STRESS POTENTIATION OF MORPHINE-INDUCED DOPAMINE EFFLUX IN THE NUCLEUS ACCUMBENS SHELL IS DEPENDENT UPON STRESSOR UNCONTROLLABILITY AND IS MEDIATED BY THE DORSAL RAPHE NUCLEUS

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Abstract—A single session of uncontrollable (inescapable tailshock, IS), but not controllable (escapable tailshock, ES), stress is known to selectively potentiate subsequent morphine-conditioned place preference in a dorsal raphe nucleus (DRN) serotonin (5-HT) dependent manner. Here, in vivo microdialysis is used to test the hypothesis that prior IS, but not ES, will potentiate morphine-induced dopamine (DA) efflux in the nucleus accumbens (NAc) shell and that this will occur by a pathway involving DRN 5-HT neurons. Male Sprague–Dawley rats were exposed to yoked IS, ES, or no stress. Twenty-four hours later, morphine (3 mg/kg s.c.) or saline was administered during microdialysis. As predicted, prior IS selectively potentiated morphine-induced DA, but not 5-HT, efflux in the NAc. This potentiation was due to morphine’s action in the DRN because it was blocked by intra-DRN microinjections of 8-hydroxy-2-di-n-propylamino)-tetralin (10 μg). IS potentiation of morphine-induced DA efflux in the NAc was also dependent upon activation of 5-HT neurons in the DRN because it was blocked by intra-DRN microinjection of the 5-HT1a autoreceptor agonist 8-hydroxy-2-di-n-propylamino)-tetralin (1 μg). No effect of IS was found on morphine-induced 5-HT or DA efflux in the ventral tegmental area. These results suggest a neural substrate for stress potentiation of morphine reward involving 5-HT neurotransmission in the DRN. © 2004 IBRO. Published by Elsevier Ltd. All rights reserved.

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A central question of research on drug abuse is the extent to which experience can predispose an individual to addiction. One aspect of experience is exposure to stressors. Clinical research has demonstrated that exposure to stressors can increase an individual’s vulnerability to substance abuse (Breslau et al., 1991), and preclinical work has shown that exposure to stressors can alter behavioral and physiological responses to abused drugs (Goeders and Guerin, 1994; Shaham and Stewart, 1994; Piazza and Le Moal, 1998; Shalev et al., 2000; Covington and Miczek, 2001; Nikulina et al., 2004). Will et al. (1998) have demonstrated that prior exposure to a single session of an intense uncontrollable stressor, tailshock (inescapable stress, IS) produces a persistent, trans-contextual potentiation of morphine conditioned place preference (CPP). This potentiation is dependent on stressor uncontrollability and fails to occur following identical amounts of controllable tailshock (escapable stress, ES) or restraint (Will et al., 1998).

We have recently shown that IS potentiation of morphine CPP requires an intact DRN (Will et al., 2004). Moreover, that study demonstrated that this potentiation requires the activation of serotonin (5-HT) neurons in the dorsal raphe nucleus (DRN) because it was blocked by intra-DRN microinjections of 8-hydroxy-2-di-n-propylamino)-tetralin (8-OH-DPAT), a 5-HT1a autoreceptor agonist that is known to inhibit DRN 5-HT neurons (Sprouse and Aghajanian, 1986). Additional support for involvement of the DRN in IS potentiation of morphine CPP is the finding that prior IS does not potentiate CPP (Will et al., 1998) or psychomotor activation (Will et al., 2002) to amphetamine, a drug that does not activate DRN 5-HT neurons (Rebec and Curtis, 1983).

Other behavioral sequelae of IS, such as impaired escape learning, are also dependent on activation and subsequent sensitization of DRN 5-HT neurons (Maier et al., 1995). IS, relative to ES, produces a prolonged increase in 5-HT levels in the DRN (Maswood et al., 1998) and preferentially activates 5-HT neurons in the DRN (Grahn et al., 1999b). Moreover, prior IS produces a potentiation of 5-HT efflux in projection regions of the DRN in response to a mild stressor (Amat et al., 1998a), which may be the proximate cause of behavioral changes following IS. Interestingly, morphine disinhibits DRN 5-HT neurons and dose-dependently increases 5-HT release both in the DRN (Tao and Auerbach, 1994) and its projection regions (Tao and Auerbach, 1995). If sensitization of 5-HT neurons in the DRN occurs following IS, then a potentiated 5-HT response to morphine after IS, but not ES, might be expected. In support of this, we have previously demonstrated that potentiation of morphine-induced 5-HT efflux occurs in the medial prefrontal cortex (mPFC) 24 h after exposure to IS, but not ES, relative to no stress (NS) controls (Bland et al., 2003b).

