



## The glial activation inhibitor AV411 reduces morphine-induced nucleus accumbens dopamine release

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### ABSTRACT

Glial activation has recently been discovered to modulate several effects of morphine, including analgesia, tolerance, and dependence. The present studies extend this line of investigation by exploring whether glial activation may also affect extracellular levels of dopamine (DA) in the nucleus accumbens (NAc) shell, a neurochemical corollary of morphine-induced drug reward, during a challenge dose of morphine in experiments both with and without precipitated withdrawal. Morphine or vehicle was administered s.c. for 4 days (starting at 15 mg/kg/day up to 20 mg/kg/day), and the glial activation inhibitor AV411 (7.5 mg/kg) or vehicle was administered twice daily. A challenge dose of morphine (22.5 mg/kg) or saline was then given during dialysis. In the first experiment, naloxone (10 mg/kg) was administered 1 h after morphine during dialysis in AV411- or vehicle-treated rats, and behavioral signs of somatic withdrawal were assessed during microdialysis. In the second experiment, using the same dosing regimen, sampling continued 3 h after morphine or saline in AV411- or vehicle-treated rats. NAc DA increased in vehicle-treated rats significantly more than in AV411-treated rats before naloxone treatment, and withdrawal symptoms were significantly reduced in AV411-treated rats. The decrease in morphine-induced NAc DA by AV411 was persistent, lasting 3+ h post-morphine. These results indicate that glial activation contributes to the effects of morphine on NAc DA, which is associated with somatic signs of precipitated withdrawal.

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### 1. Introduction

Opioids, including morphine, are critical for pain management but are highly rewarding and for some individuals their use can lead to a lifelong cycle of addiction, withdrawal, and relapse. Opioid abuse is an increasing problem worldwide and the reinforcing effects of opioid analgesics makes them susceptible to diversion and illicit use and abuse (Compton and Volkow, 2006). Although the traditional view of opioid actions is that they are neurally-mediated, recent research has suggested an important modulatory role for glia (astrocytes and microglia) in opioid actions, particularly in the areas of analgesia, tolerance, and dependence (Hutchinson et al., 2007). Moreover, glia may be involved in reward. A key finding in this regard was that of Narita et al. (2006) who demonstrated that microinjection of astrocyte conditioned media in the anterior cingulate cortex and the nucleus accumbens (NAc), but not the caudate putamen, enhanced morphine conditioned place preference (CPP), a well characterized measure of the motivational effects of various drugs (Tzschentke, 2007).

The importance of both microglia (Banati, 2002) and astrocytes (Araque et al., 1999) in modulating plasticity has been increasingly appreciated and current thinking about addiction embraces the notion of addiction as a form of experience-dependent plasticity (Hyman et al., 2006). Work in our laboratory has shown that a repeated, escalating regimen of morphine induces the activation of both microglia and astrocytes in regions associated with reward including the ventral tegmental area (VTA), prefrontal cortex, and NAc (Hutchinson et al., 2007). Furthermore, the morphine-induced activation of both microglia and astrocytes that was observed in the VTA, the source of dopamine (DA) cells that project to the prefrontal cortex and NAc, was potently inhibited by the coadministration with morphine of the glial activation inhibitor AV411 (ibudilast) (Hutchinson et al., 2007). Moreover, AV411 also reduced somatic signs of precipitated withdrawal using the same dosing regimen (Ledeboer et al., 2007). AV411 is a blood–brain barrier permeable nonspecific phosphodiesterase (PDE) inhibitor that has been used in Japan for asthma and post-stroke dizziness (Ledeboer et al., 2007) and may be an effective treatment for addiction if reward processes are attenuated. Activated glia release excitatory substances including proinflammatory cytokines (Streit et al., 1999), nitric oxide (NO) (Sparrow, 1994), prostaglandins

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(Tzeng et al., 2005), and excitatory amino acids (Araque et al., 1998) that could enhance the excitability of VTA dopamine neurons. This glial release of excitatory products might increase dopamine release in projection regions such as the NAc. AV411 is known to decrease or inhibit glial production of proinflammatory cytokines, chemokines and growth factors as well as NO (Ledeboer et al., 2007), and may thus reduce VTA excitability.

