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Septal GABAergic and hippocampal cholinergic systems modulate anxiety in the plus-maze and shock-probe tests

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Abstract

According to Gray [The neuropsychology of anxiety: an inquiry into the function of the septo-hippocampal system. Oxford: Oxford Univ. Press, 1982; Neural systems, emotion and personality. In: Madden VJ, editor. Neurobiology of learning, emotion, and affect. New York: Raven Press, 1991. p. 273–306.] the septum and hippocampus act in concert to control anxiety. In the present study we examined the roles of these structures in two animal models of anxiety: the elevated plus-maze and the shock-probe burying tests. We found that microinfusions (20 ng/0.4 μl) of the $GABA_A$ agonist muscimol into either the lateral or the medial septum increased rats' open-arm exploration in the plus-maze test, and decreased their burying behavior in the shock-probe test. We also found that infusions of the acetylcholinesterase inhibitor physostigmine (10 μg/μl) into the dorsal hippocampus, like intraseptal muscimol (20 ng/0.4 μl), increased open-arm exploration in the plus-maze test, and decreased burying behavior in the shock-probe test. Although combined infusions of intraseptal muscimol and intrahippocampal physostigmine did not increase the magnitude of anxiolysis, this may have been due to "ceiling" effects. Overall, the results confirm that septal GABAergic and hippocampal cholinergic systems are both involved in the modulation of anxiety.

Keywords: Septum; Hippocampus; Acetylcholine; GABA; Anxiety

1. Introduction

Anatomically, the septum is extensively connected to the hippocampus (e.g., Nauta and Domesick, 1984; Risold and Swanson, 1997; Swanson and Cowan, 1977), and together they form a substantial part of the limbic system. Functionally, according to Gray's theory (Gray, 1982, 1991), the septum and hippocampus act in concert to control anxiety, as evidenced in part by the remarkable correspondence between the effects of septal or hippocampal lesions in traditional aversive learning paradigms and the effects of anxiolytic drugs in the same paradigms (for reviews, see Gray, 1982; Gray and McNaughton, 1983).

With respect to the septum, we have repeatedly found that ablation or pharmacological inhibition of this area reduces rats' anxiety-related behaviors in the elevated plus-maze and the shock-probe burying tests, suggesting that the septum normally plays an excitatory role in the control of anxiety (for reviews see Menard and Treit, 1999; Treit and Menard, 2000). Briefly, we found that electrolytic or excitotoxic lesions of the septum produced anxiolytic-like effects in the plus-maze and shock-probe burying tests, i.e., open-arm exploration was increased and burying behavior was decreased (Menard and Treit, 1996a,b; Pesold and Treit, 1992; Treit and Pesold, 1990; Treit et al., 1993a). The same pattern of effects was produced when septal activity was inhibited via intraseptal infusions of the benzodiazepine-type anxiolytic, midazolam (Pesold and Treit, 1994, 1996). Although benzodiazepine anxiolytics are thought to produce their effects indirectly through an allosteric modulation of the $GABA_A$ receptor complex (e.g., Zorumski and Isenberg, 1991), there is surprisingly little evidence to suggest that directly acting $GABA_A$ agonists such as muscimol also have anxiolytic-like effects in the septum (e.g., Drugan et al., 1986). Thus one purpose of the present studies was to examine the effects of intraseptal infusions of muscimol on rats' fear behaviors in the plus-maze and shock-probe tests.

With respect to the hippocampus, there is evidence that hippocampal cholinergic systems may be particularly involved in the modulation of anxiety. For example, increases

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in rats' fear reactions have been observed in a variety of tests following intrahippocampal infusions of both muscarinic and nicotinic antagonists (File et al., 1998b; Hess and Blozovski, 1987; Smythe et al., 1998). One expectation, based on these antagonist studies, is that a general up-regulation of hippocampal cholinergic systems might result in anxiety-reduction. Thus the second purpose of the present study was to examine the effects of intrahippocampal infusions of the acetylcholinesterase inhibitor physostigmine on rats' fear responses. Finally, it is possible that septal GABAergic systems might interact with hippocampal cholinergic systems in the control of anxiety. Thus, the independent and combined effects of stimulating septal GABAergic systems and hippocampal cholinergic systems was examined.

Anxiety was assessed in the elevated plus-maze and the shock-probe burying tests. In the elevated plus-maze test, rats typically avoid the open arms of the maze and spend most of their time in the two enclosed arms (Pellow et al., 1985). In the shock-probe burying test, rats shocked from a stationary, electrified probe push bedding material from the floor of the experimental chamber toward the shock-probe (i.e., burying) while avoiding further contacts with the probe (Pinel and Treit, 1978; Treit et al., 1981, 1994). Anxiolytic drugs such as diazepam increase open-arm exploration in the plus-maze and decrease burying toward the shock-probe (De Boer et al., 1990; Pellow, 1986; Pellow et al., 1985; Pellow and File, 1986; Treit et al., 1981, 1994; Treit and Menard, 1997; Tsuda et al., 1988). Conversely, anxiogenic drugs such as yohimbine decrease open-arm exploration while increasing shock-probe burying (Johnston and File, 1989; Pellow, 1986; Pellow et al., 1985; Pellow and File, 1986; Treit, 1990; Tsuda et al., 1988). Thus, anxiolytic effects in shock-probe and plus-maze tests involve a decrease and an increase in activity, respectively. This suggests that reductions in anxiety in both tests would be difficult to explain in terms of nonspecific effects on general activity, arousal, pain sensitivity, or behavioral inhibition. Furthermore, neither test involves response learning or an explicit memory requirement, factors that can complicate the interpretation of drug effects in other animal models of anxiety (e.g., the conflict test; Treit, 1985). The combined use of rats' untrained fear reactions in two different pharmacologically validated tests of anxiety is particularly important in the present study since both septal GABAergic and hippocampal cholinergic systems have been repeatedly implicated in learning and memory (e.g., Brioni et al., 1990; Degroot and Parent, 2000; Durkin, 1992).