An extensive literature suggests a fundamental role for the mesolimbic dopamine (DA) system in reward (e.g.
Spanagel and Weiss, 1999) and in processes involved in conditioning (Salamone and Correa, 2002). Thus, it is possible that prior IS, relative to ES, potentiates morphine-induced DA efflux in the nucleus accumbens (NAC). Furthermore, it is possible that this could be mediated by the DRN, because DRN 5-HT activity can produce subsequent activation of the mesolimbic DA system (Yan and Yan, 2001). To examine these possibilities DA in the NAC shell was measured during morphine administration 24 h after exposure to IS, ES, NS using in vivo microdialysis. Although we have previously shown that prior IS potentiates morphine-induced 5-HT in one projection region of the DRN, the mPFC (Bland et al., 2003b), it is not known if IS potentiation of morphine-induced 5-HT will occur in the NAc or the ventral tegmental area (VTA). Therefore, we also measured 5-HT in the NAc shell and the VTA. We observed that prior IS selectively potentiated morphine-induced 5-HT efflux in the NAc but did not affect morphine-induced 5-HT efflux in either the NAc or the VTA. Additional experiments tested the hypotheses that this IS potentiation of morphine-induced DA in the NAc was due to morphine’s action in the DRN and was dependent on activation of DRN 5-HT neurons.

EXPERIMENTAL PROCEDURES

Subjects

Adult male Harlan Sprague–Dawley rats (250–350 g) were used. Rats were housed two per cage on a 12-h light/dark cycle (on 07:00 h; off 19:00 h) with food and water available ad libitum. Rats were allowed to acclimate to the colony for 1 week prior to experimentation. All experiments were conducted in accordance with protocols approved by the University of Colorado Animal Care and Use Committee. All procedures were within the guidelines established by the National Institutes of Health Guide for the Care and Use of Animals. All efforts were made to minimize the number of animals used and to minimize any discomfort.

Surgery

Under halothane anesthesia, CMA 12 guide cannulae (CMA Microdialysis, North Chelmsford, MA, USA) were aimed at either the right or the left NAc (AP = ± 1.7, LM = ± 0.8, DV = −6.0). Left or right hemisphere was counterbalanced. In experiments 2 and 3, guide cannulae for microinjections (26 g, 15.5 mm; Plastics One, Roanoke, VA) were implanted in the DRN (AP = −8.0, LM = 0, DV = −5.1) at the same time as dialysis guide cannula implantation. In experiment 4, CMA 12 guide cannulae were aimed at either the right or the left VTA (AP = −6.0, LM = 0.6, DV = −7.2). All coordinates were from bregma using the atlas of Paxinos and Watson (1998). The guide cannulae were anchored to the skull with three jeweler’s screws and dental cement. A tether screw (CMA Microdialysis) was also anchored in the cement. Rats were individually housed after surgery and were allowed 5–7 days of recovery before experimentation.

Drugs

Morphine sulfate (NIDA) was dissolved in sterile 0.9% saline at a concentration of 3 mg/ml for an injection volume of 1 ml/kg. This dose of morphine (3 mg/kg) was used in all of the experiments and was chosen because it has been shown to be the optimal dose for IS potentiation of morphine CPP (Will et al., 1998). Naltrexone HCl (Sigma, St. Louis, MO, USA) was dissolved in sterile 0.9% saline at a concentration of 20 mg/ml. 8-OH-DPAT (Sigma) was dissolved in sterile 0.9% saline at a concentration of 2 mg/ml.

