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Learning and memory in agmatine-treated rats

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Abstract

Agmatine, a noncompetitive *N*-methyl-D-aspartate (NMDA) antagonist, was examined for its role in water maze place learning, contextual and auditory-cued (discrete) fear learning and conditioned taste aversion learning, when administered systemically. Male Wistar rats were given saline or 1, 5, 10 or 50 mg/kg agmatine ip 20 min prior to or 30 min following daily training sessions in a hidden-platform (place learning) water maze task. Agmatine did not affect latencies to find the hidden platform or preference for the training quadrant during probe trials. When administered 20 min prior to contextual or auditory-cued fear-conditioning sessions, these doses of agmatine evoked a linear dose-dependent impairment in the magnitude of learned fear to the contextual stimuli when assessed during extinction trials 24 h later, but had no effect on the magnitude of learned fear to the auditory stimulus. Inferences of baseline motor activity and ability to respond to the presentation of footshock stimuli were not affected by the treatment. Injections of 50 mg/kg agmatine concurrently with a malaise-evoking agent following presentations to a novel sucrose solution abolished learned taste aversions; this agent did not evoke conditioned taste aversions alone. These studies indicate that systemically administered agmatine selectively impairs behavioral inferences of specific types of learning and memory.

Keywords: Agmatine; Conditioned taste aversion; Contextual fear; Discrete fear; Place learning; Rat

1. Introduction

The arginine metabolite agmatine (1-amino-4-guanidinobutane) has recently been described as a putative neurotransmitter in mammals (Reis and Regunathan, 1998, 2000). Agmatine binds to alpha-2 adrenergic and imidazoline receptors (Li et al., 1994; Piletz et al., 1995), inhibits nitric oxide (NO) synthesis (Galea et al., 1996) and can evoke a noncompetitive voltage- and concentration-dependent block of the *N*-methyl-D-aspartate (NMDA) ionophore (Yang and Reis, 1999). While few studies have addressed the behavioral effects of agmatine, we have recently reported that systemic agmatine treatment dose-dependently impaired acquisition and produced temporally graded consolidation deficits in a fear-conditioning paradigm, without affecting spontaneous motor activity or nociceptive thresholds (Stewart and McKay, 2000).

In the present study, we examined the effects of systemically delivered agmatine on the acquisition of fear conditioned to either contextual (background) cues or to a discrete auditory (tone) stimulus. The effects of agmatine on the acquisition and consolidation of a place-learning version of the Morris water maze, which required subjects to learn the location of a hidden platform using only extramaze cues (Morris, 1984), were also examined. Finally, rats were entered into a conditioned taste aversion paradigm. In addition to assessing the role that agmatine may play in modulating a very different type of memory, this paradigm enabled us to examine the possibility that the memory modulating effects of agmatine may be related to the induction of a gastrointestinal malaise. This latter outcome has been reported for several systemically administered compounds that share similar pharmacological properties to agmatine and is suspected to be a peripheral cause of some learning impairments.

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2. Methods

2.1. Subjects

A total of 104 male and 40 female Wistar rats (Charles River, Quebec, and derived from our own breeding stock), age 70–90 days, were entered into the present study. Rats were housed two to three per cage in standard colony conditions. The light/dark cycle was 12:12 with photophase onset at 0730 h local time; ambient temperature was maintained between 20 and 21°C. Food and water were available ad libitum throughout the entire study (except in Experiment 4). All procedures had been approved by the local Animal Care Committee prior to initiating the study and were in accordance with Canadian Council on Animal Care (CCAC) guidelines. Behavioral observations were completed by trained individuals blind to the treatment conditions of the rats.

2.2. Water maze apparatus and procedure

The water maze was a custom-constructed pool measuring 180 cm in diameter by 50 cm deep, filled to a depth of 35 cm. A 10-cm-diameter platform was located 1.5 cm below the surface of the water. Nontoxic blue powdered tempera paint was added to the surface of the water to visually obscure the platform location. The pool had been divided into four equally sized quadrants with the platform located in the center of one of the quadrants, halfway between the center and the wall of the pool. Various geometric shapes constructed from Bristol board were placed on the walls of the room to serve as spatial cues.

