

GLUTAMATE-ASSOCIATED PLASTICITY IN THE VENTRAL TEGMENTAL AREA IS NECESSARY FOR CONDITIONING ENVIRONMENTAL STIMULI WITH MORPHINE

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Abstract—We sought to determine if plasticity in the ventral tegmental area (VTA) of the midbrain is involved in learning to associate morphine exposure with a specific environment. For this, we tested whether activation of glutamate receptors and protein kinase A is needed for the acquisition and expression of a morphine-conditioned place preference (CPP). Rats received bilateral microinjections of either the NMDA antagonist AP5 (0.48 nmol/0.3 μ l), the AMPA antagonist CNQX (0.21 nmol/0.3 μ l), or vehicle into the VTA prior to each of three morphine-conditioning sessions. Both the AMPA and NMDA receptor antagonists blocked the development of morphine CPP when given into the VTA but not when given outside the VTA. In similar studies the protein kinase A (PKA) inhibitor, Rp-cAMPS (13 nmol/0.3 μ l), blocked the acquisition of morphine CPP when given into the VTA immediately after morphine conditioning. In separate experiments, glutamate antagonists, or Rp-cAMPS, immediately prior to the preference test blocked the expression of morphine CPP when microinjected into the VTA. These data indicate that the VTA is an important site for synaptic modifications involved in the learning and memory of environmental cues predicting reward, and that glutamate input and PKA activation are crucial to this process. © 2004 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: protein kinase A, AMPA receptor, NMDA receptor, RpcAMPS, place preference.

The mesocorticolimbic dopamine (DA) system has received a great deal of attention as a brain substrate for reinforcement and motivation. In recent years, the focus on DA mechanisms has shifted from a possible role in reinforcement per se to involvement in learning or motivation for conditioned behaviors (Beninger and Phillips, 1980; Schultz, 1993; see Robinson and Berridge, 1993; Di Chiara, 1995; Robbins and Everitt, 1996 for reviews). A pivotal role for mesocorticolimbic dopaminergic mechanisms in opiate-induced place conditioning has been shown in a

number of studies (Leone and Di Chiara, 1987; Bals-Kubik et al., 1993; Shippenberg et al., 1993). Opiates are known to interact with DA systems by suppressing GABA inhibitory input to DA neurons in the ventral tegmental area (VTA), thereby augmenting DA release (Johnson and North, 1992). In contrast, conditioned environmental stimuli are thought to act directly on VTA DA cells via excitatory inputs, possibly glutamatergic in nature (Schultz, 1998; Bespalov et al., 2000).

The association of environmental cues with drug reinforcement appears to involve plasticity within the mesocorticolimbic system (Hyman and Malenka, 2001), and glutamate transmission has been implicated in mediating this plasticity (Winder et al., 2002). Systemic administration of NMDA antagonists blocks both the acquisition (Tzschentke and Schmidt, 1995; Kim et al., 1996) and expression of a morphine place preference (Tzschentke and Schmidt, 1997). Furthermore, NMDA receptors in both the VTA and nucleus accumbens have been found to be necessary for the expression of morphine conditioned place preference (CPP; Popik and Kolasiewicz, 1999). These data indicate the importance of glutamate transmission in the learning and expression of stimulus-controlled drug associations.

Recently it was reported that many abused drugs, including morphine and cocaine, induce long-term potentiation (LTP) at glutamatergic receptors on VTA dopaminergic neurons (Saal et al., 2003). Consistent with the possibility that such glutamate-induced plasticity is associated with behavioral conditioning for drugs, we recently found that glutamate release in the VTA is essential for the acquisition of cocaine place preference (Harris and Aston-Jones, 2003a). These data indicate that glutamate inputs to the VTA play a critical role in the integration of information about stimuli that predict rewards. Furthermore, in many areas of the brain, e.g. hippocampus (Nayak et al., 1998), amygdala (Huang et al., 2000) and dorsal striatum (Spencer and Murphy, 2002), LTP-like synaptic enhancement has been shown to be dependent not only glutamate input but to also require cAMP-dependent protein kinase A (PKA) activation. In the following sets of experiments, we tested the hypothesis that synaptic enhancements in the VTA mediated by glutamate transmission are involved in the conditioning of morphine-related cues. We also tested the effect of the specific inhibitor of PKA, Rp-cAMPS (Rothermel and Parker Botelho, 1988), injected into the VTA on the acquisition or expression of morphine CPP. In the acquisition phase, the PKA inhibitor was injected immediately post-training because PKA activation is associated

