. 2003; Colom et al. 2005), because H-currents do not appear to be present on these neurons (Sotty et al., 2003).

In summary, septal infusions of ZD7288 impair spontaneous alternation performance without affecting activity levels. The impairing effects of ZD7288 are not observed in CMIA, suggesting that the septo-hippocampal H-channels are involved in short-term spatial working memory, but not in emotional long-term memory. Moreover, given the apparent exclusive presence of H-channels on the septo-hippocampal GABAergic projection, these data also suggest that this projection is involved in spatial working memory.

Materials and Methods

Subjects

Fifty-eight (n = 11-18 per group) male Sprague-Dawley rats (Charles River, Wilmington, MA) weighing 200-250 g upon arrival were used. The rats were housed individually with food and water *ad libitum* on a 12-hour light dark cycle with lights on at 7:00 a.m. All procedures involving rats were approved by the Georgia State University Institutional Animal Care and Use Committee (IACUC).

Procedure

Surgery

One week after their arrival, the rats were anesthetized in a gas chamber with 5% isoflurane delivered in medical grade oxygen at 1000 mL/min. Anesthesia was maintained through a nose piece with 3% isoflurane gas in 500 mL/min oxygen. Rats were then given injections of atropine sulfate (0.4 mg/kg, ip) and penicillin (1500 units, im, Hanford's). The rats were placed in a stereotaxic apparatus equipped with a nose piece to maintain anesthesia and secured with blunt rodent ear bars. Anesthesia was adjusted throughout surgery and rats were given the non–steroidal analgesic flunixin meglumine (2.5 mg/kg, ip, Fort Dodge, Iowa). A 2%

lidocaine/.001% epinephrine cocktail (0.5 cc, sc) was injected to reduce pain and inflammation at the incision site. The rats were implanted with one 22-gauge stainless-steel guide cannula (Plastics One, Inc., Roanoke, VA) aimed at the medial septum (0.5 mm anterior to bregma, 4.9 mm ventral to dura; Paxinos and Watston 1998). The cannula was secured to the skull with three jeweler's screws and cranioplastic cement. A dummy cannula was inserted to keep the cannula free of debris. Following the surgery, the rats were given an injection of 0.9% sterile saline (3.0 cc, sc), wrapped with a paper towel, and then kept under a warm lamp (60 W) until they recovered from anesthesia. Two days following surgery the patency of the cannula was checked and betadine was applied to the surgical wound. If signs of infection were evident, the rats were anesthetized with 5% isoflurane gas and given an additional injection of penicillin (1500 units, im).

Two days before behavioral testing, the rats were handled for 3 minutes each. The rats were allowed to habituate to the laboratory environment for a minimum of 30 minutes before or after handling and behavioral testing. At least 1 week after surgery, behavioral testing occurred between 0800 and 1800 h. Fifteen minutes prior to behavioral testing, different groups of rats were given septal infusions of saline (0.5 μl; pH = 7.2) or ZD7288 (0.2, 0.6 or 1.5 nmol) using a 22-gauge injection needle that extended 1.0 mm beyond the guide cannula. The needle was connected to a 25 μl Hamilton syringe by polyethylene tubing (PE-50), and the infusion was delivered using an infusion pump (Harvard Apparatus 11). After the completion of the injection, the needle was left in place for 1 minute to facilitate diffusion. The experimenter was blind to the identity of the drugs infusions and the order of drugs was counterbalanced across the day. The doses of ZD7288 were selected based on previous experiments showing that septal infusions of these doses affect hippocampal theta rhythm. Specifically, the 0.2 and 1.5 nmol doses of ZD7288 decrease the frequency of locomotion-stimulated theta (Kocsis and Li 2004), and the 0.6 nmol dose prevents auditory stimulus-induced hippocampal theta in conscious rats (Xu et al. 2004).

Spontaneous Alternation (SA)

SA was used as a measure of spatial working memory (Lalonde 2002). The underlying assumption is that in order to alternate successfully between spatial locations, the rat must remember its visits to previous locations. This is supported by the finding that alternation is impaired by removing extra-maze cues or by increasing the interval between arm choices (Dember and Richman 1989; Lalonde 2002). To assess SA, each rat was placed in a Y-maze that has three equally spaced arms (60%; 60 cm long X 17.5 cm high). The floor of each arm is composed of stainless steel (3.5 cm wide) and the top (14 cm wide) is covered with a transparent Plexiglas lid. Each rat was placed in the same starting arm of the Y-maze and allowed to freely explore the maze for 8 minutes. The sequence and number of arms entered were recorded and a percent alternation score was computed for rats that entered at least 10 arms. An alternation was defined as entering three different arms consecutively. The percentage alternation scores were computed by dividing the number of alternations each rat made by the number of arms entered minus two (i.e., number of alternations possible) and then multiplying the resulting quotient by 100.

