

Matthew J. Will · Andre Der-Avakian ·  
Sondra T. Bland · Ruth E. Grahn ·  
Sayamwong E. Hammack · Peter D. Sparks ·  
Julie L. Pepin · Linda R. Watkins · Steven F. Maier

## Electrolytic lesions and pharmacological inhibition of the dorsal raphe nucleus prevent stressor potentiation of morphine conditioned place preference in rats

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**Abstract** *Rationale:* Exposure to a single session of uncontrollable inescapable shock (IS), but not to identical controllable escapable shock, produces a potentiation of morphine's rewarding properties that is unusual in that the stressor can be given a number of days before the drug administration in an environment quite different from the drug context. Many other behavioral outcomes of stressors that depend on the uncontrollability of the stressor are mediated by alterations in serotonergic (5-HT) neurons within the dorsal raphe nucleus (DRN). *Objectives:* The present experiments examined the role of the DRN and 5-HT in mediating the effect of IS on the rewarding properties of morphine as assessed by conditioned place preference (CPP). *Methods:* In experiment 1, subjects received small electrolytic lesions of the DRN and were tested for morphine (3.0 mg/kg, SC) CPP after IS or control treatment. In experiment 2, subjects received an intra-DRN microinjection of the 5-HT<sub>1A</sub> agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT, 1.0 µg/0.5 µl) either before IS or before

morphine (3.0 mg/kg, SC) injections during CPP testing. *Results:* IS potentiated morphine CPP in controls, but both DRN lesion and intra-DRN 8-OH-DPAT, either before IS or before morphine administration, completely blocked this effect. *Conclusions:* These data implicate alterations in DRN 5-HT neurons in the potentiation of morphine reward produced by uncontrollable stress.

**Keywords** Reward · Uncontrollable stress · Serotonin · 5-HT · 8-OH-DPAT · Electrolytic lesion

### Introduction

The degree to which an organism can exert behavioral control over a stressor has been shown to be one of the most potent variables modulating many of the organism's reactions to a stressor (Anisman et al. 1991). Thus, exposure to uncontrollable stressors such as inescapable shock (IS) leads to a variety of behavioral changes that are not observed following exposure to the identical duration, intensity, and pattern of escapable shock (ES), a phenomenon that has been called "learned helplessness" (Maier and Seligman 1976) or "behavioral depression" (Weiss et al. 1981). Although a variety of neurotransmitters have been implicated in the mediation of stressor controllability effects (Petty and Sherman 1981; Hyson et al. 1982; Anisman and Zacharko 1986; Anisman et al. 1991; Petty et al. 1992), recent attention in our laboratory has focused on serotonergic (5-HT) systems. Exposure to IS, but not exactly equal amounts of ES or restraint, selectively increases 5-HT activity in the dorsal raphe nucleus (DRN) (Maswood et al. 1998; Grahn et al. 1999), leading to large amounts of extracellular 5-HT within the DRN and its projection regions (Amat et al. 1998a, 1998b; Maswood et al. 1998), as well as a sensitization of DRN 5-HT neurons (Amat et al. 1998b). Exposure to a stressor during the several day period of sensitization leads to exaggerated extracellular levels of 5-HT within

M. J. Will  
Department of Psychiatry,  
University of Wisconsin—Madison Medical School,  
Madison, WI 53719, USA

A. Der-Avakian · S. T. Bland · P. D. Sparks · J. L. Pepin ·  
L. R. Watkins · S. F. Maier (✉)  
Department of Psychology and Center for Neuroscience,  
University of Colorado at Boulder,  
Boulder, CO 80309-0345, USA  
e-mail: smaier@psych.colorado.edu  
Tel.: +1-303-4926275

R. E. Grahn  
Department of Psychology,  
Connecticut College,  
New London, CT 06320-4196, USA

S. E. Hammack  
Department of Psychiatry,  
Emory University School of Medicine,  
Atlanta, GA 30322, USA

DRN projection regions (Amat et al. 1998a), the putative cause of the behavioral effects that occur. If this exaggerated 5-HT activity is prevented either by lesion (Maier et al. 1993) or pharmacological inhibition (Maier et al. 1994, 1995b) of the DRN prior to IS or before later behavioral testing, IS no longer produces its usual behavioral effects of poor escape learning and exaggerated fear conditioning. Consistent with the idea that DRN 5-HT activity is crucial in mediating the behavioral effects of uncontrollable stress, pharmacologically induced activation of DRN 5-HT neurons, in the absence of IS, produces the same behavioral effects as does IS (Maier et al. 1995a).

Stressor controllability has also been shown to modulate the impact of stressors on reactions to drugs of abuse. In the earliest study of this phenomenon, Goeders and Guerin (1994) examined the acquisition of cocaine self-administration under a multiple schedule that included either contingent (controllable), non-contingent (uncontrollable), or no footshock. The non-contingent footshock increased sensitivity to cocaine, shifting the dose-response curve such that non-contingently shocked rats responded for cocaine at lower doses than did rats that were contingently shocked or not shocked at all.

The Goeders and Guerin (1994) study administered the stressor at a point in time close to drug exposure and testing, and in the same environment as was used for assessing drug responding. Indeed, the administration of the stressor in the same environment as used for drug administration or testing, and/or the administration of the stressor at a point in time close to the drug or drug testing is characteristic of experiments that have examined the impact of acute stressors on reactivity to drugs of abuse. However, in the typical learned helplessness/behavioral depression experiment the stressor is administered in a very different environment than later behavioral testing, and a point in time 24–72 h before testing (e.g. Maier et al. 1993, 1994, 1995b). That is, the effect of stressors such as IS on later escape learning, fear conditioning, etc., is trans-situational and relatively long-lasting.

Thus, we have determined whether IS might not also exert a trans-situational and persistent effect on reactivity to drugs of abuse. Exposure to a single session of IS potentiated the development of a conditioned place preference (CPP) to morphine, even though IS was administered in an environment very different from that used for morphine exposure or CPP testing, and this potentiating effect of IS persisted for between 7 and 14 days (Will et al. 1998). This persistent trans-situational effect did not follow ES, and so was specific to intense uncontrollable stress. Moreover, this IS-specific effect extended to the psychomotor response to morphine (Will et al. 2002).