The goals of the current studies were to determine if AV411 coadministered with morphine would impact nucleus accumbens dopamine (DA) and whether this would be related to withdrawal signs. First, we measured DA in the NAc shell in morphine-dependent rats treated with AV411 or vehicle. It is generally accepted that the rewarding properties of addictive drugs is due to increased DA in the NAc (see Di Chiara and Bassareo, 2007 for review), and NAc DA has also been implicated in opioid withdrawal (Harris and Aston-Jones, 1994). Dependence was induced using an escalating morphine regimen, and dependence was confirmed by measuring somatic signs of naloxone-precipitated withdrawal. Microdialysis was performed during both the morphine challenge as well as naloxone treatment, in animals receiving either AV411 or vehicle. In a separate experiment, the persistence of morphine-induced dopamine release in the NAc shell was measured in AV411- or vehicle-treated rats.

## 2. Methods

### 2.1. Animals

Adult, male Sprague–Dawley rats (Harlan, Inc., Indianapolis, IN) weighing 275–350 g were pair-housed in standard Plexiglas cages with food and water freely available. Rats were maintained in a climate-controlled colony at 21° C on a 12 h light–dark cycle. All experiments were conducted during the light phase. Rats acclimated to the colony for 2 weeks prior to any procedures. All procedures were in accordance with protocols approved by the University of Colorado Institutional Animal Care and Use Committee.

### 2.2. Experimental procedures

#### 2.2.1. Experiment 1: Effects of AV411 on NAc DA and naloxone-precipitated withdrawal

**2.2.1.1. Surgery.** Microdialysis guide cannula implantations were performed under halothane anesthesia (MWI Veterinary Supply, Denver, CO, USA). Sterile CMA 12 guide cannulae (CMA Microdialysis, Solna, Sweden) were aimed at the right or left nucleus accumbens shell (relative to bregma: AP = +1.7, ML = ±0.8, DV = –6.0; bite bar = –3) (Paxinos and Watson, 1998) in a counterbalanced fashion. A guide cannula and a tether screw (CMA Microdialysis) were anchored to the skull with jeweler's screws and dental cement. Rats were individually housed after surgery and allowed to recover for 1 week.

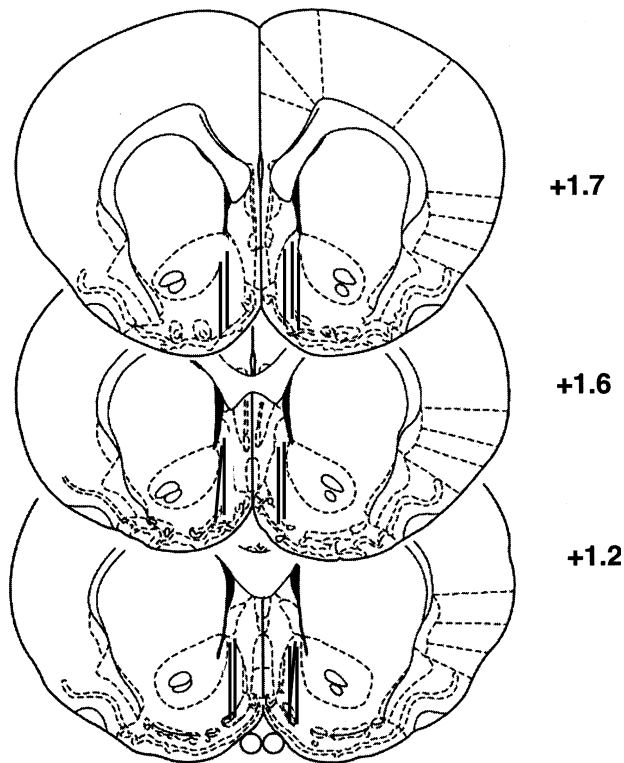
**2.2.1.2. Drug administration.** Following recovery, rats began a 7-day dosing regimen ( $n = 6/\text{group}$ ) in which they received twice-daily AV411 (7.5 mg/kg, 2 ml/kg intraperitoneally in 35% polyethylene glycol [PEG; Sigma] in saline) or equivolume vehicle. The morning injection occurred between 1:45 and 2:15 h after lights on, with the afternoon injection occurring between 9:45 and 10:15 h after lights on. On day 3, rats began a 5 day dependence regimen of morphine or equivolume saline (1 ml/kg subcutaneous saline). When morphine was administered near an AV411 administration, it occurred 45 min after the AV411 injection. The dependence regimen (times relative to lights on) consisted of the following dose escalation from 15 to 22.5 mg/kg/day: day 3: 5 mg/kg (2 h), 5 mg/kg

