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# Enhanced Retention In The Passive-Avoidance Task By 5-HT1A Receptor Blockade Is Not Associated With Increased Activity Of The Central Nucleus Of The Amygdala

Allen M. Schneider

*Swarthmore College*, [aschnei1@swarthmore.edu](mailto:aschnei1@swarthmore.edu)

Emily Wilkins , '01

Aaron Alexander Firestone , '01

E. Carr Everbach

*Swarthmore College*, [ceverba1@swarthmore.edu](mailto:ceverba1@swarthmore.edu)

J. C. Naylor

*See next page for additional authors*

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Allen M. Schneider; Emily Wilkins , '01; Aaron Alexander Firestone , '01; E. Carr Everbach; J. C. Naylor; and Peter E. Simson , '78. (2003). "Enhanced Retention In The Passive-Avoidance Task By 5-HT1A Receptor Blockade Is Not Associated With Increased Activity Of The Central Nucleus Of The Amygdala". *Learning And Memory*. Volume 10, Issue 5. 394-400. DOI: 10.1101/lm.54903  
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**Authors**

Allen M. Schneider; Emily Wilkins , '01; Aaron Alexander Firestone , '01; E. Carr Everbach; J. C. Naylor; and Peter E. Simson , '78



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*Learn. Mem.* 2003 10: 394-400

Access the most recent version at doi:[10.1101/lm.54903](https://doi.org/10.1101/lm.54903)

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# Enhanced Retention in the Passive-Avoidance Task By 5-HT<sub>1A</sub> Receptor Blockade Is Not Associated With Increased Activity of the Central Nucleus of the Amygdala

Allen M. Schneider,<sup>1,4</sup> Emily Wilkins,<sup>1</sup> Aaron Firestone,<sup>1</sup> E. Carr Everbach,<sup>2</sup> Jennifer C. Naylor,<sup>3</sup> and Peter E. Simson<sup>3</sup>

<sup>1</sup>Department of Psychology and <sup>2</sup>Department of Engineering, Swarthmore College, Swarthmore, Pennsylvania 19081, USA;

<sup>3</sup>Department of Psychology, Miami University, Oxford, Ohio 45056, USA

The effect of blockade of 5-HT<sub>1A</sub> receptors was investigated on (1) retention in a mildly aversive passive-avoidance task, and (2) spontaneous single-unit activity of central nucleus of the amygdala (CeA) neurons, a brain site implicated in modulation of retention. Systemic administration of the selective 5-HT<sub>1A</sub> antagonist NAN-190 immediately after training markedly—and dose-dependently—facilitated retention in the passive-avoidance task; enhanced retention was time-dependent and was not attributable to variations in wattages of shock received by animals. Systemic administration of NAN-190 had mixed effects on spontaneous single-unit activity of CeA neurons recorded extracellularly *in vivo*; microiontophoretic application of 5-HT, in contrast, consistently and potently suppressed CeA activity. The present findings—that 5-HT<sub>1A</sub> receptor blockade by NAN-190 (1) enhances retention in the passive-avoidance task, and (2) does not consistently increase spontaneous neuronal activity of the CeA—provide evidence that a serotonergic system tonically inhibits modulation of retention in the passive-avoidance task through activation of the 5-HT<sub>1A</sub> receptor subtype at brain sites located outside the CeA.

Memory of emotional events is modulated by neuronal activity in the amygdala (McGaugh et al. 1993, 1995). Strength of retention, in turn, is regulated by a variety of neurotransmitters, including norepinephrine (NE) and GABA, that influence the amygdala. NE agonists infused directly into the amygdala immediately after training enhance retention (Liang et al. 1986, 1990; Ferry and McGaugh 1999; Ferry et al. 1999); GABA agonists infused directly into the amygdala immediately after training impair retention (Castellano et al. 1989; Willensky et al. 2000).

There is evidence that serotonin, like GABA, inhibits amygdala activity and impairs retention. The evidence comes primarily from studies in which serotonergic agonists or antagonists are injected directly into the amygdala after passive-avoidance training. The evidence, however, is mixed: it is clear that activation of the serotonergic system via intra-amygdala injections of the 5-HT<sub>1A</sub> agonist 8-OH-DPAT impairs retention (Liang 1999); it is not clear that blockade of endogenous serotonin enhances retention. Specifically, retention is not enhanced by intra-amygdala injections of the 5-HT<sub>1A</sub> antagonist NAN-190 (Bernabeu et al. 1997; Bevilacqua et al. 1997a,b), and is only weakly enhanced by intra-amygdala injections of the 5-HT<sub>1A</sub> antagonist WAY-100635 (Liang 1999). Moreover, because cannulae implanted in the amygdala impair retention in and of themselves through neuronal damage (Gold et al. 1975, 1978; Lennartz et al. 1996; Liang 1999; Schneider et al. 2002), the effect of intra-amygdala injections of 5-HT agonists and antagonists on retention in the absence of neuronal damage remains an open question.

In the present study, we sought to determine whether a

5-HT<sub>1A</sub> antagonist administered to unimpaired animals (i.e., in the absence of cannulae-induced neuronal damage) enhances retention and, if so, whether the enhanced retention is associated with increased firing rates of amygdala neurons. To accomplish this, the effect of NAN-190, administered systemically, was determined on (1) retention in the passive-avoidance task, and (2) spontaneous single-unit activity of central nucleus of the amygdala (CeA) neurons recorded electrophysiologically *in vivo*.