Continuous Multiple Inhibitory Avoidance (CMIA)

At least 3 days after SA testing, the rats were given CMIA training. CMIA was used as a measure of emotional, long-term memory (McGaugh 2004). The rat was placed into a trough-shaped apparatus that was separated into two compartments by a retractable guillotine door. The dark compartment had a metal floor through which shock could be delivered. A 15-watt lamp was placed over the lighted compartment and was the only source of illumination in the room. The table underneath the avoidance apparatus was lined with bench paper and the apparatus was cleaned with 70% ethanol after each rat was trained. The rat was placed into the lighted compartment with his nose pointing away from the retractable door. The door was opened after the rat turned toward the door or after 12 seconds (s). Upon entering the dark compartment with

all four paws, the rat was given a 1.2 mA shock (maximum 4 s) until he returned to the lighted compartment. This sequence constituted one training trial. All rats escaped to the dark compartment within 4 s on each trial. The door remained open and if the rat re-entered the dark compartment he received another shock. Training continued until the rat remained in the lighted compartment for 100 consecutive seconds or for a maximum of 5 trials. The current level was assessed with a digital multimeter prior to every experiment and at regular intervals throughout the experiment. Forty eight hours later, the rat was placed in the light compartment and the latency (s) to enter the dark compartment (maximum of 600 s) was recorded and used as an index of retention. Footshock was not delivered on the retention test.

Histology

After behavioral testing, the rats were euthanized with an overdose of sodium pentobarbital (400mg/kg, ip) and perfused intracardially with 0.9% saline followed by 10% formalin. The brains were stored in 10% formalin for at least 2 days before they were sectioned (60 μm) on a cryostat (Leica CM 30510S) through the cannulae tracts. The sections were stained with thionin and an observer blind to treatment determined the cannula placement using a light microscope (Olympus BX41). Acceptable septal cannula placement was defined as injections tips located within the medial septum, but not within the lateral septum or the ventral diagonal band of Broca. Furthermore, the cannula must not have penetrated the fimbria-fornix. Data from rats whose cannula placements were misplaced were not included in the statistical analyses.

Data analysis

The SA data were expressed as means and standard error of the mean (SEM) and analyzed using one-way analysis of variance (ANOVA) and Tukey post hoc tests where appropriate. Due to the fact that several of the rats reached the maximum retention latency cut-off of 600 s, the retention latency data were not distributed normally. Therefore, these data were expressed as medians and inter-quartile ranges (IQ) and the non-parametric Kruskal-Wallis and

Mann-Whitney U tests were used to detect differences between treatment groups. An alpha level of 0.05 was used as the criterion for statistical significance.

