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NAN-190 potentiates the impairment of retention produced by swim stress ☆

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Abstract

Exposing rats to stress in the form of forced swim immediately after passive-avoidance training impaired retention. In contrast, exposure to the same stressor 2 h after training failed to impair retention. Systemic administration of the 5-HT_{1A} receptor antagonist NAN-190 (1 mg/kg) immediately after forced swim markedly potentiated the stress-induced impairment of retention. In contrast, NAN-190 failed to affect retention when administered 2 h after forced swim or in forced swim's absence. These findings provide evidence for a NAN-190-sensitive system modulating retention that is 1) activated during a critical period shortly after exposure to swim stress, and 2) protective of memory, thereby limiting the extent to which retention is impaired by experiential stress.

Keywords: Memory; Passive-avoidance; Swim-stress; Amygdala; Serotonin

1. Introduction

Neurobehavioral studies indicate that stress affects retention (Cahill et al., 2004; de Quervain et al., 1998; Klenerova' et al., 2003; Lui et al., 1999; Roozendaal, 2002; Shor, 2001; Woodson et al., 2004). One brain site implicated in the modulation of retention by stress is the amygdala (McGaugh, 2002), wherein agonists of the stress-related biogenic amine norepinephrine (NE) increase amygdala activity (McIntyre and Wong, 1986; Stoop et al., 2000) and enhance retention in the passive-avoidance procedure (Ferry and McGaugh, 1999; Introini-Collison et al., 1991; Liang et al., 1986).

An association between modulated strength of retention and altered neuronal activity within the amygdaloid complex is not limited to noradrenergic agents. The stress-related biogenic amine serotonin (5-HT) is also released in the amygdala during stress (Amata et al., 1998; Kawahara et al., 1993) and also modulates the strength of retention. However, whereas NE agonists enhance retention in the passive-avoidance procedure and increase amygdala activity, 5-HT agonists impair retention in the passive-avoidance procedure when administered systemically (Carli et al., 1992; Misane and Ogren, 2000; Santucci and Shaw, 2003) or via direct infusion into the amygdala (Liang, 1999). Further, 5-HT agonists suppress amygdala activity when applied microiontophoretically throughout a wide range of ejection currents (Schneider et al., 2003b; Stutzmann et al., 1998; Stutzmann and LeDoux, 1998). These findings suggest the existence of an inhibitory 5-HT-mediated system that works in opposition to an excitatory NE-mediated system to modulate amygdala activity and, ultimately, retention.

One prediction arising from a hypothesis involving opposing NE-based and 5-HT-based modulatory systems stems from the fact that pharmacological over-activation of excitatory adrenergic systems impairs retention via a well-established inverted U-shaped dose–response function, wherein low doses of adrenergic agents have no effect on retention, intermediate doses enhance retention and high doses impair retention (Gold, 2006; Gold and

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van Buskirk, 1975, 1978a,b; Koob, 1991; Liang et al., 1990). Specifically, we hypothesize that under stressful conditions sufficient to over-activate the excitatory adrenergic systems (and thus potentially impair retention), a protective inhibitory 5-HT-based modulatory system is concurrently activated. The presence of this stress-induced inhibitory system, in contrast to its absence in pharmacological studies using sympathomimetics, provides an opportunity for the inhibitory system to modulate retention by reducing the extent to which over-activation of excitatory systems impairs retention. If this hypothesis is correct, then the protective contribution of this stress-induced inhibitory 5-HT-based modulatory system should be uncovered through pharmacological blockade. That is, the blockade-induced absence of this 5-HT-based modulatory system should be evidenced by a potentiation of impaired retention.

Accordingly, in the present study direct exposure to experiential stress was combined with administration of the 5-HT_{1A} antagonist NAN-190 to investigate the potential role of an inhibitory serotonergic memory-modulation system in mediating the effect of stress on retention. In particular, using a procedure in which forced-swim stress impairs retention (presumably through over-activation of an excitatory memory-modulation system), we sought to determine if there exists a concurrently activated 5-HT_{1A}-based system that is protective of memory. We predicted that blockade of this inhibitory system by NAN-190 should, by allowing for *further* over-activation of memory-related brain sites, further impair retention.