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Abbreviations: AP5, L-(+)-2-amino-5-phosphonopentanoic acid; AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; CPP, conditioned place preference; DA, dopamine; LTP, long-term potentiation; NMDA, N-methyl-D-aspartic acid; PKA, protein kinase A; PPTg, pedunculopontine tegmental nucleus; VTA, ventral tegmental area.

with late phase but not early phase LTP in the amygdala (Huang et al., 2000). Furthermore, by giving the drug post-training we insure that the drug does not alter the direct effects of morphine. Here, we provide the first evidence that glutamate release and PKA activation in the VTA are essential for both the conditioning and expression of morphine place preference.

EXPERIMENTAL PROCEDURES

Subjects

Male Sprague–Dawley rats (200–250 g; Harlan, Indianapolis, IN, USA) were used in all experiments ($n=170$). Rats were group-housed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and all efforts were made to minimize the number of animals used and their suffering. Animals were maintained on a 12-h light/dark cycle with food and water available *ad libitum*. All animal procedures were also approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania.

Drugs

Morphine sulfate powder, provided by the National Institute on Drug Abuse (Baltimore, MD, USA), was dissolved in sterile saline and administered via i.p. injection. CNQX, D-AP5 and Rp-cAMPS were purchased from Tocris (Ellisville, MO, USA) and dissolved in lactated Ringer's solution (sodium 130 mEq, lactate 28 mEq, potassium 4 mEq, calcium 3 mEq, chloride 109 mEq, pH approximately 6.6). The doses of CNQX and AP5 were based on those we and others previously found to be effective (Cornish et al., 2001; Harris and Aston-Jones, 2003a). The dose of Rp-cAMPS was based on published reports using similar intraparenchymal microinjections (Roosendaal et al., 2002; Koh and Bernstein, 2003).

Surgery and histology

Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and implanted with bilateral chronic indwelling guide cannulae aimed 2 mm above the VTA (AP -5.3 , ML $+2.5$, DV -7.5 ; Paxinos and Watson, 1998). Cannulae (26 gauge) were angled 10° (dorsolateral to ventromedial). The tips of inner injection cannulae (30 gauge) extended 2 mm below the guide cannulae into the injection sites. Control injections were made approximately 2 mm above the VTA (AP -5.3 , ML $+2.5$, DV -5.5). Animals were given 1 week to recover from surgery before place conditioning. After each experiment, animals were killed with an overdose of sodium pentobarbital (100 mg/kg i.p.). Pontine Sky Blue dye was microinfused (0.5 μ l) at the intracerebral injection sites to allow subsequent histological confirmation.

CCP procedure

Conditioning and testing occurred in a Plexiglas apparatus that consisted of two distinct compartments that could be separated by a Plexiglas divider. Each compartment was equipped with photocells to automatically log time and activity (MED Associates, Inc.). One compartment had a grid floor with black walls, and the other compartment had a mesh floor with black and white stripes on the walls. On the first day, rats in all groups were allowed to freely explore all of the apparatus for 15 min, and the amount of time spent in each compartment was recorded. None of the animals had an initial bias for either compartment, and each was randomly assigned to a compartment for morphine conditioning in a balanced design. Conditioning began 4 days after this preconditioning day. On each of the next 3 days, rats were given injections of

either saline or morphine (8 mg/kg, i.p.) and were confined to the assigned compartment for 30 min. Morphine and saline sessions were alternated between the morning and afternoon. Rats given morphine in the morning were given saline in the opposite chamber in the afternoon, and on subsequent days received saline in the morning and morphine in the afternoon.