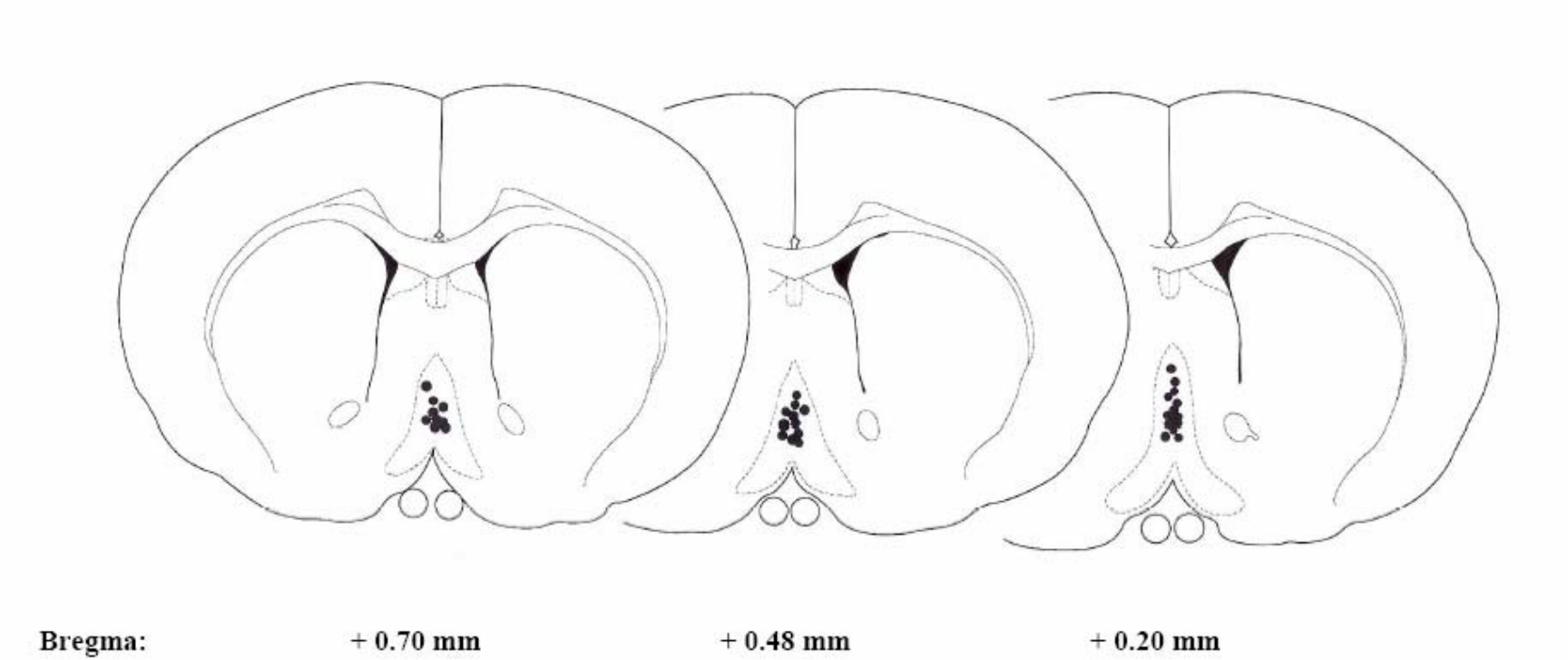

Fig.1. Schematic illustration of coronal sections of the rat brain showing the approximate location of the medial septum infusions sites. Reprinted from Paxinos and Watson 1998, with permission from Elsevier © 1998.

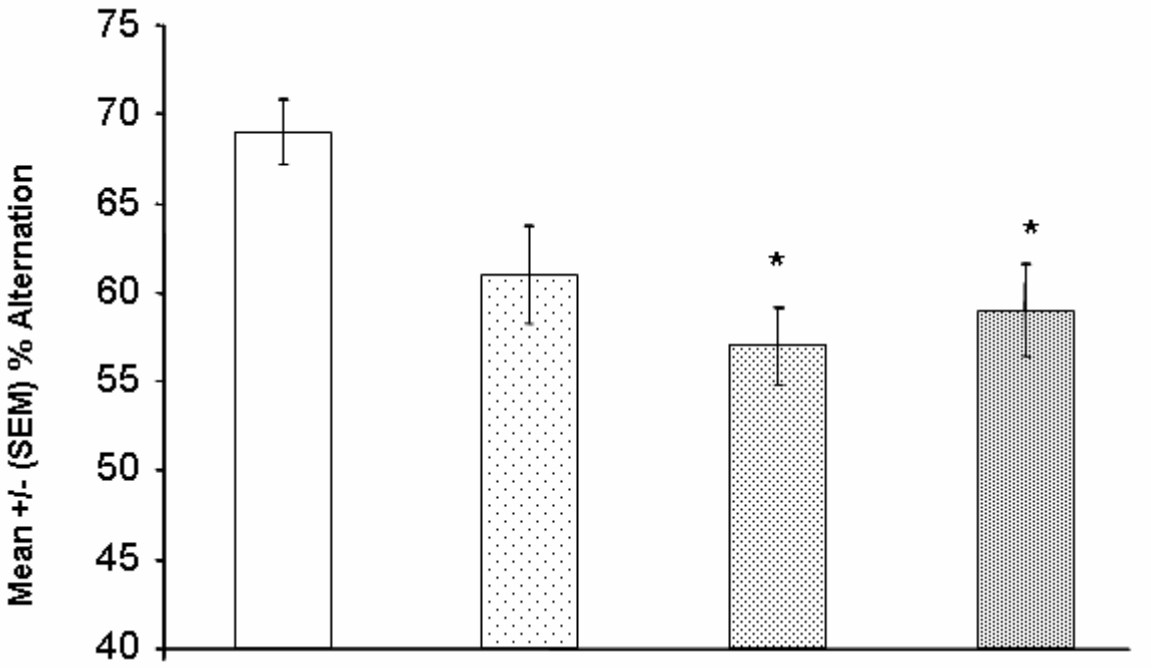

Fig.2.1. Septal infusions of the H-channel blocker ZD7288 (0.6 and 1.5 nmol) significantly decreased mean (+/-S.E.M.) spontaneous alternation scores (*p< .05 vs. vehicle).

Fig.2.2. There were no significant effects of septal drug infusions on the mean (+/-SEM) number of arm entries (n = 11-18 rats per group).

