

Amphetamine and cocaine do not increase Narp expression in rat ventral tegmental area, nucleus accumbens or prefrontal cortex, but Narp may contribute to individual differences in responding to a novel environment

W. Lu,^{1,*} M. Marinelli,^{2,†} D. Xu,³ P. F. Worley³ and M. E. Wolf¹

¹Department of Neuroscience and,

²Department of Cellular and Molecular Pharmacology, The Chicago Medical School, North Chicago, IL 60064, USA

³Departments of Neuroscience and Neurology, The Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

Keywords: addiction, AMPA receptors, immediate early genes, individual differences, neuronal plasticity

Abstract

Narp is an immediate early gene product that acts extracellularly to cluster AMPA receptors at excitatory synapses. The present study tested the hypothesis that drugs of abuse alter Narp expression and thereby influence AMPA receptor transmission in addiction-related circuits. Immunohistochemical studies demonstrated the existence of Narp-positive cells in hippocampus, prefrontal cortex (PFC) and nucleus accumbens (NAc), with lower levels of staining in the ventral tegmental area (VTA). To study the effects of psychomotor stimulants, Narp levels were quantified by Western blotting and normalized to actin. There were no differences in Narp levels in any brain region between rats treated with repeated saline injections, a single amphetamine injection (5 mg/kg), repeated amphetamine injections (5 mg/kg × 5 days), or repeated cocaine injections (20 mg/kg twice daily × 7 days). We also examined the possible role of Narp in individual differences in responding to a novel environment, a predictor of behavioural responses to psychomotor stimulant drugs including the propensity to acquire drug self-administration. Narp levels in the PFC, but not other regions, were significantly correlated with locomotor activity in a novel environment. These findings suggest that differential Narp expression in the PFC may be involved in determining individual vulnerability to drugs of abuse, perhaps by influencing the activity of its excitatory projections.

Introduction

Narp (neuronal activity-regulated pentraxin) is an immediate early gene product that is secreted into the extracellular space, where it interacts with AMPA receptor subunits to facilitate the formation of new excitatory synapses and aggregate AMPA receptors at established synapses (O'Brien *et al.*, 1999). Its expression increases in response to patterns of synaptic activity that activate NMDA receptors (Tsui *et al.*, 1996). The role of Narp in synaptic plasticity remains to be established. However, recent studies on LTP and LTD suggest that regulation of AMPA receptor trafficking in and out of postsynaptic sites is a fundamental mechanism for altering the strength of excitatory synapses (Lüscher *et al.*, 2000; Malinow *et al.*, 2000; Scannevin & Huganir, 2000; Carroll *et al.*, 2001; Sheng & Lee,

2001). Narp is an attractive candidate for linking changes in synaptic activity to the redistribution of AMPA receptor subunits.

Work over the past decade has established that neuroadaptations produced by repeated administration of psychomotor stimulants require glutamate transmission for their development and are associated with changes in glutamate receptor function (Wolf, 1998; Vanderschuren & Kalivas, 2000). Indeed, recent work suggests that psychomotor stimulants directly influence cellular mechanisms underlying LTP and LTD (Chao *et al.*, 2000, 2002; Jones *et al.*, 2000; Thomas *et al.*, 2001; Ungless *et al.*, 2001; see Wolf, 2002 for review). Such effects, by producing inappropriate plasticity in neuronal circuits mediating motivation and reward, are likely to contribute to addiction.

It is not known how psychomotor stimulants, which initially act on monoamine neurons, are able to produce changes in glutamate transmission. Based on the ability of psychomotor stimulants to induce many IEGs (Harlan & Garcia, 1998), we hypothesized that they could induce Narp and thereby produce adaptations in AMPA receptor transmission. This was examined in three brain regions that show altered AMPA receptor function after repeated cocaine or amphetamine administration: ventral tegmental area (VTA), nucleus accumbens (NAc), and prefrontal cortex (PFC).

During these studies, we became interested in the possibility that individual differences in Narp expression are involved in predispos-

Correspondence: Dr Marina E. Wolf, as above.

E-mail: marina.wolf@finchems.edu

**Present address:* Nalge Nunc International, 75 Panorama Creek Drive, Rochester, NY 14625

†*Present address:* INSERM U.259, Université Bordeaux II, Rue Camille Saint-Saëns, 33077 Bordeaux Cedex, France

Received 7 January 2002, revised 11 April 2002, accepted 23 April 2002

ing some individuals to be more sensitive to the reinforcing effects of addictive drugs. Individual differences in sensitivity to addictive drugs are well documented in both rats and humans (de Wit *et al.*, 1986; O'Brien *et al.*, 1986; Piazza *et al.*, 1998, 2000). It is important to understand their biological basis, as addiction is not a necessary consequence of drug exposure, but only develops in a subset of people that experiment with drugs of abuse.