Experimental protocol

For studies on acquisition of conditioning, rats received either bilateral vehicle ($n=9$), CNQX (0.21 nmol; $n=9$) or AP5 (0.48 nmol; $n=10$) injections into the VTA prior to each morphine conditioning trial (0.3 μ l per side). In two other groups of animals, injections of the glutamate antagonists were made outside the VTA (AP5 plus CNQX; $n=8$) prior to each morphine trial. In other animals, Rp-cAMPS (13 nmol/0.3 μ l) was microinjected bilaterally into the VTA ($n=9$; vehicle $n=8$) or dorsal to the VTA ($n=9$) immediately after each morphine conditioning trial. One day after conditioning all animals were given a preference test with the divider removed and the amount of time spent in each chamber was recorded for 15 min.

To test the effects of the microinjections alone on CPP, separate groups of animals that were not morphine conditioned received similar bilateral intra-VTA microinjections of AP5 ($n=7$), CNQX ($n=6$), or Rp-cAMPS ($n=7$) paired with one chamber; microinjections of the glutamate antagonists were given prior to being placed in the chamber, and injections of Rp-cAMPS were given after animals were removed from the chamber. Instead of morphine conditioning, systemic saline injections were paired with both chambers. The chamber paired with the intracerebral microinjections was counterbalanced among subjects. Control animals received AP5 ($n=8$) or CNQX ($n=5$) outside the VTA.

To test whether glutamate input to the VTA is necessary for the expression of a morphine CPP, separate groups of animals were given bilateral microinjections (0.3 μ l) of a cocktail containing AP5 plus CNQX (0.48 nmol and 0.21 nmol respectively; $n=10$), or Rp-cAMPS (13 nmol; $n=9$), into the VTA immediately prior to the preference test; additional animals received similar injections outside of the VTA (antagonist cocktail group $n=10$; Rp group $n=8$). Separate control groups for each compound received vehicle in the VTA prior to the preference test ($n=8$ in both cases).

Data analyses

Place conditioning data were analyzed by calculating the time spent in the morphine-paired chamber (or intracerebral microinjection-paired chamber) minus the time spent in the other chamber. The resulting difference score was compared between groups using one-way analysis of variance. In addition, a within-group measurement of CCP was assessed by comparing the difference in time spent in the morphine-paired (or intracerebral microinjection-paired) and saline paired side pre-conditioning vs. post-conditioning. Where necessary post hoc analysis was carried out with a Newman-Keuls test.

RESULTS

Effects of glutamate antagonists and Rp-cAMPS on the acquisition of morphine place preference

Rats with bilateral indwelling cannulae aimed at the VTA were trained in a morphine place preference task similar to that described in our recent publications (Harris and Aston-Jones, 2003a,b). As shown in Fig. 1a, either an AMPA or an NMDA glutamate antagonist completely blocked the acquisition of the morphine place preference when administered into the VTA prior to each morphine conditioning trial ($F(4,35)=19$, $P<0.01$). In contrast, neither antagonist administered immediately dorsal to the VTA, nor vehicle