This trans-situational and long-lasting effect of IS on morphine CPP is probably mediated by mechanisms different than other stress effects. The trans-situational persisting effects may require stressors that are sufficient to produce learned helplessness. For example, ES is also “stressful”, and indeed, the adrenocorticotrophic hor-

mone, corticosterone, and paraventricular hypothalamic corticotropin releasing hormone mRNA responses to ES are as large as those to IS (Maier et al. 1986; Helmreich et al. 1999). However, ES does not produce learned helplessness or trans-situational persistent potentiation of morphine CPP, nor does restraint (Will et al. 1998). Interestingly, IS does not produce trans-situational persistent potentiation of amphetamine CPP or psychomotor responses (Will et al. 1998, 2002), nor does it have such an effect on responses to cocaine or alcohol (unpublished data). Clearly, the effects of stressors per se, administered in the drug environment or close in time to the drug, are not restricted to morphine or other opioids and have been demonstrated with all or most drug classes.

Because DRN 5-HT neurons play a critical role in mediating the impact of uncontrollable stress on learned helplessness, it is natural to begin to examine the role of these neurons in the production of trans-situational persistent potentiation of morphine responses produced by IS. Both electrolytic lesion of the DRN (Maier et al. 1993) and inhibition of 5-HT activity within the DRN by intra-DRN microinjection of the 5-HT<sub>1A</sub> agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) (Maier et al. 1995b) have been shown to block the effects of IS on later escape behavior and fear conditioning. 5-HT<sub>1A</sub> receptors within the DRN are inhibitory somatodendritic autoreceptors (see Adell et al. 2002 for review), and so agonists such as 8-OH-DPAT inhibit both impulse activity of 5-HT DRN neurons (Sprouse and Aghajanian 1987) and 5-HT release in projection regions (Bonvento et al. 1992). Thus, the impact of both lesion of the DRN and intra-DRN 8-OH-DPAT microinjection on the trans-situational persistent potentiation of morphine CPP was explored.

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## Materials and methods

### Subjects

Adult male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, Ind., USA) weighing 300–400 g, were housed in groups of two in Plexiglas cages in a climate-controlled colony room at 22°C. The subjects were maintained on a 12-h light-dark cycle and all experiments were conducted during the light phase. They had free access to food and drinking water prior to and throughout the experiment. All subjects were naive and allowed a minimum of a week of adaptation followed by 2 days of handling before the beginning of all experiments. Group sizes were seven to ten animals in both experiments. All experimental procedures were in accord with protocols approved by the University of Colorado Institutional Animal Care and Use Committee.

### Apparatus

#### *Conditioned place preference*

The Plexiglas place preference apparatus measured 72×30×30 cm (length, width, height) and comprised two distinct conditioning environments and a neutral area. Each conditioning environment measured 30×30×30 cm. One environment was striped horizontally with alternating 2 cm black and white electrical tape on the walls,

while the other environment was striped vertically in the same manner. The floor of the apparatus was black sanded Plexiglas with 2 cm wire grid on the horizontal side, and 3 mm wire mesh on the vertical side. The neutral area measured 12×30×30 cm, was painted gray, and had no wire mesh or grid on the floor. During the conditioning phase, vertically and horizontally striped Plexiglas partition walls were inserted on the respective side of the neutral area to restrict the animals to their designated conditioning environment.

The activity of each subject was monitored by a Philips TC352A video camera (Lancaster, Pa., USA) mounted 1.5 m above the center of the CPP apparatus. The camera relayed the information of the subject's location to the Chromotrack Version 4.02 tracking software (Prototype Systems Ltd, Boulder, Col., USA) run on a PC compatible computer located in a separate room. The SA-3 tracker (San Diego Instruments, San Diego, Calif., USA) measured the subject's time spent within each of the three compartments.

### *Stressor*

The stressor environment was a dimly lit room with dimensions of approximately 3×2.5×2.5 m. Inescapable shock or restraint occurred in Plexiglas restraining tubes which were 17.5 cm in length and 7.0 cm in diameter. The rat's tail extended from the rear of the tube and was taped at the base to a Plexiglas rod 4.0 cm in length. The front end of the tube was blocked by a Plexiglas plunger containing several air holes. Unscrambled shocks were delivered by a source modeled after Grason-Stadler Model 700. Electrodes, coated with a small amount of electrode paste, were taped to the midsection of the tail.

### Experiment 1

#### *DRN lesion surgery*

Following halothane anesthesia, lesions were administered with an epoxy coated tungsten electrode (diameter of 0.5 mm). A current of 1.0 mA was delivered for a 10-s duration. Lesions were placed relative to interaural zero (IA) based on coordinates from the atlas of Paxinos and Watson (1986). The coordinates were AP 1.0 mm, ML 0.0 mm, and DV 4.0 mm. Sham surgery was performed in exactly the same manner except that no current was delivered. Following the lesion or sham surgery, the outer portion of a screw cap from a 15 ml conical centrifuge tube was fixed upside down to the skull with four screws and acrylic for later use as part of the tracking system. Subjects were given 1 week to recover prior to beginning the experiment.

#### *Behavioral testing*

Prior to each testing session, a light assembly consisting of a red LED and two 1.5 V watch batteries encased in a half-inch portion of plastic tubing was threaded into the screw cap previously mounted on the subjects' heads for tracking purposes. On day 1, between 1000 and 1400 hours, all subjects were individually exposed to the CPP apparatus. Subjects were initially placed in the neutral area and allowed to explore the entire preference apparatus for 30 min. This day served to assess the subjects' initial preferences and any possible box bias. Any rat that spent less than 6 min (20% of total time) in either side was eliminated from the study. On day 2, half of the rats received 100 inescapable tailshocks in Plexiglas restrainer tubes (5 s, 1 min inter-trial interval, 1.0 mA) in a different room, while the other half remained in their home cages. On day 3, all subjects were weighed and given random counter-balanced assignments so that half were conditioned with morphine in the vertically striped side and half in the horizontally striped side. There were two conditioning trials per day, one with morphine and one with saline. Morning conditioning occurred

between 0900 and 1100 hours, while afternoon conditioning occurred 4 h later. Half the animals received morphine conditioning in the morning and half in the afternoon. Animals were first injected and then 5 min later placed into the conditioning environment for 45 min. Subjects were conditioned with 3 mg/kg SC morphine to the drug-paired side and 1 ml/kg SC saline vehicle in the other side at the other time of day. On day 4, animals again were conditioned in the same manner except the order of presentation was reversed. On day 5, testing of CPP was conducted between 1000 and 1400 hours exactly as was performed on day 1. Subjects were simply placed in the neutral area of the preference apparatus and their presence in each compartment was measured for 30 min. The experimenter was blind to all aspects of this procedure.

