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Previous Exposure to VTA Amphetamine Enhances Cocaine Self-Administration under a Progressive Ratio Schedule in an NMDA, AMPA/Kainate, and Metabotropic Glutamate Receptor-Dependent Manner

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Previous exposure to amphetamine (AMPH) in the ventral tegmental area (VTA) enhances cocaine self-administration in a D_1 dopamine receptor-dependent manner. The present study examined the contribution of VTA NMDA, AMPA/kainate, and metabotropic glutamate (mGlu) receptors to this effect. Rats in different groups received three intra-VTA injections, one every third day, of either saline (0.5 μl/side), AMPH (2.5 μg/0.5 μl/side), AMPH+CPP (NMDA receptor antagonist; 10 μM or 100 μM/0.5 μl/side), AMPH+CNQX (AMPA/kainate receptor antagonist; 0.3 mM or 1 mM/0.5 μl/side), AMPH+MCPG (mGlu receptor antagonist; 0.5 mM or 50 mM/0.5 μl/side), or the glutamate receptor antagonists alone. Starting 7–10 days after the last pre-exposure injection, rats were trained to self-administer cocaine (0.3 mg/kg/infusion) and then tested under a progressive ratio (PR) schedule of reinforcement for 6 consecutive days. As reported previously, VTA AMPH pre-exposed rats worked more and obtained more infusions of cocaine than saline pre-exposed animals. Coadministration of CPP, CNQX, or MCPG with AMPH during pre-exposure dose-dependently blocked this enhancement of cocaine self-administration. Rats pre-exposed to the glutamate receptor antagonists alone did not differ on the test days from the saline pre-exposed controls. These results indicate that, in a manner paralleling the induction of sensitization of the locomotor stimulating effects of AMPH, activation of NMDA, AMPA/kainate, and mGlu receptors during pre-exposure to AMPH in the VTA is necessary for the enhancement of cocaine self-administration to develop.
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INTRODUCTION

Repeated exposure to psychostimulants leads to an enduring augmentation of the behavioral and neurochemical effects of these drugs, termed sensitization. This phenomenon may be relevant for psychostimulant addiction because it comprises neuroadaptational processes in specific brain areas initiated by previous exposure to abused drugs (Robinson and Berridge, 1993). Consistent with this view, repeated exposure to amphetamine (AMPH) in the ventral tegmental area (VTA), but not in other brain areas such as the prefrontal cortex (PFC) or the nucleus accumbens (NAcc), leads to sensitization of its locomotor and neurochemical effects (Vanderschuren and Kalivas, 2000) and also to enhanced self-administration of AMPH (Vezina *et al*, 2002) and cocaine (Suto *et al*, 2002).

Dopamine (DA) neurotransmission in the VTA has been particularly implicated in the induction of sensitization by AMPH. Sensitization of the locomotor (Stewart and Vezina, 1989; Bjijou *et al*, 1996) and NAcc DA releasing (Vezina, 1996) effects of AMPH is prevented by coadministrating a D_1 receptor antagonist with AMPH into the VTA during pre-exposure. D_2 receptor antagonists, whether administered systemically (Vezina and Stewart, 1989) or into the VTA (Bjijou *et al*, 1996), have been found to be without effect on the induction of locomotor sensitization by AMPH. The enhancement of cocaine self-administration produced by previous exposure to VTA AMPH also requires activation of D_1 receptors in this site (Suto *et al*, 2002). In addition, previous exposure to a D_1, but not D_2, receptor agonist in the VTA leads to sensitization of the locomotor effects of cocaine (Pierce *et al*, 1996).

Importantly, activation of D_1, but not D_2, receptors in the VTA increases extracellular levels of glutamate in this site

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(Kalivas and Duffy, 1995; Wolf and Xue, 1998, 1999), possibly by a mechanism ultimately involving reversal of glutamate transporters and reactive oxygen species (Wolf *et al*, 2000). Indeed, induction of locomotor sensitization by AMPH has been shown to be dependent on activation of *N*-methyl-D-aspartate (NMDA: Cador *et al*, 1999; Vezina and Queen, 2000) and metabotropic glutamate (mGlu: Kim and Vezina, 1998) receptors in the VTA. Systemic administration of NMDA (Karler *et al*, 1989; Wolf *et al*, 1995) or α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA)/kainate receptor antagonists (Karler *et al*, 1991a; Li *et al*, 1997) also prevents the development of locomotor sensitization by systemic AMPH. Moreover, induction of locomotor sensitization by AMPH is blocked by lesions of the PFC, which provides major glutamatergic afferentation to the VTA (Wolf *et al*, 1995; Cador *et al*, 1999).

Exposure to AMPH in the VTA initiates neuroadaptational processes in this site that lead to a long-lasting enhancement of cocaine self-administration. Based on the above evidence, it was hypothesized that these processes require the activation of NMDA, AMPA/kainate, or mGlu receptors in the VTA. To test this hypothesis, AMPH was infused into the VTA alone or in combination with (±)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP: NMDA receptor antagonist), 6-cyano-7-nitroquinoxaline-2,3-dione disodium (CNQX: AMPA/kainate receptor antagonist), or (±)-alpha-methyl-4-carboxyphenylglycine (MCPG: mGlu receptor antagonist). Starting 9–15 days later, cocaine self-administration was assessed.

METHODS

Subjects

Male Long-Evans rats (Harlan Sprague–Dawley, Madison, WI; Toconic, German Town, NY) weighing 250–275 g on arrival were used. They were individually housed with food and water freely available in a reverse cycle room (12-h light : 12-h dark) for the duration of the experiment. Animals were always tested during the dark period of the light cycle.

Apparatus

A total of 15 chambers, each measuring $22 \times 43 \times 33\,cm^3$, were used for cocaine self-administration. Each chamber was made of stainless steel (rear and two side walls), a Plexiglas front hinged door, and a tubular stainless-steel ceiling and floor. These chambers were placed in a plastic box that shielded animals from extraneous disturbances. White noise was supplied in each box by a ventilating fan. A lever (5 cm above the floor) and a stimulus light (13.5 cm above the lever) were positioned on the right side wall. Each chamber was equipped with a liquid swivel system comprised of a steel-spring tether, a liquid swivel, and an infusion pump (Razel Scientific Inc., Model, A. E) that allowed free movement of the animal in the chamber and delivery of drug upon depression of the lever. The tether was connected to the animal by screwing its captive collar onto the threaded portion of a custom-designed L-shaped Plastics One cannulae (20 gauge) secured to the animals skull (see Pierre and Vezina, 1997). Lever presses and drug infusions were recorded and controlled via an electrical interface by a computer using locally developed software.