2. Experiment 1

2.1. Materials and methods

2.1.1. Subjects

The subjects were naive, male albino Sprague–Dawley rats, purchased from Charles River, Canada, weighing 300–350 g at the time of surgery. Following surgery, rats were individually housed in polycarbonate cages ($47 \times 25 \times 20.5$ cm) and maintained on a 12:12 h light/dark cycle (lights on at 0700 h), with food and water available ad libitum. Behavioral testing occurred between 0900 and 1900 h. The treatment of all animals was in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animals.

2.1.2. Surgery and histology

The rats were given an oral administration of the analgesic acetaminophen (Tylenol 120 mg/1.5 ml) followed 1 h later by atropine sulfate (0.1 mg/0.2 ml ip) to reduce respiratory complications due to the anaesthetic. The rats were then anaesthetized with pentobarbital (Nembutal 50 mg/kg ip), hydrated with saline (3 cc sc), and given the antibiotic penicillin (Crystiben, Rhone Merieux Canada, 15,000 IU/0.05 cc im). Stereotaxic procedures were used to implant 45 rats with one 22-gauge stainless-steel guide cannula (Plastics One, Roanoke, VA) aimed at the medial septum (0.5 mm anterior to bregma [AP], 4.9 mm ventral to dura [DV], using flat skull coordinates (Paxinos and Watson, 1986) and 45 rats with two 22-gauge cannulae aimed bilaterally at the lateral septum (0.7 mm AP, 3.0 mm lateral to bregma, 4.6 mm DV, with the cannula angled 22° medially). The cannulae were attached to the skull with four jeweler's screws and cranioplastic cement. A dummy cannula was inserted into each guide cannula to keep the cannula tract clear. Immediately after surgery the rats were placed into a warm environment until they regained consciousness. Two days after surgery, each cannula was checked for obstructions, and Betadine was applied to the surgical wound. Following behavioral testing, rats were sacrificed with an overdose of chloral hydrate and perfused intracardially with 0.9% saline followed by 10% formalin. The brains were removed and placed in 10% formalin solution. After at least 48 h had elapsed, the brains were frozen with dry ice, sectioned (60 μm), mounted onto glass slides and stained with thionin. The location of the cannulae for each rat was examined microscopically by an observer who was "blind" to the behavioral results. The location of the cannulae tips were then transcribed onto the appropriate atlas plates (Paxinos and Watson, 1986). The behavioral data for animals with necrosis of brain tissue at the site of implantation ($n = 1$) or misplaced cannulae ($n = 13$) were discarded. It is important to note that any cannula tip (bilateral or unilateral) observed outside of its intended target was deemed "misplaced." In addition, one rat failed to contact the probe in the shock-probe burying test and therefore was not included in the analyses of the burying data.

2.1.3. Infusion procedures

Rats in each of the two surgical groups were randomly assigned to one of the following two drug conditions: (1) a control condition, infused with vehicle, or (2) a drug

condition, infused with muscimol. The tame, hand-held rats were given an infusion of muscimol (20 ng/0.4 μl/1 min) or its vehicle (phosphate buffered saline; PBS, pH 7.4; 0.4 μl/1 min) into the medial or lateral septum through a 26-gauge stainless steel internal cannula lowered 1.0 mm below the tip of the guide cannula. The internal cannula was connected to a 10-μl constant rate Hamilton microsyringe with polyethylene tubing and the infusions were delivered using an infusion pump (Harvard Apparatus 22). The internal cannula was left in place for 1 min following the infusions in order to allow for diffusion. The muscimol dose was selected based on pilot work in our laboratory.

2.1.4. Behavioral testing

The behavioral testing procedures were the same as those used in previous experiments (e.g., Menard and Treit, 2000; Pesold and Treit, 1996; Treit et al., 1993b, 1994). All behavior was recorded on videotape for ensuing analysis. Plus-maze testing occurred at least 7 days postsurgery and shock-probe testing occurred at least 12 days postsurgery. This order of testing is based on earlier work (Treit and Pesold, 1990) showing no effect of a 5-min exposure to the plus-maze on subsequent behavior in the shock-probe test, but some disruption of plus-maze behavior when preceded by a shock-probe test. Drug conditions (vehicle versus muscimol) were counterbalanced across the two behavioral tests.