It is well established that rats exhibiting a high locomotor response to a novel environment have a greater propensity to acquire and maintain psychostimulant self-administration (Piazza *et al.*, 1989, 2000; Grimm & See, 1997; Pierre & Vezina, 1997; Marinelli & White, 2000). High responders (HRs) also develop behavioural sensitization more readily than low responders (LRs) (Hooks *et al.*, 1991a, b, 1992b; Pierre & Vezina, 1997). We therefore examined whether individual differences in Narp expression (in VTA, NAc and PFC) are correlated with individual differences in the locomotor response to a novel environment.

Materials and methods

Animals

Male Sprague–Dawley rats (Harlan, Indianapolis, IN, USA), weighing 200–225 g at the beginning of experiments, were used for these studies. Food and water were available *ad libitum*. A 12-h light : 12-h dark cycle was maintained, with lights on from 07.00 to 19.00 h, in a temperature and humidity controlled environment. All procedures were performed in strict accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee of the Chicago Medical School.

Drug treatment

To examine the effect of psychostimulant treatment on Narp levels, 23 rats were divided into four treatment groups. (i) Saline control group; rats were injected with saline (1 mL/kg, *i.p.*) twice daily on days 1–14. (ii) Acute amphetamine group; saline was injected twice daily on days 1–13, and amphetamine sulphate (5 mg/kg, *i.p.*) was injected on day 14. (iii) Repeated amphetamine group; saline was injected twice daily on days 1–9, and amphetamine sulphate (5 mg/kg, *i.p.*) was injected once daily on days 10–14. (iv) Repeated cocaine group; saline was injected twice daily on days 1–7, and (–)-cocaine HCl (20 mg/kg) was injected twice daily on days 8–14. All rats were killed on day 15, 12 h after the last injection. Tissue was processed for Western blotting. (+)-Amphetamine sulphate was obtained from Sigma Chemical Co. (St. Louis, MO, USA). (–)-Cocaine hydrochloride was obtained from the Research Technology Branch of the National Institute on Drug Abuse.

Response to a novel environment

Two identical experiments were conducted ($n = 12$ rats per experiment). In each experiment, animals were housed individually and allowed 9–10 days to acclimate to the animal room before testing for individual differences in responding to a novel environment. The novel environment consisted of a shoe box-type cage (floor, 41 × 20 cm; height, 21 cm). Three photoelectric beams placed equidistant along the long axis of the cage (PAS monitoring system; San Diego Instruments, San Diego, CA, USA) enabled determination of locomotor activity for each animal (expressed as ambulation counts, which are defined as breaks of consecutive beams). While the test cage was physically similar to the home cage, it was placed in a distinct testing room, contained a minimal amount of bedding, and

had a different top. We have shown previously that screening in this environment yields activity scores that correlate with vulnerability to develop self-administration behaviour (Marinelli & White, 2000). Activity was measured for 2 h (13.00–15.00 h), with data collected in 10 min bins. On the next day, rats were decapitated between 09.00 and 11.00 h. Brain tissue from each rat was processed for Western blotting to examine possible correlations between Narp levels in VTA, NAc and PFC and activity scores in response to a novel environment. To combine data from the two experiments, activity levels were normalized to percentage of the mean activity level in each experiment, such that all rats below the mean were <100% and all rats above the mean were greater than 100%. Narp : actin ratios were normalized in the same manner. Pearson correlation coefficients were used to examine correlations between Narp : actin ratios and locomotor activity. In addition, rats were classified as high (HRs) or low (LRs) responders to the novel environment (according to their activity scores above or below the sample median) and data were further analyzed by comparing the two groups with respect to Narp : actin data that were not normalized. HR : LR classification was the same regardless of whether it was performed using activity scores summed over the first hour of the test or over the entire 2 h duration of the test, although locomotor activity was mainly confined to the first hour of exposure to the novel environment.

Preparation of rat brain sections

Rats were anaesthetized with pentobarbital and perfused with 200 mL of ice-cold saline, followed by 400 mL of fixative solution containing 4% paraformaldehyde (Sigma, Sigma-Aldrich, St Louis, MO, USA), 1.5% sucrose and 0.1 M phosphate buffer at pH 7.2 (PB). After perfusion, rat brains were immediately removed and immersed in the above fixative solution for another hour. Then, rat brains were immersed sequentially in solutions containing 0.1 M PB, 0.1% sodium azide and either 10, 20 or 30% sucrose at 4 °C. Coronal sections (40 µm) of rat brain were cut frozen on a sliding microtome and stored free-floating in cryoprotectant solution (30% sucrose, 30% ethylene glycol (Fisher Scientific, Pittsburgh, PA, USA) and 0.1 M PB (pH 7.2)) at –20 °C.