We recorded from CeA neurons because of previous findings (Schneider et al. 2000; Simson et al. 2001) that systemically administered propranolol increased CeA activity and enhanced retention in the passive-avoidance task. Because propranolol has both adrenergic and 5-HT<sub>1A</sub> antagonist action (Middlemiss 1984; Oksenberg and Peroutka 1988; Kreiss and Lucki 1994), the present study—by utilizing the selective serotonergic antagonist NAN-190—provided an opportunity to determine whether propranolol enhanced retention and increased amygdala activity, at least in part, through 5-HT<sub>1A</sub>-blockade.

The passive-avoidance task was identical to the commonly used one-trial passive-avoidance procedure (in which rats are given a single foot-shock for stepping from a lighted to a dark compartment), save for two modifications: (1) to investigate enhancing effects of drugs on retention (which requires weak retention in the absence of drugs), mildly aversive shock was used, and (2) to monitor individual differences in reactions to the mildly aversive shock, wattages actually received by animals were recorded.

Animals' reactions to shock are frequently monitored when drugs are administered *prior* to passive-avoidance training in order to determine the extent to which drugs may affect retention by altering the reactions to shock during training (e.g., Decker et al. 1990; Lennartz et al. 1996). In the present study, although drugs were administered after training, wattages received were

<sup>4</sup>Corresponding author.

E-MAIL [Aschnei1@swarthmore.edu](mailto:Aschnei1@swarthmore.edu); FAX (610) 328-7814.

Article and publication are at <http://www.learnmem.org/cgi/doi/10.1101/lm.54903>.

nonetheless recorded owing to the low level of shock used to investigate enhancement. That is, when delivering a low level of shock—to which animals tend to show varied reactions—it could not be assumed that animals in all groups would respond to shock similarly. Consequently, to rule out that differences in shock reactions—instead of drug effects—could account for differences in strength of retention between groups, wattages received were monitored.

## RESULTS

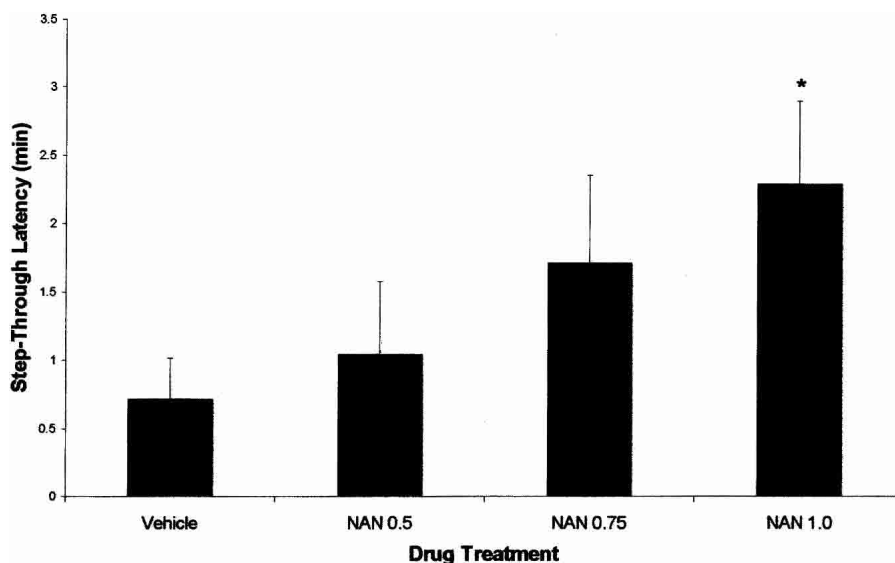
### *When administered immediately after training, NAN-190 (1 mg/kg, i.p.) enhances retention.*

The effect of NAN-190 (0.5, 0.75, and 1.0 mg/kg, i.p.) and its vehicle was determined on retention in the passive-avoidance task. When administered systemically at a dose of 1.0 mg/kg immediately after training, the 5-HT<sub>1A</sub> antagonist significantly facilitated retention. As shown in Figure 1, step-through latencies (STLs) (mean  $\pm$  SEM) on the test day increased with increases in the dose of NAN-190: compared to the 0.5 mg/kg dose ( $n = 10$ ), 0.75 mg/kg dose ( $n = 10$ ), or vehicle ( $n = 10$ ), the 1.0 mg/kg dose ( $n = 10$ ) significantly increased STLs (1-tailed  $t = 2.07$  [ $K = 4$ ; 36df],  $P < .025$ ). The mean STL on the test day for control animals receiving vehicle was  $0.72 \pm 0.30$  min, whereas animals receiving 0.5 mg/kg, 0.75 mg/kg, or 1.0 mg/kg of NAN-190 showed mean STLs of  $1.05 \pm .53$  min,  $1.72 \pm .64$  min, and  $2.30 \pm .61$  min, respectively.

### *When administered in the absence of shock or 2 h or 6 h after training, NAN-190 (1 mg/kg, i.p.) does not enhance retention.*

That the ability of NAN-190 (1.0 mg/kg) to enhance retention could not be attributed to aversive properties of the drug is evidenced by results from the no-shock control animals: in the absence of shock, the STLs (mean  $\pm$  SEM) on the test day for no-shock control animals receiving 1.0 mg/kg NAN-190 ( $0.13 \pm 0.003$  min;  $n = 5$ ) did not differ significantly ( $t$  [one-tailed] = 1.08 [8 df],  $P = .16$ ) from STLs for no-shock control animals receiving vehicle ( $0.07 \pm 0.002$  min;  $n = 5$ ).