### Experiment 2

#### *Intra-DRN cannulation surgery*

Following halothane anesthesia, a guide cannula (26 gauge, 15.5 mm, Plastics One, Roanoke, Va., USA) was stereotaxically implanted at the site of the DRN based on coordinates from the atlas of Paxinos and Watson (1986). The coordinates were AP 1.0 mm, ML 0.0 mm, DV 4.5 mm relative to IA. The end of the cannula was placed 1.0 mm dorsal to the target region to prevent tissue damage. The cannula was held in place with four dental screws and acrylic.

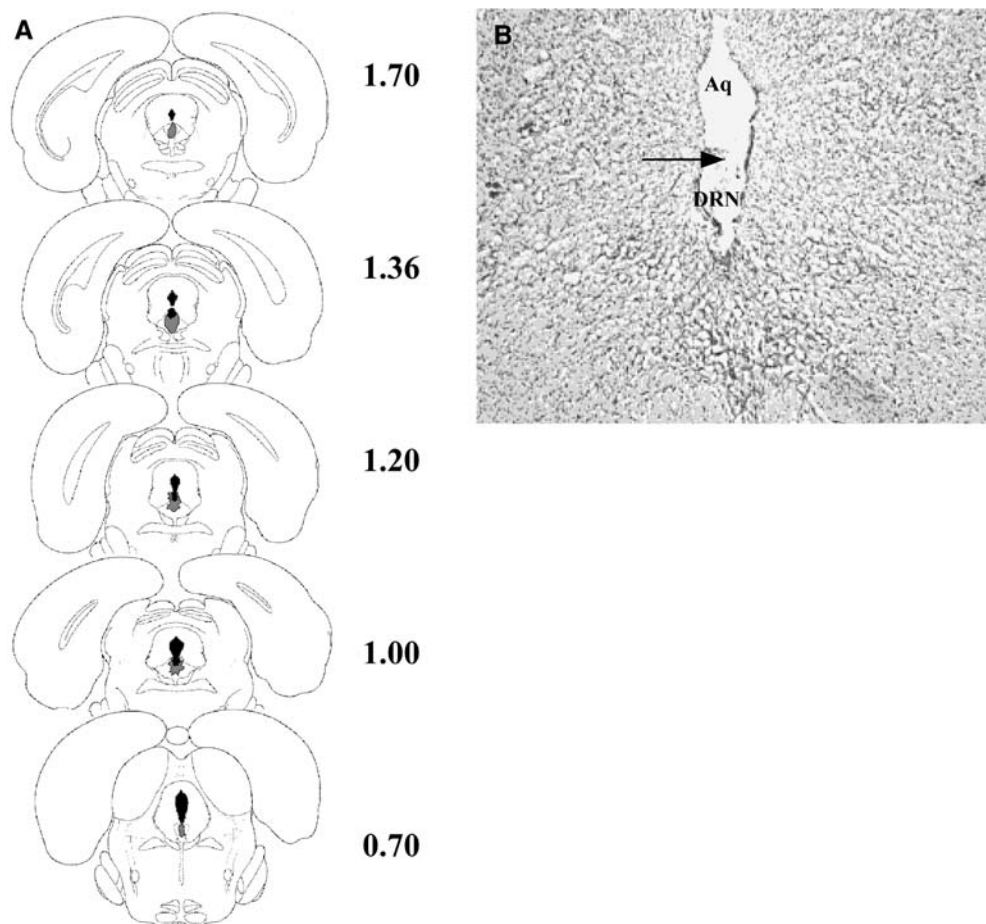
#### *Intra-DRN microinjections*

Each subject was held gently in a towel during the injection procedure. The cannula dummy was removed from the cannula and a microinjector (33gauge; Plastics One) was inserted into the guide cannula. The microinjector extended 1.0 mm beyond the end of the guide cannula into the DRN. The microinjector was attached to 500 mm of PE-10 tubing, which was connected to a 10  $\mu$ l Hamilton syringe (Reno, Nev., USA). 0.5  $\mu$ l of either 8-OH-DPAT (1.0  $\mu$ g) or an equal volume of saline was injected by hand over a period of 30 s, using a small air bubble introduced to the tubing for measurement. The microinjector was left in place for 2 min to allow for drug diffusion, after which the microinjector was removed and the cannula dummy was reinserted.

#### *Behavioral testing*

One day prior to testing, a 2×2 cm piece of reflective tape was attached to a rat collar (BAS, West Lafayette, Ind., USA), which was then loosely collared around each rat's neck for detection by the tracking system. The reflective tape eliminated the need for additional surgery to implant the LED assembly used in experiment 1. The testing procedure was similar to experiment 1, except that 8-OH-DPAT was microinjected into the DRN either before IS or before CPP conditioning trials. Thus one IS and one home cage control group received intra-DRN 8-OH-DPAT 10 min before these treatments and intra-DRN vehicle 10 min before each CPP conditioning trial. A second set of IS and control groups received vehicle before IS or home cage treatment and 8-OH-DPAT before each CPP conditioning trial, while a final set of IS and control groups received vehicle at each point in the experiment. Thus, all subjects received five microinjections, one before IS or control treatment, and one before each of the four conditioning trials (two with morphine and two with saline). In the groups given 8-OH-DPAT during CPP training, 8-OH-DPAT was given before both the morphine and vehicle conditioning trials so that any rewarding or aversive effects of the 8-OH-DPAT itself would be equally associated with the two environmental contexts. Thus, the design was a 2 (IS versus control)×3 (8-OH-DPAT before IS, 8-OH-DPAT before conditioning, vehicle-vehicle) factorial.