The water maze task consisted of six sessions conducted once daily over 6 successive days. Each session consisted of four trials separated by approximately 30 s. Rats were placed manually into the pool, facing the pool wall in the center (and along the periphery) of one of the quadrants not containing the platform. Each rat was trained with a unique release schedule wherein release locations were pseudorandomized. The latency to find the platform was recorded manually with a maximum of 120 s allowed for each trial. For rats that did not find the platform in this amount of time, the value of 120 s was recorded as the time for that trial, and the rat was guided onto the platform. The rat was allowed to remain on the platform for the duration of the intertrial interval.

A 120-s probe trial (transfer test, e.g., platform removed from pool) was conducted on the seventh day of the study (24 h after the last hidden-platform session). Rats were released into the pool into the quadrant opposite to that previously associated with the escape platform. A manual time-sampling procedure was utilized to record the time spent swimming in each of the four quadrants of the pool (e.g., one observation per second corresponding to the location of the rat in the pool).

2.3. Experiment 1a. Effects of pretraining agmatine on place-learning acquisition

Rats (38; $n = 6–8$/group) received intraperitoneal (ip) injections of one of four doses of agmatine (1, 5, 10 or 50 mg/kg; 1 cc/kg) or physiological saline (0.9%; 1 cc/kg) 20 min prior to each first daily trial in the water maze (Agmatine, Sigma, St. Louis, MO). For the probe trials, rats were injected 20 min prior to testing with the same dose of agmatine that they received during the hidden-platform trials.

2.4. Experiment 1b. Effects of posttraining agmatine on place-learning consolidation

Rats (30; $n = 6$/group) received one of four doses of agmatine or physiological saline (ip) 30 min following each daily session in the water maze. The probe test was conducted drug free. All rats were injected following the probe test with the same dose of agmatine that they had received during hidden-platform trials in order to standardize the total number of injections per rat with Experiment 1a.

2.5. Experiment 2. Contextual fear conditioning

The fear-conditioning procedure has been described in detail elsewhere (Stewart and McKay, 2000). Briefly, 72 h after water maze testing, rats were placed in the conditioning chamber for a 3-min baseline period and were then delivered three unsignaled footshocks through a metal grid floor (2-s, 0.5 mA, 60-s interstimulus interval). Rats were returned to their home cages 60 s after the last footshock. During the baseline period, spontaneous motor activity (chamber crossovers) was recorded for each rat. Rats were scored for defensive freezing (Bolles, 1970) following each footshock presentation and 24 h later during an extinction test using a time-sampling procedure where behavior (*immobility* (1) or *movement* (0)) was recorded every 8 s for each 1-min period (7 observations) following the footshocks and every 8 s for a total of 8 min (60 observations) during extinction testing. Nominal freezing measures were converted to a percentage of total observations. The conditioning apparatus was cleaned with a 0.4% acetic acid solution.

Twenty minutes prior to conditioning, rats were treated with the same dose of agmatine administered during water maze trials ($n = 30$). A second group of rats that received repeated agmatine injections during water maze testing were also examined but were not administered agmatine prior to conditioning ($n = 38$). A third group of naïve rats ($n = 12$; 4/group) not used in the water maze study served as a reference group and received a saline vehicle, or 10 or 50 mg/kg agmatine. The small sample size was selected for the naïve group of rats as we have previously demonstrated the consistency of the agmatine-mediated contextual fear learning impairment in naïve rats.

2.6. Experiment 3. Auditory-cued (discrete) fear conditioning

The procedure and conditioning environment were identical to that described above for contextual fear conditioning with the addition of three 10-s presentations of a 90-dB, 2000-Hz tone. Each 10-s tone terminated with the presentation of a footshock stimulus. A total of 24 adult (90 days) Wistar rats ($n = 6$/group) were administered a saline vehicle or 1, 5 or 10 mg/kg agmatine ip 20 min prior to conditioning sessions. On the testing day, rats were placed singly into a novel Plexiglas environment of equal dimensions to the conditioning box in a room distant from the conditioning room. Rats were given a 2-min habituation interval during which time they were scored, by the procedure described above, for freezing to the novel environment (total of 15 observations per rat). The tone stimulus was then initiated for 8 min, and rats were scored for freezing during this interval. The apparatus was cleaned with a dilute solution of ethanol and dried prior to testing each rat.