Immunohistochemistry

Rat brain sections were transferred from cryoprotectant solution into a net in a glass dish containing 0.1 M phosphate buffer with 0.3% Triton-X 100 (PB/T). After rinsing in PB/T for 6 × 10 min at room temperature with agitation, sections were preincubated in 1% bovine serum albumin (BSA) and PB/T for 30 min to block background staining. Sections were transferred into wells of cell culture plates and incubated with rabbit anti-Narp antibody (1 : 4000) in PB/T containing 1% BSA at 4 °C overnight with agitation. The Narp antibody was raised against full-length Narp protein and recognizes residues 115–200. After rinsing 4 × 10 min in PB/T, staining was visualized using an ABC kit (Vector, Burlingame, CA, USA). Briefly, sections were incubated with biotinylated anti-rabbit IgG antibody for 1 h at room temperature, rinsed in PB/T 4 × 10 min, incubated with avidin and biotinylated horseradish peroxidase for 1 h at room temperature, and stained with diaminobenzidine (DAB; Sigma-Aldrich, St Louis, MO, USA). Sections were mounted onto gelatin-coated microslides. To verify the specificity of Narp immunostaining, GST-Narp bound agarose beads were used to preabsorb anti-Narp antibody for 4 h at 4 °C before incubation with rat brain sections. Images from immunohistochemical studies were obtained and analyzed using a system consisting of a Nikon microscope, an ORCA ER digital camera (Hamamatsu Photonics, Japan) and

MetaMorph Imaging software (Universal Imaging, West Chester, PA, USA).

Western blots

Rats were decapitated and brains removed on ice. Coronal sections (2 mm) were prepared using a brain mold (Activational Systems; Detroit, MI, USA). Regions of interest were dissected from appropriate coronal sections (PFC, Bregma \sim 2.50–4.50; NAc, Bregma \sim 0.50–2.50; VTA, Bregma \sim –4.50 to –6.50), according to Paxinos & Watson (1986). In dissecting out the VTA, care was taken to avoid the interpeduncular nucleus, which contains high levels of AMPA receptor subunits (Lu *et al.*, 2002) and Narp (data not shown). Brain tissue was sonicated for 2×5 s in ice cold modified Laemmli sample buffer (Bio-Rad) containing 3% SDS, leupeptin (20 μ g/mL, Sigma) and PMSF (0.1 mM). Protein concentrations were determined with the BCA Protein Assay Kit (Pierce, Rockford, IL, USA). For most experiments, samples were heated at 65 °C for 1 min just before loading onto 8% polyacrylamide gels. In some control experiments, samples were heated at 100 °C in 2-mercaptoethanol (5%) for 3 min to dissociate the Narp complex. A Trans-Blot Cell System was used for blotting onto PVDF membrane (Pall, Ann Arbor, MI, USA). Anti-Narp antibody (1 : 4000), HRP-labelled anti-rabbit IgG antibody (1 : 10 000), and ECL reagents were employed for determination of Narp immunoreactivity. Blots were also probed with anti-actin antibody (1 : 1000; Chemicon, Temecula, CA, USA). To verify the specificity of Narp staining on Western blots, GST-Narp bound agarose beads were used to preabsorb anti-Narp antibody for 4 h at 4 °C before use in some experiments. Western blots were analyzed using a Power Macintosh G3, an Apple-One Scanner (Macintosh, Cupertino, CA, USA) and NIH Image software. To adjust for any variations in loading, Narp levels were normalized to actin levels. Data from drug treated groups were expressed as a percentage of the saline group run on the same gel. Experimental groups were compared by one-way analysis of variance (ANOVA).

Results

Distribution of Narp protein

Immunohistochemical techniques have demonstrated Narp staining in adult rat hippocampus (O'Brien *et al.*, 1999) but its distribution in other brain regions has not been examined previously. We used immunohistochemistry to study Narp expression in the hippocampus and in mesocorticolimbic brain regions that are important in the effects of psychomotor stimulants (Fig. 1). In the hippocampus, darkly staining cells were distributed in the dentate gyrus, and Narp-positive cells were also found in CA1, CA2 and CA3 areas. This is consistent with previous results (O'Brien *et al.*, 1999). In the PFC, Narp-positive cells were found in layers II–VI, with the highest density of darkly stained cells in layer VI. In the NAc, Narp-positive cells were located in both core and shell areas, and the density of Narp-positive cells was higher in the ventral portion of the core than in the dorsal portion. In the VTA, light staining for Narp protein was found in both cell bodies and processes. In all of these brain regions, most Narp-positive cells exhibited a lightly stained core with dark staining in the surrounding area (Figs 1 and 2), suggesting that most Narp protein is located in the cytoplasm.

The specificity of Narp immunostaining was evaluated by preabsorption of the Narp antibody with GST-Narp bound agarose beads. Pre-absorption of the Narp antibody almost completely eliminated cellular staining in rat hippocampus and VTA (Fig. 2).

Specific staining was also eliminated if the Narp antibody was inactivated prior to use (100 °C for 5 min; data not shown).