Drugs

S(+)-amphetamine sulfate (AMPH), (−)-cocaine hydrochloride (cocaine), CPP, CNQX, and MCPG were obtained from Sigma, Inc. (Saint Louis, MO). Drugs were dissolved in sterile saline (0.9% w/v) for both i.p. and i.c. routes of administration. Doses refer to the weight of the salt.

Surgery

For all surgical procedures, rats were anesthetized with a mix of ketamine (100 mg/kg, i.p.) and xylazine (6 mg/kg, i.p.). For intracranial implantation of cannulae, animals were placed in a stereotaxic instrument with the incisor bar positioned 5.0 mm above the interaural line (Pellegrino *et al*, 1979). They were, then, implanted with chronic bilateral guide cannulae (22 gauge, Plastics One, Roanoke, VA) aimed either at the VTA (A/P, −3.6; L, ±0.6; DV, −8.9 from bregma and skull) or areas surrounding this site in all three planes. Cannulae were angled at 16° to the vertical and positioned 1 mm above the final injection site. After surgery, 28 gauge obturators were placed in the guide cannulae and rats were returned to their home cages for a 7–10-day recovery period.

For cocaine self-administration, rats were surgically implanted with an i.v. catheter into their right external jugular vein as described by Pierre and Vezina (1997). The intravenous catheter used was made of silastic tubing (Dow Corning, Inc.). Catheters were flushed daily with a 0.9% sterile saline solution containing 30 IU/ml heparin and 250 mg/ml ampicillin in order to promote patency. Seven rats (saline, 1; CNQX, 1; AMPH+CPP, 3; AMPH+CNQX, 1; AMPH+MCPG, 1) were dropped because their catheters became nonpatent or developed leaks.

All surgical procedures were conducted using aseptic techniques according to an approved IACUC protocol.

Design and Procedure

The experiment consisted of three phases: pre-exposure, cocaine self-administration training, and cocaine self-administration testing. Animals were randomly assigned to different groups depending on the VTA pre-exposure condition (AMPH, saline, receptor antagonist, and AMPH+receptor antagonist).

Pre-exposure. Starting 7–10 days after implantation of bilateral guide cannulae into the VTA, animals received a total of three microinjections corresponding to their pre-exposure condition: AMPH (2.5 μg/0.5 μl/side), saline (0.5 μl/side), CPP (10 or 100 μM/0.5 μl/side), CNQX (0.3 or 1.0 mM/0.5 μl/side), MCPG (0.5 or 50 mM/0.5 μl/side), AMPH+CPP, AMPH+CNQX, or AMPH+MCPG. Microinjections were made once every third day with injection cannulae (28 gauge) connected to 1 μl syringes (Hamilton, Reno, NV) via PE-20 tubing and inserted to a depth 1 mm below the guide cannula tips. Injections were made in a volume of 0.5 μl/side over 30 s. After 60 s, the injection cannulae were withdrawn and the obturators were replaced.

The dose of AMPH was selected based on the findings of Vezina (1996), Kim and Vezina (1998), Cador *et al* (1999), Vezina *et al* (2002), and Suto *et al* (2002) firmly establishing a critical role for actions of AMPH in the VTA in the induction of sensitization. In these studies, this dose of AMPH was sufficient to induce not only sensitization of its locomotor activating effects and its ability to increase extracellular levels of DA in the NAcc, but also enhanced self-administration of AMPH and cocaine. The two doses of CPP and MCPG were selected based on the findings of Kim and Vezina (1998) and Cador *et al* (1999), where the higher dose of each antagonist blocked the development of locomotor sensitization by AMPH in the VTA. In these studies, neither CPP nor MCPG, when administered alone during pre-exposure, influenced the locomotor effects of the subsequent AMPH challenge. The two doses of CNQX were selected based on the findings of Mathe *et al* (1998). In this study, intra-VTA infusion of CNQX dose-dependently blocked the locomotor and NAcc DA activating effects of dizocilipine without affecting locomotion and extracellular levels of NAcc DA by itself.

Cocaine self-administration training. Training for cocaine self-administration began 7–10 days after the final drug pre-exposure injection and at least 3 days after animals received their i.v. catheters. Cocaine self-administration sessions were held daily and lasted for 3 h. In all cases, reinforced presses on the lever delivered an infusion of cocaine through the i.v. catheter (0.3 mg/kg/infusion). The cocaine solution was injected in volumes of 0.10–0.13 ml/infusion at a rate of 1.6 ml/min. For 15 s immediately following reinforced depressions of the lever, a stimulus light above the lever was lit and lever presses were recorded but did not lead to further infusions.

An experimenter-delivered priming infusion of cocaine (0.3 mg/kg, i.v.) was given at the beginning of each session. The initial schedule used was a fixed ratio 1 (FR1) and it was increased to an FR2 once animals successfully administered an additional nine infusions within the 3 h session. Rats were then again required to self-administer an additional nine infusions within a 3 h session under the FR2 schedule. Animals that did not satisfy each of the FR1 and the FR2 criteria (ie nine infusions in a 3-h session) within 5 days were excluded from the study. The following numbers of rats were thus excluded (AMPH, 3; saline, 2; CPP, 6; CNQX, 5; MCPG, 3; AMPH+CPP, 4; AMPH+CNQX, 4; AMPH+MCPG, 4). Each training session lasted until animals self-administered nine infusions or until 3 h elapsed. Days to satisfaction of the training criteria under each FR schedule were recorded.

Cocaine self-administration testing. Upon satisfactory completion of self-administration training under the FR schedules, rats were tested daily under a progressive ratio (PR) schedule of reinforcement for 6 days. Under this schedule, the number of responses required to obtain each successive infusion of cocaine was determined by ROUND $(5 \times \mathrm{EXP}(0.25 \times \text{infusion number}) - 5)$ to produce the following sequence of required lever presses: 1, 3, 6, 9, 12, 17, 24, 32, 42, 56, 73, 95, 124, 161, 208, etc. (Richardson and Roberts, 1996). The daily PR sessions lasted 3 h or until 1 h elapsed without a drug infusion. Priming infusions were not given during these sessions. The number of infusions obtained in each PR session was recorded.