2.1.5. Plus-maze

This wooden, plus-shaped apparatus was elevated to a height of 50 cm, and consisted of two 50×10 cm open arms, and two $50 \times 10 \times 50$ cm enclosed arms, each with an open roof. The maze was in the center of a quiet and dimly lit room. The rats' behavior was observed using a mirror that was suspended at an angle above the maze. Behavioral data were collected by a "blind" observer who quietly sat 1 m behind one of the closed arms of the maze. Five minutes following their respective drug treatment, rats were placed individually in the center of the plus-maze, facing one of the closed arms. The observer measured (1) time spent in the open arms, (2) time spent in the closed arms, (3) number of entries into the open arms, and (4) number of entries into the closed arms during the 5-min test period. An entry was defined as all four paws in the arm. The maze was cleaned with distilled water after each rat was tested. For the purpose of analysis (Pellow et al., 1985; Pellow and File, 1986), open-arm activity was quantified as the amount of time that the rat spent in the open arms relative to the total amount of time spent in any arm (open/total $\times$ 100), and the number of entries into the open arms was quantified relative to the total number of entries into any arm (open/total $\times$ 100). The total number of arms entered as well as the total number of closed arms entered were used as indexes of general activity (for details see Pellow et al., 1985; Rodgers and Johnson, 1995).

2.1.6. Shock-probe

The shock-probe burying apparatus consisted of a $40 \times 30 \times 40$ cm Plexiglas chamber, evenly covered with approximately 5 cm of bedding material (odor-absorbent kitty litter). The shock-probe was inserted through a small hole on one wall of the chamber, 2 cm above the bedding material. The Plexiglas shock-probe (6.5 cm long and 0.5 cm in diameter) was helically wrapped with two copper wires through which an electric current was administered. Current from the 2000 V shock source (60 cycle AC RMS) was varied with potentiometers and set at 2 mA. Rats were habituated in pairs to the test chamber without the shock-probe, for 30 min on each of four consecutive days prior to the test day. On the test day, 5 min following their respective drug treatments, rats were individually placed in one corner of the testing chamber, facing away from the shock-probe. The probe was not electrified until the rat touched it with its snout or forepaws, at which point the rat received a brief 2-mA shock. The 15-min testing period began once the rat received its first shock and the probe remained electrified for the remainder of the testing period. Following the first shock, the duration of time each rat spent spraying bedding material toward or over the probe with its snout or forepaws (i.e., burying behavior) was measured, as was the total number of contact-induced shocks each rat received from the probe. An index of the rat's reactivity to each shock was scored according to the following four-point scale (Pesold and Treit, 1992): (1) flinch involving only head or forepaw, (2) whole body flinch, with or without slow ambulation away from the probe, (3) whole body flinch, and/or jumping, followed by immediate ambulation away from the probe, and (4) whole body flinch and jump (all four paws in the air), followed by immediate and rapid ambulation (i.e., running) to the opposite end of the chamber. A mean shock reactivity score was calculated for each rat by summing its shock reactivity scores and dividing by the total number of shocks it received. The total time that the rat spent immobile (e.g., resting on the chamber floor) during the 15-min testing period was used as an index of general activity. All behavior measures were made by a "blind" observer who was watching the rat via a video monitor in a room adjacent to the testing room.

2.1.7. Statistical analysis

Results are expressed as means and standard errors of the mean (S.E.M.). The plus-maze data and the shock-probe data were analyzed using analysis of variance (ANOVA). In order to correct for nonnormality and heterogeneity of variance, the burying scores and the immobility scores were transformed to their square roots prior to ANOVA. Measures of anxiety (i.e., open-arm exploration and shock-probe burying) were analyzed by a priori F tests between the muscimol drug groups and their respective vehicle controls. Control measures (e.g., shock-reactivity, total arm entries) were analyzed by overall between-groups ANOVAs (α=.05).

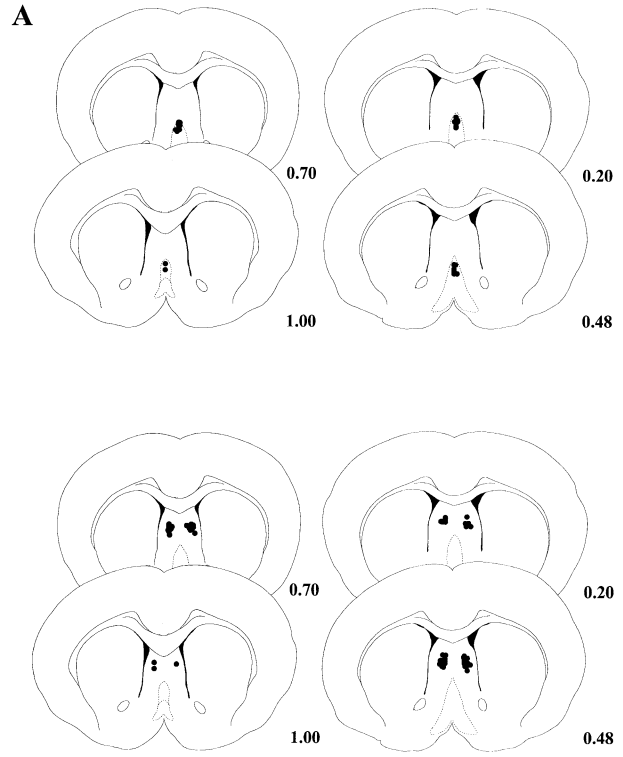

Fig. 1. Schematic illustration of coronal sections of the rat brain showing the approximate location of (A) medial septal and (B) lateral septal infusion sites in Experiment 1. The numbers indicate sections anterior to bregma. Atlas plates adapted from Paxinos and Watson (1986).