The time-dependent effect of NAN-190 on retention is evi-



**Figure 1** NAN-190 (1.0 mg/kg) enhanced retention in the passive-avoidance task. NAN-190 was administered intraperitoneally immediately after training at 0.50 mg/kg ( $n = 10$ ), 0.75 mg/kg ( $n = 10$ ), or 1.0 mg/kg ( $n = 10$ ). Control animals received vehicle ( $n = 10$ ). \* $P < .025$ .

denced by the finding that STLs on the test day for animals receiving NAN-190 (1 mg/kg) 2 h after training ( $0.69 \pm 0.20$  min;  $n = 8$ ) did not differ significantly (one-tailed- $t = 0.32$  [14df],  $P = .38$ ) from STLs for animals receiving vehicle 2 h after training ( $0.85 \pm 0.07$  min;  $n = 8$ ). Similarly, STLs on the test day for animals receiving NAN-190 (1 mg/kg) 6 h after training ( $1.28 \pm 0.39$  min;  $n = 6$ ) did not differ significantly (one-tailed- $t = 0.94$  [10df],  $P = .18$ ) from STLs for animals receiving vehicle 6 h after training ( $0.49 \pm 0.12$  min;  $n = 6$ ). This finding indicates that enhancement of retention produced by NAN 190 is indeed time-dependent and, therefore, most likely the result of blockade of 5-HT<sub>1A</sub> receptors occurring during a critical period shortly after training when strength of retention is regulated (inhibited) by serotonergic activity.

### *NAN-190's ability to enhance retention cannot be attributed to differences in wattage of shock received.*

Wattage of shock received during training was generally highly correlated with STLs during testing: as wattage received increased, STLs increased. In the vehicle control group, the correlation between wattage received and STLs was  $r = 0.64$  ( $F = 5.6$  [1,8 df],  $P = .04$ ). In the NAN 0.5 group, NAN 0.75 group, and NAN 1.0 group, the correlations between wattage received and STLs were  $r = 0.23$ ,  $r = 0.71$ , and  $r = 0.59$ , respectively. Given these correlations between wattage received and STLs, the potential clearly existed for wattage to confound drug effects if different groups received—purely by chance—differing wattages: in the present experiment, wattage received did not differ among the vehicle and drug groups:  $F(3,36) = 1.00$ ,  $P = .40$ .

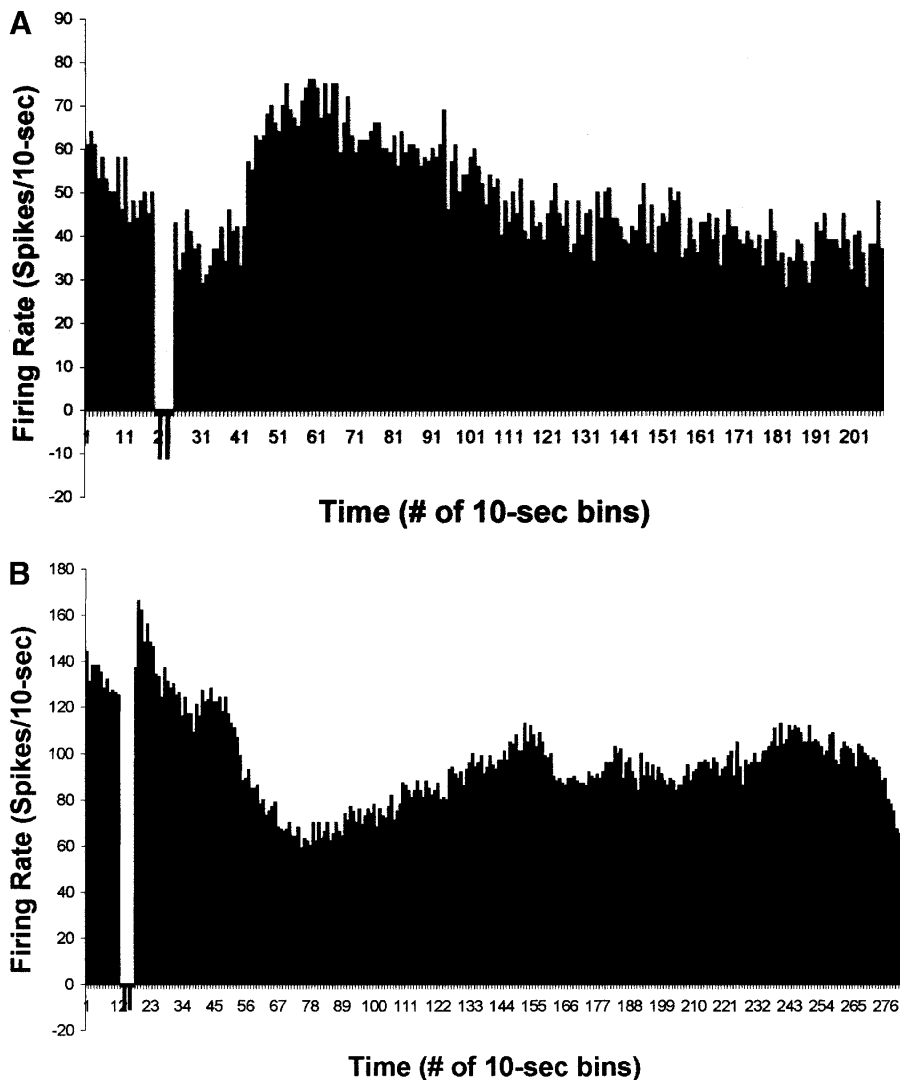
### *In contrast to locally applied 5-HT, systemically administered NAN-190 does not consistently affect spontaneous single-unit activity of neurons in the CeA.*