2.7. Experiment 4. Conditioned taste aversion learning

Ninety-day-old female Wistar rats (sex selected on the basis of availability) were singly housed and restricted to 20-min/day access to tap water for 10 days. On Day 11, rats were given 20-min presentations of a novel sucrose solution (10% sucrose in tap water). Immediately following the sucrose presentation, half the rats were given 10 cc/kg ip of either 0.15 M lithium chloride (LiCl) to evoke gastrointestinal malaise, or 0.15 M sodium chloride (NaCl) as a control, and half the rats in each of these groups were given 1 cc/kg sc of either physiological (0.9%) saline or 50 mg/kg agmatine. The two injections were given to each rat successively. The four treatment groups ($n = 10$/group) were thus NaCl/NaCl, NaCl/Agmatine (to assess the possibility that agmatine evokes a conditioned taste aversion alone), LiCl/NaCl and LiCl/Agmatine. On Days 12 and 13, rats were given 20-min/day access to tap water, and on Day 14, the sucrose solution was returned. The volume of fluid (to the nearest milliliter) was quantified each day.

2.8. Statistical analyses

All statistical analyses were completed with SPSS software on a VAX 4000 computer. Repeated-measures multivariate analysis of variance (MANOVA) was the primary statistical tool to analyze escape latency and probe test (quadrant preference) water maze data and conditioned taste aversion learning data. Fear-conditioning data were analyzed by univariate analysis of variance (ANOVA). Post hoc analyses included correlated t tests and Student–Neuman–Keuls ($P < .05$) where appropriate.

3. Results

3.1. Experiments 1a and 1b. Water maze acquisition and consolidation

MANOVA with two levels repeated (six sessions (days) with four trials per session) and two levels not repeated (pretraining agmatine injection (acquisition) versus post-training agmatine injection (consolidation); saline plus four doses of agmatine) discerned statistically significant differences between sessions [$F(5,290) = 93.5$, $P < .001$] and between trials [$F(3,174) = 45.8$, $P < .001$]. Correlated t tests discerned the source of the session effect to be the decrease in escape latency across sessions; this expected learning effect was not systematically related to time of injection (pre/post) [$F(5,290) = 2.20$, n.s.], dose of agmatine

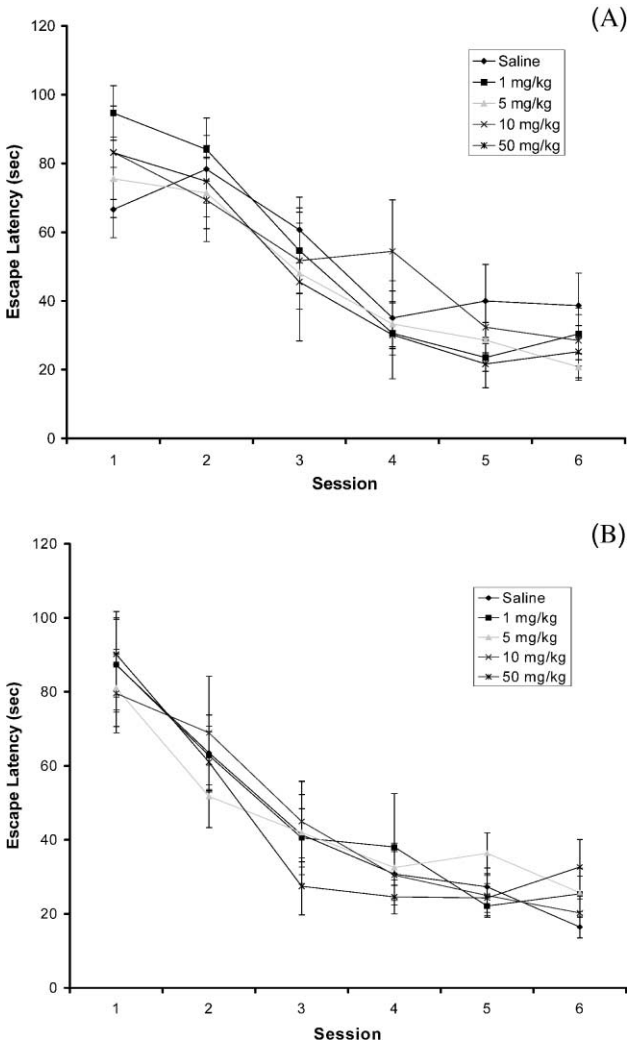

Fig. 1. Effects of agmatine on place-learning acquisition (A) and consolidation (B): Mean escape latency (s) per session (average of four daily trials) for rats given physiological saline or 1–50 mg/kg agmatine 20 min preceding place-learning sessions. Error bars indicate standard errors of the mean.