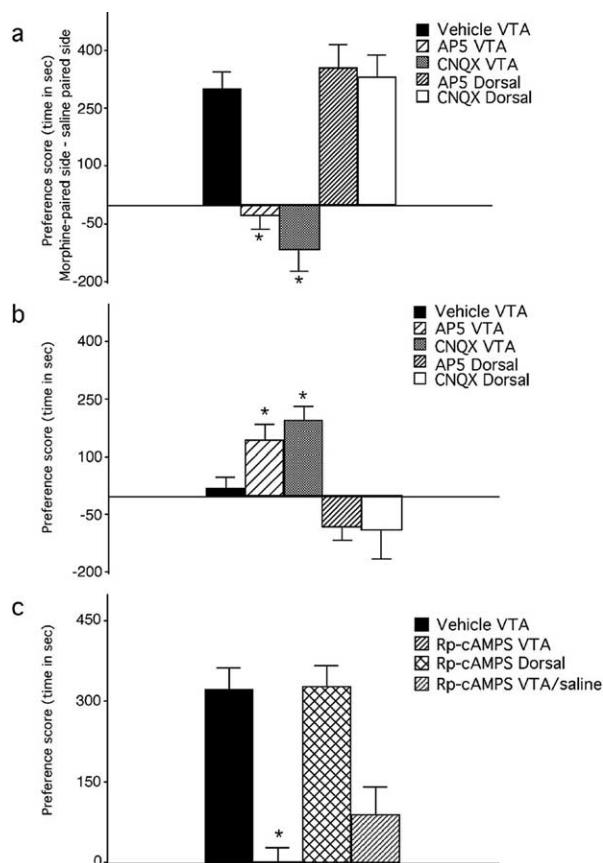


Fig. 1. Inhibition of glutamate receptors or PKA in the VTA blocked the acquisition of morphine CPP. (a) Preference scores for the morphine-paired environment expressed as the mean time in seconds spent in the morphine-paired side minus the mean time in seconds spent on the saline-paired side on the test day. Groups include Vehicle, CNQX or AP5 administered into the VTA, and CNQX or AP5 administered dorsal to the VTA. (b) Preference scores for the antagonist-paired environment, expressed as the mean time in seconds spent in the antagonist-paired side minus the mean time spent in the sham-paired side on the test day. (c) Preference scores for Rp-cAMPS groups were calculated as described above. Morphine-conditioned groups included vehicle and Rp-cAMPS microinjections in the VTA, and Rp-cAMPS microinjections outside the VTA, immediately after morphine conditioning. In addition, the fourth group received Rp-cAMPS injections immediately after removal from one chamber that was paired with saline injections (non-conditioned, morphine-naïve animals). * $P < 0.01$.

injections inside the VTA, significantly affected morphine conditioning (Fig. 1a). CNQX injected into the VTA of morphine-conditioned animals appeared to produce a slight aversion, but the preconditioning versus post-conditioning preference scores were not significantly different ($P = 0.18$).

When injected into the VTA of animals that received no morphine conditioning, both glutamate antagonists produced a modest place preference ($F(4,29) = 5.4$, $P < 0.01$; Fig. 1b). The antagonist-induced place preference was specific for sites in the VTA and not due to non-specific damage caused by the microinjection because neither vehicle injections into the VTA nor antagonist injections outside the VTA of non-morphine conditioned rats produced a place preference (Fig. 1b). An overall 2×5 way ANOVA