Microdialysis

On the afternoon before an experiment, rats were transferred to the dialysis room that was on the same light/dark cycle as the colony. Microdialysis probes (CMA 12, MW cutoff 20,000 Da, 2 mm active membrane for the NAc and 1 mm active membrane for the VTA) were inserted into the guide cannulae and rats were placed in separate Plexiglas infusion bowls with food and water available. Ringer’s solution (147 mM NaCl, 2.97 mM CaCl, 4.02 mM KCl, Baxter, Deerfield, IL, USA) was perfused through the probes using a CMA infusion pump at a flow rate of 0.2 μl/min overnight. The next morning the flow rate was increased to 1.5 μl/min. After a 2-h equilibration period sample collection began. Collection tubes were prefilled with 3 μl of 0.02% of the antioxidant EDTA in 1% ethanol. Dialysates were collected manually every 20 min and immediately placed in −80 °C until analysis. After collection of baseline samples, either morphine (3 mg/kg) or saline (1 ml/kg) was injected s.c. Since the logic of the experiment was to determine what the DA and 5-HT response to morphine would be like in a CPP experiment, the morphine CPP experiment was emulated as closely as possible. Thus, rats were placed in the same conditioning boxes as were used in the CPP experiments for 40 min after the injections. These boxes were 30 × 30 × 30 cm Plexiglas boxes with alternating 2 cm black and white stripes, either horizontal or vertical, and a wire mesh floor. Rats had been familiarized with these boxes by preexposing them for 30 min the day before the stress session. Sampling continued for 160 min after morphine or saline injections.

Stressor controllability procedure

Stressor exposure took place between 09:00 and 11:00 h. For both IS and ES exposure, each rat was placed in a Plexiglas box (14 × 11 × 17 cm) with a wheel mounted in the front and a Plexiglas rod extending out the back. The rats’ tails were taped to the Plexiglas rod and affixed with copper electrodes. Rats received shocks in yoked pairs. One rat (ES) was in a box equipped with a wheel that, when turned as described below, terminated the shock to both rats. The other rat (IS) was in a box in which the wheel could not be turned. Each session consisted of 100 trials on a 1 min variable-interval schedule and a shock intensity of 1.0 mA. Shocks began simultaneously for each rat in a pair and terminated for both rats when the escape requirement was met by the ES rat. The following procedure was used to insure that the ES rat had to learn an operant response to terminate the shock. Initially the shock was terminated by a one-quarter turn of the wheel. The response requirement was increased by a one-quarter turn when each of three consecutive trials was completed in less than 5 s. Subsequent latencies under 5 s increased the requirement by 50% up to a maximum of four full turns. The requirement was reduced if the trial was not completed in less than 5 s. If the requirement was not reached in less than 30 s, the shock was terminated and the requirement was reduced to one-quarter turn of the wheel. We have previously demonstrated using microdialysis that the loose restraint used here does not increase 5-HT efflux in the DRN (Maswood et al., 1998), amygdala (Amat et al., 1998a), or hippocampus (Amat et al., 1998b). Moreover, loose restraint does not potentiate morphine CPP (Will et al., 1998). Therefore, unhandled NS controls were used.

Inescapable stress

Because there was no effect of ES on either morphine-induced DA or 5-HT in experiment 1, this group was not included in experiments 2–4. IS was produced with 100 tail shocks (5 s, 1.0 mA) on
a 1 min variable-interval schedule (30–90 s ITI) while rats were restrained in Plexiglas tubes (23.4 cm long and 7.0 cm in diameter). Stressor exposure took place between 09:00 and 11:00 h. NS controls remained in the home cage.

Intra-DRN microinjections

Each rat was held gently in a towel and the stylet was removed from the injection cannula. A microinjector (33 g; Plastics One) attached to a cannula connector (Plastics One, Roanoke, VA, USA) was inserted into the cannula. Drugs were injected manually using PE-20 tubing and a 10 µl Hamilton syringe. Injection volumes were 0.5 µl. An air bubble introduced to the tubing was used to verify flow through the injector. Rats were placed back in the dialysis bowls and injectors were left in place for 2 min to allow diffusion to occur. Sampling resumed immediately after the microinjections were completed.

HPLC

Dialysates were analyzed within 2 weeks of collection. DA and 5-HT in the dialysates were determined simultaneously using an ESA 5600A Coularray detector with an ESA 5014B analytical cell and an ESA 5020 guard cell connected to an ESA MD-150 column (C18, 3 µm, 150×3.2 mm) which was maintained at 26 °C. The mobile phase was 90 mM sodium dihydrogen phosphate monohydrate, 50 mM citric acid monohydrate, 1.7 mM 1-octanesulfonic acid sodium salt, 50 µM EDTA, and 10% acetonitrile, pH=3 (MD-TM, ESA, Chelmsford, MA, USA). The potentials were set at −75 and +250 mV, and the guard cell potential was set at +300 mV. Injections were performed with an ESA 542 autosampler using an injection volume of 27 µl. Quantitative comparisons were made with external standards (Sigma) that were run each day.