2. Method

The experimental protocol was approved by Swarthmore College's *Institutional Animal Care and Use Committee* and was in compliance with the *National Institutes of Health Guide for Care and Use of Laboratory Animals* (Publication No. 85-23, revised 1985). In the four experiments to follow, animals received one-trial passive-avoidance training (in which they received a single foot-shock for stepping from a lighted to dark compartment) followed (with the exception of animals in control groups) by exposure to forced swim. The next day, animals underwent a retention test. Since forced swimming administered immediately after passive-avoidance training produces varying effects depending on any number of experimental factors–the stressor has been reported to impair retention in mice (Jodar et al., 1995) and enhance retention in rats (Flint et al., 1997)–the present study first established the effect of forced swimming on retention in our hands. After determining that forced swimming administered immediately after training *impairs* retention in the passive-avoidance procedure (Experiments 1 and 2), forced swim stress in combination with pharmacological intervention was employed to investigate the potential role of a serotonergic memory-modulation system on retention (Experiments 3 and 4).

2.1. Subjects

The subjects ($n=134$) were male Long–Evans hooded rats (obtained from Harlan Sprague Dawley, Indianapolis, IN) weighing 240–280 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 30 s and was in the laboratory for at least 8 days, but not more than 16 days, before the start of the experiment. All experiments were conducted between 10:00 a.m. and 12:00 p.m.

2.2. Apparatus

The rats were trained in a standard trough-shaped passive-avoidance apparatus that consisted of a small lighted compartment (20W × 28H × 18L cm at the top; 8W × 28H × 18L cm at the base) illuminated by a 95 W bulb, connected to a larger dark compartment (20W × 28H × 42L cm at the top; 8W × 28H × 42L cm at the base). 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; Lafayette, IN) was connected to the floor of the large compartment. The apparatus was located in a quiet, dimly illuminated room.

2.3. Training/testing pocedure

As previously described (Schneider et al., 2003b), the animals received a single training trial and a single test trial the next day. In each case the animals were transported to the training–testing room in a new cage while their littermates were left in their home cage.

On the training trial, all animals received shock (0.5 mA, 0.5 s duration) for stepping from the lighted to dark compartment. Step-through latencies (STLs) on the training trial provided a measure of the animals' *inherent* (i.e., baseline) aversion to the dark compartment. Step-through latencies on the test trial provided a measure of the animals' *learned* aversion of the dark compartment (i.e., a measure of retention). The shock parameters were based on findings of an earlier study in which the effect of swim stress on retention was related to the intensity of shock during training: as intensity increased, the effect of swim stress on retention decreased (Schneider et al., 2003a).

The training trial consisted of the following: Each rat was placed in the lighted compartment facing away from the sliding door. After 15 s the door was raised, the animal was allowed to step into the dark compartment, the door was lowered and shock was delivered to the floor of the compartment. The animal was left in the dark compartment for 15 s, then removed and immediately administered the experimental treatment (except for Experiment 2, in which in one group there was a 2-hr delay before treatment). After each animal completed the trial the apparatus was cleaned.

The test trial was identical to the training trial except shock was omitted and the experimental treatment was not administered. Step-through latencies on the test trial served as the measure of retention, i.e., as STLs increased, retention was taken to increase. If STLs reached 600 s, the trial was terminated and the animal was retired from the experiment.

2.4. Monitoring wattage received

Despite the use of a constant-current shock source, differences exist among animals in the behavior that they exhibit in response to shock (e.g., pausing, flinching, running). Preliminary investigation showed these differences were highly correlated with wattage of shock received (owing, for example, to individual differences in resistance and/or small, unavoidable variations in shock current). In the typical one-trial passive-avoidance procedure utilizing relatively high levels of shock, the effect on the behavioral response–and thus on the experimental outcome–of these relatively small variations in wattage received by animals is insignificant. However, these wattage differences (and the resultant differences in behavioral responses to shock) can become significant when delivering low to moderate levels of shock. For example, Schneider et al. (2003a) have reported that variations in wattage produced by low to moderate levels of shock were positively correlated with strength of retention; furthermore, as wattage of shock received by animals increased, the effect of swim stress on retention decreased.