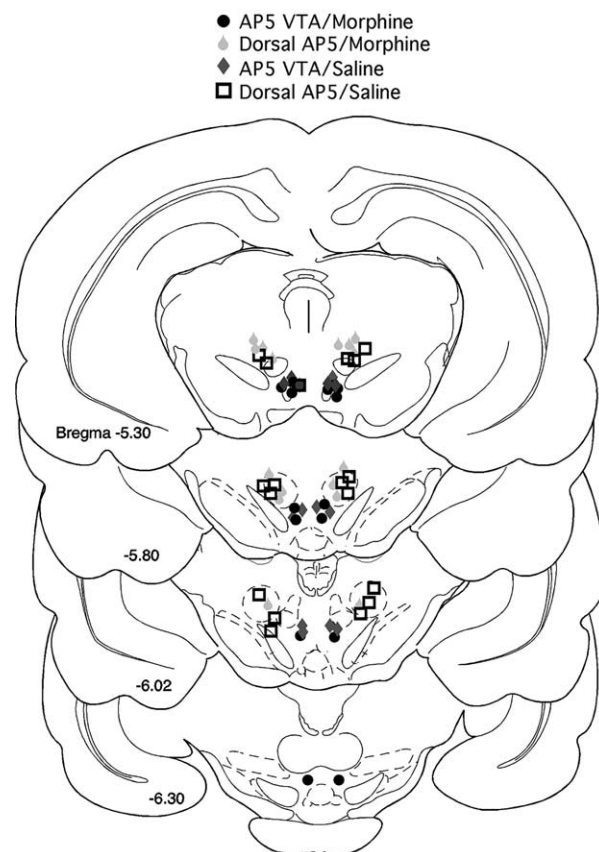


Fig. 2. Schematic representations of frontal sections showing the effective (black circles and gray diamonds) and ineffective (white squares and black drops) sites for AP5 administration. Injection sites for other groups were similar and are not plotted. Drawings were adapted from Paxinos and Watson (1998).

(drug treatment: morphine vs no morphine during conditioning, and groups: AP5–VTA, CNQX–VTA, Veh–VTA, AP5–dorsal, CNQX–dorsal) resulted in a significant treatment effect ($F(1,63) = 14$, $P < 0.01$; morphine conditioning was different than non-morphine conditioning) and a significant group by treatment interaction ($F(4,63) = 21$, $P < 0.01$) which confirmed that the glutamate antagonists blocked morphine conditioning but produced a place preference when given without morphine ($P < 0.05$, Newman-Keuls post hoc comparisons).

Fig. 2 shows the locations of effective and ineffective sites for AP5 administration. These sites are representative of those used for the other VTA microinjections.

No significant differences were found in locomotor activation among the animals that received morphine conditioning in the presence of the antagonists ($F(2,23) = 17$, $P = 0.84$; results not shown). In animals that received the glutamate antagonists in the VTA without morphine conditioning, AP5 produced a significant increase in locomotor activity that was seen on all three days of conditioning ($P < 0.05$; Cornish et al., 2001).

In additional experiments we found that the PKA inhibitor, Rp-cAMPS, blocked morphine conditioning when injected into the VTA immediately after each morphine con-

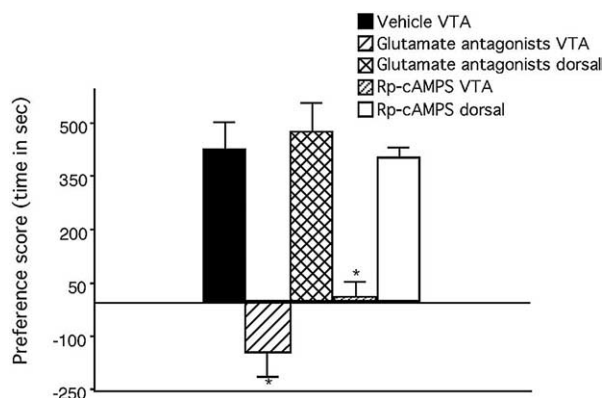


Fig. 3. Inhibition of glutamate receptors or PKA in the VTA blocked the expression of morphine CPP. Preference scores for the morphine-paired environment expressed as the mean time in seconds spent in the morphine-paired side minus the mean time in seconds spent on the saline-paired side on the test day. Groups with microinjections in the VTA include vehicle, AP5+CNQX cocktail, or Rp-cAMPS, and groups with injections dorsal to the VTA include AP5+CNQX cocktail or Rp-cAMPS. All of these microinjections occurred immediately prior to the CPP test. * $P < 0.01$.

conditioning trial ($F(2,23)=28$, $P < 0.01$; Fig. 1c). Rp-cAMPS injections given in the VTA immediately after removing animals from a chamber paired with saline injections did not produce aversion or preference for that chamber ($t(6)=29$, $P < 0.69$; Fig. 1c).