All CeA neurons fired at a steady rate. NAN-190, when administered systemically at the dose that had significant effects on retention (1.0 mg/kg, i.p.), had mixed effects on spontaneous single-unit activity of CeA neurons (Fig. 2) without altering the steady pattern of firing: it increased ( $n = 2$ ; Fig. 2A), decreased ( $n = 4$ ; Fig. 2B), and had no effect on ( $n = 3$ ) CeA firing rates. However, the failure of NAN-190 to consistently affect CeA neuronal activity was not due to the inability of serotonin to affect the CeA: when applied microiontophoretically, serotonin inhibited spontaneous activity of the CeA in all neurons tested ( $n = 12$ ; Figs. 3,4).

## DISCUSSION

The behavioral results show that blockade of 5-HT<sub>1A</sub> receptors immediately after training by systemic administration of NAN-190 enhances retention in the passive-avoidance task. Enhancement of retention, as measured by STLs, increased as a function of the dosage of NAN-190 administered, could not be attributed to aversive properties of the serotonergic antagonist (i.e., did not occur in the absence of shock), and depended on time of administration (occurring with immediate, but not delayed, administration).

The electrophysiological results show that blockade of 5-HT<sub>1A</sub> receptors by systemic administration of NAN-190 is not associated with consistent



**Figure 2** Rate histograms of two representative CeA neurons demonstrating increases (Panel A) and decreases (Panel B) in spontaneous firing rates to a single dose of NAN-190 (1 mg/kg, i.p.). After a baseline (predrug) period of spontaneous activity, NAN-190 was administered (as indicated by the two tic marks extending below the histograms). The ordinate represents the number of action potentials per 10-sec bin; the abscissa represents the number of 10-sec bins from the start of the recording. At the same dose (1 mg/kg) that had significant effects on retention in the passive-avoidance task, NAN-190 increased ( $n = 2$ ; Panel A), decreased ( $n = 4$ ; Panel B), and had no effect on ( $n = 3$ ) spontaneous activity of single units of the CeA.

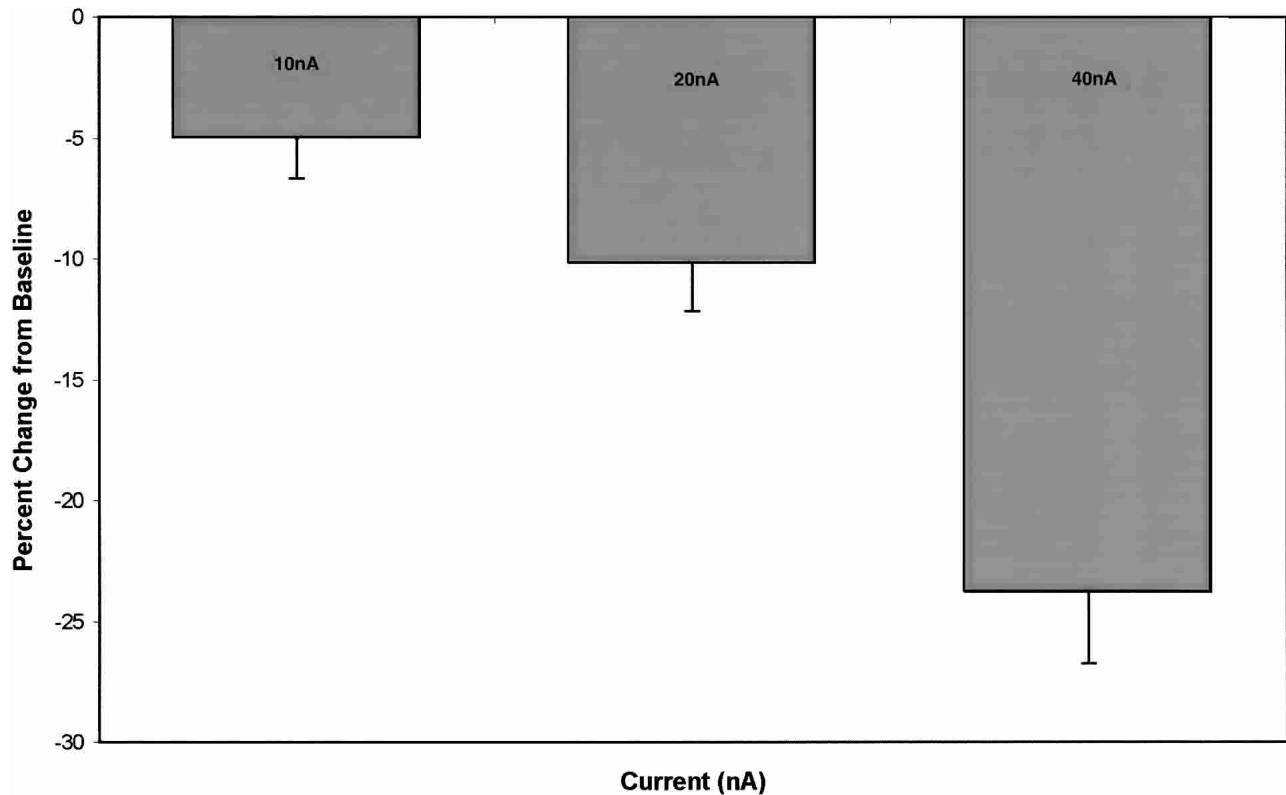
changes in spontaneous CeA activity. This finding suggests that the increased retention produced by systemically administered NAN-190 is not associated with increased activity of the CeA. Taken together, the behavioral and electrophysiological results suggest that NAN-190 antagonizes a 5-HT<sub>1A</sub>-mediated modulatory system located outside the CeA that tonically inhibits retention.