[$F(20,290) = 0.90$, n.s.] or the interaction between time of injection and dose of agmatine [$F(20,290) = 0.98$, n.s.] (Fig. 1A and B; figures for acquisition and consolidation presented separately for clarity). Latencies to the platform for Sessions 1 through 5 were all significantly different from one another; an asymptote in escape latency was noted between Sessions 5 and 6. Correlated *t* tests revealed a trend for reduced escape latency across trials within each training session. The mean time in seconds (standard deviation in parentheses) for Trials 1 through 4 averaged across all six training sessions were 64.3 (23.1), 46.7 (16.7), 43.0 (19.2) and 38.9 (21.4), respectively. Statistically significant main effects for time of agmatine injection or dose of agmatine injected, and all interactions between these dependent variables and the session and trial repeated measures, were not evident (all F's < 2.20).

MANOVA with one level repeated (quadrant) and two levels not repeated (pre/posttraining agmatine injection; dose of agmatine) indicated a statistically significant difference between quadrants for searching time during the 2-min probe tests [$F(3,174) = 27.5$, $P < .001$]. Post hoc analysis (correlated *t* tests) discerned the source of this effect to be the significantly greater amount of time spent in the quadrant previously associated with escape from the water relative to all other quadrants. A slight spatial bias for quadrant adjacent-right was noted over quadrant adjacent-left. Mean quadrant search times (in percent; standard deviation in parentheses) for the four quadrants were goal: 34.4 (12.0), adjacent-left: 19.2 (8.7), adjacent-right: 24.5 (7.5), opposite-goal: 21.7 (10.1). A statistically significant interaction between quadrant and dose of agmatine was noted [$F(12,174) = 3.0$, $P < .01$]. Correlated *t* tests discerned the source of the interaction to be the absence of a goal-quadrant preference for rats receiving 1 mg/kg agmatine. However, the preference for the goal quadrant, although statistically significant, was not robust in any group when each group was evaluated independently.

3.2. Experiment 2. Contextual fear conditioning

The number of midline chamber crossovers prior to context/shock pairings and the average amount of postshock freezing following context/shock pairings (mean of three postshock periods) did not differ significantly between the three treatment history conditions (chronic agmatine during water maze testing without preconditioning agmatine treatment; chronic agmatine during water maze testing with preconditioning agmatine treatment; naïve rats with preconditioning agmatine treatment) [$F(2,77) = 0.01$, n.s., and $F(2,77) = 0.35$, n.s., respectively], and did not differ as a function of pretraining agmatine dose or chronic agmatine dose within each of the three treatment history conditions (all F's < 1.41, n.s.) (Table 1).

Statistically significant differences in freezing duration during contextual extinction testing between groups of rats chronically treated with different dosages of agmatine, but left untreated prior to contextual fear-conditioning trials, was not evident [$F(4,33) = 0.17$] (Table 1). However, a statistically significant linear trend for chronic agmatine-treated rats receiving preconditioning injection of saline or 1, 5, 10 or 50 mg/kg agmatine was evident [$F(1,22) = 11.46$, $P < .01$] as was a statistically significant group difference [$F(4,22) = 3.01$, $P < .05$]. Post hoc analysis (Tukeys, $P < .05$) discerned the source of the group effect to be the significantly reduced freezing-to-context for rats treated with 10 mg/kg agmatine relative to saline-treated rats. A statistically significant difference in freezing-to-context between groups of naïve rats treated prior to conditioning sessions with saline, or 10 or 50 mg/kg agmatine was evident [$F(2,8) = 6.89$, $P < .05$], the source of which was a significantly reduced duration of freezing for 10 and 50 mg/kg treated rats (which did not differ significantly from one another) relative to saline-treated rats. The linear trend was also statistically significant [$F(1,8) = 9.84$, $P < .05$].