The dose of cocaine used and the procedures followed in the self-administration training and testing phases of the experiment were previously used to confirm this drug's reinforcing effects: rats self-administering cocaine obtained significantly more infusions than rats self-administering saline under all three schedules (FR1, FR2, and PR) of reinforcement used (Suto *et al*, 2002).

Histology

After completion of the experiments, rats were anesthetized with sodium pentobarbital and perfused via intracardiac infusion of saline and 10% formalin. Brains were removed and postfixed in 10% formalin. Coronal sections (40 μm) were mounted onto gelatin-coated slides and subsequently stained with cresyl violet for verification of cannulae tip placements. The brains of three additional rats were postfixed in saline solution containing 10% formalin and 30% sucrose and prepared for tyrosine hydroxylase (TH) immunohistochemistry using procedures adapted from those described by Bencsics *et al* (1996).

In order to assess the neuroanatomical specificity of the VTA AMPH pre-exposure infusions, rats in an additional group (AMPH outside VTA: $N = 6$) were pre-exposed to bilateral infusions of AMPH in areas adjacent to the VTA. These animals received infusions into the red nucleus, the substantia nigra, or other sites dorsal and caudal to the VTA (see Figure 6a). Remaining procedures were as described above.

Only data obtained from animals with both cannula tips placed in the VTA and from animals specifically in the AMPH outside the VTA group were retained for statistical analyses. After histological verification, the following numbers of animals were excluded from each group because one or both cannula tips were found to be outside the VTA: AMPH, 1; saline, 1; CPP, 2; CNQX, 7; MCPG, 6; AMPH+CPP, 5; AMPH+CNQX, 4; AMPH+MCPG, 1. As a result, the final number of animals tested in the different experimental groups was: AMPH, 10; saline, 12; CPP (10), 7; CPP (100), 11; CNQX (0.3), 6; CNQX (1.0), 6; MCPG (0.5), 6; MCPG (50), 8; AMPH+CPP (10), 9; AMPH+CPP (100), 12; AMPH+CNQX (0.3), 7; AMPH+CNQX (1.0), 7; AMPH+MCPG (0.5), 8; AMPH+MCPG (50), 8.

Data Analyses

The data obtained during self-administration training (days to criterion) were analyzed with two-way between analyses of variance (ANOVA) with pre-exposure (2 levels: AMPH and saline) and glutamate receptor blockade (7 levels: CPP, 2 doses; CNQX, 2 doses; MCPG, 2 doses and saline) as the between factors. The data obtained during self-administration testing (number of infusions obtained under the PR schedule of reinforcement) were analyzed with two-way between one-way within ANOVA with the two above between factors and days of testing (6) as the within factor. Thus, all these cocaine self-administration PR test data were analyzed with one ANOVA even though they are illustrated in different figures below (see Figures 3–5). The data used to assess the neuroanatomical specificity of the VTA AMPH

infusions were analyzed with one-way between ANOVA with pre-exposure condition (3 levels: AMPH, saline and AMPH outside VTA) as the between factor. The number of infusions obtained in a PR session was used for statistical analysis rather than the number of presses emitted or the final ratios obtained since the later were, by definition, generated from an exponential function (Richardson and Roberts, 1996). *Post hoc* Tukey HSD tests were made according to Kirk (1968).

RESULTS

Cocaine Self-Administration Training

In agreement with previous reports (Mendrek *et al*, 1998; Lorrain *et al*, 2000; Suto *et al*, 2002; Vezina *et al*, 2002), rats in all 14 groups readily satisfied the self-administration training criteria and did so in similar fashion regardless of pre-exposure condition. Thus, previous exposure to AMPH or any of the glutamate receptor antagonists either alone or with AMPH in the VTA did not affect, relative to saline pre-exposed animals, the number of days to achieve the criterion under either the FR1 or the FR2 schedules of reinforcement. On average, rats in the different groups satisfied each of the FR1 and FR2 criteria in 1–2 days (Figures 1 and 2). The ANOVA conducted on these data revealed no significant effects. Days to achieve FR1 criterion: pre-exposure ($F_{1,103} = 3.05$, NS), glutamate receptor blockade ($F_{6,103} = 0.70$, NS), interaction ($F_{6,103} = 1.53$, NS). Days to achieve FR2 criterion: pre-exposure ($F_{1,103} = 1.09$, NS), glutamate receptor blockade ($F_{6,103} = 0.51$, NS), interaction ($F_{6,103} = 0.58$, NS).

Cocaine Self-Administration Testing: Facilitation of Cocaine Self-Administration by VTA AMPH Requires NMDA, AMPA/Kainate, and mGlu Receptor Activation During Pre-Exposure

Consistent with previous reports (Suto *et al*, 2002), rats pre-exposed to VTA AMPH alone worked more and obtained significantly more cocaine infusions than saline pre-exposed control rats when tested under the PR schedule of reinforcement. All three glutamate receptor antagonists when coinfused with AMPH into the VTA during pre-exposure were able to prevent the development of this effect. Previous exposure to any of the glutamate receptor antagonists alone did not affect subsequent