Consequently, to control for the possibility that differences in wattage received by animals factored into differences in strength of retention among groups in the present experiments–that is, to rule out the possibility that differences in shock reaction instead of, or in addition to, swim and/or drug effects could account for differences in strength of retention among groups–the wattage received by individual animals was recorded. Specifically, the time integral of instantaneous current × the voltage of shock received by each animal was monitored via a custom-designed LabView computer program and circuit board (PCI 6023E National Instruments, Austin, TX). In the present study, wattages received did not differ among groups within any of the four experiments; thus, variations in wattage received were not a factor in the present experiments.

2.5. Forced-swim procedure

Forced swimming was used as the stressor because its neurochemical and hormonal effects are well defined and meet the criteria of a stress-inducing agent. Animals exposed to forced-swim stress of short durations show elevated levels of plasma corticosterone (Ahmed et al., 2006; Kirby et al., 1997; Rittenhouse et al., 2002) and significant alterations in NE levels throughout the amygdala, medial prefrontal cortex, and other limbic nuclei (Gotoh et al., 1998; Jordan et al., 1994). In addition to these effects on glucocorticoids and sympathomimetics, forced-swim stress has also been shown to target the serotonergic system; specifically, forced swimming alters 5-HT release (Adell et al., 1997; Linthorst et al., 2002; Roche et al., 2003) and 5-HT_{1A} receptor function in the amygdala and hippocampus (Briones-Aranda et al., 2005). Thus, exposure to forced swimming not only meets the criteria of a stressor, but produces neurochemical effects consistent with a potential modulator of retention.

The forced-swim procedure occurred in a quiet, dimly lit room and consisted of placing rats in a cylindrical tank (46 cm tall × 20 cm in diameter) with water (~20 °C) filled to a depth of 30 cm. The water depth of 30 cm forced the rats to swim or float without their tails touching the bottom of the tank. All experiments included a *No Swim* control group in which animals were, in lieu of exposure to swim stress, placed in a quiet, dimly lit room before being returned to the animal colony. An immersion-in-water group (unique to Experiment 2) was placed in the cylindrical tank with water (~20 °C) filled to a depth of 4 cm, thereby allowing the animals to stand in water but not swim. Since immersion-in-water, unlike forced swim, does not appear to be associated with significant increases in glucocorticoids (Curtis et al., 1999), animals in this group served to control for factors (e.g., exposure to a novel situation) other than the *stress* of forced-swim affecting retention.

2.6. Drug administration and drug doses

The rats were injected intraperitoneally with vehicle or the 5-HT_{1A} antagonist NAN-190 (Sigma Chemical). The vehicle was comprised of 25% DMSO and 75% saline (0.9%). The doses of NAN-190 used–0.5 mg/kg, 1.0 mg/kg, or 2.0 mg/kg dissolved in vehicle to a concentration of 0.5, 1.0 or 2.0 mg/ml, respectively–have been shown to be behaviorally relevant in that doses in this range affect locomotion, freezing behavior, and modulation of memory (Hashimoto et al., 1997; King et al., 1993; Schneider et al., 2003b).

As with other systemically administered putative 5-HT_{1A} antagonists, NAN-190's blockade of postsynaptic 5-HT_{1A} receptors should be offset to some degree by increased serotonin transmission resulting from concurrent blockade of inhibitory presynaptic 5-HT_{1A}-autoreceptors at the dorsal raphe nucleus and its projections (Mundey et al., 1996). For this reason, the dosages of NAN-190 employed in the present study were centered around 1.0 mg/kg—this dosage was previously shown to enhance retention in the absence of swim stress, most plausibly through net blockade of (inhibitory) postsynaptic 5-HT_{1A} receptors (Schneider et al., 2003b).

2.7. Statistics

Data were analyzed with one-way analyses of variance (ANOVAS) as well as protected-t and Dunnett multiple comparison tests. p values (two-tailed) of less than 0.05 were taken as statistically significant.