Effects of glutamate antagonists and Rp-cAMPS on the expression of morphine place preference

In separate experiments, drugs were bilaterally microinjected into the VTA just prior to the preference test to examine effects on expression of CPP after conditioning. Fig. 3 shows that a cocktail containing both AMPA and NMDA glutamate antagonists completely blocked the expression of the morphine place preference when injected into the VTA ($F(2,25)=20.95$, $P < 0.01$). Similarly, VTA microinjections of Rp-cAMPS blocked the expression of morphine preference ($F(2,22)=23.53$, $P < 0.01$, Fig. 3). Neither microinjections of the glutamate antagonist cocktail nor Rp-cAMPS outside of the VTA, nor vehicle injections into the VTA, affected the expression of morphine preference (Fig. 3). Furthermore, none of the drug treatment conditions significantly affected locomotor activity on the test day (glutamate antagonists cocktail ($F(2,25)=.95$, $P=0.64$), Rp-cAMPS ($F(2,22)=0.07$, $P=0.93$)).

DISCUSSION

Our results indicate that activation of glutamate receptors as well as PKA in the VTA are required for both the acquisition and the expression of morphine-associated place preference. The finding that the administration of glutamate antagonists into the VTA blocked the acquisition of morphine place conditioning is similar to our previous results with cocaine CPP (Harris and Aston-Jones, 2003a), and similar to what others have reported for morphine CPP with systemic administration of glutamate antagonists

(Tzschentke and Schmidt, 1995). The present results with the PKA inhibitor, Rp-cAMPS, provide the first evidence that the PKA pathway in VTA cells is required for a classically conditioned instrumental approach response. Together, these data indicate that synaptic plasticity in the VTA is necessary for both acquisition and expression of morphine CPP.

Our results are unlikely to be explained by performance deficits or changes in DA neuronal activity. None of the drugs changed locomotor activity during conditioning, indicating that they did not block the locomotor activating effects of morphine, nor did they prevent exploration of the environments when given on the test day. Although DA release in the nucleus accumbens is necessary for the acquisition of morphine CPP (Shippenberg et al., 1993), it is unlikely that the effects of glutamate or PKA antagonists observed here are mediated by blockade of DA release. For example, glutamate antagonists have minimal effects on baseline DA neuronal activity. Thus, some authors have reported no effects of these compounds on VTA DA neurons (French et al., 1993), while others have reported slight decreases in firing rate (Georges and Aston-Jones, 2002) or bursting activity of these cells (Overton and Clark, 1992). Furthermore, *in vivo* microdialysis studies found that glutamate antagonists administered into the VTA do not alter extracellular DA levels in the VTA's primary terminal fields (i.e. the nucleus accumbens and prefrontal cortex; Svensson et al., 1998). Although there is no information on the effects of Rp-cAMPS on DA cell activity, other PKA inhibitors have not been found to alter basal firing rates in VTA neurons (Shi and Bunney, 1992). It is important to note in this regard that the PKA inhibitor in our experiments was given after the morphine conditioning session, and therefore would have only interfered with the consolidation of the conditioning. Although the abovementioned studies were conducted while animals were in a resting state and may not measure changes in DA release during a stimulated state (e.g. in the CPP paradigm), it seems unlikely that the effects of the glutamate antagonists or PKA inhibitor observed here were due to alterations in morphine-induced DA release. This conclusion is also consistent with previous studies revealing that the excitatory effects of morphine on VTA DA neurons are produced by decreased GABAergic inhibition, not increased glutamate input (Johnson and North, 1992). Furthermore, μ opioid agonists have been reported to decrease (rather than increase) glutamate-mediated excitatory post-synaptic potentials on VTA DA neurons, further indicating that morphine's excitatory effects on these cells are not mediated by glutamate (Manzoni and Williams, 1999). However, it is noteworthy that the effects of morphine on VTA DA neuronal activity has not been tested in the presence of glutamate antagonists, and such studies are necessary to rule out the possibility that our antagonist injections altered VTA responses to morphine administration.