2.2. Results

2.2.1. Plus-maze

Fig. 1 shows the infusion sites in the medial and lateral septum. Fig. 2 indicates that muscimol infusions into either the medial or lateral septum produced clear anxiolytic-like effects in the plus-maze. Specifically, rats infused with 20 ng of muscimol into the medial septum showed a significantly greater percentage of open-arm entries [$F(1,34)=8.19$, $P=.01$] and open-arm time [$F(1,34)=4.11$; $P=.05$] than their vehicle-infused controls. Similarly, rats infused with 20 ng of muscimol into the lateral septum displayed a significantly greater percentage of open arm entries [$F(1,34)=6.91$; $P=.01$] and open-arm time [$F(1,34)=5.90$; $P=.02$] compared to their vehicle-infused controls. There was no indication of nonspecific changes in general activity, as the total number of arms entered did not differ between groups [$F(3,68)=0.77$; $P=.51$; see Table 1]. Although the number of closed arms entered did differ significantly between groups [$F(3,68)=3.54$; $P=.02$; see Table 1], post hoc comparisons (Bonferroni test, $\alpha=.05$) revealed no significant differences between rats infused with muscimol in the medial or lateral septum and their respective vehicle-infused controls.

2.2.2. Shock-probe

Fig. 3 indicates that muscimol infusions also produced anxiolytic-like effects in the shock probe burying test. Rats infused with 20 ng of muscimol in the medial septum [$F(1,34)=8.04$, $P=.01$] or the lateral septum [$F(1,34)=4.85$, $P=.03$] each displayed significantly lower burying levels than their respective vehicle-infused controls. This anxiolytic-like effect occurred in the absence of any significant changes in immobility [$F(3,68)=0.33$, $P=.80$], number of shocks received [$F(3,68)=0.57$, $P=.64$], or shock reactivity [$F(3,68)=0.47$, $P=.70$; see Table 1]. Taken together, these data suggest that stimulating GABAergic receptors with a 20 ng dose of muscimol, in either the medial or lateral septum, produces behaviorally specific anxiolytic-like effects in both the plus-maze and shock-probe burying tests.

3. Experiment 2

Experiment 2 examined the individual and combined effects of stimulating the septal GABAergic and hippocampal cholinergic systems. Based on the anxiogenic effects previously found after intrahippocampal administration of cholinergic antagonists (see Introduction), we expected that up-regulation of hippocampal cholinergic systems should have anxiolytic-like effects, possibly equivalent to those of intraseptal GABAergic receptor stimulation. In addition,

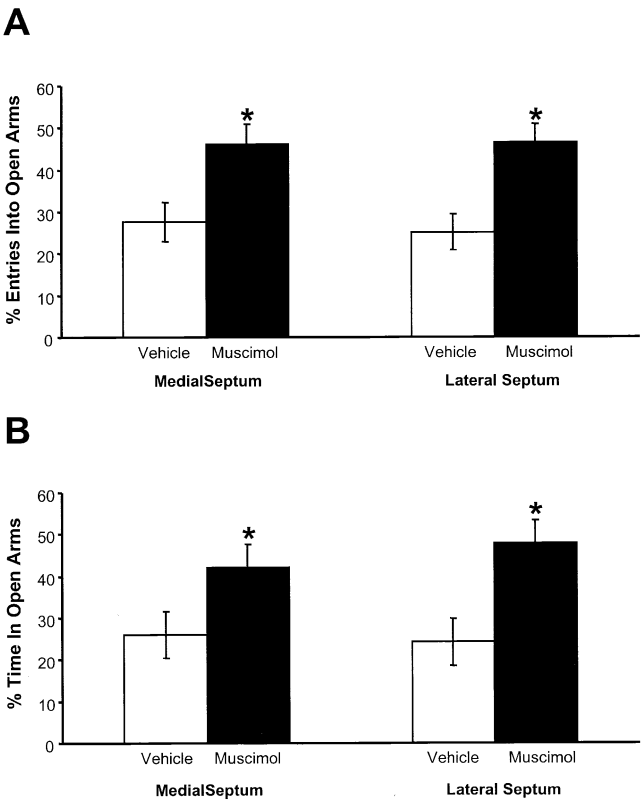

Fig. 2. Mean (±S.E.M.) percent open arm entries (A) and percent open arm time (B) in the elevated plus maze after infusions of muscimol (20 ng/0.4 μl) or vehicle (0.4 μl) into the medial or lateral septum. *$P<.05$ compared with the vehicle control group.