3. Results

3.1. Experiment 1: Exposure to forced-swim stress impaired retention in the passive-avoidance task

The effect of forced swimming on retention was determined in the passive-avoidance task. Animals were trained and, immediately after training, randomly assigned to one of three groups: *No Swim*, *5-min Swim*, and *15-min Swim*. Animals in the two experimental groups (*5-min Swim; 15-min Swim*) were then immediately exposed to forced-swim stress. Animals in the *No Swim* control group were, in lieu of exposure to swim stress, placed in a quiet, dimly lit room for 5 or 15 min before being returned to the animal colony.

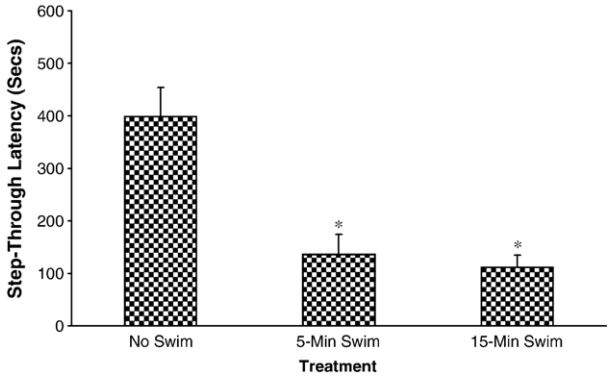

Fig. 1. Exposure to forced swim impaired retention in the passive-avoidance task. Mean STLs (±SEM) on the test trial for the No Swim group (n=7), 5-min Swim group (n=9) and 15-min Swim group (n=9). *p<0.01 compared to *No Swim*.

While there were no significant differences in STLs among any of the groups on the training trial (F(2,22)=1.41, p=0.26), exposure to forced swim significantly impaired retention in the passive-avoidance task on the test trial (F(2,22)=15.40, p< 0.01). As shown in Fig. 1, mean STLs on the test trial were significantly lower in both the *5-min Swim* (136.4 s±37.8; Dunnett-t (3,22 df)=4.76, p<0.01) and *15-min Swim* (111.2 s± 23.4; Dunnett-t (3,22 df)=5.22, p<0.01) groups when compared to the *No Swim* group (398.3 s±55.9).

3.2. Experiment 2: Neither immersion in water in the absence of swimming nor delayed forced swim impaired retention in the passive-avoidance task

The results of Experiment 1–that exposure to forced-swim stress impaired retention in the passive-avoidance task–do not preclude the possibility that STLs were reduced in the experimental groups through means other than exposure to the stressor of swim per se, and other than through disruption of memory modulation. For instance, exposure to water in and of itself (e.g., as a result of its novelty) might be sufficient to reduce STLs in a subsequent retention test; similarly, STLs might be reduced through any number of means unrelated to modulation of memory. If, on the other hand, forced-swim stress did in fact impair retention in Experiment 1 via disruption of memory modulation, then delaying exposure to the stressor beyond the critical period shortly after training when strength of retention is modulated (as opposed to presenting the stressor shortly after training during the critical period of modulation as in Experiment 1) should not impair retention. Similarly, if the stressor of forced swimming, and not simply exposure to water per se, is required to impair retention, then immersion in water in the absence of forced swimming should not impair retention.

In the following control experiment, animals received passive-avoidance training and then, immediately after training, were randomly assigned to one of four groups: 1) *No Swim*, 2) *Immediate Swim*, 3) *2-hr Delay Swim*, and 4) *Immersion-in-Water* in the absence of swim. Animals assigned to the *No Swim* group were, in lieu of exposure to swim stress, immediately placed in a quiet, dimly lit room for 5 min before being returned to the animal colony. Animals assigned to the *Immediate Swim* group underwent 5 min of forced swim. Animals assigned to the *2-hr Delay Swim* group (i.e., those exposed to swim stress after the critical period of post-training memory-modulation had presumably passed) were immediately returned to the animal colony; 2 h later they underwent 5 min of forced swim. Animals assigned to the *Immersion-in-Water* group were immediately placed in the swim tank for 5 min, but, because the tank was filled with water to a level of only 4 cm, the animals could stand but not swim.