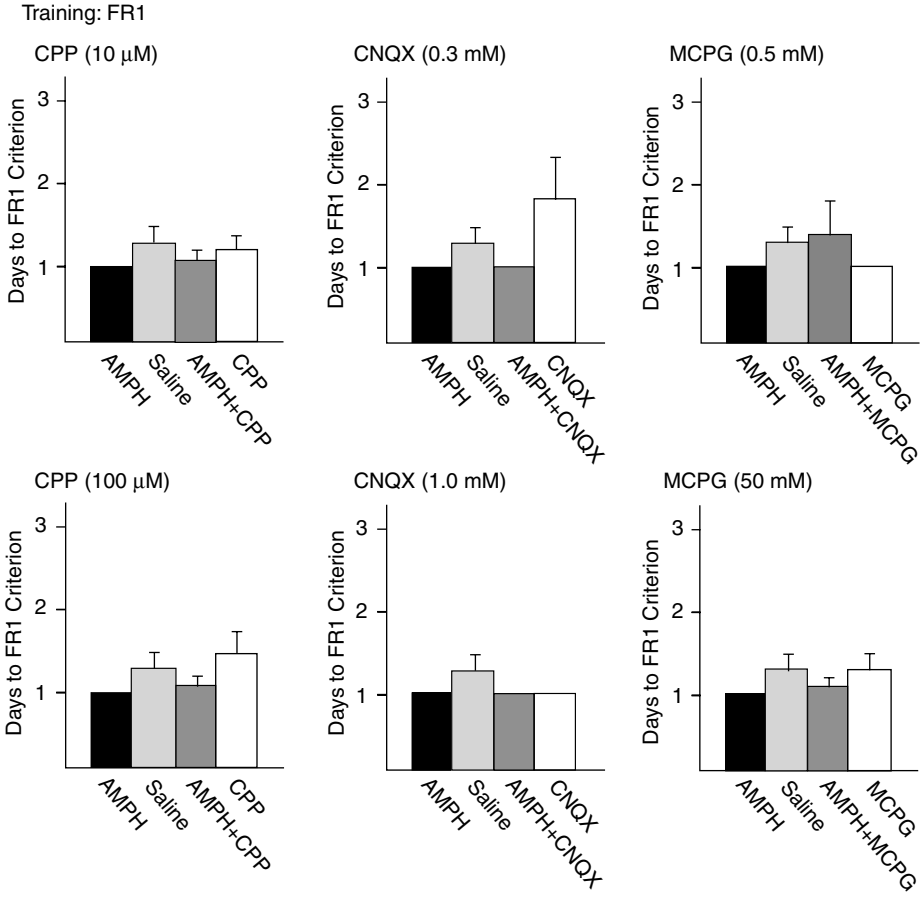

Figure 1 Cocaine self-administration training under the FR1 schedule of reinforcement. Data are shown as the mean (+SEM) number of days animals took to reach the criterion (nine self-administered infusions within a 3-h session) for this schedule. Following a single priming infusion of cocaine (0.3 mg/kg), rats were allowed to self-administer nine additional infusions of the drug. Pre-exposure condition (AMPH, saline, CPP, CNQX, MCPG, AMPH+CPP, AMPH+CNQX, and AMPH+MCPG) did not significantly influence the number of days to achieve the criterion under the FR1 schedule of reinforcement. No receptor antagonists were administered during cocaine self-administration training.

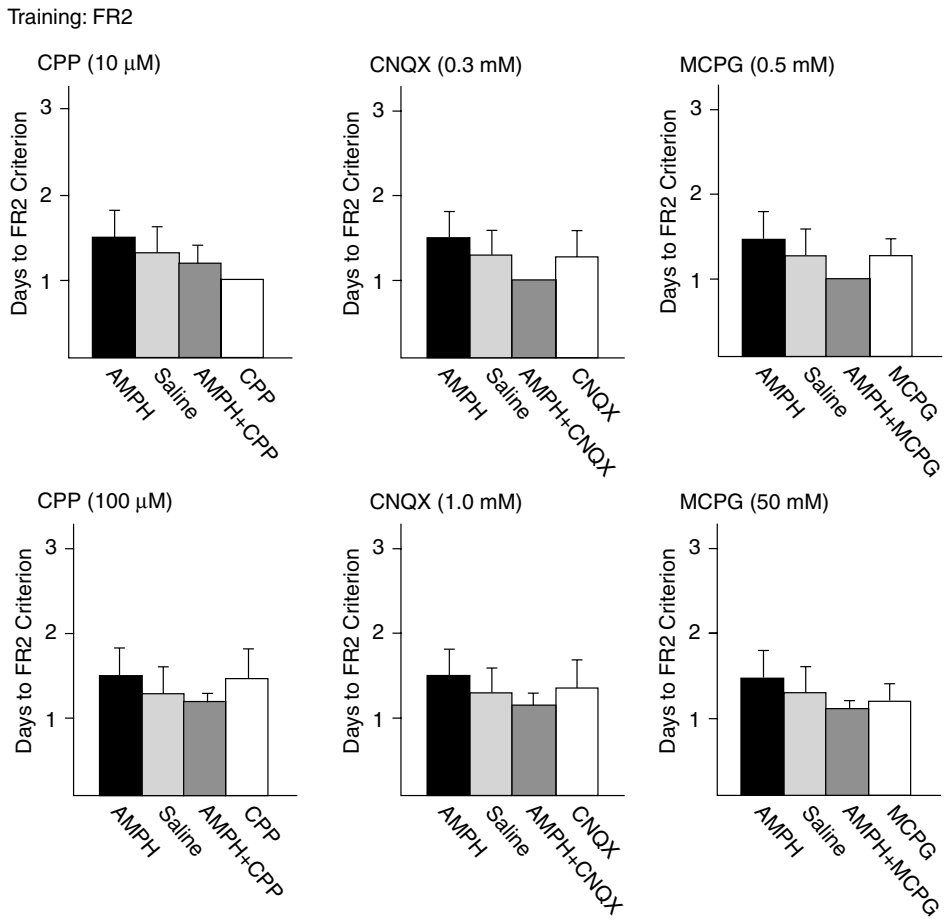

Figure 2 Cocaine self-administration training under the FR2 schedule of reinforcement. Data are shown as the mean (+SEM) number of days animals took to reach the criterion (nine self-administered infusions within a 3-h session) for this schedule. Once animals satisfied the FR1 criterion, they were switched to an FR2 schedule, once again given a single priming infusion (0.3 mg/kg) and then allowed to self-administer nine additional infusions. As with the FR1 schedule, the pre-exposure condition did not significantly influence the number of days to achieve the criterion under the FR2 schedule of reinforcement. Again, no receptor antagonists were administered during cocaine self-administration training.

performance during PR testing in comparison to that observed in the saline pre-exposed control rats. The ANOVA conducted on all of the PR test data revealed significant effects of pre-exposure ($F_{1,103} = 4.63$, $P < 0.05$), glutamate receptor blockade ($F_{6,103} = 2.93$, $P < 0.05$) and day ($F_{5,515} = 4.86$, $P < 0.001$), and a significant pre-exposure × glutamate receptor blockade interaction ($F_{6,103} = 2.43$, $P < 0.05$). The remaining interactions (pre-exposure × day ($F_{5,515} = 0.54$, NS), glutamate receptor blockade × day ($F_{30,515} = 1.40$, NS), and pre-exposure × glutamate receptor blockade × day ($F_{30,515} = 0.99$, NS)) did not achieve statistical significance.