To examine the possible role of tolerance effects for freezing-to-context, chronic agmatine-treated rats given preconditioning agmatine were compared to naïve agmatine-treated rats using a two-way analysis of variance (chronic versus naïve; saline, 10 or 50 mg/kg agmatine). A statistically significant effect for dose of agmatine was noted [$F(2,21) = 11.24$, $P < .001$], but the main effect for chronic versus naïve treatment [$F(1,21) = 3.83$] and the interaction between dose of agmatine and chronicity of treatment [$F(2,21) = 0.47$] were not statistically significant. Conse-

Table 1
Means and S.E.M. for numbers of midline chamber crossovers prior to context/shock pairings, mean percent freezing for the average of the three post context/shock pairing periods, and mean percent freezing during extinction testing (contextual or discrete stimuli) for rats chronically or acutely treated with saline or one of several doses of agmatine

	Midline crossovers		Postshock freezing		Extinction test freezing	
Treatment	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.
Contextual fear: chronic agmatine						
Saline	7.1	0.7	53.0	5.7	71.7	6.4
1 mg/kg	9.0	0.9	57.7	8.7	76.3	8.4
5 mg/kg	8.0	1.1	60.0	6.2	77.3	4.5
10 mg/kg	8.6	1.2	50.0	4.0	78.1	5.3
50 mg/kg	8.3	1.9	59.5	9.4	77.2	5.9
Contextual fear: acute agmatine						
Saline	8.3	1.2	68.1	6.4	81.2[a]	3.3
1 mg/kg	8.5	1.5	45.2	11.1	74.2	4.2
5 mg/kg	9.0	0.8	58.1	6.5	68.3	5.7
10 mg/kg	9.5	0.9	60.0	5.8	53.5[b]	5.8
50 mg/kg	7.0	0.9	52.4	4.4	53.6[b]	5.9
Discrete fear						
Saline	6.3	1.2	67.5	9.8	53.3	10.0
1 mg/kg	7.0	1.0	50.0	8.7	46.9	13.7
5 mg/kg	5.8	2.2	58.7	11.1	70.0	13.8
10 mg/kg	4.2	1.0	53.2	6.6	78.1	6.5

[a] vs. [b] Post hoc, $P < .05$.

quently, the chronic versus naïve treatment groups were collapsed to facilitate a dose-dependent analysis for all rats receiving precontext conditioning agmatine treatment. One-way analysis of variance with Tukey's post hoc ($P<.05$) discerned a statistically significant [$F(4,33)=6.68$, $P<.001$] reduction in freezing-to-context for 10 and 50 mg/kg agmatine-treated rats (which did not differ significantly from one another) relative to saline-treated rats. Trend analysis indicated a linear dose-dependent relationship between freezing-to-context and ascending doses of agmatine [$F(1,33)=25.14$, $P<.0001$] (Table 1).

3.3. Experiment 3. Discrete fear conditioning

Differences between saline-treated rats, and rats given one of three doses of agmatine 20 min prior to tone/shock pairings for numbers of midline crossovers prior to tone/shock pairings [$F(3,20)=0.72$, n.s.], freezing following tone/shock pairings [$F(3,20)=0.69$, n.s.] and freezing during extinction testing in a novel environment with tone presentation 24 h later [$F(3,20)=1.60$, n.s.] were not evident (Table 1). MANOVA indicated that there was a statistically significant increase in freezing during the 8-min tone presentation period (mean = 62.1%, S.E.M. = 5.9) compared to the 2-min habituation period wherein no tone was applied (mean = 23.9%, S.E.M. = 4.9) on the testing day [$F(1,20)=55.62$, $P<.001$]. The interaction between treatment group and pretone/during tone period was not evident.

3.4. Experiment 4. Conditioned taste aversion learning

MANOVA with one level repeated (10 days of habituation to the 20-min/day drinking schedule) and one level not repeated (treatment group) indicated that there were no statistically significant differences between the four groups for water consumption during the habituation period [$F(3,36)=0.40$, n.s.], and statistically significant interactions between treatment group and day of the habituation period were not evident [$F(27,324)=0.43$, n.s.]. A statistically significant difference between days of the habituation period [$F(9,324)=30.58$, $P<.001$] was most parsimoniously explained by a progressive increase in the volume of fluid consumed as the habituation period progressed. Daily fluid consumption stabilized by the eighth day.