As expected, on the training trial there were no significant differences in STLs among any of the groups (F(3,25)=0.28, p=0.84). On the test trial (Fig. 2), neither the *2-hr Delay Swim* group nor the *Immersion-in-Water* group showed impaired retention: mean STLs in the *2-hr Delay Swim* group (417.6 s± 63.2) were not significantly different from those in the *No Swim* group (310.6±78.5; Dunnett-t (4,25 df)=1.19, p>0.05), but were significantly greater than those in the *Immediate Swim* group (95.0 s±42.6; Dunnett-t (4,25 df)=3.58, p<0.01); mean STLs in the *Immersion-in-Water* group (373.9 s±64.3) were not significantly different from those in the *No Swim* group (Dunnett-t (4,25 df)=0.49, p>0.05), but were significantly greater than those in the *Immediate Swim* group (Dunnett-t (4,25 df)=3.10, p<0.01).

3.3. Experiment 3: NAN-190 potentiated impairment of retention produced by forced-swim stress

If an inhibitory serotonergic memory-modulation system is in fact activated by exposure to swim stress, thereby decreasing the extent to which retention in a subsequent passive-avoidance test is impaired, then NAN-190, to the extent that it blocks postsynaptic 5-HT$_{1A}$ receptors, might be expected to at least partially block this modulatory system and further impair retention. To test this hypothesis, animals received passive-avoidance training and then, immediately after training, underwent 5-min of forced-swim stress (or, if randomly assigned to the *No Swim* control group, bypassed exposure to swim stress). Animals exposed to forced-swim stress were then

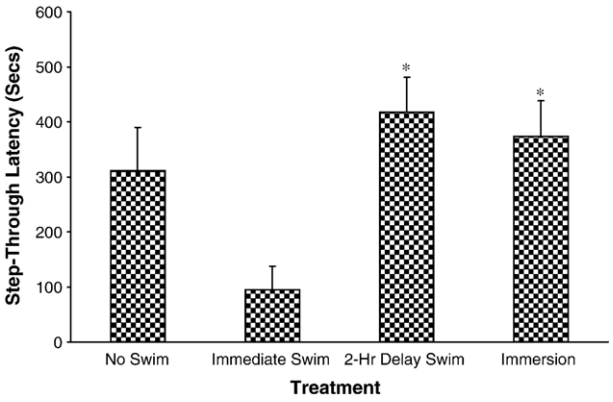

Fig. 2. Neither immersion in water in the absence of swimming nor delayed forced swim impaired retention in the passive-avoidance task. Mean STLs (±SEM) on the test trial for the No Swim group (n=7), Immediate Swim group (n=7), 2-hr Delay Swim group (n=8) and Immersion-in-Water group (n=7). *p<0.01 compared to *Immediate Swim*.

randomly assigned to one of four subgroups–*Swim-Vehicle*, *Swim-NAN 0.5*, *Swim-NAN 1.0*, and *Swim-NAN 2.0*–that received vehicle or the 5-HT_{1A} blocker NAN-190 (at a dose of 0.5, 1.0 or 2.0 mg/kg, i.p.) *immediately after the forced swim procedure*. Animals in the *No Swim* control group were, in lieu of exposure to swim stress, placed in a quiet, dimly lit room for 5-min, after which they received an injection of vehicle.

On the training trial, none of the groups differed significantly in STLs ($F(4,39)=1.41$, $p=0.25$). In contrast, on the test trial (Fig. 3), compared to the *No Swim* control group, STLs in each of the experimental groups decreased significantly ($F(4,39)=6.17$, $p<0.01$). Moreover, while mean STLs in the *Swim-NAN 0.5* group (198.6 s±49.4) and the *Swim-Vehicle* group (230.6 s± 63.0) did not differ significantly (Dunnett-*t* (5,39)=0.46, $p>0.05$), mean STLs in the *Swim-NAN 1.0* group (79.2 s± 19.3) were significantly lower than those in the *Swim-Vehicle* group (Dunnett-*t* (5,39)=2.24, $p<0.05$), indicating that the 5-HT_{1A} blocker potentiated the impairment of retention produced by swim stress. Importantly, the ability of NAN-190–at the 1.0 mg/kg dose–to potentiate the impairment of retention produced by swim stress did not extend to the highest dose tested: the *Swim-NAN 2.0* group did not differ significantly from the *Swim-Vehicle* group (Dunnett-*t* (5,39)=0.09, $p>0.05$).