(6 h), 5 mg/kg (10 h); day 4: 7.5 mg/kg (2 h), 12.5 mg/kg (10 h); day 5: 15 mg/kg (2 h); day 6: 17.5 mg/kg (2 h); and day 7: 22.5 mg/kg (2 h). Body weights were recorded prior to each dose. Rats were habituated to the microdialysis bowls twice (60 min each) in both a drug free and a morphine state before microdialysis experiments began.

**2.2.1.3. Microdialysis and withdrawal behavior.** On the afternoon before microdialysis (day 6 of the 7 day dosing regimen), rats were transferred to a dialysis room that was on the same light–dark cycle as the colony room. Microdialysis probes (CMA 12, MW cut-off 20,000 Da, 2 mm active membrane) were inserted and rats were placed in separate Plexiglas infusion bowls with chip bedding as well as food and water freely available. Artificial cerebrospinal fluid (145 mM NaCl, 2.7 mM KCl, 1.2 mM CaCl<sub>2</sub>, 1.0 mM KCl) was perfused through the probes using a CMA infusion pump at a flow rate of 0.2 µl/min overnight. The flow rate was increased to 1.5 µl/min the next morning and after AV411 or vehicle dosing and a 2 h equilibration period sample collection began. Three samples were collected before the final subcutaneous dose of morphine was administered (22.5 mg/kg). Sixty minutes later, naloxone (10 mg/kg in 1 ml/kg) was administered subcutaneously to precipitate opioid withdrawal. Withdrawal behaviors were scored by two investigators (3 rats/investigator) blinded to treatment for 6 × 10 min blocks. These behaviors are rarely observed in normal animals (Hutchinson et al., 2009; Ledeboer et al., 2007) thus due to the technical difficulty of performing behavioral analysis during microdialysis, scoring of behaviors was limited to the precipitated withdrawal phase in morphine-dependent rats. Behaviors that were scored included: jumping, rearing, exploration (movement greater than one body length), teeth chattering, wet dog shakes, abnormal posture, ptosis, diarrhea, penis licking, oral stimulation (filling of mouth with bedding), paw chewing, cleaning, salivation, vocalization, chewing (large jaw movements including masseter muscle contraction, to include paw chewing), abnormal posture, and writhing (small shifts in body position). Each rat's score consisted of one point for each observed behavior, except in cases where the response was prolonged, for example ptosis, where counts were made every 30 s. These behaviors have previously been described as associated with opioid withdrawal and are the set of behaviors routinely measured in studies of precipitated morphine withdrawal (Fdez Espejo et al., 1995; Gellert and Holtzman, 1978). Each behavior was analyzed separately and as a combined score to provide an overall assessment of elicited withdrawal behavior, as is standard in the field (Fdez Espejo et al., 1995; Gellert and Holtzman, 1978). There was no interaction of drug group by time, so scores of individual behaviors were summed across the 10 min blocks for a total withdrawal score.

Collection tubes were pre-filled with 3 µl of 0.02% EDTA (anti-oxidant) in 1% ethanol. Dialysates were collected manually every 20 min for a total of 4 h (1 h baseline, 1 h post-morphine, 2 h post-naloxone) and placed on dry ice throughout the experiment then stored in –80 °C until analysis. Dialysates were analyzed by high performance liquid chromatography (HPLC) coupled with electrochemical detection within 2 weeks of collection as previously described in detail (Bland et al., 2004).