Post hoc Tukey HSD comparisons were subsequently made on the group mean number of infusions obtained averaged over the 6 days of PR testing (bar graphs in Figures 3–5). These revealed that only the AMPH pre-exposed rats obtained significantly more ($P < 0.01$) cocaine infusions compared to the saline pre-exposed animals. These *post hoc* comparisons also revealed that coadministration of the NMDA, AMPA/kainate, and mGlu receptor antagonists with AMPH into the VTA during pre-exposure produced significant effects on performance during PR testing. The NMDA receptor antagonist, CPP, dose-dependently blocked the facilitation of cocaine self-administration by VTA AMPH (Figure 3). Rats coadministered the higher concentration of this antagonist (100 μM) with VTA AMPH obtained significantly fewer ($P < 0.01$) cocaine infusions during PR testing than rats pre-exposed to VTA AMPH alone. Similarly, the AMPA/kainate receptor antagonist, CNQX, dose-dependently blocked the development of the enhanced cocaine self-administration by VTA AMPH (Figure 4). The number of cocaine infusions obtained by rats coadministered the higher concentration of this antagonist (1.0 mM) with VTA AMPH was significantly lower ($P < 0.01$) than that obtained by rats pre-exposed to VTA AMPH alone. Both concentrations (0.5 and 50 mM) of the mGlu receptor antagonist, MCPG, when coadministered with AMPH into the VTA during pre-exposure, lead to significantly lower levels ($Ps < 0.05$–0.01) of cocaine self-administration compared to levels obtained in rats previously exposed to VTA AMPH alone (Figure 5). None of the glutamate receptor antagonists when administered alone at either dose during pre-exposure produced levels of cocaine self-administration during PR testing that differed

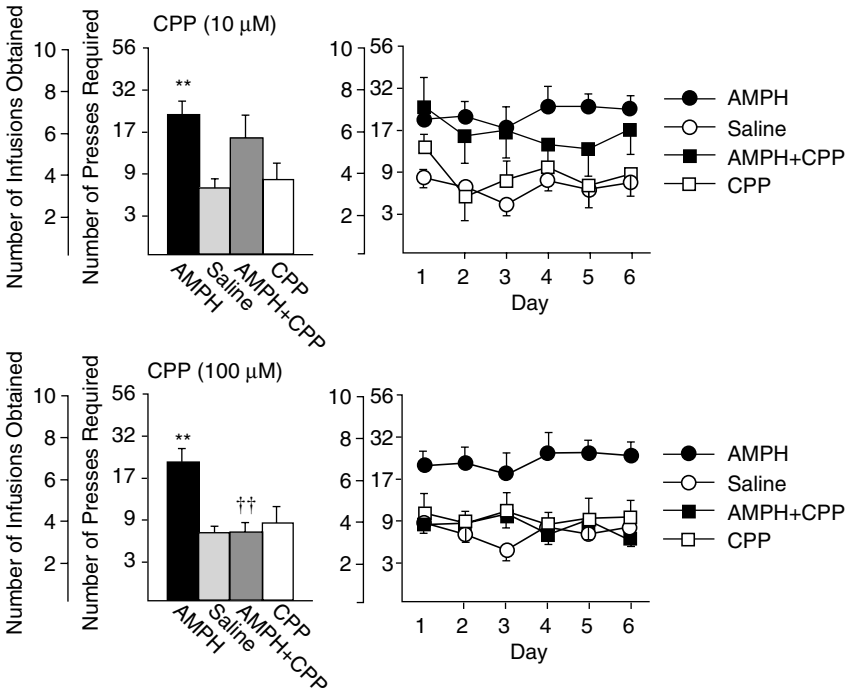

Figure 3 Previous exposure to AMPH in the VTA enhances the self-administration of cocaine under a PR schedule of reinforcement: NMDA receptor dependence. Data are shown as mean (± SEM) number of cocaine infusions obtained. The number of presses required under the PR schedule to obtain successive infusions of cocaine (0.3 mg/kg/infusion) is also shown. The bar graphs to the left were derived from means of the values obtained for each subject on each of the 6 PR test days. These are shown to the right as group means. Group names indicate pre-exposure condition. No receptor antagonists were administered during cocaine self-administration testing. $^{**}P<0.01$ *vs* saline pre-exposed rats; $\dagger\dagger P<0.01$ *vs* AMPH pre-exposed rats; as revealed by *post hoc* Tukey HSD comparisons following ANOVA. $N=7$–12/group.

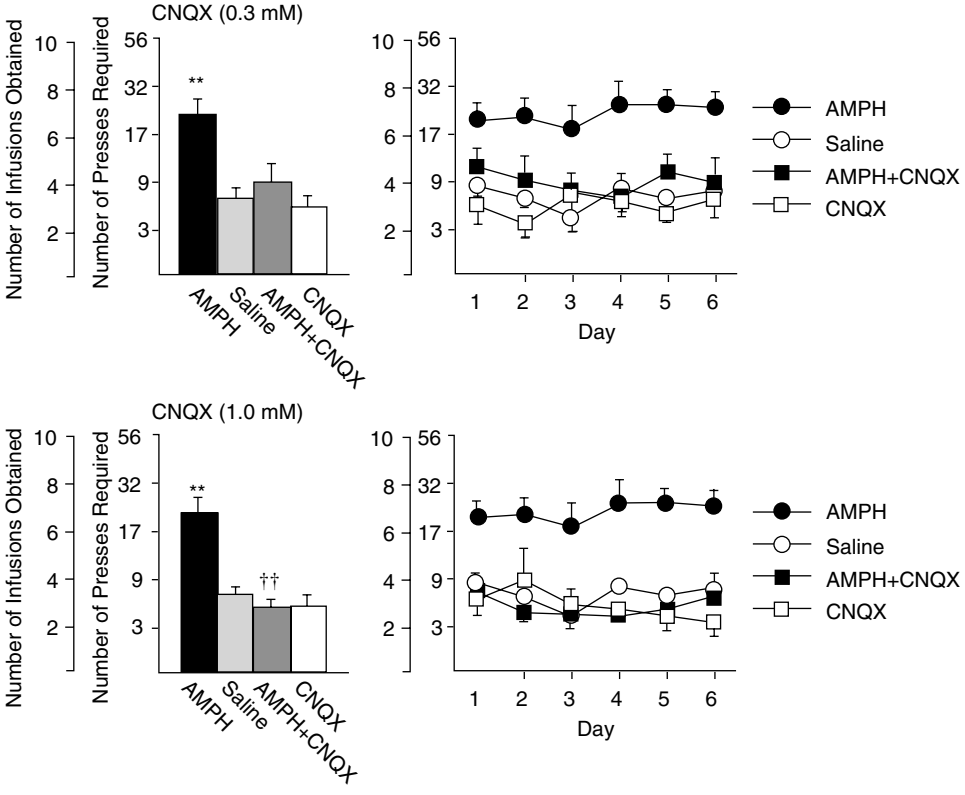

Figure 4 Previous exposure to AMPH in the VTA enhances the self-administration of cocaine under a PR schedule of reinforcement: AMPA/kainate receptor dependence. Procedures and illustration of data are as in Figure 3. $^{**}P<0.01$ *vs* saline pre-exposed rats; $\dagger\dagger P<0.01$ *vs* AMPH pre-exposed rats; as revealed by the *post hoc* Tukey HSD comparisons following ANOVA. $N=6$–12/group.