**Fig. 1.** **A** Histological localization of DRN lesion sites. In experiment 1, subjects received either 1.0 mA electrolytic lesions or sham surgery 1 week prior to CPP testing. The maximum lesion (depicted in *gray*) extended from interaural (IA) 1.70 mm to 0.7 mm, while the minimum lesion (depicted in *black*) extended from IA 1.36 mm to 1.00 mm. Anterior-posterior coordinates are in mm with respect to interaural zero. **B** A representative photomicrograph of a minimal DRN lesion (*arrow*). *Aq* aqueduct, *DRN* dorsal raphe nucleus



## Drugs

Morphine (Mallinkrodt, St Louis, Mo., USA) was dissolved in sterile 0.9% saline. Injection volume of morphine and saline was 1.0 ml/kg body weight. We have previously shown that a 3.0 mg/kg dose of morphine is optimal in inducing IS potentiation of CPP (Will et al. 1998). 8-OH-DPAT (Sigma, St Louis, Mo., USA) was dissolved in sterile 0.9% saline and microinjected at a dose of 1  $\mu\text{g}/0.5 \mu\text{l}$ . We have previously shown an intra-DRN injection of this dose to block IS mediated behaviors (Maier et al. 1995b).

## Histology

Following behavioral testing, subjects were overdosed with sodium pentobarbital and perfused transcardially with heparinized saline (200 ml). The brains were then removed and placed in 10% formalin-30% sucrose for 1 week. Frozen serial sections (40  $\mu\text{m}$ ) were collected through the entire extent of the DRN, mounted on gelatinized slides, and counter-stained with cresyl violet. Lesion and cannulation profiles were then analyzed blind with respect to the behavioral results. Lesions (Fig. 1) and cannula placements (Fig. 2) typically extended from 1.70 to 0.70 mm IA, as described by the atlas of Paxinos and Watson (1986). Subjects with lesions or cannula placements that did not fit the intended criteria were excluded from analysis.

## Statistical analysis

Data were analyzed by analysis of variance (ANOVA) followed by post hoc Newman-Keuls tests (alpha set at 0.05) or by orthogonal contrasts. The dependent variable for measuring each subject's preference score was expressed as the difference in time spent on

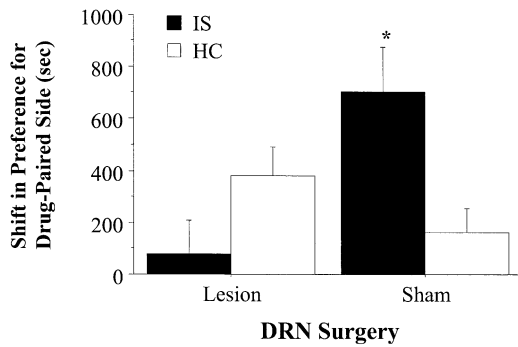
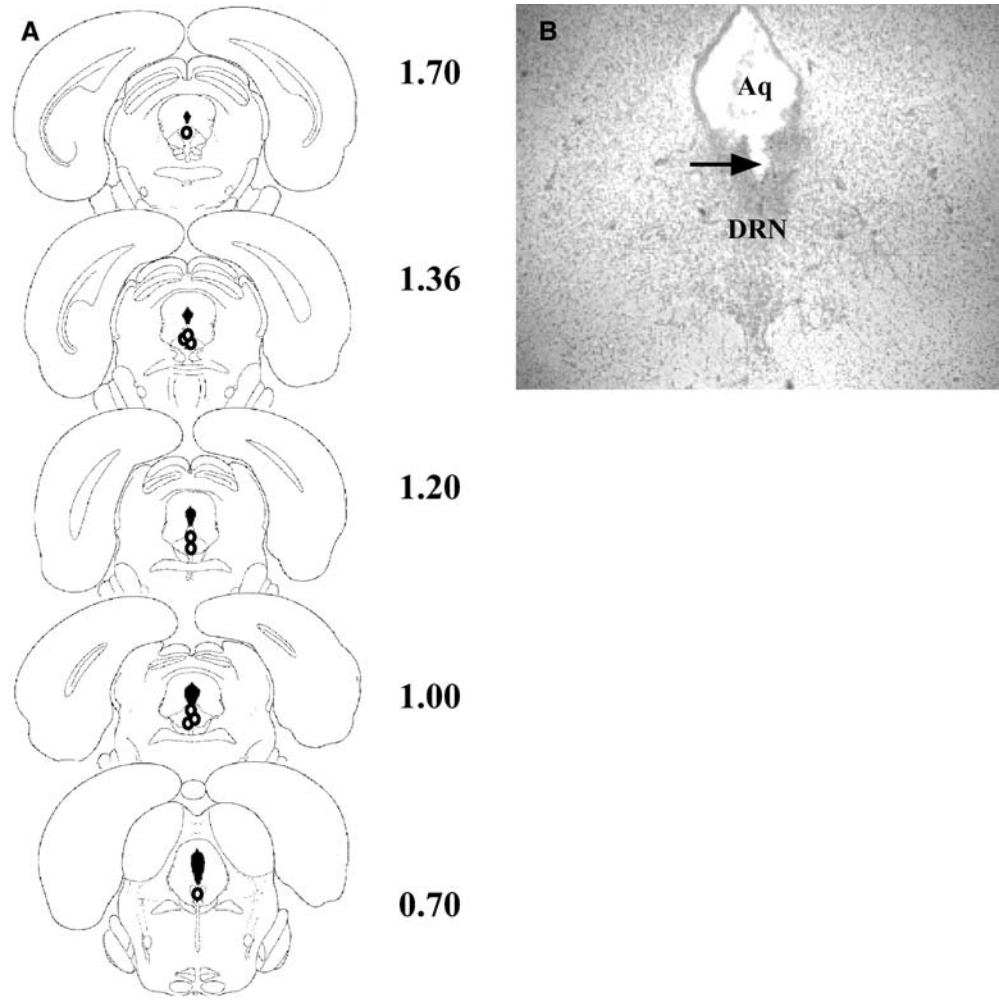
the drug-paired side between the 30-min pre-exposure session and the 30-min conditioned preference session.