Table 1
Mean (±S.E.M.) total arm entries and closed arm entries in the plus-maze task, and mean (±S.E.M.) activity and reactivity in the shock-probe burying test

	Medial septum		Lateral septum	
	Vehicle	Muscimol	Vehicle	Muscimol
Plus-maze				
n	18	18	17	19
Total arm entries	11.83	11.28	12.18	10.53
	(0.88)	(0.81)	(0.82)	(0.78)
Closed arm entries	8.39	6.39	8.94	6.21
	(0.66)	(0.84)	(0.70)	(0.72)
Shock-probe				
n	17	19	19	17
Immobility	4.59	6.48	4.96	5.31
(SQR; s)	(1.39)	(1.79)	(1.25)	(1.16)
Shock reactivity	2.29	2.16	2.22	2.41
	(0.15)	(0.15)	(0.17)	(0.16)
Shock number	1.59	1.68	1.63	1.94
	(0.21)	(0.15)	(0.19)	(0.26)

given the extensive interconnections of the hippocampus and the septum, it seemed possible that an interaction could arise from the simultaneous stimulation of the two systems. In order to examine these hypotheses, rats were implanted with guide cannulae in both the medial septum and the dorsal hippocampus and infused with muscimol in the septum and physostigmine in the hippocampus.

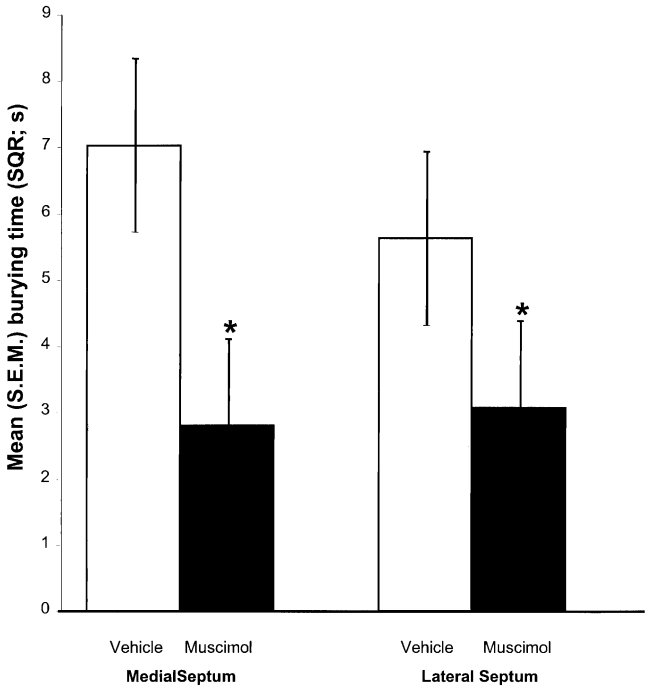

Fig. 3. Mean (±S.E.M.) bury time (SQR; s) in the shock probe apparatus after infusions of muscimol (20 ng/0.4 μl) or vehicle (0.4 μl) into the medial or lateral septum. *$P < .05$ compared with the vehicle control group.

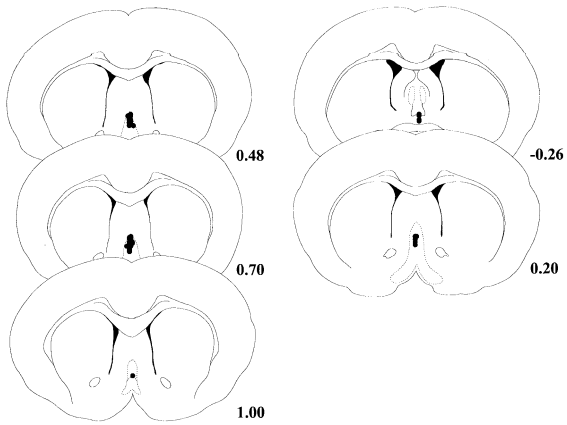

Fig. 4. Schematic illustration of coronal sections of the rat brain showing the approximate location of medial septal infusion sites in Experiment 2. The numbers indicate sections anterior to bregma. Atlas plates adapted from Paxinos and Watson (1986).

3.1. Materials and methods

The methods and procedures used in this experiment were basically the same as those used in Experiment 1. Stereotaxic procedures were used to implant 120 rats with one 22-gauge cannula aimed at the medial septum and one 22-gauge cannula aimed at the dorsal hippocampus (−4.2 mm AP, 2.0 mm DV, 4.1 mm lateral to the midline). We decided to implant guide cannulae in the medial septum based on the positive results found in Experiment 1 and because it was surgically more convenient. Half of the rats ($n = 60$) had the hippocampal cannulae implanted in the left hemisphere. We chose to examine the effects of unilateral hippocampal infusions for surgical convenience, and because it has been demonstrated that perfusions into one hippocampal hemisphere affects acetylcholine in both hippocampal hemispheres (Ragozzino et al., 1998). The behavioral data for animals with necrosis of brain tissue at the site of implantation ($n = 25$) or misplaced cannulae ($n = 23$) were discarded. "Misplacements" occurred when either one or both of the two cannulae were outside of their intended targets. The tame, hand-held rats were given an infusion of muscimol (20 ng/0.4 μl/1 min) or vehicle (PBS; 0.4 μl/1 min) into the medial septum through a 26-gauge stainless steel internal cannula lowered 1.0 mm below the tip of the guide cannula, or an infusion of physostigmine (10 μg/1 μl/1 min) or its vehicle (PBS; 1 μl/1 min) into the dorsal hippocampus through a 26-gauge stainless steel internal cannula lowered 0.8 mm below the tip of the guide cannula. The infusions were delivered using an infusion pump (Harvard Apparatus 22). The 20 ng dose of muscimol was selected based on the findings from Experiment 1. The 10 μg dose for physostigmine was based on previous results (Degroot and Parent, 2000), as well as pilot experiments in our laboratory. Rats were randomly assigned to one of the four drug conditions: (1) vehicle in both the hippocampus and the medial septum (Veh–Veh), (2) phys-