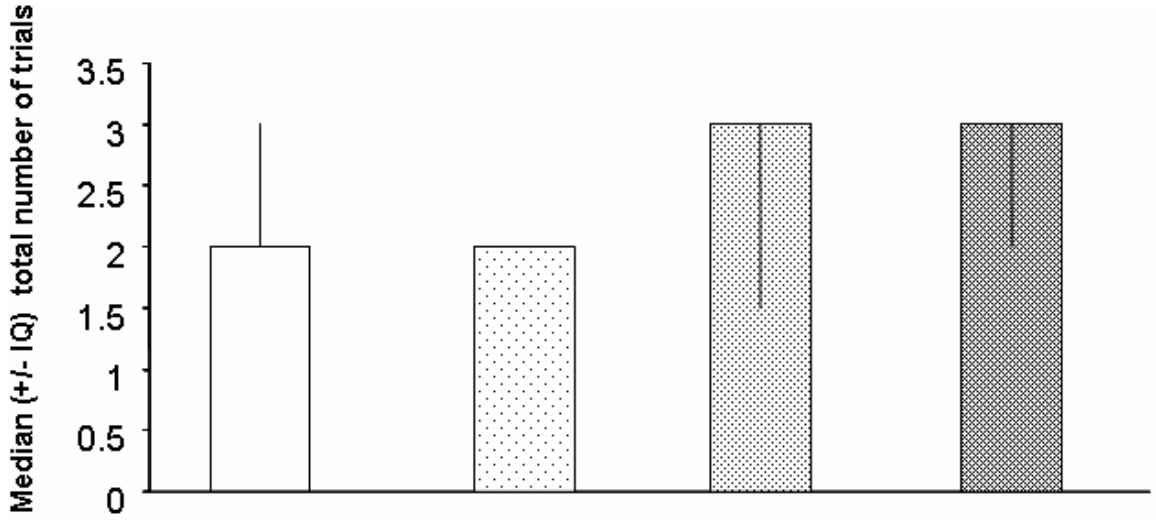

Fig.3.1. Septal drug infusions did not significantly affect the median (+/-IQ) number of trials to reach criterion during training.

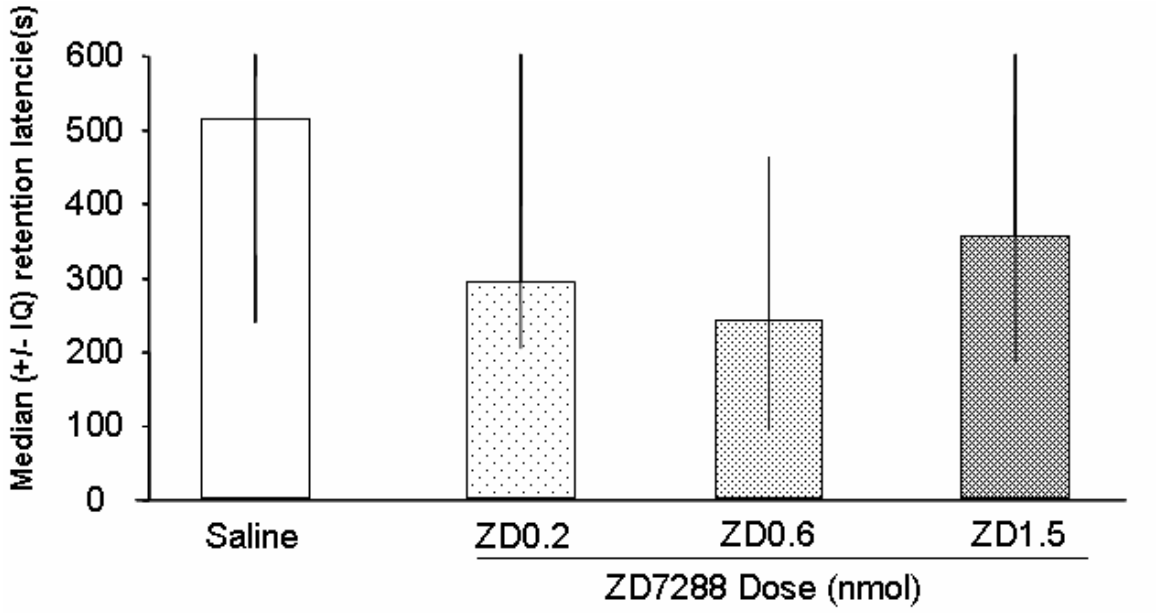

Fig.3.2. Septal drug infusions did not affect median (+/-IQ) retention latencies ($p > .05$ vs. vehicle; n = 11-23 rats per group).

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