## Results

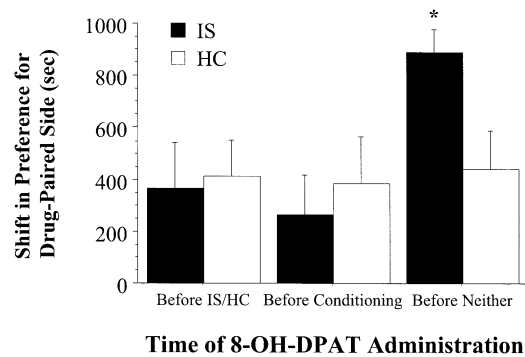
### Experiment 1

Only subjects that showed no initial bias during testing and with accurate lesions were included in the study. Therefore, the final sample sizes were seven for the lesion-control group, eight for the sham-control group, and seven for both the lesion-IS and sham-IS groups. As shown in Fig. 3, morphine conditioning led to a modest CPP, with the sham-control subjects spending roughly 3 min more on the morphine-paired side after conditioning. IS strongly potentiated the morphine CPP, such that sham-IS subjects spent almost the entire test session on the morphine-paired side. The DRN lesions completely blocked this potentiation, but did not interfere with morphine CPP in control subjects. A 2 $\times$ 2 ANOVA revealed no main effect of stressor treatment or surgery, but did reveal a significant interaction between stressor treatment and surgery [ $F(1,25)=10.5, P<0.01$ ]. Post-hoc orthogonal contrasts revealed a significantly higher CPP score for the sham-IS treated group, relative to the sham-controls and the lesion-IS group ( $P<0.01$ ).

**Fig. 2.** **A** Histological localization of intra-DRN 8-OH-DPAT microinjection sites. In experiment 2, subjects received either 1.0  $\mu\text{g}$  8-OH-DPAT in 0.5  $\mu\text{l}$  physiological saline or an equal volume of vehicle either before inescapable shock, control treatment, or conditioning trials during CPP testing. Each *circle* represents a microinjection site. Anterior-posterior coordinates are in mm with respect to interaural zero. **B** A representative photomicrograph of a microinjection site (*arrow*) in the DRN. *Aq* aqueduct, *DRN* dorsal raphe nucleus



**Fig. 3** The mean ( $\pm$ SEM) difference in the time spent on the drug-paired side after and before conditioning (time after minus time before) for groups that had received DRN or sham lesion 1 week prior to testing and inescapable shock (*IS*) or control treatment (*HC*) 24 h before the first day of conditioning. \*Different from sham-*HC* and lesion-*IS* groups ( $P < 0.05$ )



**Fig. 4** The mean ( $\pm$ SEM) difference in the time spent on the drug-paired side after and before conditioning (time after minus time before) for groups that had received intra-DRN 8-OH-DPAT microinjection either before inescapable shock (*IS*), before control treatment (*HC*), before conditioning trials, or only vehicle. \*Different from *HC*-vehicle group and all other *IS* groups ( $P < 0.05$ )

Experiment 2

Only subjects that showed no initial bias during testing and with correct cannula placements were included in the study. There were nine subjects receiving vehicle before *IS* and 8-OH-DPAT before each conditioning trial and ten

subjects in each of the other five groups. As in experiment 1, *IS* strongly potentiated morphine CPP (Fig. 4). This effect of *IS* was completely blocked by intra-DRN 8-OH-DPAT administered either before *IS* or before the morphine and saline conditioning trials. The effects of

8-OH-DPAT were significant [ $F(2,53)=6.27, P=0.05$ ]. In the absence of a significant interaction between the effects of 8-OH-DPAT and stressor treatment, we tested our a priori hypotheses using orthogonal contrasts, which revealed that the vehicle-IS group differed significantly from all of the others ( $P<0.01$ ).

## Discussion

The results of these studies confirm the previous finding that exposure to IS potentiates morphine reward as measured by CPP (Will et al. 1998). Lesion of the DRN completely blocked this potentiation, as IS-DRN lesioned subjects failed to demonstrate a potentiation of morphine CPP. This effect was particularly striking since DRN lesion had no effect in control subjects. However, electrolytic lesions eliminate both fibers of passage as well as non-5-HT neurons within the DRN. In addition, this procedure cannot distinguish between whether the neurons eliminated are important at the time of IS, the time of morphine administration, or both. The 8-OH-DPAT microinjection study addressed these issues. 8-OH-DPAT acts as a selective agonist at the 5-HT<sub>1A</sub> receptor. This receptor is located on the soma and dendrites of 5-HT neurons within the DRN (Kia et al. 1996) and is coupled to a K<sup>+</sup> channel via a G-protein (Aghajanian and Lakoski 1984). Thus, 5-HT<sub>1A</sub> receptors within the DRN act as inhibitory autoreceptors, and so intra-DRN 8-OH-DPAT inhibits DRN 5-HT neurons (Sprouse and Aghajanian 1987). Importantly, intra-DRN 5-HT<sub>1A</sub> receptors are expressed almost exclusively on 5-HT neurons (Sotelo et al. 1990), and so the inhibition produced by 8-OH-DPAT is selective to 5-HT neurons within the DRN. 8-OH-DPAT administered before either IS or morphine administration completely blocked the effects of IS on morphine CPP. This would suggest that the activation of 5-HT neurons within the DRN at the time of exposure to IS, and at the time of morphine administration, is critical.