Microdialysis probe and microinjection cannula placement verification

Animals were anesthetized with 65 mg/kg sodium pentobarbital. The brains were removed, snap frozen in isopentane, and cryostat sectioned (40 µm) at −20 °C. Sections were mounted on gelatin-treated slides, stained with Cresyl Violet, and coverslipped. Fig. 1A shows microdialysis probe placements in the NAc of rats included in experiments 1–3. Fig. 1B shows microinjection cannula placements in the DRN of rats included in experiments 2 and 3. Fig. 1C shows microdialysis probe placements in the VTA of rats included in experiment 4.

Experimental design and data analysis

For the analysis of treatment effects, dialysate data were normalized as a percent of basal levels consisting of the mean of the first three samples. Data were analyzed using mixed ANOVA with Groups as a between variable and Time as a repeated measure. α was set at 0.05 for all effects. Student Newman-Keuls tests were
used for post hoc analyses of between groups effects, and simple effects tests were used for post hoc analyses of within groups effects at each time point. For the analysis of basal levels, data were expressed as absolute values.

**Experimental protocols**

*Experiment 1: effects of stressor controllability on morphine-induced DA and 5-HT in the NAc.* In this experiment only, rats were transferred to the dialysis room the day before stressor exposure, and microdialysis was performed in a separate room during yoked IS, ES, or NS. These data have been published separately (Bland et al., 2003a). Rats were then returned to the original dialysis room. The next day, after baseline samples were collected, morphine or saline was administered s.c. There were no significant Group differences in DA efflux between saline treated rats after prior IS, ES, or NS and no Group × Time interaction, so the data from these groups were pooled and labeled SALINE. The experimental design was Stress (IS, ES, NS) × Drug (morphine, saline) × Time. Final group sizes were six to eight rats.

*Experiment 2: effect of DRN opioid receptor blockade on IS modulation of morphine-induced DA and 5-HT in the NAc.* On the first day of the experiment animals received either IS or NS treatments. Dialysis was conducted the next day. After collection of three baseline samples, naltrexone (10 μg) or vehicle was administered intra-DRN. Intra-DRN doses of naltrexone in this range have been shown to block the escape deficit and the enhancement of fear conditioning produced by IS (Grahn et al., 1999a). Twenty minutes later, morphine was administered s.c. and rats were placed in the conditioning boxes for 40 min. Because there were no effects of saline injection in any groups in experiment 1, all rats received morphine. The experimental design was Stress (IS, ES, NS) × Drug (NAX, VEH) × Time. Final group sizes were six to eight rats.

*Experiment 3: effect of DRN 5-HT1A receptor stimulation on IS modulation of morphine-induced DA and 5-HT in the NAc.* Experimental procedures were the same as in experiment 2. However, in this experiment 8-OH-DPAT (1 μg) or vehicle was administered intra-DRN. This dose of intra-DRN 8-OH-DPAT has been shown to block the escape deficit and the enhancement of fear conditioning produced by IS (Maijer et al., 1999). Twenty minutes later, morphine was administered s.c. The experimental design was Stress (IS, ES, NS) × Drug (DPAT, VEH) × Time and final group sizes were five to seven rats.

*Experiment 4: effect of IS on morphine-induced DA and 5-HT in the VTA.* On the first day rats received either IS or NS. On day 2, after collection of three baseline samples, morphine was administered to all rats. The experimental design was Stress (IS, NS) × Time. Final group sizes were five to six rats.

**RESULTS**

**Effects of stressor controllability on morphine-induced DA and 5-HT in the NAc**

Prior IS potentiated morphine-induced DA efflux in the NAc (Fig. 2A). There were no significant differences in DA efflux between saline treated rats after IS, ES, or NS, and no Group × Time interaction, so the data from these groups were pooled and labeled SALINE. Morphine produced a roughly 50% increase in DA in NS rats and rats with prior ES. However, prior IS strongly potentiated this response, as morphine produced a 309% increase in DA in IS rats. There was a significant main effect of Group, \(F(3, 24)=7.706, P<0.001\). Post hoc tests indicated that IS animals were significantly different from ES, NS, and SALINE. There was a significant Group × Time interaction, \(F(30, 24)=3.082, P<0.001\). Post hoc tests indicated that IS rats were significantly different from ES, NS, and SALINE at 20, 60, 80, 100, 120, 140, and 160 min after morphine. There were no differences between stress groups in basal DA levels (pg/27 μl, mean±S.E.M.): IS: \(1.22±0.34\); ES: \(1.87±0.51\); NS: \(1.82±0.59\).