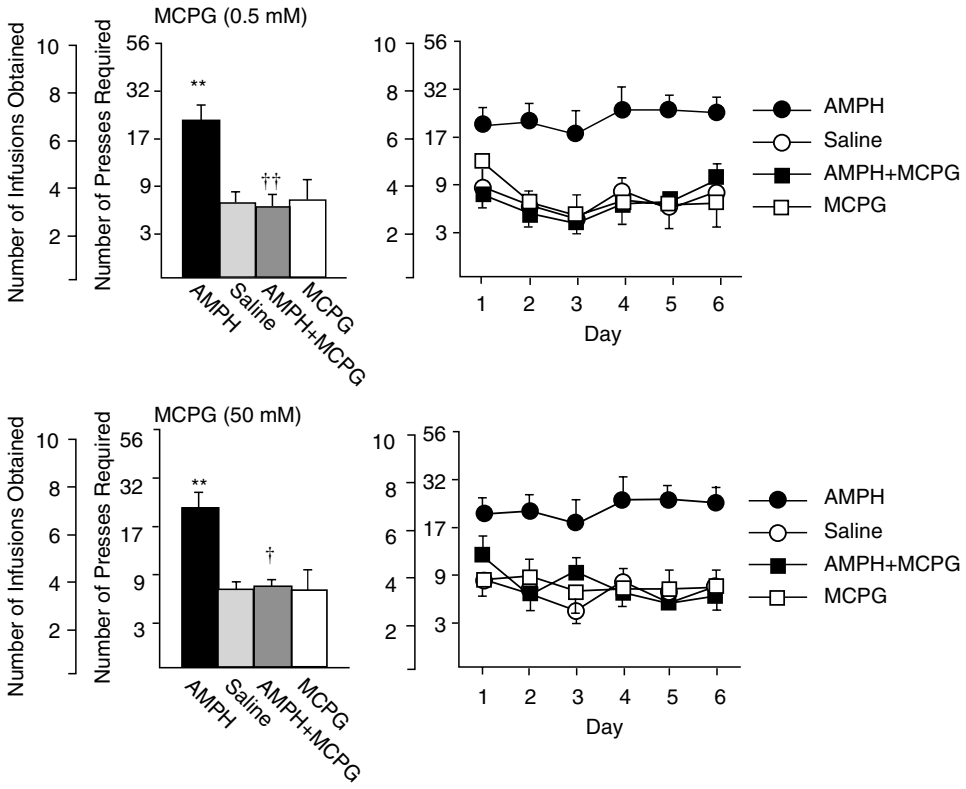

Figure 5 Previous exposure to AMPH in the VTA enhances the self-administration of cocaine under a PR schedule of reinforcement: mGlu receptor dependence. Procedures and illustration of data are as in Figure 3. $^{**}P<0.01$ *vs* saline pre-exposed rats; †$P<0.05$, ††0.01 *vs* AMPH pre-exposed rats; as revealed by the *post hoc* Tukey comparisons following ANOVA. $N=6$–12/group.

significantly from levels obtained in saline pre-exposed animals.

Histology

Figure 6a illustrates the location of the injection cannula tips in the VTA for rats in the AMPH and saline pre-exposed groups as well as those for animals that received bilateral AMPH infusions into sites outside the VTA. While these latter animals received the same number of AMPH infusions as rats in the AMPH pre-exposed group, these infusions into sites peripheral to the VTA failed to enhance cocaine self-administration during PR testing (Figure 6b). These animals together with the VTA-saline pre-exposed rats obtained significantly fewer cocaine infusions than animals previously exposed to VTA AMPH. The ANOVA conducted on these data averaged over the 6 PR test days revealed a significant effect of groups ($F_{2,25}=12.41$, $P<0.001$), and *post hoc* Tukey HSD comparisons showed that only VTA AMPH pre-exposed animals obtained significantly more cocaine infusions ($P<0.001$) than saline pre-exposed rats. The remaining two groups did not differ significantly from one another. Also shown is a photomicrograph of a cresyl-violet-stained brain section with a representative injection cannula tip in the VTA (arrow in Figure 6c). The higher power magnification photomicrograph in Figure 6d shows TH immunolabeled cells in close proximity to the injection cannula tip. Little evidence of neurotoxicity beyond the mechanical damage produced by penetration of the cannulae was detected.

DISCUSSION

Consistent with previous reports, repeated exposure to AMPH in the VTA led in the present study to enhanced self-administration of cocaine. Importantly, it was found that this enhancement of cocaine self-administration requires the activation of NMDA, AMPA/kainate, and mGlu receptors in the VTA during pre-exposure to AMPH in this site.

AMPH Acts in the VTA to Induce Psychostimulant Sensitization and Enhanced Self-Administration

A number of studies have now shown that repeated exposure to systemic AMPH leads to augmentations in the locomotor and NAcc DA responses to AMPH (Vanderschuren and Kalivas, 2000), the locomotor activating effects of cocaine (Schenk *et al*, 1991; Hooks *et al*, 1992) as well as enhanced self-administration of AMPH (Piazza *et al*, 1989; Pierre and Vezina, 1997; Mendrek *et al*, 1998; Lorrain *et al*, 2000) and cocaine (Horger *et al*, 1992; Valadez and Schenk, 1994). AMPH appears to produce these long-lasting effects by acting in the VTA. Infusions of the drug into this site have been shown to produce locomotor sensitization to AMPH (Perugini and Vezina, 1994; Cador *et al*, 1995) and cocaine (Kalivas and Weber, 1988), NAcc DA sensitization to AMPH (Vezina, 1993, 1996), as well as enhanced AMPH (Vezina *et al*, 2002) and cocaine (Suto *et al*, 2002; present study) self-administration. Conversely, sensitized locomotor responding to AMPH and cocaine (Kalivas and Weber,