Although DRN lesion and intra-DRN 8-OH-DPAT microinjection eliminated the exaggeration of morphine CPP produced by IS, neither manipulation influenced the level of morphine CPP in normal control subjects. It is difficult to compare this result in the controls to the existing literature. There are only a very small number of studies that have lesioned or inhibited 5-HT systems and examined CPP with morphine. Spyraiki et al. (1988) found that 5,7-DHT lesion of the nucleus accumbens reduced morphine CPP, while the manipulations used in the present study had no effect in non-IS controls. This difference would not be surprising if the nucleus accumbens is the critical site at which 5-HT acts to modulate CPP. This is because the intra-accumbal 5,7-DHT injection produced a greater than 80% depletion of 5-HT in the nucleus accumbens, a level of depletion within the accumbens that is unlikely to be produced by the restricted DRN lesions and 8-OH-DPAT microinjections employed in the present study. In addition, conditions were chosen in the present study that would produce only

a weak CPP in control subjects. This was done to minimize the possibility of "ceiling effects" with regard to potential enhancement of CPP by IS. Indeed, non-IS subjects only shifted their time in the morphine-paired box by 175–400 s during the 30-min test. Thus, there was only a weak CPP in non-IS controls against which the DRN lesion or 8-OH-DPAT could act. In contrast, Spyraiki et al. (1988) used conditions (four morphine pairings rather than two) that produced a very strong morphine CPP (a shift of 450 s in time on the morphine-paired side during a 15-min test), and so there was ample opportunity to observe a decrease produced by the 5,7-DHT lesion. Moreover, the DRN projects to numerous other structures, and so identical effects of DRN lesions and depletion of 5-HT in the nucleus accumbens should, perhaps, not be expected. However, it has often been concluded that 5-HT plays only a minor role in CPP *per se* (Tzschentke 1998), and the present results do not dispute this conclusion.

The finding that the DRN lesion and pharmacological blockade of DRN 5-HT activation blocked the potentiation of morphine CPP by IS is consistent with prior evidence demonstrating that a variety of other effects produced by IS, such as poor escape learning and exaggerated fear conditioning, are blocked by DRN lesion (Maier et al. 1993) and pharmacological blockade of DRN 5-HT activity (Maier et al. 1994, 1995b). The available evidence suggests that IS sensitizes DRN neurons for a period of time. Thus, input to the DRN during the sensitization period, such as that produced by the shock during escape testing or fear conditioning, produces exaggerated release of 5-HT in projection regions of the DRN (Amat et al. 1998a, 1998b). These projection regions (Steinbusch 1981; Vertes 1991) are known to regulate escape (the dorsal periaqueductal gray) and fear (the amygdala), with this exaggerated release of 5-HT being the proximate cause of the behavioral changes. Importantly, morphine inhibits GABAergic neurons within the DRN that themselves exert tonic inhibition on the 5-HT neurons (Jolas and Aghajanian 1997), thereby disinhibiting the 5-HT neurons. In addition, morphine inhibits GABA neurons within the DRN that inhibit glutamatergic excitatory afferent input to the DRN (Tao and Auerbach 2000), again producing activation of DRN 5-HT neurons. Both of these mechanisms lead to morphine-induced increases in extracellular levels of 5-HT within the DRN and its projection regions (Tao et al. 1996; Jolas and Aghajanian 1997). It is therefore noteworthy that the DRN projects to regions involved in mediating drug reward such as the shell of the nucleus accumbens and the medial prefrontal cortex (Van Bockstaele et al. 1993; Phelix and Broderick 1995). Indeed, morphine produces release of 5-HT in these regions (Tao and Auerbach 1995). Thus, IS should be expected to potentiate morphine-induced increases in extracellular 5-HT in the nucleus accumbens and medial prefrontal cortex.

Interestingly, elevations of 5-HT above basal levels within the nucleus accumbens and medial prefrontal

cortex increase dopamine efflux in these structures. Thus, systemic administration of selective 5-HT reuptake inhibitors or infusion of these substances directly into the medial prefrontal cortex (Tanda et al. 1995) and nucleus accumbens (Yoshimoto et al. 1996) produce large (200–400% above baseline) increases in dopamine efflux in these sites, as does infusion of 5-HT itself (Iyer and Bradberry 1996). Indeed, either chemical (Yoshimoto and McBride 1992) or electrical (De Deurwaerdere et al. 1998) stimulation of the DRN produces increased levels of dopamine in the nucleus accumbens. This increase in extracellular dopamine is known to be mediated by 5-HT neurons in the DRN, since the increase is blocked by 5,7-DHT lesion of the DRN (De Deurwaerdere et al. 1998). This 5-HT/DRN-mediated increase in dopamine efflux in the nucleus accumbens and medial prefrontal cortex is at least in part mediated by 5-HT<sub>3</sub> receptors located within these regions because intra-accumbal and/or intra-medial prefrontal cortex infusion of 5-HT<sub>3</sub> antagonists, such as ondansetron, blocks or reduces the increase in dopamine efflux produced by a) elevated 5-HT (Pei et al. 1993; Tanda et al. 1995; Bassareo et al. 1996), b) electrical stimulation of the DRN (De Deurwaerdere et al. 1998), and c) morphine and other drugs of abuse (Carboni et al. 1989a; McNeish et al. 1993; Pei et al. 1993; Bassareo et al. 1996; Yoshimoto et al. 1996). Consistent with these data, systemic administration of 5-HT<sub>3</sub> antagonists reduces or blocks CPP (Carboni et al. 1989b; Higgins et al. 1992) and locomotor responses (Pei et al. 1993) to morphine. It should be noted that other 5-HT receptor subtypes might be involved as well. For example, 5-HT<sub>2</sub> agonists, such as DOI, infused into the medial prefrontal cortex have been reported to increase DA efflux, while selective 5-HT<sub>2A</sub> antagonists decrease extracellular DA levels (Pehek et al. 2001).

These data suggest the possibility that IS produces a trans-situational and persistent potentiation of the rewarding effects of morphine because a) IS sensitizes 5-HT neurons in the DRN, b) morphine activates DRN 5-HT neurons, c) 5-HT efflux within the nucleus accumbens and/or medial prefrontal cortex is therefore exaggerated in subjects that have recently been exposed to IS, and d) this enhanced release of 5-HT in IS subjects potentiates the dopamine release produced by morphine by action at 5-HT<sub>3</sub> receptors or other 5-HT receptor subtypes located within these structures. The present findings that DRN lesion and intra-DRN microinjection of 8-OH-DPAT before either IS or morphine administration completely blocked the facilitation of morphine CPP produced by IS is fully consistent with this proposal. Inhibition of DRN 5-HT activity during IS should prevent the sensitization of these neurons so that later morphine would now act on DRN 5-HT neurons with normal sensitivity, while inhibition of DRN 5-HT neurons at the time of morphine should prevent the excessive activation of these neurons that would otherwise occur in IS subjects. A crucial role for DRN 5-HT neurons in IS-induced trans-situational persistent potentiation of drug responses is also consistent with the findings that such an effect does not occur with

amphetamine conditioned place preference or psychomotor responding (Will et al. 1998, 2002). This is because sensitization of 5-HT DRN neurons should only be of consequence with regard to drugs that activate DRN 5-HT neurons. The available evidence suggests that amphetamine does not activate this population of neurons, and may even inhibit them (Rebec and Curtis 1983; Penington and Reiffenstein 1986).