To verify probe placement, rats were euthanized with 65 mg/kg intraperitoneal sodium pentobarbital (Abbott Laboratories, North Chicago, IL, USA). Brains were frozen in chilled isopentane and sectioned (40 µm) at –20 °C. Sections mounted on gelatin-treated slides were cresyl violet stained, cover-slipped, and viewed under a light microscope. Only rats with at least 75% of the probe placed within the nucleus accumbens shell were included in the analysis. Fig. 1 is a composite of drawings of probe tracts used to verify placements.



**Fig. 1.** Probe placements in the NAc shell in microdialysis experiments using plates adapted from Paxinos and Watson (1998). Not all probes can be seen due to overlapping placements.

#### 2.2.2. Experiment 2: Effects of AV411 on morphine-induced NAc DA without withdrawal

Experimental procedures were the same as for Experiment 1. However, no naloxone was administered and no behaviors were scored, and dialysis continued for 180 min after morphine or saline injections.

#### 2.3. Statistical analysis

DA in the dialysates was expressed as the percent of basal (the average of the first three samples). *Experiment 1:* Differences in DA were determined using mixed ANOVA with drug treatment (AV411, vehicle) as the between groups variable and time as the within subjects variable. Differences in withdrawal behavior were determined using mixed ANOVA with drug treatment (AV411, vehicle) as the between groups variable and specific behaviors (e.g. teeth chattering, wet dog shakes) as the within subjects variable. The relationship between NAc DA and withdrawal scores was determined using a Pearson product-moment correlation, with peak DA response (levels attained at 60 min post-morphine) as the y variable and total withdrawal score (the sum of the individual behaviors across the 1 h post-naloxone period) as the x variable. An *r* to *z* transform was used to determine the significance of the correlation. *Experiment 2:* There was no difference between the saline treated groups so their data were pooled and titled "Saline". Differences in DA were determined using mixed ANOVA with drug treatment (AV411 + morphine, vehicle + morphine, Saline) as the between groups variable and time as the within subjects variable. For all experiments, statistically significant effects were followed with Fisher's LSD post hoc tests ( $\alpha$  set at 0.05). All statistics were performed using StatView for Windows (SAS Institute, Inc.).