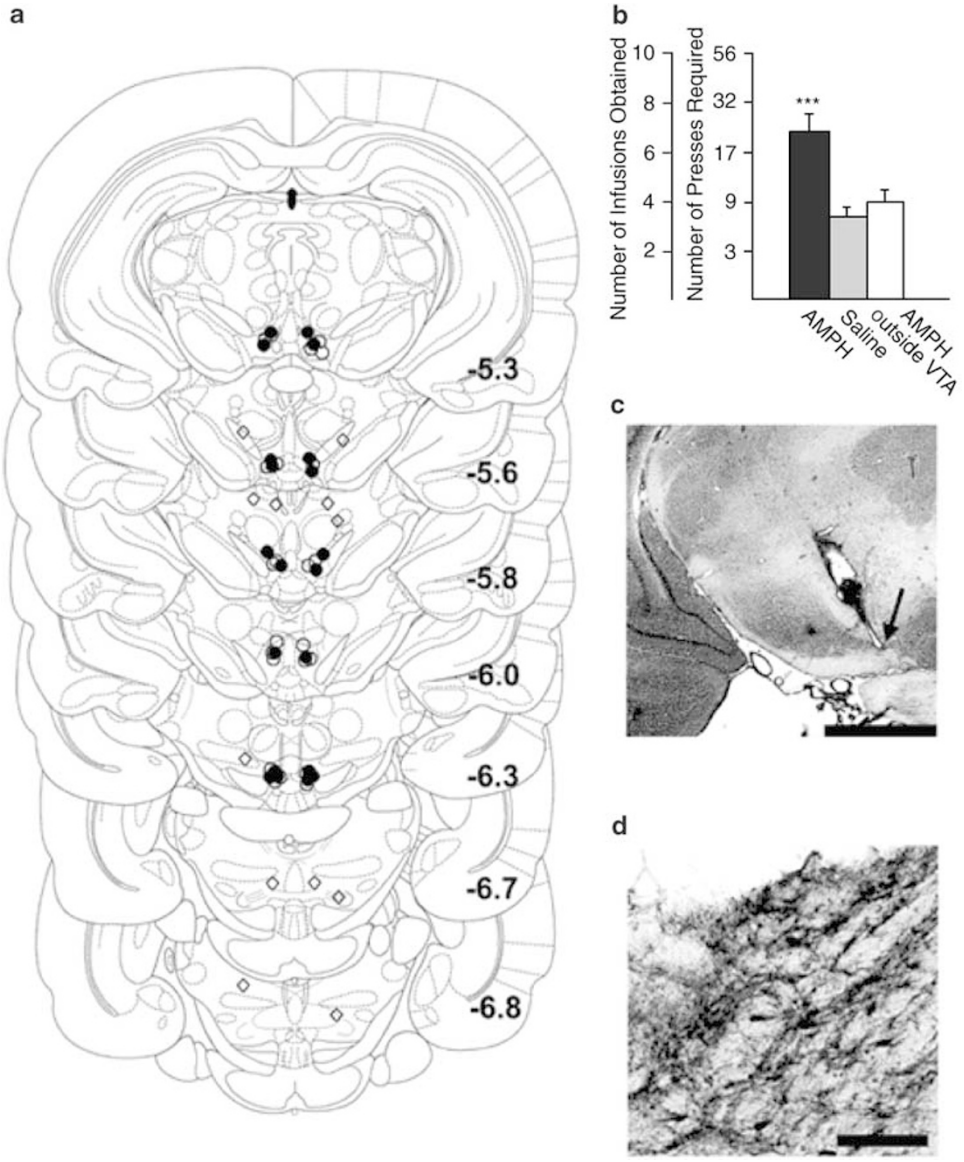

Figure 6 Injection cannula tip placements in the VTA. Location of the injection cannula tips of rats previously exposed to VTA AMPH (●) or saline (○) or to AMPH in sites outside the VTA (◇) is illustrated in (a). Line drawings are from Paxinos and Watson (1997). Numbers to the right indicate mm from bregma. (b) Previous exposure to AMPH in sites outside the VTA did not enhance cocaine self-administration during PR testing. Data are shown as group mean (+SEM) number of cocaine infusions obtained averaged over the 6 PR test days. The number of presses required under the PR schedule to obtain successive infusions of cocaine (0.3 mg/kg/infusion) is also shown. ***$P<0.001$, significantly different from saline pre-exposed rats as revealed by *post hoc* Tukey HSD comparisons following one-way ANOVA. $N=6$–12/group. The photomicrographs illustrate a representative injection cannula tip in the VTA (arrow in c) and TH positive cells in close proximity to it (d). Scale bars: 2 mm in (c) and 100 μm in (d).

1988; Hooks *et al*, 1992; Cador *et al*, 1995) and enhanced AMPH self-administration (Vezina *et al*, 2002) are not produced when animals are pre-exposed to AMPH in DA neuron terminal regions such as the NAcc. Overall, this evidence indicates that AMPH acts in the VTA not only to produce sensitized locomotor and NAcc DA responding to psychostimulants but also to enhance the self-administration of these drugs. Thus, the enhanced cocaine self-administration observed following pre-exposure to VTA AMPH in the present study may represent an instance of AMPH-induced sensitization that can be used to model psychostimulant addiction, as suggested by some (Robinson and Berridge, 1993).

Induction of Sensitization by AMPH Requires Activation of Glutamate Receptors in the VTA

The systemic administration of either NMDA (Karler *et al*, 1989; Wolf *et al*, 1995) or AMPA/kainate (Karler *et al*, 1991a; Li *et al*, 1997; cf Akiyama *et al*, 1998) receptor antagonists as well as the application into the VTA of either NMDA (Cador *et al*, 1999; Vezina and Queen, 2000) or mGlu (Kim and Vezina, 1998) receptor antagonists has been shown to prevent the induction of AMPH-induced locomotor sensitization. Moreover, systemically administering NMDA receptor antagonists with AMPH during pre-exposure also prevents cellular correlates of locomotor

sensitization normally observed in the VTA such as D_2 DA autoreceptor subsensitivity (Wolf *et al*, 1994) and increased basic fibroblast growth factor, which through its effects on astrocytic function may affect the function of dopaminergic neurons (Flores and Stewart, 2000). The present results are consistent with these findings. Taken together, they indicate that activation of glutamate receptors in the VTA is necessary for the induction of sensitization by AMPH and its subsequent enhancement of cocaine self-administration.