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## References

- Adell A, Celada P, Abellan MT, Artigas F (2002) Origin and functional role of the extracellular serotonin in the midbrain raphe nuclei. *Brain Res Brain Res Rev* 39:154–180
- Aghajanian GK, Lakoski JM (1984) Hyperpolarization of serotonergic neurons by serotonin and LSD: studies in brain slices showing increased K<sup>+</sup> conductance. *Brain Res* 305:181–185
- Amat J, Matus-Amat P, Watkins LR, Maier SF (1998a) Escapable and inescapable stress differentially alter extracellular levels of 5-HT in the basolateral amygdala of the rat. *Brain Res* 812:113–120
- Amat J, Matus-Amat P, Watkins LR, Maier SF (1998b) Escapable and inescapable stress differentially and selectively alter extracellular levels of 5-HT in the ventral hippocampus and dorsal periaqueductal gray of the rat. *Brain Res* 797:12–22
- Anisman H, Zacharko RM (1986) Behavioral and neurochemical consequences associated with stressors. *Ann NY Acad Sci* 467:205–225
- Anisman H, Zalcman S, Shanks N, Zacharko RM (1991) Multi-system regulation of performance deficits induced by stressors: an animal model of depression. In: Boulton AA, Baker GB, Martin-Iverson MT (eds) *Animal models in psychiatry*, II. Humana Press, Clifton, N.J., pp 1–59
- Bassareo V, Tanda G, Petromilli P, Giua C, Di Chiara G (1996) Non-psychostimulant drugs of abuse and anxiogenic drugs activate with differential selectivity dopamine transmission in the nucleus accumbens and in the medial prefrontal cortex of the rat. *Psychopharmacology* 124:293–299
- Bonvento G, Scatton B, Claustre Y, Rouquier L (1992) Effect of local injection of 8-OH-DPAT into the dorsal or median raphe nuclei on extracellular levels of serotonin in serotonergic projection areas in the rat brain. *Neurosci Lett* 137:101–104
- Carboni E, Acquas E, Frau R, Di Chiara G (1989a) Differential inhibitory effects of a 5-HT<sub>3</sub> antagonist on drug-induced stimulation of dopamine release. *Eur J Pharmacol* 164:515–519
- Carboni E, Acquas E, Leone P, Di Chiara G (1989b) 5HT<sub>3</sub> receptor antagonists block morphine- and nicotine- but not amphetamine-induced reward. *Psychopharmacology* 97:175–178
- De Deurwaerdere P, Stinus L, Spampinato U (1998) Opposite change of in vivo dopamine release in the rat nucleus accumbens and striatum that follows electrical stimulation of dorsal raphe nucleus: role of 5-HT<sub>3</sub> receptors. *J Neurosci* 18:6528–6538
- Goeders NE, Guerin GF (1994) Non-contingent electric footshock facilitates the acquisition of intravenous cocaine self-administration in rats. *Psychopharmacology* 114:63–70
- Grahn RE, Will MJ, Hammack SE, Maswood S, McQueen MB, Watkins LR, Maier SF (1999) Activation of serotonin-immunoreactive cells in the dorsal raphe nucleus in rats exposed to an uncontrollable stressor. *Brain Res* 826:35–43
- Helmreich DL, Watkins LR, Deak T, Maier SF, Akil H, Watson SJ (1999) The effect of stressor controllability on stress-induced neuropeptide mRNA expression within the paraventricular nucleus of the hypothalamus. *J Neuroendocrinol* 11:121–128