The interpretation of place conditioning studies such as ours, in which animals are trained and tested in different drug-states, is complicated by the possible role of state-

dependent learning in the results obtained. However, it seems unlikely that state-dependent effects play a major role in the results obtained here. First, the nature of the CPP paradigm indicates that state-dependent effects are probably not a factor as animals are always trained in a drug state but tested in a non-drug state. Second, studies by others show that morphine CPP can be blocked when glutamate antagonists are administered both on conditioning days and on the test day, ruling out state-dependent changes due to the antagonists (Tzschenke and Schmidt, 1997). Finally, the idea that glutamate antagonists in the VTA create state dependent effects is discounted by the fact that when these drugs were administered alone in the VTA without morphine conditioning they produced a place preference even when the animals were tested in a non-drug state (without glutamate antagonists in the VTA).

It is also unlikely that the glutamate antagonists blocked morphine CPP by decreasing the rewarding properties of morphine. Administration of glutamate antagonists into the VTA have rewarding, not aversive, properties (David et al., 1998). This is consistent with results from the present experiments, as well as those in our previous study (Harris and Aston-Jones, 2003a) where we found that glutamate antagonist administration into the VTA elicited a significant place preference. These findings indicate that glutamate antagonists do not alter the learning or expression of morphine CPP because of aversive properties. NMDA antagonists injected into the VTA have been reported to increase locomotor activity in a non-DA dependent fashion (Cornish et al., 2001). It was postulated that these locomotor activating effects may be mediated through inhibition of GABA cells that project from the VTA to forebrain areas, thereby releasing these regions from inhibition (David et al., 1998; Cornish et al., 2001). GABA containing neurons in the VTA project to both the nucleus accumbens (Van Bockstaele and Pickel, 1995) and the prefrontal cortex (Carr and Sesack, 2000), areas important for reward-related learning (Wise, 2002). Therefore, the reinforcing effects of intra-VTA glutamate antagonist administration observed here may be mediated outside the VTA in one of many VTA projection areas. Because both glutamate antagonists and morphine produce rewarding effects, one would expect that administration of the glutamate antagonists would enhance the rewarding efficacy of morphine and enhance the acquisition of morphine CPP. In contrast, we found that the antagonists abolished the acquisition of morphine CPP. We hypothesize therefore, that the glutamate and PKA antagonists interfere with synaptic plasticity in VTA neurons which is necessary for an environmental cue to become associated with morphine and elicit an approach response.

The VTA is reported to receive major glutamatergic inputs from a number of brain areas including the prefrontal cortex (Christie et al., 1985), bed nucleus of the stria terminalis (Georges and Aston-Jones, 2002), and pedunculopontine tegmental nucleus (PPTg; Clements et al., 1991). The PPTg is of particular interest because lesions of this nucleus block the acquisition of CPP with morphine (Bechara and van der Kooy, 1989) and other drugs (Olm-

stead and Franklin, 1994), indicating that it is a good candidate for supplying the glutamate that is necessary for CPP conditioning to the VTA.

Both NMDA and AMPA receptor activation were found to be equally necessary for the acquisition of morphine CPP. This may reflect the fact that NMDA receptors are necessary for the increase in synaptic strength found in VTA DA neurons after morphine or cocaine treatment, and that this increase in synaptic strength reflects an increase in the number of AMPA receptors (Ungless et al., 2001; Saal et al., 2003).