Properties of Narp protein in VTA, NAc and PFC

Narp is a member of the pentraxin family, secreted proteins that covalently self-multimerize through disulphide bonds to form pentamers and may further aggregate into larger complexes (Tsui *et al.*, 1996). Accordingly, rat brain Narp migrates as a multimer when analyzed by SDS-PAGE with a nonreducing sample buffer (O'Brien *et al.*, 1999). Our results are consistent with these prior findings. Under nonreducing conditions, the Narp antibody detected a slowly migrating band (> 200 kDa) in all three of the brain regions examined (PFC, NAc and VTA; Fig. 3). In the VTA, another band (\sim 56 kDa) was present, which may be the glycosylated monomer of Narp (Tsui *et al.*, 1996; O'Brien *et al.*, 1999). To test this possibility, and to verify that the high molecular weight band dissociates upon incubation with reducing agents, 2-mercaptoethanol (5%) was added to VTA tissue samples and samples were incubated at 100 °C for 3 min. This treatment greatly reduced the optical density of the high molecular weight band (Fig. 4, left) and increased the intensity of the 56 kDa band (not shown), indicating that the high molecular weight band is the Narp multimeric complex. To further examine the specificity of the Narp antibody in Western blotting studies, the antibody was preabsorbed with GST-Narp bound agarose beads, as described above for immunohistochemical studies. This treatment also reduced the intensity of the high molecular weight band (Fig. 4, right). Based on these results, we used the high molecular weight band to measure Narp protein expression in subsequent experiments. This is the same approach taken in prior studies showing increased Narp protein expression after electroconvulsive seizures (O'Brien *et al.*, 1999; Reti & Baraban, 2000).

Repeated treatment with cocaine or amphetamine does not alter Narp levels in rat VTA, NAc and PFC

In an initial experiment (not shown), we treated rats ($n = 6$ –7 rats per group) with a single amphetamine injection (5 mg/kg) or with repeated injections of saline (1 mL/kg \times 6 days), cocaine (20 mg/kg \times 7 days) or amphetamine (5 mg/kg \times 5 days). Robust behavioural sensitization is produced by this amphetamine regimen (Wolf & Jeziorski, 1993) and by similar cocaine regimens (e.g. Kalivas & Duffy, 1993). Narp levels were quantified by Western blotting and normalized to actin levels. No significant effects of drug treatment were observed in the VTA, NAc, or PFC (data not shown). However, these studies revealed a surprising level of individual variability in Narp levels, particularly in the VTA. We therefore performed a second study in which rats received 5–7 days of twice daily saline injections prior to beginning repeated treatment with cocaine or amphetamine (as described in Materials and Methods) in the hope that habituating rats to the injection procedure might reduce variability in the VTA to a level that would enable detection of potential drug effects. The results of these studies, shown in Fig. 5, did not differ from the initial study performed without saline preinjections. Thus, there was no significant effect of drug treatment on Narp levels in VTA, NAc or PFC (Fig. 5). Furthermore, the saline preinjections did not reduce individual variability in Narp levels (Fig. 6).

Relationship between locomotor response to novelty and Narp expression in VTA, NAc and PFC

Two identical experiments were conducted ($n = 12$ rats each) in which rats were tested for response to a novel environment and then killed the next day for analysis of Narp : actin ratios by Western