Although the most likely explanation for results showing that NAN-190 is not associated with consistent changes in spontaneous CeA activity is that NAN-190 antagonizes a 5-HT<sub>1A</sub>-mediated modulatory system located outside the CeA that tonically inhibits retention, it is also conceivable that NAN-190 failed to produce consistent changes in CeA activity because it was administered to anesthetized animals. This explanation is unlikely, however, because our laboratories (Simson et al. 2001) previously showed that systemically administered propranolol, adminis-

tered to anesthetized animals under precisely the same conditions as NAN-190 in the present study, does indeed consistently change CeA activity. Combined with studies demonstrating that urethane does not produce qualitative changes in neural activity—including responsiveness to pharmacological challenge—in a variety of brain sites compared to activity in awake, behaving animals (Givens and Breese 1990), it is unlikely that the anesthetic per se prevented consistent changes in CeA activity from occurring.

It also might be argued that a change in spontaneous activity by a small subset of neurons in the CeA (those showing an increase or decrease in activity to NAN-190) rather than outside the CeA mediates NAN-190's behavioral effects, but this, too, seems unlikely in light of the neuropharmacological literature. For example, studies have shown that NAN-190, infused directly into the CeA at relatively low volumes (Bernabeu et al. 1997; Bevilacqua et al. 1997a,b) fails to enhance retention, whereas the 5HT<sub>1A</sub>-agonist 8-OH-DPAT and the 5HT<sub>1A</sub>-antagonist WAY-100635, infused directly into the amygdala at relatively high volumes—volumes capable of spreading to areas outside the amygdala (Myers 1966)—impair and enhance retention, respectively (Liang 1999). If 5HT<sub>1A</sub>-mediated modulation indeed occurs at sites outside the CeA, as our data suggest, infusion of relatively small volumes of drug confined to the CeA would not be expected to affect retention; infusion of relatively high volumes of drug that spread to areas outside the CeA, in contrast, would be expected to affect retention. In this regard, neuropharmacological studies suggest at least two brain sites outside the CeA involved in serotonergic—and in particular 5-HT<sub>1A</sub>—modulation of retention: the basolateral nucleus of the amygdala (Ferry and McGaugh 1999; Hatfield and McGaugh 1999; Rainnie 1999) and the hippocampus (Carli et al. 1995; Belcheva et al. 1997; Levkovitz and Segal 1997; Liang et al. 1998).

That serotonin tonically inhibits retention through a 5-HT<sub>1A</sub>-mediated modulatory system located outside the CeA does not rule out the possibility that serotonin also tonically inhibits CeA firing rates through action on 5-HT<sub>1A</sub> receptors within the CeA, particularly in light of the present findings that CeA activity is potently suppressed by microiontophoretically applied 5-HT. For example, the effect of systemically administered NAN-190 on spontaneous CeA activity could be the net result of competing actions of 5-HT<sub>1A</sub>-blockade both within the CeA (Kia et al. 1996) and on afferents projecting to the CeA (Verge et al. 1986; Tork 1988; Sotelo et al. 1990; Riad et al. 2000). Blockade of inhibitory postsynaptic 5-HT<sub>1A</sub> receptors within the CeA would be expected to increase, via disinhibition, CeA firing rates (if these receptors were tonically activated by 5-HT); block-