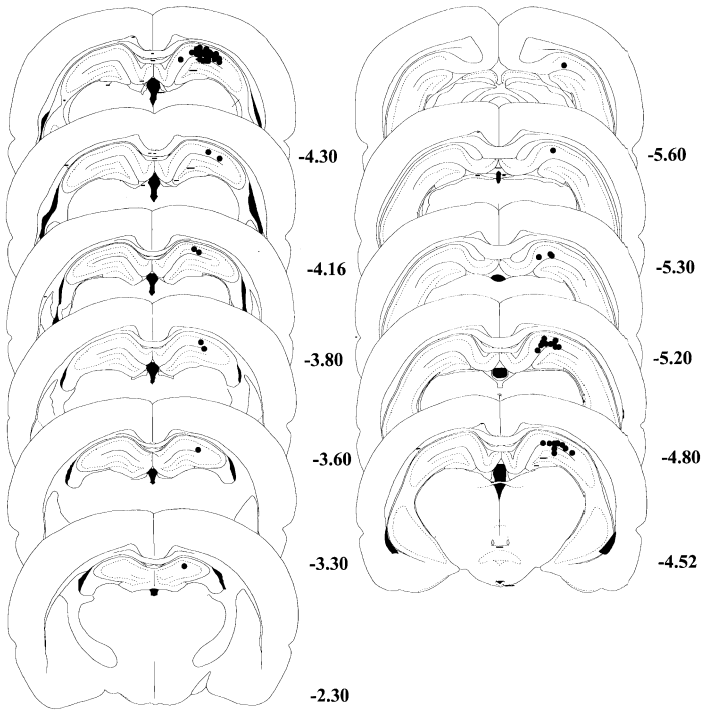

Fig. 5. Schematic illustration of coronal sections of the rat brain showing the approximate location of hippocampal infusion sites in Experiment 2. The numbers indicate sections posterior to bregma. Atlas plates adapted from Paxinos and Watson (1986).

ostigmine in the hippocampus and vehicle in the medial septum (Phys−Veh), (3) vehicle in the hippocampus and muscimol in the medial septum (Veh−Mus), or (4) physostigmine in the hippocampus and muscimol in the medial septum (Phys−Mus).

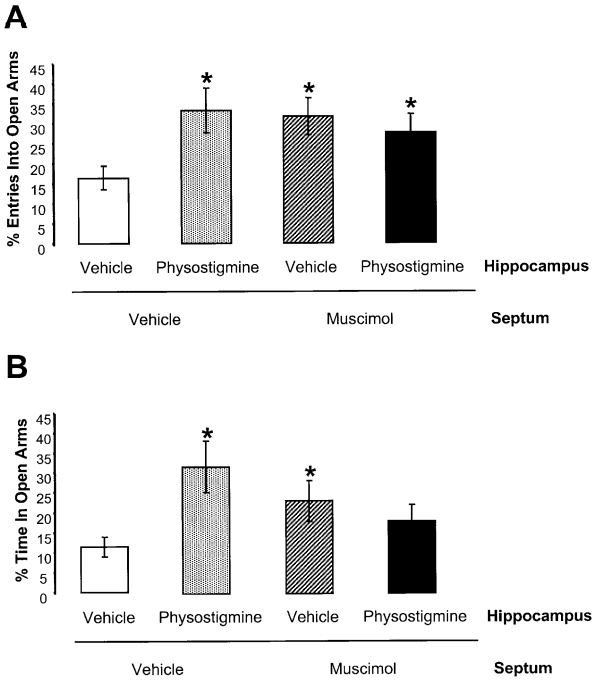

Fig. 6. Mean (±S.E.M.) percent open arm entries (A) and percent open arm time (B) in the elevated plus maze after infusions of muscimol (20 ng/0.4 μl) into the medial septum, physostigmine (10 μg/1 μl) into the dorsal hippocampus, or combined infusions. * $P < .05$ compared with the vehicle control group.