Prior IS did not potentiate morphine-induced 5-HT efflux in the NAc, nor was there a significant 5-HT response to morphine (Fig. 2B). There was no significant Group difference in 5-HT efflux between saline-treated rats after IS, ES, or NS and no Group × Time interaction, so the data from these groups were pooled and labeled SALINE. There was no significant main effect of Group, and no significant Group × Time interaction. There were no differences between groups in basal 5-HT levels (pg/27 μl, mean±S.E.M.): IS: \(1.12±0.48\); ES: \(1.59±0.35\); NS: \(1.35±0.49\).

**Effect of DRN opioid receptor blockade on IS modulation of morphine-induced DA and 5-HT in the NAc**

IS potentiation of morphine-induced DA efflux in the NAc was again observed, and this potentiation was blocked by intra-DRN microinjections of naltrexone (Fig. 3A). This was indicated by a significant Stress × Drug interaction, \(F(1, 19)=6.729, P<0.05\). Post hoc tests indicated that IS-VEH was significantly different than IS-NAX, NS-NAX, and NS-VEH. DA levels peaked at 266% of basal in the IS-VEH group. Post hoc tests indicated that IS-VEH was different from the other groups at 20, 60, 80, 100, and 120 min after morphine. There were no differences between groups in basal DA levels (pg/27 μl, mean±S.E.M.): IS-NAX: \(2.68±0.53\); IS-VEH: \(1.40±0.21\); NS-NAX: \(1.70±0.43\); NS-VEH: \(2.45±0.71\).

As in the first experiment, prior IS did not potentiate morphine-induced 5-HT efflux in the NAc (Fig. 3B). There was no significant main effect of Stress on 5-HT, and no significant Stress × Drug or Stress × Drug × Time interaction. There were no differences between groups in basal 5-HT levels (pg/27 μl, mean±S.E.M.): IS-NAX: \(3.40±0.98\); IS-VEH: \(3.20±1.56\); NS-NAX: \(3.20±1.82\); NS-VEH: \(2.91±0.87\).

**Effect of DRN 5-HT1A autoreceptor stimulation on IS modulation of morphine-induced DA and 5-HT in the NAc**

IS potentiation of morphine-induced DA efflux in the NAc was again observed, and this potentiation was blocked by intra-DRN 8-OH-DPAT (Fig. 4A). This was indicated by a significant Stress × Drug interaction, \(F(1, 18)=4.223, P<0.05\), as well as a significant Stress × Drug × Time interaction, \(F(11, 197)=2.359, P<0.01\). Post hoc tests indicated that IS-VEH was significantly different than IS-DPAT, NS-DPAT, and NS-VEH. DA levels peaked at 310% of basal in the IS-VEH group. Post hoc tests indicated that IS-VEH differed from all the other groups at 20, 40, 60, 80, 100, 120, and 140 min after morphine. There
were no differences between groups in basal DA levels (pg/27 μl, mean±S.E.M.): IS-DPAT: 1.79±0.74, IS-VEH: 2.34±1.19, NS-DPAT: 1.11±0.19, NS-VEH: 2.56±0.94.

Again, prior IS did not potentiate morphine-induced 5-HT efflux in the NAc (Fig. 4B). One statistical outlier was excluded from the 5-HT analysis. There was no significant main effect of Stress, and no significant Stress×Drug or Stress×Drug×Time interactions. There was a significant Drug×Time interaction $F(11, 187)=2.56$, $P<0.01$ indicating that 5-HT efflux was lower after DPAT relative to vehicle pretreatment across stress groups. There were no differences between groups in basal 5-HT levels (pg/27 μl, mean±S.E.M.): IS DPAT: 4.46±2.06, IS VEH: 3.38±1.88, NS DPAT: 1.55±0.98, NS VEH: 1.29±0.43.

**Effect of IS on morphine-induced DA and 5-HT in the VTA**

Morphine produced an increase in VTA DA (Fig. 5A), as indicated by a significant effect of Time, $F(10,100)=5.06$, $P<0.001$. Prior IS did not potentiate morphine-induced DA in the VTA. There were no differences between groups in basal DA levels in the VTA (pg/27 μl, mean±S.E.M.): IS-MORPH: 1.15±0.44, NS-MORPH: 0.76±0.21.