MANOVA with one level repeated (pairing day versus testing day) and one level not repeated (treatment group) revealed a statistically significant interaction between treatment group and the repeated level [$F(3,36)=4.40$, $P<.01$] for amount of sucrose solution consumed on these 2 days. The source of this interaction was (1) the significantly decreased sucrose consumption on the testing day compared to the pairing day for all rats receiving LiCl injections, and (2) the significantly decreased sucrose consumption for rats receiving both 0.15 M LiCl and physiological saline relative to all other groups (which did not differ significantly from one another) on the testing day (Table 2).

Table 2
Means and S.E.M. for volume (ml) of 10% sucrose solution consumed on the day of pairing the malaise-evoking agent with the novel sucrose solution (pairing day) and 72 h later during a representation of the sucrose solution (testing day)

	Pairing day		Testing day	
Treatment	Mean	S.E.M.	Mean	S.E.M.
NaCl/NaCl	16.2	1.1	14.6[b]	0.9
NaCl/Agmatine	15.1	1.0	14.5[b]	0.7
LiCl/NaCl	16.9[c]	0.7	10.5[a,d]	0.7
LiCl/Agmatine	16.7[c]	0.9	12.9[b,d]	0.6

[a] vs. [b] post hoc, $P<.01$, and [c] vs. [d] post hoc, $P<.01$.

4. Discussion

Peripheral (systemic) administration of agents, which affect NO, NMDA or adrenergic transmission are known to impair a variety of behavioral inferences of learning and memory and often evoke peripheral effects. Nitric oxide synthase (NOS), whose activation generates the diffusible retrograde neurotransmitter NO, may at some synapses function to reinforce the strength of NMDA-mediated increases in synaptic efficiency (Kandel, 2000). Interestingly, acute NO inhibition does not impair learning in either contextual conditioned fear (Maren, 1998; Johnson et al., 2000) or place-learning water maze tasks (Blokland et al., 1999), although contradictory results have been reported for the latter task (Holscher et al., 1996; Prendergast et al., 1997). NO synthase inhibition has been shown to reduce locomotor activity (Maren, 1998) and induce conditioned taste aversions (Prendergast et al., 1997) in rodents.

Antagonists of alpha-2 adrenoreceptors apparently have no effect on spatial learning in a water maze task; however, increases in locomotor behavior have been reported (Niittykoski et al., 1998). Working memory impairments in monkeys have been ameliorated by clonidine, a nonspecific agonist at alpha-2 adrenergic and imidazoline sites (Birnbaum et al., 2000). Although agmatine was originally described as a clonidine-displacing substance (Li et al., 1994), ostensibly suggesting a possible role for agmatine in working memory impairments, in the present study, we found that rats progressively decreased their escape latency to the platform between trials within each session, a finding independent of dose of agmatine or pre/postinjection interval, and an indication that working memory was not affected by agmatine treatment. Furthermore, the requirement for working memory in the contextual fear-conditioning task is not clear, although equivalent amounts of postshock freezing (an inference that the short-term memory trace of the relationship between context and shock is retained) between all groups of rats may suggest an equivalent to working memory for this task. Neurotoxic lesions of the adrenergic fibers projecting to the bed nucleus of the stria terminalis are

known to impair acquisition of contextual fear stimuli (Onaka and Yagi, 1998). Although this latter treatment did not discriminate between the contributions of discrete receptor subtypes, Zou et al. (1998) demonstrated that alpha-1 adrenoreceptor activity was required at least for the neuroendocrine responses associated with fear conditioning, although no results were reported for alpha-2 activity. The effects of systemically delivered alpha-2 antagonists on learned fear, and the effects of imidazoline agents on all paradigms in the present study, have not been examined.

Several compounds that antagonize NMDA receptors are known to impair water maze learning (for a review, see McNamara and Skelton, 1993). The NMDA antagonist MK-801 (dizolcipine) has been shown to evoke conditioned taste aversions (Jackson and Sanger, 1989) and impair contextual fear conditioning (Bordi et al., 1996) in rodents. These latter authors demonstrated that 80 μg/kg sc impaired both place learning in the water maze and learned fear to contextual stimuli. Interestingly, almost four times this dose (0.3 mg/kg) was required to evoke a conditioned taste aversion (Jackson and Sanger, 1989). The possibility thus emerges that the highest dose of agmatine examined in the present study (50 mg/kg), which was sufficient to reduce learned fear to contextual stimuli, was insufficient to evoke a conditioned taste aversion. This line of reasoning may account for the negative results obtained in the place learning water maze study as well.