### 3. Results

#### 3.1. Experiment 1: AV411 attenuated morphine-induced NAc DA and naloxone-precipitated withdrawal behaviors

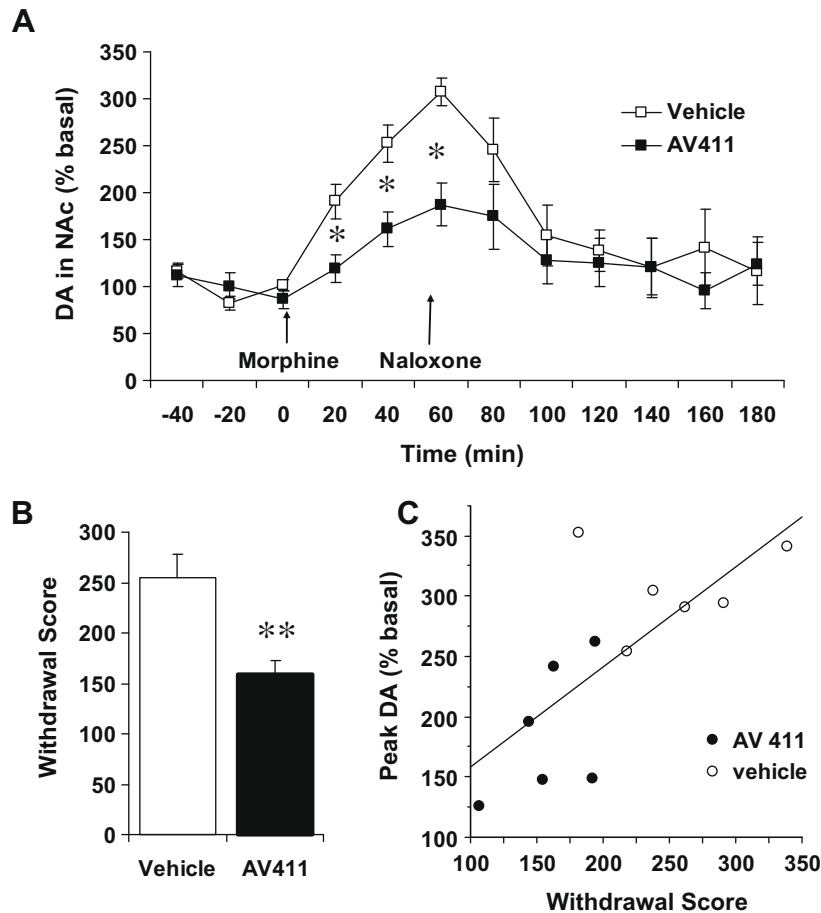
Morphine produced an increase in NAc DA efflux, and this increase was reduced by AV411 (Fig. 2A). Naloxone precipitated potent withdrawal, and this was blunted by AV411 (Fig. 2B). Finally, there was a relationship between peak DA levels and magnitude of somatic withdrawal (Fig. 2C). With regard to extracellular DA, ANOVA revealed a significant drug group  $\times$  time interaction,  $F(11,110) = 2.31, p = .01$ . Post hoc tests indicated that DA levels were greater in vehicle-treated rats at 20, 40, and 60 min post-morphine,  $p < .05$ , indicating that AV411 attenuated NAc DA before naloxone treatment (Fig. 2A). Post-naloxone DA levels did not differ between groups. There was no group difference in basal levels of NAc DA. Basal DA levels (pg/27  $\mu$ g, mean  $\pm$  SEM) were vehicle:  $0.313 \pm 0.058$ , AV411:  $0.395 \pm 0.045$ . With regard to withdrawal behavior, mixed ANOVA revealed a significant main effect of drug treatment,  $F(1,10) = 13.23, p < .01$ , AV411-treated rats had significantly fewer somatic signs of withdrawal (Fig. 2B shows the main effect of drug treatment). The drug  $\times$  behavior interaction was marginally significant  $F(9,90) = 1.71, p = .09$ . Scores and *p* values for the specific behaviors are shown in Table 1, only teeth chattering was statistically significant. There was a significant correlation between peak DA (levels attained at 60 min post-morphine) and total withdrawal score (the sum of all withdrawal behaviors across the 1 h test),  $r(12) = .71, p < .01$  (Fig. 2C).

#### 3.2. Experiment 2: AV411 attenuation of morphine-induced NAc DA is persistent

Morphine produced a sustained increase in extracellular NAc DA that persisted for the 180 min of testing (Fig. 3). AV411 reduced this morphine-induced increase in DA efflux for the entire 180 min period of measurement. ANOVA revealed a significant drug group  $\times$  time interaction,  $F(22,143) = 8.91, p < .0001$ . Post hoc tests indicated that AV411-treated rats had less morphine-induced NAc DA at all time points beginning 40 min post-morphine throughout the 180 min post-morphine sampling period ( $p < .05$ ) with the exception of the 160 min time point ( $p = .08$ ). In addition, vehicle-treated rats were significantly different than saline at all time points 40 min post-morphine and later while AV411-treated rats were not different than saline at 40, 100, and 180 min post-morphine. There were no group differences in basal levels of NAc DA. Basal DA levels (pg/27  $\mu$ g, mean  $\pm$  SEM) were vehicle + morphine:  $0.339 \pm 0.12$ , AV411 + morphine:  $0.385 \pm 0.09$ , vehicle + saline:  $0.398 \pm 0.11$ , AV411 + saline:  $0.374 \pm 0.02$ .