3.4. Experiment 4: NAN-190 neither impaired retention in the absence of forced-swim stress nor potentiated impairment when administered 2-h after forced-swim stress

If NAN-190 potentiated impairment of retention in the passive-avoidance task (Experiment 3) by altering the effect of exposure to forced-swim stress on memory modulation, then NAN-190 should *not* impair retention in the absence of forced-swim stress nor should it potentiate impairment when administered after the critical memory-modulation period has passed (e.g., 2 h after exposure to swim stress).

Animals received passive-avoidance training and were then randomly assigned to one of five groups: two no-swim groups and three 5-min swim groups. Animals in the three swim groups received either vehicle or NAN-190 (1.0 mg/kg) immediately after forced swimming or NAN-190 (1.0 mg/kg) 2 h after forced swimming. Animals in the two no-swim groups were, in lieu of exposure to swim stress, placed in a quiet, dimly lit room for 5 min immediately after training; they then received either vehicle or NAN-190 (1.0 mg/kg).

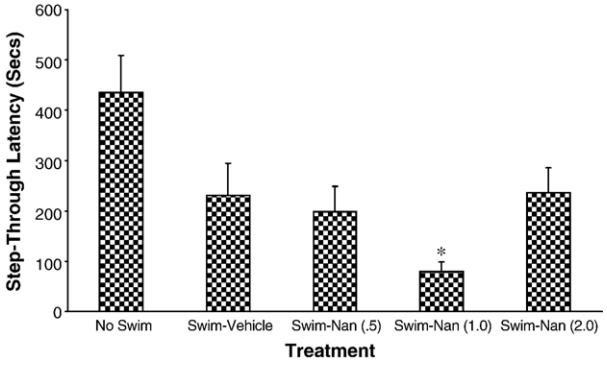

Fig. 3. NAN-190 (1.0 mg/kg) potentiated the impairment of retention produced by forced swimming. Mean STLs (±SEM) on the test trial for the No Swim group ($n=7$), Swim-Vehicle group ($n=8$), Swim-Nan (0.5) group ($n=7$), Swim-NAN (1.0) group ($n=11$) and Swim-NAN (2.0) group ($n=11$). *$p<0.05$ compared to *Swim-Vehicle*.

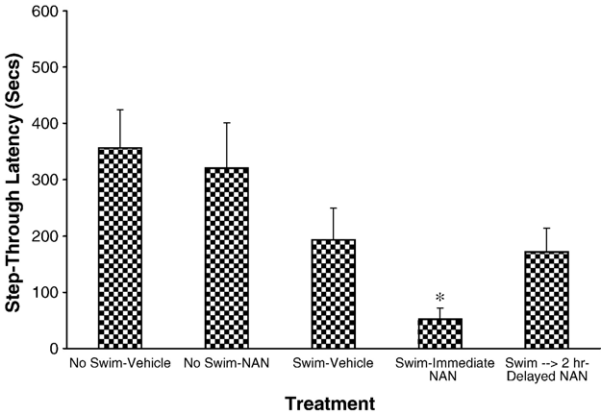

Fig. 4. NAN-190 (1.0 mg/kg) neither impaired retention in the absence of forced swim nor potentiated impairment when administered 2 h after forced swim. Mean STLs (±SEM) on the test trial for the No Swim-Vehicle group ($n=7$), No Swim-NAN group ($n=7$), Swim-Vehicle group ($n=7$), Swim-Immediate NAN group ($n=8$), and Swim-2 hr-Delayed NAN group ($n=8$). *$p<0.02$ compared to *Swim-Vehicle*.

None of the groups differed significantly in STLs on the training trial ($F(4,30)=0.47$, $p=0.76$). On the test trial, as shown in Fig. 4, NAN-190 failed to affect retention when administered in the absence of forced swim: not only were mean STLs in the *No Swim-NAN* group (320.0 s±80.6) not significantly different from mean STLs (356.0 s±68.0) in the *No Swim-Vehicle* control group (Dunnett-*t* (5,31)=0.45, $p>0.05$), but mean STLs in the *Swim-Immediate NAN* group (52.4 s± 20.0) were markedly lower (by 72.9%, replicating the 65.7% decrease observed in groups receiving the same treatments in Experiment 3; $t=2.59$, 13 *df*, $p<0.02$) than mean STLs in the *Swim-Vehicle* group (193.6 s±55.4).