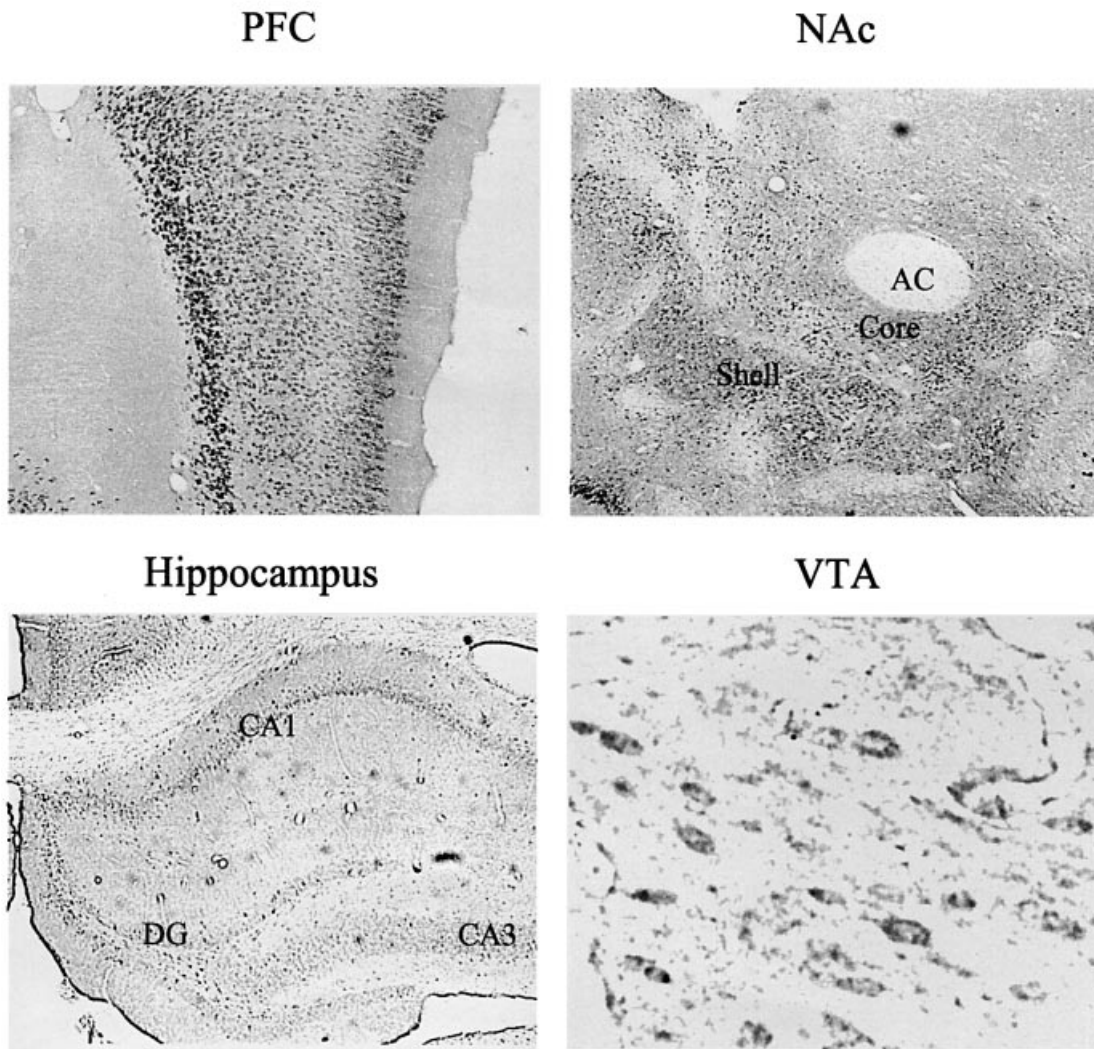


FIG. 1. Immunoreactivity detected with Narp antibody in hippocampus, prefrontal cortex (PFC), nucleus accumbens (NAc) and ventral tegmental area (VTA).

blotting. To combine data from the two experiments, activity levels were normalized to percentage of the mean activity level from each experiment. Narp : actin ratios were normalized in the same manner. Activity data were collected for 120 min in 10 min bins, and correlations between Narp : actin and activity were determined for cumulative activity counts at all time-points (10–120 min). Correlations between Narp : actin and activity counts during the first 60 min of the test were selected for presentation (Fig. 7), because locomotor activity was mainly confined to the first hour of exposure to the novel environment.

In the VTA, there was no statistically significant correlation between locomotor activity and Narp levels at any of the time-points (Fig. 7, left). Similarly, no significant correlation was found for the NAc (Fig. 7, middle). For the PFC, however, the correlation between locomotor activity and Narp levels was statistically significant at all time-points between 10 and 120 min of the test (r^2 values between 0.24 and 0.37, all P -values < 0.05) (Fig. 7, right).

To further analyse PFC data, rats were classified as high responders (HRs) or low responders (LRs) to the novel environment according to their activity scores above or below the sample median (see Materials and methods). ANOVA was conducted (using locomotor activity and

Narp : actin data that had not been normalized) using two between factors (HR vs. LR, and experiment 1 vs. experiment 2). There was a significant effect for both HR vs. LR ($F_{1,30} = 6.29$, $P < 0.002$) and experiment ($F_{1,20} = 94.40$, $P < 0.0001$) but no significant interaction ($F_{1,20} = 0.97$, $P > 0.33$). Thus, overall, rats with higher locomotor response to novelty have greater Narp expression in the PFC.

Discussion

The initial goal of this study was to examine the possible role of Narp in mediating adaptations in AMPA receptor transmission that occur following the repeated administration of psychomotor stimulants. Although we had hypothesized that Narp protein expression would be increased by psychomotor stimulants, we found no effect of repeated amphetamine or cocaine administration (or acute amphetamine) on Narp expression in the VTA, NAc or PFC as measured by Western blotting. However, individual differences in levels of Narp expression observed during these studies prompted us to examine, for individual rats, the correlation between Narp expression and the locomotor response to a novel environment, a predictor of vulnerability to