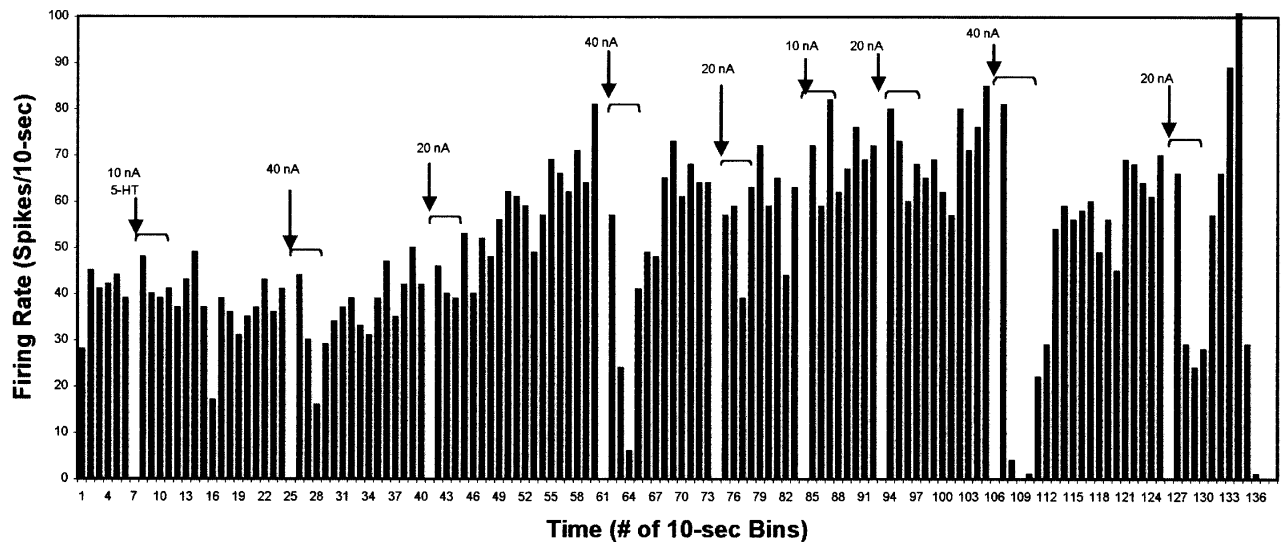


**Figure 3** Current-response bar graph demonstrating inhibition of spontaneous activity in 12 CeA neurons by microiontophoretically applied 5-HT. Bars represent mean percent change in firing rates from baseline (predrug)  $\pm$  SEM at three ejection currents: 10 nA (*left*), 20 nA (*middle*), and 40 nA (*right*). Each of the 12 neurons contributing to the current-response bars received multiple challenges with microiontophoretically applied 5-HT at each of the three ejection currents (see Fig. 4, below).

ade of presynaptic 5-HT<sub>1A</sub>-autoreceptors (Kreiss and Lucki 1994) on terminals projecting from the dorsal raphe to the CeA (Tork 1988; Sotelo et al. 1990) would be expected to inhibit CeA activity (by increasing release of serotonin onto CeA neurons). The net effect of systemic NAN-190 administration on activity of a single CeA neuron—*increase, decrease, or no change*—would

then depend on the relative contributions of the presynaptic 5-HT<sub>1A</sub> autoreceptors and postsynaptic 5-HT<sub>1A</sub>-receptors influencing the firing rate of the individual neuron.

One could test this model by restricting 5-HT<sub>1A</sub> blockade to the CeA via local administration of NAN-190: the drug should increase CeA firing rates because there would be no opposing



**Figure 4** Rate histogram showing the current-response relationship of an individual CeA neuron to microiontophoretically applied 5-HT. The horizontal brackets above the histogram indicate the duration of current applied to the 5-HT containing pipette; numbers above the brackets indicate the amount of ejection current.

effect of blockade of inhibitory afferents influencing spontaneous CeA activity (assuming no 5-HT<sub>1A</sub> autoreceptors reside within the CeA). Since intra-amygdala administration of NAN-190 does not enhance retention (Bernabeu et al. 1997; Bevilacqua et al. 1997a,b), such a finding would lend further support to a dissociation between CeA activity and serotonergic modulation of retention.

It appears, then, that a tonically active 5-HT<sub>1A</sub>-mediated modulatory system inhibiting retention, when blocked, is not associated with increased activity of the CeA. This contrasts with previous reports that blockade of a tonically active  $\beta$ -adrenergic modulatory system inhibiting retention is clearly associated with increased activity of the CeA (Simson et al. 2001). Specifically, using behavioral and electrophysiological procedures identical to those in the present study, we reported that—like NAN-190—systemic administration of the  $\beta$ -adrenergic receptor antagonist propranolol enhances retention in the passive-avoidance task (Schneider et al. 2000); unlike NAN-190, however, systemically administered propranolol consistently increased CeA activity (Simson et al. 2001).

Although propranolol has 5-HT<sub>1A</sub> antagonist properties (Middlemiss 1984; Oksenberg and Peroutka 1988; Kreiss and Lucki 1994), the present finding that NAN-190 did not increase CeA firing rates discounts the possibility that propranolol increased CeA activity through 5-HT<sub>1A</sub>, rather than  $\beta$ -adrenergic, blockade. This does not rule out the possibility, however, that propranolol's effect on retention, in contrast to its effect on CeA activity, is mediated by 5-HT<sub>1A</sub>-blockade: propranolol's ability to enhance retention (Schneider et al. 2000), like NAN-190's, may depend on neither blockade of the  $\beta$ -adrenergic modulatory system nor increased activity of the CeA.

Delayed administration of NAN-190 failed to enhance retention in the passive-avoidance task, thus supporting memory modulation as the means by which NAN-190 enhanced retention when administered immediately after training. However, in addition to showing that systemically administered NAN-190 failed to modulate retention when administered 2 h after training, the present findings also indicate that the 5-HT<sub>1A</sub> antagonist failed to enhance retention when administered 6 h after training. This latter finding is of particular interest given that, when applied locally in the hippocampus 6 h after training, NAN-190 enhances retention (Bernabeu et al. 1997; Bevilacqua et al. 1997a,b). Of course, blocking 5-HT<sub>1A</sub> receptors through systemic administration of NAN-190 is vastly different than doing so through local administration. Indeed, that NAN-190 in our hands enhances retention when administered immediately, but not 6 h, after training may well be due to ambiguous signals when the hippocampus and amygdaloid sites modulating memory, by virtue of systemic drug administration, are concurrently affected at a critical time period (i.e., immediately but not 6 h after training). Other aspects of methodology differed between Bevilacqua et al.'s study (1997a,b) and the present study, as well. For example, although both studies used inhibitory-avoidance training, Bevilacqua et al. used platform training and we used light-dark training. Bevilacqua et al. necessarily cannulated animals—and we as well as others have shown that cannulae-produced brain damage in and of itself affects retention in an inhibitory-avoidance task (Gold et al. 1975, 1978; Lennartz et al. 1996; Liang 1999; Schneider et al. 2002). Finally, wattage of shock actually received by each animal was not monitored in the Bevilacqua et al. study; differences among groups could have resulted in false positives and/or false negatives.