Fig. 4 also shows that NAN-190 failed to affect retention when administered 2 h after exposure to forced swim: mean STLs in the *Swim-2 hr-Delayed NAN* group (172.0 s±42.5) were not significantly different (Dunnett-*t* (5,31)=0.27, $p>$ 0.05) from mean STLs in the *Swim-Vehicle* group (193.6 s± 55.4), providing evidence that impairment of retention by NAN-190 is indeed time-dependent and most likely the result of action during a critical period shortly after exposure to swim stress.

4. Discussion

The present study utilized exposure to a forced-swim stressor–combined with pharmacological blockade–to investigate the role of an inhibitory serotonergic memory-modulation system in mediating the effect of stress on retention. The results show that a) impairment of retention in the passive-avoidance procedure occurs when training is followed immediately, but not 2 h later, by exposure to swim stress, and b) the impairment is potentiated by NAN-190 when systemically administered

immediately, but not 2 h, after exposure to swim stress. These results provide evidence for an inhibitory memory-modulation system sensitive to blockade by NAN-190 that serves to limit the extent to which retention is impaired by exposure to stress. Furthermore, since NAN-190 produced neither impairment of retention in the absence of swim-stress nor potentiation of impairment when administered 2 h after swim-stress, it appears that blockade of this NAN-190-sensitive system at a critical period shortly after swim-stress–when strength of retention is modulated by exposure to stress–potentiates impaired retention.

The finding that NAN-190 potentiated stress-induced impairment of retention at the 1.0 mg/kg dose–but not the higher 2.0 mg/kg dose–is consistent with the neuropharmacology literature regarding NAN-190's intrinsically high potency and decreasing selectivity, as the dosage is increased, as an antagonist at the postsynaptic 5-HT_{1A} receptor (Greuel and Glaser, 1992; Sharp et al., 1996). Thus, at the highest dose used in the present study (2.0 mg/kg), the increasing contribution of the drug's agonist action (Greuel and Glaser, 1992; Sharp et al., 1996) may have been sufficient to offset the antagonist action responsible for potentiation of impaired retention. Alternatively, a sufficient blockade of α_1-adrenergic receptors (Claustre et al., 1991) at the 2 mg/kg dose might explain the lack of potentiation.

The deleterious effect of NAN-190 on retention in the present study suggests that a 5-HT_{1A}-mediated system plays a "protective" role in memory modulation by serving to decrease the extent of the impairment of retention produced by stress in the passive-avoidance procedure. The finding of such an inhibitory serotonergic memory-modulation system that opposes impairment of retention during stress may have implications for other memory-modulation systems shown to be inhibitory in the absence of stress. For example, opioid and GABA-ergic agonists, administered shortly after training, have been reported to impair retention (Castellano et al., 1989, 1990; Gallagher and Kapp, 1978). Like the NAN-190-sensitive system uncovered in the present study, however, the opioid and GABA-ergic memory-modulation systems (or subsystems mediated by their respective receptor subtypes) may protect retention during stress. If so, pharmacological blockers of these systems (or subsystems) should, like NAN-190 in the present study, potentiate impairment of retention produced by stress.