The conclusion that synaptic plasticity in the VTA is necessary for the acquisition of morphine CPP is further supported by our result showing that the PKA inhibitor Rp-cAMPS blocked the acquisition of CPP when given immediately after each morphine conditioning session. Post-conditioning PKA activation has been implicated in the establishment of long-term memories for both fear-related conditioning (Schafe and LeDoux, 2000) and reward-related learning (Baldwin et al., 2002; Beninger et al., 2003). PKA activation can have a wide range of effects on the development of synaptic plasticity. For instance, activation of PKA is important for the formation of LTP in hippocampal circuitry (Kandel, 1989) and enhances neurotransmission necessary for LTP in corticostriatal synapses (Spencer and Murphy, 2002). Furthermore, PKA is known to increase transcription of NMDA receptor subunits (Lau et al., 2004), regulate the translocation of stored NMDA receptors (Scott et al., 2003), and phosphorylate the NR1 NMDA receptor subunit, allowing enhanced flow of calcium through this channel (Nijholt et al., 2000). In terms of AMPA receptors, PKA-mediated phosphorylation of AMPA receptor subunits is thought to contribute to diverse mechanisms underlying synaptic plasticity (Esteban et al., 2003). For example, PKA-induced phosphorylation helps determine the expression of AMPA receptors on the cell surface (Wolf et al., 2003). This is potentially important here because VTA synaptic enhancements due to morphine administration are thought to result from increased numbers of AMPA receptors (Saal et al., 2003). Synthesis of AMPA receptor subunits is increased after LTP and this process is PKA dependent (Nayak et al., 1998).

Carlezon et al. (2000) reported that overexpression of the AMPA receptor subunit GluR1 in the caudal VTA produced aversion to morphine. This apparent inconsistency with our results may occur because in their study the GluR1 was artificially induced, whereas in our study we propose that increased AMPA receptors in the VTA are induced as a result of conditioning. Saal et al. (2003) found that a single exposure to morphine increases AMPA receptor function. We speculate that repeatedly pairing morphine with an environmental stimulus could further augment this process and link increased glutamate function to the associated stimulus. In this scenario, blockade of this increased synaptic strength in the VTA by pretreatment with glutamate antagonists or post-treatment with a PKA inhibitor would decrease conditioned behavioral responses to morphine-associated stimuli, as we observed.

We used a cocktail of NMDA and AMPA receptor antagonists to test the role of glutamate receptors overall in the plasticity associated with the expression of morphine conditioning. Our results indicate that glutamate receptor activation, as well as PKA activation, is critical for the expression of morphine preference. However, the use of a glutamate cocktail precludes us from ascertaining whether NMDA or AMPA receptors, or both, are necessary for this plasticity. Previous studies indicate that NMDA receptors in the VTA are critical for expression of morphine preference (Popik and Kolasiewicz, 1999). Additional studies with individual antagonists would be needed to test the role of VTA AMPA receptors in this conditioning.

Peripheral or central administration of glutamate antagonists has been shown to block the expression of morphine CPP (Tzschentke and Schmidt, 1997; Popik and Kolasiewicz, 1999), revealing the importance of glutamate transmission for CPP expression. A wealth of data indicates the importance of plasticity in the onset of learning, but recent work also shows that similar plasticity is involved in the retrieval of learned behaviors. For example, inhibition of protein synthesis attenuates the expression of conditioned fear (Nader et al., 2000), and PKA activation is necessary for the maintenance of conditioned taste aversion (Koh and Bernstein, 2003). Our results are consistent with these findings and indicate that the memory of morphine-associated stimuli, or motivation to approach them, requires reactivation of glutamate receptors and PKA. It is possible that glutamate inputs and subsequent PKA activation in the VTA is a necessary part of the circuit involved in the retrieval of the CPP memory.

Our data indicate that synaptic plasticity in the VTA involving activation of glutamate receptors and the PKA pathway is necessary for both acquisition and expression of morphine CPP. It is tempting to speculate that the VTA may be an important site where synaptic modifications occur allowing environmental stimuli to be associated with appetitive reward. This information may then be integrated with plasticity-induced changes in other areas such as the nucleus accumbens, amygdala or prefrontal cortex which are also integral parts of networks involved in the acquisition or expression of conditioned reward (Kelley and Berridge, 2002; Wise, 2002). Conditioned effects of drugs are integral to craving and relapse in human drug addicts (Ehrman et al., 1992). The present results reveal neural mechanisms that could contribute to such conditioned effects of drugs, and subsequently lead to human drug addiction.

Acknowledgments—PHS Merit Award DA 06214.

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