In this regard, wattages of shock received by animals from a constant-current shock source varied in the present study. These variations in wattage received, presumably due to small variations in current or the resistance of the animal, were highly cor-

related with retention in the passive-avoidance task. Although not a factor in the present experiments, these results indicate that, if by chance shock wattage were to differ among treatment groups, animals might well show different strengths of retention as a result of different reactions to shock, and not different reactions to treatment. To avoid such a potential confounding, studies using drugs and low levels of shock to investigate memory-related processes such as enhancement of retention should routinely monitor wattage received by animals, regardless of whether drugs are administered prior to or after training.

## MATERIALS AND METHODS

### Behavioral Experiment: Retention of the Passive-Avoidance Task

#### *Experimental Design*

The rats were randomly assigned to one of four groups: vehicle, NAN 0.5 mg/kg, NAN 0.75 mg/kg, and NAN 1.0 mg/kg. They were then trained and immediately given their designated treatment. Because the highest (1.0 mg/kg) but not the lower doses (0.5, 0.75 mg/kg) of NAN-190 was found to increase retention, two control experiments were conducted.

In one experiment, for the assessment of the time-dependent effects of the higher dose of NAN-190, animals received vehicle or NAN-190 (1 mg/kg) 2 h or 6 h after the training trial. Ordinarily, a 2-h delay group is sufficient to assess time-dependent effects (Schneider et al. 2000) but, because there is evidence that NAN-190 injected locally into the hippocampus 6 h after training enhances retention (Bernabeu et al. 1997; Bevilacqua et al. 1997a,b), a 6-h group was included to determine whether similar effects occur with systemically administered NAN-190.

In a second experiment, for the assessment of the potentially aversive effect of the higher dose of NAN-190 in and of itself, animals received vehicle or NAN-190 (1 mg/kg) immediately after the training trial in the absence of shock (no-shock control groups).

#### *Animals*

The subjects ( $n = 78$ ) were male Long-Evans hooded rats (obtained from Harlan Sprague Dawley) weighing 250–325 g at the start of the experiment. The rats were housed two to a cage with access to food and water ad libitum. The colony room was maintained at 20°C and was illuminated on a 12-h light-dark cycle (lights on at 9:00 a.m.). Each rat was handled daily for 15 sec and was in the laboratory for at least 9 d, but not more than 18 d, before the start of the experiment. All experiments were conducted between 10:00 a.m. and 3:00 p.m.

#### *Apparatus*

The rats were trained in a standard trough-shaped passive-avoidance apparatus that consisted of a small lighted compartment (20 × 28 × 8 cm), illuminated by a 95-W bulb, connected to a larger dark compartment (20 × 28 × 42 cm). A manually operated sliding door separated the two compartments. The top of each compartment was hinged, and the floor of each compartment was made of stainless steel plates. A constant-current Lafayette Master Shocker (Model 2400SS) was connected to the floor of the large compartment. The apparatus was located in a quiet, dimly illuminated room.

#### *Drug Administration and Drug Doses*

The rats were injected intraperitoneally with 1.0 mL of vehicle or NAN-190 (Sigma Chemical). The vehicle was comprised of 25% DMSO and 75% saline (0.9%). The doses of NAN-190 used were 0.5 mg/kg (NAN 0.5), 0.75 mg/kg (NAN 0.75), and 1.0 mg/kg (NAN 1.0), dissolved in vehicle to a concentration of 0.5, 0.75, or 1.0 mg/mL, respectively. These doses of NAN-190 have been shown to be behaviorally relevant (King et al. 1993; Hashimoto et al. 1997).



### Training/Testing Procedure

The animals received a single training trial and a single test trial the next day. On the training trial, all animals (except those in the no-shock control groups) received shock (0.36 mA, 1-sec duration) for stepping from the lighted to dark compartment. STLs on the training trial provided a measure of the animals' inherent (i.e., baseline) aversion to the dark compartment. STLs on the test trial provided a measure of the animals' learned aversion of the dark compartment (i.e., a measure of retention). NAN-190 or vehicle was administered immediately, 2 h, or 6 h after the training trial.

The training trial consisted of the following: Each rat was placed in the lighted compartment facing away from the sliding door. After 15 sec the door was raised, the animal was allowed to step into the dark compartment, the door was lowered and shock (if the animal was not in a no-shock control group) was delivered to the floor of the compartment. The animal remained in the dark compartment for 15 sec and was then removed and administered NAN-190 or vehicle. After each animal completed the trial, the apparatus was cleaned.

The test trial was identical to the training trial except that shock was omitted and drug was not administered. STLs on the test trial served as the measure of retention, that is, as the STLs increased, retention was taken to increase. If an STL reached 300 sec, the trial was terminated and the animal was retired from the experiment. It should be noted that only four animals (one from the NAN 0.5 group, two from the NAN 0.75 group, and one from the NAN 1.0 group) reached the 300-sec cutoff.