- Higgins GA, Joharchi N, Nguyen P, Sellers EM (1992) Effect of the 5-HT<sub>3</sub> receptor antagonists, MDL72222 and ondansetron on morphine place conditioning. *Psychopharmacology* 106:315–320
- Hyson RL, Ashcraft LJ, Drugan RC, Grau JW, Maier SF (1982) Extent and control of shock affects naltrexone sensitivity of stress-induced analgesia and reactivity to morphine. *Pharmacol Biochem Behav* 17:1019–1025
- Iyer RN, Bradberry CW (1996) Serotonin-mediated increase in prefrontal cortex dopamine release: pharmacological characterization. *J Pharmacol Exp Ther* 277:40–47
- Jolas T, Aghajanian GK (1997) Opioids suppress spontaneous and NMDA-induced inhibitory postsynaptic currents in the dorsal raphe nucleus of the rat in vitro. *Brain Res* 755:229–245
- Kia HK, Miquel MC, Brisorgueil MJ, Daval G, Riad M, El Mestikawy S, Hamon M, Verge D (1996) Immunocytochemical localization of serotonin<sub>1A</sub> receptors in the rat central nervous system. *J Comp Neurol* 365:289–305
- Maier SF, Seligman MEP (1976) Learned helplessness: theory and evidence. *J Exp Psychol [Gen Sect]* 105:3–46
- Maier SF, Ryan SM, Barksdale CM, Kalin NH (1986) Stressor controllability and the pituitary-adrenal system. *Behav Neurosci* 100:669–674
- Maier SF, Grahn RE, Kalman BA, Sutton LC, Wiertelak EP, Watkins LR (1993) The role of the amygdala and dorsal raphe nucleus in mediating the behavioral consequences of inescapable shock. *Behav Neurosci* 107:377–388
- Maier SF, Kalman BA, Grahn RE (1994) Chlordiazepoxide microinjected into the region of the dorsal raphe nucleus eliminates the interference with escape responding produced by inescapable shock whether administered before inescapable shock or escape testing. *Behav Neurosci* 108:121–130
- Maier SF, Busch CR, Maswood S, Grahn RE, Watkins LR (1995a) The dorsal raphe nucleus is a site of action mediating the behavioral effects of the benzodiazepine receptor inverse agonist DMCM. *Behav Neurosci* 109:759–766
- Maier SF, Grahn RE, Watkins LR (1995b) 8-OH-DPAT microinjected in the region of the dorsal raphe nucleus blocks and reverses the enhancement of fear conditioning and interference with escape produced by exposure to inescapable shock. *Behav Neurosci* 109:404–412
- Maswood S, Barter JE, Watkins LR, Maier SF (1998) Exposure to inescapable but not escapable shock increases extracellular levels of 5-HT in the dorsal raphe nucleus of the rat. *Brain Res* 783:115–120
- McNeish CS, Svingos AL, Hitzemann R, Strecker RE (1993) The 5-HT<sub>3</sub> antagonist zacopride attenuates cocaine-induced increases in extracellular dopamine in rat nucleus accumbens. *Pharmacol Biochem Behav* 45:759–763
- Paxinos G, Watson C (1986) *The rat brain in stereotaxic coordinates*. Academic Press, New York
- Pehek EA, McFarlane HG, Maguschak K, Price B, Pluto CP (2001) M100,907, a selective 5-HT<sub>2A</sub> antagonist, attenuates dopamine release in the rat medial prefrontal cortex. *Brain Res* 888:51–59
- Pei Q, Zetterstrom T, Leslie RA, Grahame-Smith DG (1993) 5-HT<sub>3</sub> receptor antagonists inhibit morphine-induced stimulation of mesolimbic dopamine release and function in the rat. *Eur J Pharmacol* 230:63–68
- Penington NJ, Reiffenstein RJ (1986) Direct comparison of hallucinogenic phenethylamines and D-amphetamine on dorsal raphe neurons. *Eur J Pharmacol* 122:373–377
- Petty F, Sherman AD (1981) GABAergic modulation of learned helplessness. *Pharmacol Biochem Behav* 15:567–570
- Petty F, Kramer G, Wilson L (1992) Prevention of learned helplessness: in vivo correlation with cortical serotonin. *Pharmacol Biochem Behav* 43:361–367
- Phelix CF, Broderick PA (1995) Light microscopic immunocytochemical evidence of converging serotonin and dopamine terminals in ventrolateral nucleus accumbens. *Brain Res Bull* 37:37–40
- Rebec GV, Curtis SD (1983) Reciprocal changes in the firing rate of neostriatal and dorsal raphe neurons following local infusions or systemic injections of D-amphetamine: evidence for neostriatal heterogeneity. *J Neurosci* 3:2240–2250
- Sotelo C, Cholley B, El Mestikawy S, Gozlan H, Hamon M (1990) Direct immunohistochemical evidence of the existence of 5-HT<sub>1A</sub> autoreceptors on serotonergic neurons in the midbrain raphe nuclei. *Eur J Neurosci* 2:1144–1154
- Sprouse JS, Aghajanian GK (1987) Electrophysiological responses of serotonergic dorsal raphe neurons to 5-HT<sub>1A</sub> and 5-HT<sub>1B</sub> agonists. *Synapse* 1:3–9
- Spyraki C, Nomikos GG, Galanopoulou P, Daifotis Z (1988) Drug-induced place preference in rats with 5,7-dihydroxytryptamine lesions of the nucleus accumbens. *Behav Brain Res* 29:127–134
- Steinbusch HW (1981) Distribution of serotonin-immunoreactivity in the central nervous system of the rat-cell bodies and terminals. *Neuroscience* 6:557–618
- Tanda G, Frau R, Di Chiara G (1995) Local 5HT<sub>3</sub> receptors mediate fluoxetine but not desipramine-induced increase of extracellular dopamine in the prefrontal cortex. *Psychopharmacology* 119:15–19
- Tao R, Auerbach SB (1995) Involvement of the dorsal raphe but not median raphe nucleus in morphine-induced increases in serotonin release in the rat forebrain. *Neuroscience* 68:553–561
- Tao R, Auerbach SB (2000) Regulation of serotonin release by GABA and excitatory amino acids. *J Psychopharmacol* 14:100–113
- Tao R, Ma Z, Auerbach SB (1996) Differential regulation of 5-hydroxytryptamine release by GABA<sub>A</sub> and GABA<sub>B</sub> receptors in midbrain raphe nuclei and forebrain of rats. *Br J Pharmacol* 119:1375–1384
- Tzschentke TM (1998) Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. *Prog Neurobiol* 56:613–672
- Van Bockstaele EJ, Biswas A, Pickel VM (1993) Topography of serotonin neurons in the dorsal raphe nucleus that send axon collaterals to the rat prefrontal cortex and nucleus accumbens. *Brain Res* 624:188–198
- Vertes RP (1991) A PHA-L analysis of ascending projections of the dorsal raphe nucleus in the rat. *J Comp Neurol* 313:643–668
- Weiss JM, Goodman PA, Losito BA, Corrigan S, Charry JM, Bailey WH (1981) Behavioral depression produced by an uncontrollable stressor: relationship to norepinephrine, dopamine, and serotonin levels in various regions of rat brain. *Brain Res Rev* 3:167–205
- Will MJ, Watkins LR, Maier SF (1998) Uncontrollable stress potentiates morphine's rewarding properties. *Pharmacol Biochem Behav* 60:655–664
- Will MJ, Der-Avakian A, Pepin JL, Durkan BT, Watkins LR, Maier SF (2002) Modulation of the locomotor properties of morphine and amphetamine by uncontrollable stress. *Pharmacol Biochem Behav* 71:345–351
- Yoshimoto K, McBride WJ (1992) Regulation of nucleus accumbens dopamine release by the dorsal raphe nucleus in the rat. *Neurochem Res* 17:401–407
- Yoshimoto K, Yayama K, Sorimachi Y, Tani J, Ogata M, Nishimura A, Yoshida T, Ueda S, Komura S (1996) Possibility of 5-HT<sub>3</sub> receptor involvement in alcohol dependence: a microdialysis study of nucleus accumbens dopamine and serotonin release in rats with chronic alcohol consumption. *Alcohol Clin Exp Res* 20:311A–319A