3.1.1. Statistical analysis

Results are expressed as means and S.E.M. The plus-maze data and the shock-probe data were analyzed using analysis of variance (ANOVA). As for Experiment 1, in order to correct for nonnormality and heterogeneity of variance, the burying scores as well as the immobility scores were transformed to their square roots prior to ANOVA. Measures of anxiety (i.e., open-arm exploration and shock-

Table 2
Mean (±S.E.M.) total arm entries and closed arm entries in the plus-maze task, and mean (±S.E.M.) activity and reactivity in the shock-probe burying test

	PBS/PBS	PHYS/PBS	PBS/MUSC	PHYS/MUSC
Plus-maze				
n	18	21	19	14
Total arm entries	12.39	12.76	12.63	11.64
	(1.05)	(1.02)	(0.96)	(1.03)
Closed arm entries	10.22	8.33	7.74	8.29
	(0.91)	(0.88)	(0.89)	(0.85)
Shock-probe				
n	17	20	19	16
Immobility	6.30	6.51	7.81	11.52
(SQR; s)	(1.07)	(1.06)	(1.78)	(1.70)
Shock reactivity	2.08	1.73	2.09	2.30
	(0.10)	(0.12)	(0.13)	(0.14)
Shock number	2.06	2.10	1.63	2.19
	(0.30)	(0.28)	(0.17)	(0.25)

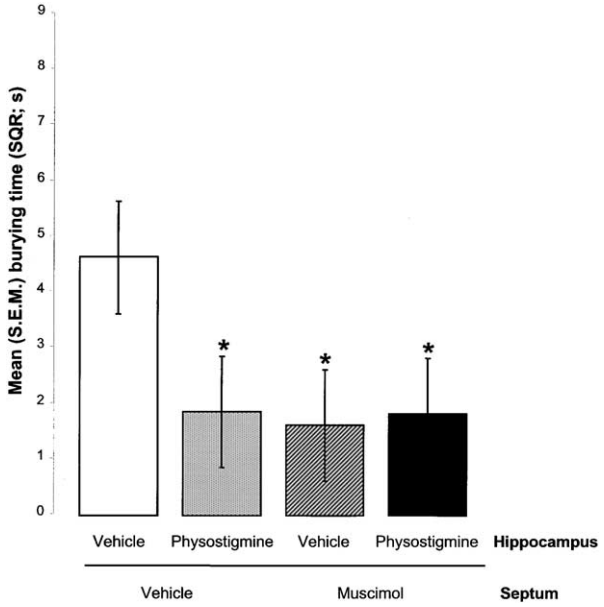

Fig. 7. Mean (±S.E.M.) bury time (SQR; s) in the shock probe apparatus after infusions of muscimol (20 ng/0.4 μl) into the medial septum, physostigmine (10 μg/1 μl) into the dorsal hippocampus, or combined infusions. * $P < .05$ compared with the vehicle control group.

probe burying) were analyzed by a priori F tests between the drug groups (muscimol and physostigmine) and their respective vehicle controls. Control measures (e.g., shock-reactivity, total arm entries) were analyzed by overall between-groups ANOVAs ($\alpha = .05$).

3.2. Results

3.2.1. Plus-maze

Figs. 4 and 5 show the location of the infusion sites in the medial septum and the hippocampus, respectively. Fig. 6 shows that all three drug conditions (Phys–Veh, Veh–Mus and Phys–Mus) produced an increase in the percentage of open arm entries (panel A) and open arm time (panel B) compared to the Veh–Veh control condition. Concurrent infusions of physostigmine in the hippocampus and vehicle in the medial septum (Phys–Veh) increased the percentage of entries into the open arms [$F(1,37) = 6.40$, $P = .02$] and the percentage of time spent in the open arms [$F(1,37) = 7.57$, $P = .01$] compared to Veh–Veh controls. Veh–Mus rats also demonstrated significantly more open-arm entries [$F(1,35) = 7.67$, $P = .01$] and open-arm time [$F(1,35) = 4.15$, $P = .05$] compared to Veh–Veh controls. Similarly, Phys–Mus rats entered significantly more open arms than did Veh–Veh rats [$F(1,30) = 4.64$, $P = .04$], although their time spent on the open arms failed to reach significance [$F(1,30) = 2.04$, $P = .16$]. Neither the total number of arms entered [$F(3,68) = 0.22$, $P = .88$; see Table 2] nor the number of closed arms entered [$F(3,68) = 1.49$, $P = .23$; see Table 2] differed significantly between the groups.

3.2.2. Shock-probe

Fig. 7 shows a large anxiolytic effect in all three drug groups in comparison to Veh–Veh control. Statistical analysis confirmed that all drug groups buried the shock-probe at significantly lower levels than Veh–Veh controls [Phys–Veh: $F(1,35) = 8.07$, $P = .01$; Veh–Mus: $F(1,34) = 9.64$, $P = .01$; Phys–Mus: $F(1,31) = 6.59$, $P = .02$]. These anxiolytic effects were not confounded by between-group differences in general activity [$F(3,68) = 2.63$, $P = .06$], or number of shocks received [$F(3,68) = 1.12$, $P = .35$]. Although there was a significant between-group difference in shock-reactivity [$F(3,68) = 5.08$, $P = .01$; see Table 2], subsequent correlational analysis showed there was no systematic relationship between rats' shock-reactivity scores and their burying scores (Pearson's $r = .07$, $P = .5$).