### Monitoring Wattage of Shock Actually Received

Preliminary observations indicated that even though shock current of 1-sec duration was set at 0.36 mA for all animals, there were variations during training in the wattages received by animals. This raised the possibility that wattages received might differ, purely by chance, among the groups; differences in reactions to shock resulting from differences in wattage received—instead of, or in addition to, drug effects—might then account for differences in retention. Consequently, the wattage (time integral of instantaneous current  $\times$  voltage) received by each animal was monitored via a custom-designed LabView computer program and circuit board (PCI 6023E National Instruments).

### Statistics

Data were analyzed with Dunnett t-tests (when comparing multiple treatment groups to a control group), Student's t-test, or one-way analyses of variance (ANOVAS) followed by protected-t tests. *P*-values (two-tailed unless otherwise noted) of less than .05 were taken as significant.

## Electrophysiological Experiment: Spontaneous Single-Unit Activity of CeA Neurons

### Preparation of Animals

Animals ( $n = 21$ ) were male Long Evans hooded rats (Harlan Sprague Dawley) weighing 250–400 grams. Stereotaxic surgery was performed under urethane anesthesia (4.0 g/kg, i.p.). After adjusting the incisor bar so that bregma and lambda suture landmarks lay in the same horizontal plane (skull flat), a burr hole was made 2.1 mm posterior to bregma and 4.0 mm lateral to the sagittal suture.

### Single-Unit Recordings

When administering NAN-190 systemically, a single-barrel glass recording electrode was produced by pulling a single-barrel micropipette (1.5 mm outside diameter; A&M Systems). The tip of the pipette was then broken back to approximately 1.0  $\mu$  to obtain a recording impedance of 5–10 M $\Omega$ .

When administering 5-HT microiontophoretically, the single-barrel recording electrode (above) was attached to a four-barrel micropipette with epoxy cement such that the recording electrode protruded 20  $\mu$  beyond the four-barrel pipette. Once the epoxy had set, one of the barrels of the four-barrel micropi-

pette was filled with a solution of 5-HT dissolved in distilled water to a concentration of 50 mM, and a second barrel (for the current balance channel) was filled with 0.9% saline.

Whether administering drug systemically or iontophoretically, the recording electrode was filled with 0.9% NaCl saturated with Sky Blue dye. The recording electrode (and four-barrel pipette assembly for iontophoresis) was then advanced into the CeA via a hydraulic microdrive (Trent Wells). CeA neurons were found at a depth of 6.5–8.0 mm below the dura at the coordinates presented above. Action potentials from spontaneously firing, single units were amplified by a high-impedance preamplifier and a secondary amplifier (Fintronics), and then filtered and displayed on a Tektronix oscilloscope after being processed through a window discriminator (Fintronics). Signals from the amplifier also drive an audiomonitor. Individual spikes were isolated by the window discriminator, then integrated over 10-sec periods by a data collection program (Brainstorm Systems) and displayed in real time on a computer monitor. Data were also stored on computer in real time. Interspike intervals were recorded to determine whether the pattern of neural firing remained consistent.

Neurons were isolated at random and were not preselected based on firing rate, with one exception: a small percentage (fewer than 10%) of neurons in the CeA displayed a strongly rhythmic, bimodal bursting firing pattern. Neurons displaying this rhythmically bursting firing pattern—a pattern described by others and encountered similarly infrequently (Martina et al. 1999)—were not recorded from in the present study. Neurons recorded from the CeA fired at a steady rate ranging between 4.4 and 13.6 spikes/sec, with a mean rate of  $8.9 \pm 0.84$  spikes/sec. Although difficult to encounter and isolate, waveforms were exceptionally homogenous and mostly positive-going, and of only moderate amplitude. Only neurons meeting a criterion of at least a 3:1 signal/noise (i.e., signal to background activity) ratio during the baseline recording period were used. Only a single neuron was recorded from each rat to avoid residual drug effects. Neurons showing signs of injury or marked irritability during the recording procedure, as evidenced by marked alterations in waveform and/or amplitude, were not included in the study. If a neuron showed signs of injury after drug administration, the experiment was terminated, the rat was sacrificed, and the data were discarded.

As neurons of the CeA were difficult to hold longer than 40–45 min (i.e., movement of the electrode and/or brain eventually resulted in smaller-than-optimal signal/noise ratios), recordings were limited to a 35-min postdrug recording period.

### Drug Administration

Animals ( $n = 9$ ) were injected intraperitoneally with vehicle or 1.0 mL of NAN-190 (Sigma Chemical). The vehicle was comprised of 25% DMSO and 75% saline (0.9%). NAN-190 was administered systemically at a dose of 1 mg/kg because these were the dose and route utilized in the behavioral experiment demonstrating significant facilitation of retention.

Animals ( $n = 12$ ) received microiontophoretic application of 50 mM 5-HT (Sigma Chemical) at ejection currents of 10 nA, 20 nA, and 40 nA.

### Histology

Histological verification of electrode placement was obtained by passing 5  $\mu$ A of negative-going current through the recording electrode for 5 min, thereby depositing a spot of Sky Blue at the electrode tip. The brains were removed, frozen and cut in 20- $\mu$  sections, and then analyzed for placement of the dye deposit. Only animals from which recording electrodes were verified to be located in the CeA were included in the study.

## ACKNOWLEDGMENTS

This work was supported by grants from the Howard Hughes Medical Institute and Eugene M. Lang Professorship to A.M.S. and the Alcoholic Beverage Medical Research Foundation to P.E.S.

The publication costs of this article were defrayed in part by

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Received August 23, 2002; accepted in revised form February 25, 2003.