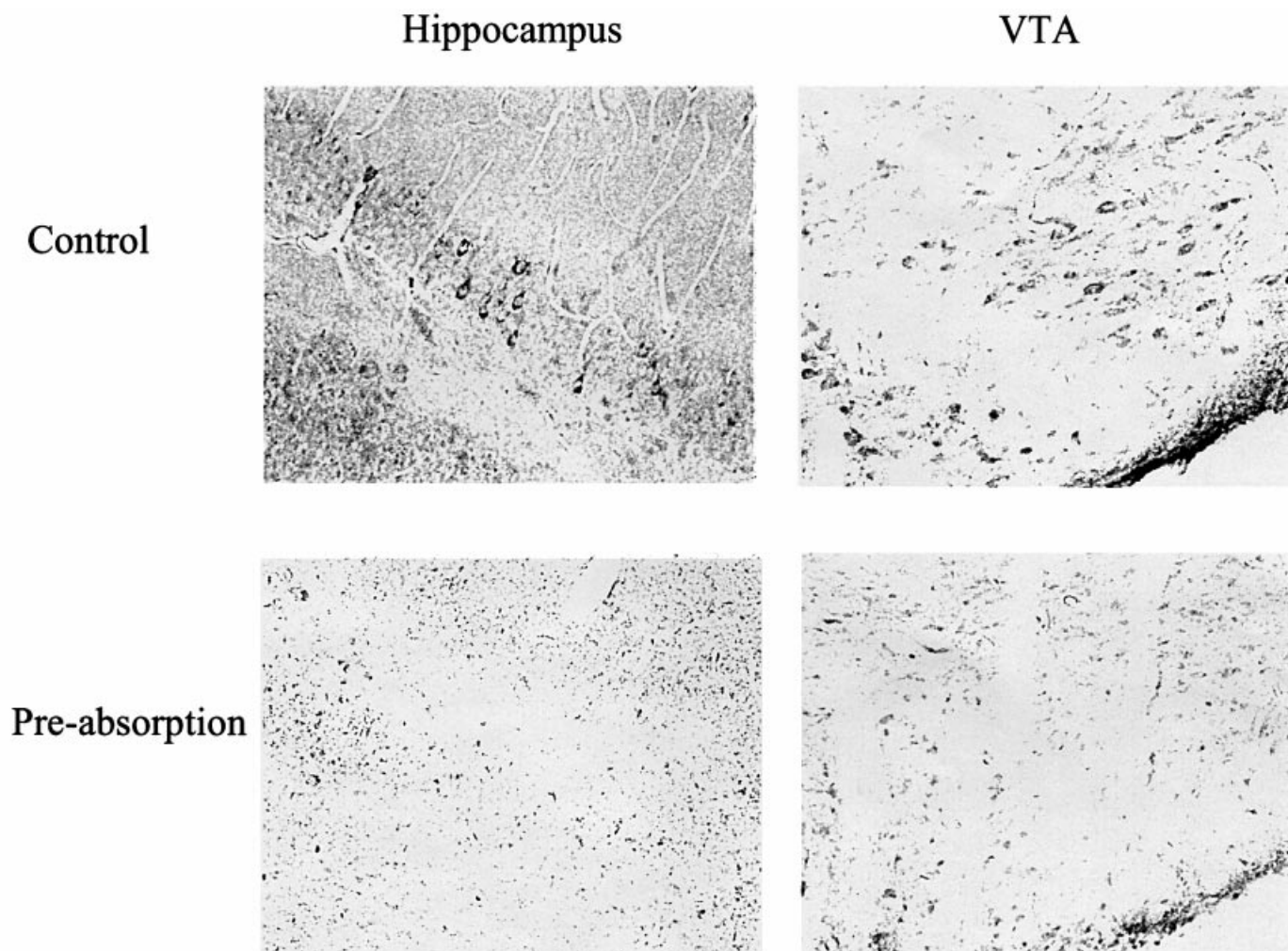


FIG. 2. Pre-absorption of the Narp antibody with GST-Narp bound agarose beads significantly reduces Narp-like immunostaining in hippocampus and VTA. Upper panels, Narp immunostaining with normal methods. Lower panels, GST-Narp bound agarose beads were used to preabsorb anti-Narp antibody for 4 h at 4 °C before incubation with rat brain sections.

acquire drug-taking behaviour (see Introduction). A significant correlation was found in the PFC, but not the VTA or the NAc.

Distribution of Narp protein

Immunohistochemical studies confirmed expression of Narp in many neurons throughout the hippocampus, and demonstrated modest Narp immunostaining in the PFC, NAc and the VTA. Staining in PFC was highest in layer VI. In the NAc, staining was present in neurons of both core and shell. In the VTA, light staining was observed in cell bodies and neuropil. The localization of VTA staining is of particular interest for evaluating hypothesized roles for Narp (below). Some of the labelled processes may correspond to afferent pathways innervating the VTA, as labelling was observed in some processes with a beaded appearance and dopamine (DA) dendrites are generally smooth (Oades & Halliday, 1987). Moreover, in preliminary double labelling studies, we did not observe coincidence of immunostaining for Narp and tyrosine hydroxylase in the VTA (data not shown). Narp labelling in afferent pathways is most likely to occur in glutamatergic axons. This would be consistent with results in hippocampal cultures showing Narp immunostaining in excitatory axons and presynaptic to excitatory synapses, but not in GABAergic axons or synapses

(although synaptic Narp can also be derived from postsynaptic neurons; O'Brien *et al.*, 1999). It is possible that some Narp staining in the VTA is localized to afferent pathways originating in the PFC, because the PFC contains Narp-positive neurons and sends glutamatergic fibers to the VTA (e.g. Sesack & Pickel, 1992). Narp-positive axon terminals could also originate from other brain regions that send excitatory projections to the VTA, such as the pedunculopontine nucleus or laterodorsal tegmentum (Phillipson, 1979; Jackson & Crossman, 1983).