Morphine also produced an increase in VTA 5-HT (Fig. 5B), as indicated by a significant effect of Time, $F(10,100)=6.82$, $P<0.001$. Prior IS did not potentiate morphine-induced 5-HT in the VTA. There were no differences between groups in basal 5-HT levels (pg/27 μl,
DISCUSSION

The present study examined the role of stressor controlability in mediating the effects of prior stressor exposure on DA and 5-HT efflux in the NAc in response to morphine. It was observed in the first experiment that morphine-induced DA efflux was potentiated by prior IS, but not ES. IS potentiation of morphine-induced DA efflux was again observed in 2 subsequent studies. Because sensitization of the DRN has been shown to be involved in many of the sequelae of IS, the role of the DRN was investigated. In the second experiment, antagonism of opioid receptors in the DRN by microinjections of naltrexone blocked the IS potentiation of morphine-induced DA efflux in the NAc, suggesting that morphine’s action in the DRN mediates this potentiation. The third experiment demonstrated that inhibition of DRN 5-HT neurons by microinjections of the 5-HT1A autoreceptor agonist 8-OH-DPAT also blocked this potentiation. Thus, morphine-induced activation of 5-HT neurons in the DRN appears to mediate this potentiation, but not likely by the direct projection of DRN neurons to the NAc since 5-HT efflux was not potentiated within the NAc in any of the experiments. However, a role for NAc 5-HT cannot be ruled out based on the present results. In the
fourth experiment, IS did not potentiate either the 5-HT or the DA response to morphine in the VTA, suggesting that a direct DRN-VTA interaction may not be involved in IS potentiation of DA in the NAc. Taken together, these results suggest a neural pathway involved in IS potentiation of DA efflux in the NAc, and thus possibly of morphine reward, in which morphine acting on opioid receptors in the DRN activates 5-HT neurons in the DRN that are sensitized by prior IS.

The present experiments are the first to demonstrate that uncontrollable, but not controllable, stress selectively increases morphine-induced DA efflux in the NAc. This suggests a mechanism for the previous findings that uncontrollable, but not controllable stress potentiates morphine CPP (Will et al., 1998) and psychomotor activation (Will et al., 2002). The present results are also the first to report a long-lasting, trans-contextual effect of a single exposure to a stressor on drug-induced NAc DA efflux, because testing occurred 24 h after exposure to the stressor and in a very different environment. DA efflux in response to morphine consistently increased to about 300% above baseline in rats exposed to IS and about 50% above baseline in rats exposed to NS.
baseline levels in rats exposed to ES or NS. We used a low dose of morphine (3 mg/kg) in these and in previously published studies (Will et al., 2004) in order to avoid possible ceiling effects of higher doses, and observed morphine-induced DA levels in control animals similar to those of several reports (Maisonneuve et al., 2001; Steinmiller et al., 2003), although lower than in some other reports. However, the experimental procedures used here were different from those used in some of the prior studies (Di Chiara and Imperato, 1988; Rouge-Pont et al., 2002). For example, we administered morphine after overnight habituation to the microdialysis chamber, and in an environment to which rats had been familiarized because it has been shown that environmental novelty can potentiate morphine’s effects (Badiani et al., 1998).

The present data suggest that morphine acts on opioid receptors in the DRN to mediate IS-potentiated NAc DA. Thus, we observed that intra-DRN naltrexone before morphine blocked this potentiation. However, an inherent limitation of microinjection studies is the possibility of diffusion of the drug from the site of injection, thus we cannot rule out the possibility that distal sites were affected. Nevertheless, it can be noted that μ-opioid receptors are expressed on GABA interneurons in the DRN (Mansour et al., 1994).
and that these interneurons synapse on 5-HT neurons (Wang et al., 1992). Thus, opioids inhibit GABA interneurons (Wang and Nakai, 1993), disinhibiting 5-HT neurons in the DRN. If 5-HT neurons within the DRN are sensitized by IS, then it might be expected that opioid receptor-mediated disinhibition of 5-HT neurons would be enhanced due to reduced feedback inhibition by 5-HT1A autoreceptors, leading to increased release in terminal regions such as the mPFC (Bland et al., 2003b).