The amygdala has been strongly implicated in the modulation of memory by stress, particularly as it relates to the adrenergic system: sympathomimetics increase amygdala activity (McIntyre and Wong, 1986; Stoop et al., 2000) and enhance retention in the passive-avoidance procedure (Ferry and McGaugh, 1999; Ferry et al., 1999; Liang et al., 1986, 1990). In addition to the adrenergic system, the serotonergic system also appears to play a role in stress-induced modulation of memory via the amygdala. Indeed, serotonin is released in the amygdala during stress (Amata et al., 1998; Kawahara et al., 1993), and the central and lateral nuclei of the amygdala are inhibited by iontophoretically applied 5-HT agonists throughout a wide range of ejection currents (Schneider et al., 2003b; Stutzmann et al., 1998; Stutzmann and LeDoux, 1998). At the 5-HT receptor level, the central and basolateral nuclei of the amygdala contain high densities of various 5-HT receptor subtypes, including the NAN-190-sensitive 5-HT_{1A} receptor subtype (Kia et al., 1996; Narita et al., 2005; Rainnie, 1999; Riad et al., 2000; Tork, 1988; Verge' et al., 1986). The density of the 5-HT_{1A} receptor subtype, in particular, is significantly altered in the amygdala by exposure to stress (Briones-Aranda et al., 2005). Paralleling these receptor subtype findings are behavioral pharmacology studies showing that the 5-HT_{1A} receptor agonist buspirone impairs retention (Liang, 1999), and the 5-HT_{1A} receptor antagonists NAN-190 (Schneider et al., 2003b) and WAY-100635 (Liang, 1999) enhance retention, in the passive-avoidance procedure.

Yet, despite considerable evidence suggesting that blockade of 5-HT_{1A} receptors in the amygdala mediates the potentiation of impaired retention by NAN-190 in the present study, other brain sites may well be involved. For example, the hippocampus also plays a role in memory consolidation of the passive-avoidance task (Roozendaal and McGaugh, 1997) and this brain site, like the amygdala, also contains a high density of 5-HT_{1A} receptors (Patel and Zhoe, 2005; Burnet et al., 1995) that is altered by exposure to stress (Briones-Aranda et al., 2005). Further, hippocampal activity, like amygdala activity, appears to be regulated by the concurrent activation of adrenergic and serotonergic systems (Matsumoto et al., 1995; Tao and Hjorth, 1992). Thus, whether the amygdala, the hippocampus, or some other memory-related brain site mediates the attenuation of stress-induced impairment of memory demonstrated in the present study, excitatory and inhibitory modulation of retention at a single brain site via adrenergic and serotonergic action, respectively, is certainly plausible.

The functional significance of an inhibitory memory-modulation system becomes most apparent when viewed in terms of its combined action with the excitatory adrenergic system and the impaired retention that results from its over-stimulation (Gold, 2006; Gold and van Buskirk, 1978a,b; Koob, 1991; Liang et al., 1990). That is, an inhibitory modulatory system, if activated under stressful conditions, may serve to prevent–or reduce the extent of–over-activation of the amygdala produced by an excitatory NE-based modulatory system. In this model, depending on the relative levels of activity of the two opposing modulatory systems, one expects to find either a) less impairment of retention than would otherwise occur, owing to activation of the inhibitory system (i.e., in this scenario, the excitatory NE-based system is hypothesized to be activated by exposure to stress to such an extent that the amygdala is over-stimulated, resulting in impaired retention; concurrent activation of the inhibitory system reduces the extent of this over-stimulation and thus decreases the magnitude of the impaired retention), or b) lack of impairment–or even enhancement–of retention (i.e., in this scenario, the inhibitory system actually prevents over-stimulation of the amygdala by the excitatory NE-based system).

The results of the present study, in which swim-stress produced impairment of retention and in which NAN-190 potentiated the impairment, are in accordance with the first scenario above, wherein the NAN-190-sensitive system, activated by swim-stress, *decreases* the extent of over-stimulation of the amygdala (and thus the degree of impaired retention) produced by strong activation of the NE-based system. This model can similarly account for

the *enhancement* of retention by stress reported by others (Cahill et al., 2004; Lui et al., 1999), in that the results of these studies (in which stress, either in the form of exposure to cold water in humans or exposure to conditioned emotional stimuli in rats, enhances rather than impairs retention) are in accordance with the second scenario, wherein an inhibitory system prevents *over*-stimulation of the amygdala by an excitatory NE-based system.

In conclusion, the present results provide evidence for a NAN-190-sensitive system modulating retention that is activated by experiential stress (forced swimming) and is inhibitory in nature. These results suggest that a 5-HT_{1A}-mediated system, activated during stressful conditions, attenuates impairment of retention, possibly by protecting the amygdala from over-stimulation.

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