The absence of a rigorous comparison of these classes of compounds given at equivalent doses across several different behavioral paradigms makes it difficult to suggest whether one or more of the binding sites of agmatine was implicated in the present results. However, in the light of the results of Bordi et al. (1996) that contextual fear conditioning and water maze place learning were impaired by equivalent doses of MK-801, the possibility emerges that peripherally administered agmatine may preferentially affect specific sites within the CNS, perhaps classes of NMDA receptors with as yet unknown subunit compositions and thus binding affinities, which are localized within CNS sites that anatomically distinguish place learning from context learning. This central-acting and site-specific hypothesis for systemic agmatine treatment is further supported by the negative results for all our measures of nonspecific or peripheral side effects.

During conditioning sessions to contextual cues, a configural representation of the background or nonspecific cues encountered in the environment associated with the pairing of aversive footshock stimuli is formed in circuits within the hippocampus (Maren and Fanselow, 1995), whereas information relevant to tone stimuli are integrated by the auditory thalamus (Iwata et al., 1986); both inputs are propagated to the basolateral amygdala (BLA) (Iwata et al., 1986; Maren and Fanselow, 1995). The BLA acts as a coincidence detector for predictive information from the environment (context or tone) and ascending information coding the footshock stimuli and is the source of descending outputs through the brainstem that effect a response to the footshock stimuli. The circuitry underlying conditioned fear (acquisition, consolidation and expression) is thus common to both variants of the paradigm once the predictive contextual or discrete cues integrated by the hippocampus or auditory thalamus, respectively, have been propagated to the BLA (Fendt and Fanselow, 1999).

Here, we have reported that agmatine dose-dependently impaired the acquisition of learned fear to contextual cues but had no effect on learned fear to a discrete auditory stimulus. As the neural pathways underlying these forms of learning are very similar, yet, only the conditioning to contextual cues was affected by agmatine treatment, it may be reasonable to suggest that the hippocampus was selectively, or at least predominately, affected by peripheral doses of agmatine. We have also shown that place learning in a water maze task was refractory to the doses of agmatine we employed. From a hippocampal perspective, water maze learning requires the physiological integrity of the dorsal hippocampus (Moser et al., 1995), whereas contextual fear learning appears to be based upon ventral hippocampal functioning (Richmond et al., 1999). Although speculative at this point, it may be of interest to note that Otake et al. (1998), who have shown immunolabeling of agmatine at numerous central sites, have proposed that the highest concentrations of endogenous agmatine may be within the subiculum (ventral hippocampus) as the axonal transport inhibitor colchicine was not required to label agmatine in this region.

If indeed endogenous agmatine plays a greater role in synaptic transmission within the ventral hippocampus, relative to other parts of the central nervous system, as may be inferred by its putative preferential distribution within the ventral hippocampus, then our finding that ventral hippocampus-mediated contextual fear, but not dorsal hippocampus mediated place learning, or hippocampal independent fear learning to tone stimuli, may be rooted in a selective effect of endogenous agmatine administration within this region. Confirmation of this hypothesis could come from the direct release of endogenous agmatine within the ventral hippocampus, and an assessment of the effects of this treatment on learning, or microelectrode recordings from both ventral and dorsal hippocampal sites to discern the effects of agmatine on cellular events, including long-term potentiation, which have known correlates to the magnitude of learned fear to contextual stimuli (Maren et al., 1994).

We have further shown that agmatine reduces the magnitude of a taste aversion to sucrose solutions and does not evoke a taste aversion when administered singly. The former finding may be related to the putative role of agmatine in visceral functions (Otake et al., 1998). The latter finding is crucial in that it suggests that peripheral injections of agmatine (50 mg/kg) do not evoke gastrointestinal malaise, suggesting that peripherally mediated illnesses known to be evoked by compounds with similar pharmacological profiles to agmatine, did not confound a learning or memory

interpretation of the other behavioral data that we collected. The lack of peripheral effects of agmatine at the doses examined is additionally suggested by the absence of agmatine-mediated modulation of the sensitivity to graded electrical footshock stimuli (Stewart and McKay, 2000) and latencies to respond to thermal footpad stimulation (Kolesnikov et al., 1996), both inferences of drug-evoked modulation of nociceptive processing, and the lack of change in locomotor behaviors and immediate (postshock) freezing responses to footshock stimuli.

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