### 4. Discussion

Products of glial activation can modulate diverse effects of opioids (Watkins et al., 2005, 2007), and it has previously been reported that the glial activation and PDE inhibitor AV411 (ibudilast) reduced the somatic signs of withdrawal (Ledeboer et al., 2007). The present results show that AV411 attenuates neurochemical and behavioral indicators of both morphine dependence and reward. A novel finding here is that morphine-induced DA release in the NAc shell is reduced by concurrent AV411 treatment in morphine-dependent rats, and that this reduction in DA is associated with a reduction in somatic signs of naloxone-precipitated withdrawal. Although it has long been known that there is in large part a dissociation between opioid dependence and reward (Wise and Bozarth, 1985), with opioid dependence involving primarily the periaqueductal gray area (Laschka et al., 1976) and opioid reward relying primarily on the



**Fig. 2.** Glial inhibition decreases morphine-induced NAc DA and naloxone-precipitated withdrawal in morphine-dependent rats. Rats received escalating morphine for 5 days with concurrent AV411 or vehicle treatment and dialysis was performed on day 5. Naloxone (10 mg/kg) was injected 1 h after morphine (22.5 mg/kg). (A) Morphine-induced DA in the NAc shell was attenuated in AV411-treated rats compared to vehicle controls prior to naloxone. Data are means  $\pm$  SEMs of 6 rats/group. (B) Somatic signs of naloxone-precipitated withdrawal were attenuated in AV411-treated rats compared to vehicle controls. Data are means  $\pm$  SEMs of 6 rats/group. (C) There was a significant correlation between peak DA and total behavioral score. \*AV411 + morphine less than vehicle + morphine,  $p < .05$ . \*\*AV411 + morphine less than vehicle + morphine,  $p < .01$ .

**Table 1**

Specific behaviors scored during naloxone-precipitated morphine withdrawal for 1 h during microdialysis. Values are means (SEMs) of 6 rats/group.

Specific behavior	Vehicle	AV411	<i>p</i> value
Teeth chattering	52.5 (16.9)	11.5 (3.2)	.009**
Rearing	3.67 (1.5)	0.67 (0.3)	.07
Ptosis	61.5 (10.9)	36.17 (10.4)	.12
Grooming	3.50 (1.2)	1.33 (0.7)	.15
Exploration	1.83 (0.5)	9.0 (4.6)	.16
Abnormal posture	12.5 (4.7)	5.17 (1.7)	.17
Chewing/oral stimulation	50.5 (16.9)	37.83 (9.8)	.53
Writhing	45.33 (13.0)	36.33 (7.7)	.56
Wet dog shake	21.83 (3.9)	18.67 (5.7)	.65
Penis licking	1.5 (0.2)	2.0 (1.3)	.71

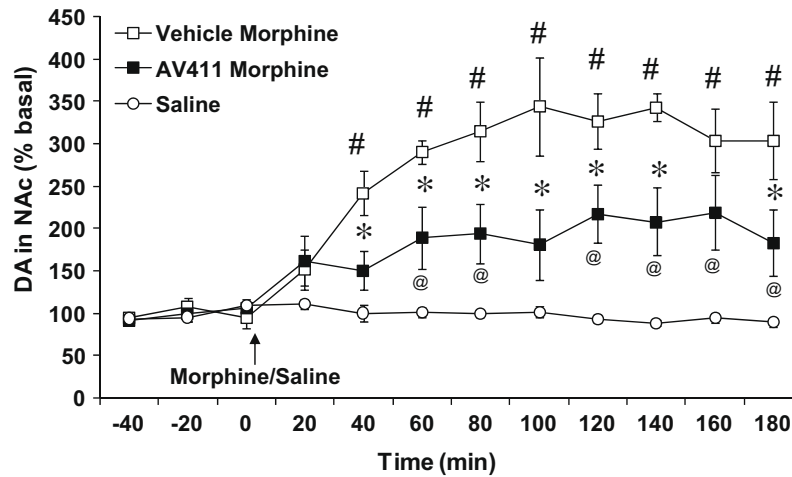
There were no observations of jumping, diarrhea, salivation, or vocalization.