4. General discussion

The present results indicate that increasing ACh levels in the hippocampus or stimulating GABAergic receptors in the medial or the lateral septum decreases anxiety as measured in the elevated plus-maze and the shock-probe burying tests. Moreover, the data suggest that stimulating hippocampal cholinergic and septal GABAergic receptors simultaneously does not perturb the anxiolytic effect obtained by stimulating either receptor system independently. These anxiolytic-like effects are difficult to explain in terms of nonspecific drug effects on general activity, arousal, or behavioral inhibition since anxiety-reduction in the plus-maze is indicated by a selective increase in a specific activity while anxiety-reduction in shock-probe is indicated by a selective decrease in a specific activity. All rats avoided the shock-probe to a similar extent, suggesting they were quite capable of associating the shock with the probe. Furthermore, both tests utilize rats' untrained fear reactions to clear and present anxiogenic stimuli, making interpretations in terms of learning or memory processes even less likely. In one instance rats did differ in their reactivity to the shock-probe, but this effect was not correlated with burying behavior. Thus, our study may be the first to provide clear evidence that increasing hippocampal ACh levels results in a decrease in anxiety. In addition, the study confirmed that stimulating septal GABAergic receptors with a directly acting $GABA_A$ agonist, muscimol, can have effects that are nearly indistinguishable from traditional benzodiazepine anxiolytics, which are indirect modulators of the $GABA_A$ receptor site.

It was our original intention to demonstrate site specificity by comparing the data obtained from rats with misplaced cannulae with that from rats whose cannulae were correctly placed. However, in Experiment 1, animals whose cannulae were placed outside of the intended target area (either medial or lateral septum) still received a (unilateral) infusion within the septal area. Since one of the targets (medial septum or lateral septum) was very likely stimulated, comparisons between "hits" and "complete misses" could

not be made. Furthermore, in Experiment 2, at least one cannula was always located within the intended target area. Therefore, in the case of combined drug infusions, one of the target areas (hippocampal cholinergic or septal GABAergic receptors) was likely stimulated. In the case of single infusions, "misplaced" cannulae were still not totally outside of the target region. This problem prevented unambiguous comparisons of "hits" and "misses."

The anxiolytic effects induced by stimulating the hippocampal cholinergic system are consistent with previous data indicating that intrahippocampal infusions of cholinergic antagonists increase anxiety (File et al., 1998b; Hess and Blozovski, 1987; Smythe et al., 1998). Our results are also consistent with data indicating that cholinergic agonists such as nicotine induce anxiolytic effects under certain test conditions (Ouagazzal et al., 1999). The nicotine effect is complex and appears to depend on dose, the type and level of "basal" anxiety, as well as the model of anxiety that is used (File et al., 1998a; Ouagazzal et al., 1999). Our study produced more consistent results, since we found that a general up-regulation of hippocampal cholinergic activity resulted in a clear anxiolytic effect in two different tests of anxiety. Overall, these results suggest that increasing acetylcholine activity in the dorsal hippocampus reduces anxiety.

The similarity of the anxiolytic effects of muscimol infused into the lateral and medial septum is consistent with lesion studies (e.g., Menard and Treit, 1996b), but contrasts with a study using intraseptal infusion of the benzodiazepine anxiolytic midazolam, where a dissociation was found (Pesold and Treit, 1996). In the latter study, anxiolytic effects occurred only when midazolam was infused into the lateral septum, not the medial septum. It is possible that muscimol, because it is a directly acting $GABA_A$ agonist, is more potent than midazolam, and thereby more likely to display anxiolytic effects in both septal areas. Diffusion of muscimol from the medial septum to the lateral septum seems unlikely since we used a small injection volume (0.4 μl).

It seems curious that simultaneous stimulation of both hippocampal cholinergic and septal GABAergic receptors did not result in a larger anxiolytic effect, compared to the independent stimulation of each of these receptor systems. One possibility is that separate stimulation of each of these systems already produced maximal anxiolytic effects in both tests (i.e., nearly 50% open-arm exploration in the plus-maze test and a substantial suppression of burying in the shock-probe test). Thus, these behavioral "ceiling" and "floor" effects could have masked any further reduction in anxiety that might otherwise have been observed when both cholinergic and GABAergic systems were simultaneously stimulated. It is also possible that the independent stimulation of each receptor system in our study resulted in asymptotic receptor activation. Thus, a further stimulation of either or both systems would not result in a further reduction in anxiety. For these reasons it may be premature to conclude that the hippocampal cholinergic and septal GABAergic receptor systems do not act in concert in the control of anxiety. Studies in which subthreshold doses of muscimol and physostigmine are infused into the septum and hippocampus, respectively, might indicate whether and how the two systems cooperate in the control of anxiety.

It is unlikely that the hippocampus influenced anxiety via an interaction with the amygdala, a structure that is also involved in fear and anxiety (e.g., Davis, 1992; LeDoux et al., 1990). Although the hippocampus is extensively connected to the amygdala (e.g., Pikkarainen et al., 1999), there is little evidence that the amygdala regulates open-arm exploration in the plus-maze or burying behavior in shock-probe tests (Pesold and Treit, 1994; Treit et al., 1993b; Treit and Menard, 1997; but see Pesold and Treit, 1995).

In summary, the present findings demonstrate that stimulating the GABAergic system of the medial or the lateral septum as well as increasing hippocampal acetylcholine levels reduces anxiety. Stimulating hippocampal cholinergic and septal GABAergic receptors simultaneously does not increase the anxiolytic effect obtained by stimulating either receptor system independently.

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