There is a good deal of evidence pointing to the activation and sensitization of the DRN as a mediator of the long-lasting sequelae of IS (Maier et al., 1995; Amat et al., 1998; Maswood et al., 1998; Grahn et al., 1999b; Will et al., 2004). Here, direct evidence for DRN 5-HT mediation of IS potentiation of morphine-induced NAc DA is provided by the finding that this potentiation was blocked by intra-DRN injections of the selective 5-HT1A agonist 8-OH-DPAT. The present results support those of Will et al. (2004). In that study, intra-DRN microinjections of 8-OH-DPAT blocked IS potentiation of morphine CPP. The 5-HT1A receptor is an inhibitory autoreceptor (Sprouse and Aghajanian, 1986) located on somata and dendrites of 5-HT neurons within the DRN (Kia et al., 1996) and are almost exclusively expressed on 5-HT neurons (Sotelo et al., 1990). Thus, 8-OH-DPAT selectively inhibits 5-HT neurons (Sprouse and Aghajanian, 1986). Here, we have observed attenuation of morphine-induced 5-HT efflux in the NAC after 8-OH-DPAT pretreatment relative to vehicle controls in both the IS and NS groups, indicating that 8-OH-DPAT treatment successfully blocked activation of 5-HT neurons in the DRN produced by morphine. Morphine-induced 5-HT efflux in the NAC was slight and was not was potentiated by IS in the NAC in any of the experiments. Tao and Auerbach (1995) have shown that both systemic and intraraphe morphine produce increased 5-HT efflux in DRN projection regions, including the NAC. However, the effect was dose-dependent and increased 5-HT efflux was only induced by doses of morphine in the range of 10–20 mg/kg. Consistent with the present results in both stressed and unstressed rats, morphine at doses of 5 mg/kg or lower did not produce significant increases in 5-HT in that study (Tao and Auerbach, 1995).

In contrast with the absence of IS potentiation of morphine-induced 5-HT efflux in the NAC and in the VTA found here, we have previously reported that prior IS, but not ES, potentiates morphine-induced 5-HT efflux in the mPFC. The DRN is known to be composed of distinct subregions with different afferent and efferent connections (Moore et al., 1978; Sesack et al., 1989), and different DRN 5-HT neurons may project to the NAC, VTA, and mPFC (Valentino et al., 2001; Lowry, 2002). Furthermore, IS selectively activates only a subpopulation of DRN 5-HT neurons (Grahn et al., 1999b). Therefore, IS may sensitize DRN 5-HT neurons that project to regions that indirectly regulate the NAC, such as the mPFC. It is thus possible that this enhanced 5-HT in the mPFC may modulate DA efflux in the NAC via afferents from the mPFC to either the NAC or the VTA, both of which are known to exist (Berendse et al., 1992; Carr and Sesack, 2000). Future work will explore this possibility.

Because IS did not increase morphine-induced NAC 5-HT, but inhibition of 5-HT neurons within the DRN nevertheless blocked IS potentiation of morphine-induced NAC DA, it was possible that the DRN might potentiate NAC DA via the 5-HT projection from the DRN to the VTA that has been described (Moore et al., 1978). This seemed plausible because electrical stimulation of the DRN activates DA neurons in the VTA (Gervais and Rouillard, 2000), and 5-HT in the VTA can increase NAC DA efflux (Yan and Yan, 2001). Although we did observe significant morphine-induced increases in both 5-HT and DA in the VTA across stress groups, IS did not modulate either morphine-induced 5-HT or DA release in the VTA. Therefore, potentiated 5-HT in the VTA does not appear to be responsible for IS potentiation of morphine-induced DA in the NAC. However, it is still possible that the VTA is involved in this potentiation, perhaps via glutamatergic afferents. Consistent with this notion, several lines of research indicate that glutamate’s actions in the VTA are involved in sensitization induced both by stress and drugs of abuse (Fitzgerald et al., 1996; Wolf, 1998; Carlezon and Nestler, 2002; Saal et al., 2003).

In conclusion, the present results suggest a possible neural substrate for the potentiation of morphine reward produced by prior uncontrollable, but not controllable, stress. They show that exposure to a single session of uncontrollable stress can produce a robust and reliable potentiation of morphine-induced DA efflux in the NAC long after stressor exposure in a very different environment than that in which the stressor occurred. The experiments reported here also suggest that this potentiation involves opioid receptors and 5-HT neurotransmission in the DRN.

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