\*\* Statistically significant,  $\alpha$  set at .05.

mesolimbic DA system (Wise, 1989), there also seems to be a role for DA transmission in the NAc shell in opioid withdrawal (Harris and Aston-Jones, 1994). In agreement with a previous report (Pothos et al., 1991), we observed decreases in NAc DA release after naloxone-precipitated withdrawal. It is intriguing that we observed a strong relationship between NAc DA and somatic signs of withdrawal, suggesting that AV411 similarly affects neural systems regulating both the motivational (reward) and somatic (physical dependence) properties of opioids. Thus, the more rewarding mor-

phine is in terms of a neurochemical marker, NAc DA, the greater the magnitude of behavioral withdrawal. Little is known about the neurochemical substrates of specific withdrawal behaviors. Here we only observed statistically significant differences in teeth chattering, with trends in several of the other behaviors. However, Stella et al. (2003) have shown that adenosine antagonists reduced both NAc DA and teeth chattering, writhing, jumping, and diarrhea, the last two of which were not observed in our studies. This inconsistency may be due to the different morphine dependence regimens used. However, it is clear that overall, withdrawal is reduced by AV411 and this is associated with a reduction in a neurochemical marker of reward, NAc DA. Thus, rats that received AV411 had lower peak NAc DA and lower withdrawal scores, while rats that received vehicle had both higher peak NAc DA and higher withdrawal scores. This is important because NAc dopamine is known to be involved in drug craving (De Vries et al., 1999; Di Chiara and Bassareo, 2007; Wise and Bozarth, 1985), which is a strong predictor of relapse.

PDE inhibitors have previously been shown to attenuate dependence produced by abused drugs, including opioids. For example, coadministration of rolipram, a specific PDE4 inhibitor, reduced naloxone-precipitated morphine withdrawal (Hamdy et al., 2001; Mamiya et al., 2001). PDE4 inhibitor, pentoxifyllin reduced expression of the microglial activation marker CD11b/c and reduced behavioral tolerance in morphine-tolerant mice, suggesting that the behavioral effects of pentoxifyllin on morphine tolerance involve microglia (Mika et al., 2009). The PDE4 inhibitor propentofyl-



**Fig. 3.** Glial inhibition attenuates morphine-induced DA in the NAc shell for at least 3 h. Rats received escalating morphine or saline control injections (there was no difference between saline AV411 and saline vehicle groups so they were pooled) for 5 days with concurrent AV411 or vehicle treatment. Dialysis was performed on day 5 with a challenge dose of morphine (22.5 mg/kg) or equivalent saline. Data are means  $\pm$  SEMs of 5 or 6 rats/group. #Vehicle + morphine greater than saline,  $p < .05$ , \*AV411 + morphine less than vehicle + morphine,  $p < .05$ , @AV411 + morphine greater than saline,  $p < .05$ .

line reduced morphine tolerance and associated increases in proinflammatory cytokines in the spinal cord (Raghavendra et al., 2004). Theophylline also attenuated signs of morphine withdrawal, but was much less effective than rolipram (Itoh et al., 1998). In terms of reward, rolipram attenuated CPP produced by both morphine and cocaine, but not food (Thompson et al., 2004), and reduced the discriminative-stimulus effects of morphine and methamphetamine (Yan et al., 2006). However, the use of rolipram is severely limited by side effects such as nausea and emesis (Dyke and Montana, 2002). Therefore the development of PDE inhibitors with the potential for reducing opioid dependence and reward, but with a reduced side effect liability, is of great clinical importance. AV411 (ibudilast) has been used clinically in Japan and elsewhere for asthma and post-stroke dizziness, and is well tolerated, orally available, and with few side effects (Ledeboer et al., 2007), and is known to inhibit morphine-induced glial activation (Hutchinson et al., 2009).