Drugs of abuse do not alter Narp expression

Although it is clear that repeated administration of cocaine or amphetamine alters glutamate transmission, the mechanism(s) remain unknown. Drugs of abuse induce many immediate early genes (Harlan & Garcia, 1998). If they also induce Narp, it would provide a mechanism whereby drug-induced changes in synaptic activity could be coupled to alterations in AMPA receptor transmission.

We were particularly interested in the potential role of Narp in the development of behavioural sensitization, an animal model for intensification of drug craving. The development of sensitization in response to repeated administration of amphetamine or cocaine

requires NMDA receptor stimulation within the VTA and involves a transient increase in the responsiveness of VTA DA neurons to the excitatory effects of AMPA. The resultant increase in DA cell activity is hypothesized to result in longer-lasting adaptations in limbic and

cortical brain regions innervated by VTA DA neurons, explaining the persistence of sensitization (White *et al.*, 1995; Zhang *et al.*, 1997; Giorgetti *et al.*, 2001). This transient enhancement of AMPA receptor transmission in the VTA does not result from a generalized increase in AMPA receptor expression (Lu *et al.*, 2002). An alternative possibility is that it results from more subtle mechanisms akin to those operative in LTP, such as redistribution of AMPA receptors to synaptic sites. In fact, a recent study showed that a single high dose of cocaine (which resulted in behavioural sensitization) produced NMDA receptor-dependent LTP in VTA DA neurons (Ungless *et al.*, 2001). The requirement for NMDA transmission in the VTA for the initiation of sensitization and related cellular changes is compatible with a role for Narp, because Narp is induced by conditions that enhance NMDA receptor transmission (Tsui *et al.*, 1996). Thus, one goal of this study was to test the hypothesis that psychomotor stimulants increase Narp expression in the VTA, and that this couples stimulant-induced changes in synaptic activity to augmented AMPA receptor transmission. We also investigated the effect of psychomotor stimulants on Narp expression in the NAc and PFC, brain regions that also show adaptations in AMPA receptor function after repeated psychostimulant administration (see below).

We found no changes in Narp expression in any of these brain regions in rats killed 12 h after an acute injection of amphetamine or the last injection in a regimen of repeated amphetamine or cocaine administration. It is unlikely that we failed to detect an effect on Narp expression because we examined an inappropriate time after drug administration. Narp resembles other immediate early genes in that it is induced rapidly (within 30 min) but it is unusual in that protein levels remain elevated for more than 24 h. For example, Narp levels were elevated from 30 min to 36 h after a single episode of electroconvulsive shock, with maximal induction at 12 h (O'Brien *et al.*, 1999). A similar time-course was reported in a subsequent study of repeated electroconvulsive seizures (Reti & Baraban, 2000).

Narp was one of the IEGs identified in a differential display PCR study that screened for genes whose expression increased in response to striatal DA D1 receptor stimulation (Berke *et al.*, 1998). It is difficult to compare our results to that study for two reasons. First, although Berke *et al.* (1998) found elevated Narp mRNA levels in the

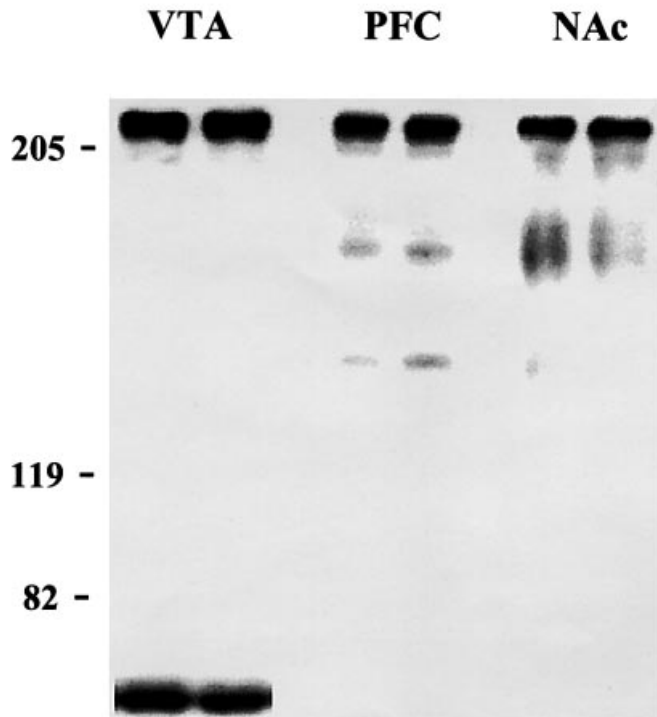


FIG. 3. The Narp antibody detects a slowly migrating band (> 200 kDa) under nonreducing conditions in all brain regions examined, consistent with prior studies showing that Narp forms a multimeric complex in the rat brain (O'Brien *et al.*, 1999). A lower molecular weight band (~56 kDa) was also detected in tissue prepared from the VTA, corresponding to the monomeric form of Narp (see Results).