AMPH promotes the somatodendritic release of DA in the VTA (Kalivas and Duffy, 1991). This DA likely increases extracellular levels of glutamate in this site by activating local D_1, but not D_2, receptors (Kalivas and Duffy, 1995; Wolf and Xue, 1998) including those expressed on glutamate afferent terminals (Lu *et al*, 1997). Although the precise localization of all subtypes of glutamate receptors in the VTA remains unknown, this glutamate would at least be available to activate those glutamate receptors (NMDA, AMPA/kainate, and mGlu) known to be expressed by DA perikarya in this site (Wang and French, 1993). Importantly, activation of VTA D_1 receptors has been shown to be necessary for the induction of the locomotor and NAcc DA sensitization (Stewart and Vezina, 1989; Bjijou *et al*, 1996; Vezina, 1996) as well as the enhanced cocaine self-administration (Suto *et al*, 2002) observed in VTA AMPH pre-exposed animals. D_2 receptor blockade fails to prevent the induction of locomotor sensitization by VTA AMPH (Bjijou *et al*, 1996). Thus, by increasing extracellular levels of glutamate in the VTA via local D_1 receptors, AMPH may initiate neuroadaptational processes leading ultimately to the expression of locomotor and NAcc DA sensitization as well as enhanced drug self-administration.

The VTA receives afferent glutamatergic projections from various brain areas, including the PFC, pendunculopontine nuclei, amygdala, and subthalamic nucleus (Kalivas, 1993). Of these, the descending excitatory amino acid projection from the PFC to the VTA has been particularly implicated in the induction of psychostimulant sensitization, because lesions of the PFC prevent the induction of locomotor sensitization by systemic (Wolf *et al*, 1995) and intra-VTA (Cador *et al*, 1999) AMPH. Interestingly, recent anatomical (Carr and Sesack, 2000), neurochemical (Takahata and Moghaddam, 2000), and electrophysiological (Lokwan *et al*, 1999) reports suggest that the PFC-VTA glutamatergic projection does not regulate VTA-NAcc DA neurons directly but rather via polysynaptic midbrain circuits using GABA, glutamate, acetylcholine, and perhaps other transmitters originating in the VTA and sites (eg pedunculopontine and laterodorsal nuclei; Lokwan *et al*, 1999; Forster and Blaha, 2000) projecting to it. The contribution of these local circuits as well as non-PFC originating glutamatergic afferents to the VTA to the enhancement of psychostimulant self-administration produced by previous exposure to AMPH remains to be elucidated.

Consequences for the Induction of Sensitization of NMDA, AMPA/Kainate, and mGlu Receptor Activation

In the present experiment, it was found that activation of NMDA, AMPA/kainate, and mGlu receptors in the VTA is necessary for the induction of sensitization by AMPH in this site. The mechanisms underlying the contribution of these receptors to the development of AMPH-induced sensitization remain unknown, however. Interestingly, induction of long-term potentiation (LTP) in different brain regions also requires activation of NMDA, AMPA/kainate, and mGlu receptors (Little *et al*, 1995; Anwyl, 1999; Malenka and Nicoll, 1999). Previous exposure to AMPH or cocaine has been reported to produce transient LTP or LTP-like effects in the VTA (White *et al*, 1995; Zhang *et al*, 1997; Giorgetti *et al*, 2001; Ungless *et al*, 2001), suggesting that sensitization and LTP may reflect related or interacting forms of neuroplasticity. To date, however, only NMDA receptor-dependent LTP has been reported in DA neurons of the VTA (Bonci and Malenka, 1999; Ungless *et al*, 2001).

Ca^{2+} entry into the intracellular space, where it can recruit a number of second-messenger pathways, may constitute one of the key neuronal events leading to induction of sensitization. Systemic administration of an L-type Ca^{2+} channel antagonist during AMPH pre-exposure prevents the induction of locomotor sensitization (Karler *et al*, 1991b), while repeated administration of an L-type Ca^{2+} channel agonist into the VTA produces locomotor sensitization to cocaine (Licata *et al*, 2000). NMDA, AMPA/kainate, and mGlu receptors are known to interact to influence this influx of Ca^{2+}. For example, Ca^{2+} entry through NMDA receptors generally requires concurrent activation of both NMDA and AMPA/kainate receptors for removal of Mg^{2+} blockade. Moreover, mGlu receptors modulate both NMDA and AMPA receptor-mediated Ca^{2+} influx (Anwyl, 1999), while activation of the mGluR1-subtype releases Ca^{2+} from intracellular stores (Pin and Duvoisin, 1995). Interestingly, direct application of NMDA into the VTA failed to produce cross-sensitization to the locomotor activating effects of cocaine (Schenk and Partridge, 1997; Licata *et al*, 2000), indicating that, as in the case of LTP (Anwyl, 1999; Malenka and Nicoll, 1999), induction of psychostimulant-induced sensitization may require concurrent activation of more than one type of glutamate receptor.

The function of ionotropic glutamate receptors is known to be regulated by Ca^{2+}-dependent kinases. For example, AMPA receptor function is enhanced by phosphorylation of its GluR1 subunit by Ca^{2+}/calmodulin-dependent protein kinase II (Derkach *et al*, 1999) as well as cAMP-dependent protein kinase and protein kinase C (Roche *et al*, 1996; Banke *et al*, 2000). Importantly, repeated exposure to psychostimulants has been shown to transiently increase the function of these receptors in the VTA (Zhang *et al*, 1997; Giorgetti *et al*, 2001). This transient enhancement of AMPA receptor transmission in the VTA has been suggested by some to be critical for the induction of sensitization (White, 1996; Giorgetti *et al*, 2001). Given the above evidence, it is conceivable that phosphorylation of the GluR1 subunit may play a role. Consistent with this possibility, infusion into the VTA of selective and nonselective protein kinase inhibitors blocks the induction of locomotor sensitization by cocaine and AMPH (Steketee, 1994; Tolliver *et al*, 1996, 1999). By thus enhancing AMPA receptor function and transiently increasing the responsiveness of DA neurons in the VTA, AMPH pre-exposure may promote the further entry of Ca^{2+} into these neurons and the recruitment of additional Ca^{2+}-dependent intracellular cascades ultimately leading to the expression of

sensitization, including the enhanced cocaine self-administration observed in the present study.

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