To the best of our knowledge, this is the first report of effects of PDE inhibitors on morphine-induced DA in the NAc shell. A previous study reported that rolipram decreased behavioral sensitization to methamphetamine, while having no effect on methamphetamine-stimulated DA release in the striatum (Iyo et al., 1996). It is unknown whether the discrepancy between these results and the present finding of AV411 reduction of morphine-induced NAc DA is due to differential effects of PDE inhibitors in the mesolimbic vs mesostriatal pathways, or due to differential effects on opioid vs psychostimulant signaling pathways.

PDEs act by hydrolyzing the cyclic nucleotides cyclic adenosine monophosphate (cAMP) or cyclic guanosine monophosphate (cGMP), or both. They comprise a family of 11 different subfamily members with various isoforms existing in each subfamily, most of which are found in the CNS (Menniti et al., 2006) and some of which, particularly PDE4, are expressed in astrocytes (Madelian and La Vigne, 1996) and microglia (Madelian and La Vigne, 1996). AV411 is a nonspecific PDE inhibitor with reports of inhibition of PDE1, 4, 3, 10, and 11 (Ledeboer et al., 2007). It is important to note that microglial activation has been shown to be regulated by a cAMP-specific PDE4 isoform and is inhibited by rolipram (Sebastiani et al., 2006). Moreover, there is a good deal of evidence for PDE inhibitor-induced decreases in cAMP mediated glial activation and decreased release of products that might increase morphine reward. For example, enhancing cAMP signaling in

cultured microglia with the PDE inhibitor propentofylline inhibits the release of the cytokines tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-1 beta (IL-1 $\beta$ ) (Schubert et al., 2000). Si et al. (1998) found that propentofylline reduced LPS-induced TNF- $\alpha$  and IL-1 $\beta$  production in microglial cell culture. TNF- $\alpha$  and IL-1 $\beta$  both upregulate inducible nitric oxide synthase (iNOS), the synthetic enzyme that produces NO, as well as increase the subsequent production of NO, and these effects are attenuated by the PDE inhibitors theophylline and pentoxifylline (Saud et al., 2005). NO has been implicated in morphine's effects in the mesolimbic DA system. Thus, L-arginine, a nitric oxide (NO) precursor, potentiates morphine CPP when infused directly into the VTA, and intra VTA infusions of N(G)-nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthase (NOS) inhibitor, attenuates morphine CPP (Gholami et al., 2003). Because NO production is known to be inhibited by AV411 (Suzumura et al., 1999), the possibility exists that the effects of AV411 on morphine-induced DA release may be produced by a reduction of NO by AV411.

Alternatively, AV411 could be acting by decreasing levels of extracellular adenosine, a product of cAMP hydrolyzation by PDEs (a major source of extracellular adenosine). Adenosine inhibits GABA mediated synaptic transmission in the VTA (Wu et al., 1995) which would likely result in disinhibition of DA cells as they are under tonic GABAergic inhibition. It is important to note that adenosine receptors are found on both astrocytes and microglia (van Calker and Biber, 2005) and stimulation of glial adenosine A<sub>2A</sub> receptors enhances the levels of several substances that could contribute to morphine reward and morphine-induced NAc DA release by stimulating DA cell excitability in the VTA. For example, the specific A<sub>2A</sub> agonist CGS21680 increases glutamate efflux from cultured glia (Li et al., 2001), and glutamate signaling in the VTA has long been known to be associated with morphine reward as well as cross-sensitization with stress and other drugs of abuse (Fitzgerald et al., 1996). In light of the finding that AV411 decreased morphine-induced activation of astrocytes and microglia in the VTA (Hutchinson et al., 2009) it is possible that decreased VTA excitability, possibly mediated by glial NO, glutamate, or adenosine, may mediate AV411's attenuation of morphine-induced DA neurotransmission and withdrawal signs. Future work will address these possibilities.

It has been recognized that glia modulate morphine's analgesic effects and this understanding is leading to efforts to improve pain

management pharmacotherapies (Hutchinson et al., 2007; Watkins et al., 2005, 2007). The present results implicate glia in the rewarding effects of morphine. Thus the opportunity exists for improving pharmacotherapies for addiction to opioids and other abused drugs by targeting glia, cell types whose importance in CNS function is increasingly appreciated.

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