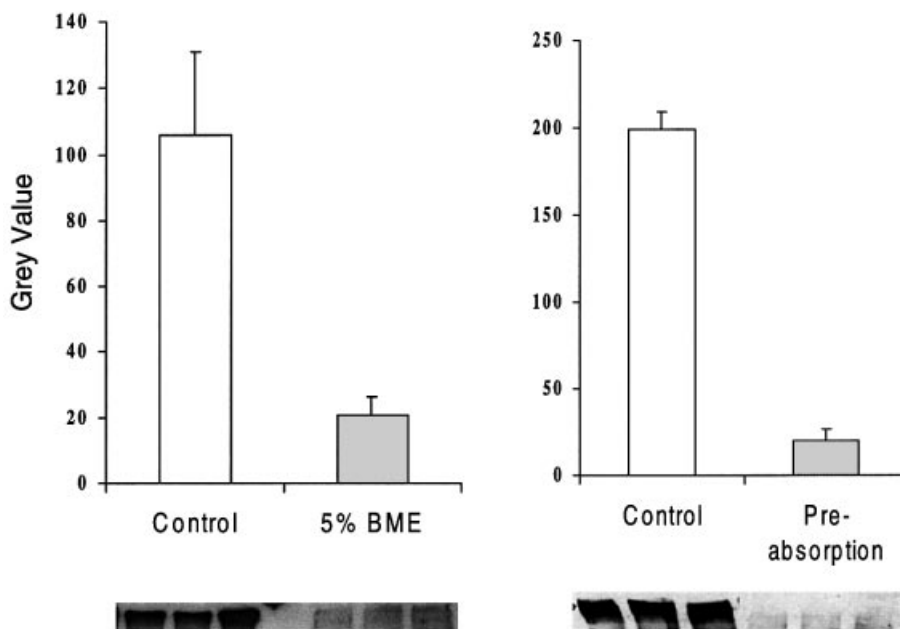


FIG. 4. The slowly migrating band (> 200 kDa; see Fig. 3) corresponds to the Narp multimeric complex. Left, reducing conditions, which dissociate the Narp multimeric complex (O'Brien *et al.*, 1999), decreased the optical density of the slowly migrating band. For each of three rats, one aliquot of VTA tissue was processed normally (control lanes 1–3) and the other was incubated with 5% 2-mercaptoethanol at 100 °C for 3 min prior to SDS-PAGE and immunoblotting (BME lanes 4–6). Right, pre-absorption of the Narp antibody with GST-Narp bound agarose beads (4 h at 4 °C) decreased the optical density of the slowly migrating band. For each of three rats, one aliquot of VTA homogenate was subjected to SDS-PAGE and immunoblotting with normal Narp antibody (control lanes 1–3), while another aliquot was probed with preabsorbed Narp antibody (preabsorbed lanes 4–6). Bars indicate mean ± SD.

dorsal striatum, the brain regions examined in the present study (VTA, NAc, PFC) were not specifically evaluated. Second, and perhaps more importantly, the pharmacological treatments were very different. Rats used to measure D1 receptor-induced gene expression received 6-hydroxydopamine lesions of the medial forebrain bundle to induce postsynaptic DA receptor supersensitivity. Three weeks later, rats were administered the D2 agonist quinpirole for 5 days via an osmotic minipump, to increase selectivity for D1 receptor supersensitivity, and then challenged with a high dose of the D1 agonist SKF 81297 (5 mg/kg).

Alternative mechanisms by which drugs of abuse may alter AMPA receptor function

Our results suggest that Narp is not involved in drug-induced changes in AMPA receptor transmission in the VTA, NAc, or PFC. It remains

possible that drugs of abuse influence other, as yet unidentified, IEGs that play direct roles in regulating AMPA receptor transmission in these regions. Alternatively, drugs of abuse may induce IEGs that serve as transcription factors, or other signalling pathways that modulate transcription, and thereby influence the expression of AMPA receptor subunits. For example, PFC neurons show a transient increase in GluR1 expression at mRNA and protein levels during early withdrawal from repeated amphetamine administration (Lu *et al.*, 1997; Lu & Wolf, 1999) and a corresponding increase in electrophysiological responsiveness to the excitatory effects of glutamate (Peterson *et al.*, 2000). In the NAc, withdrawal from repeated amphetamine leads to a more persistent decrease in GluR1 and GluR2 mRNA and protein levels (Lu *et al.*, 1997; Lu & Wolf, 1999) and a corresponding decrease in electrophysiological responsiveness to glutamate (White *et al.*, 1995). A problem with relating these results to behavioural sensitization is that other drug regimens, which also elicit sensitization, lead to different changes in the expression of AMPA receptor subunits (for examples relating to the NAc, see Churchill *et al.*, 1999; Ghasemzadeh *et al.*, 1999).

Drugs of abuse may also influence AMPA receptor transmission through mechanisms that are unrelated to either IEGs or transcriptional regulation. In some instances, DA receptors may activate signal transduction pathways that directly alter AMPA receptor function. For example, stimulation of D1 receptors in the striatal complex results in phosphorylation of GluR1 at the protein kinase A site and thereby enhances AMPA receptor currents (Price *et al.*, 1999; Yan *et al.*, 1999; Snyder *et al.*, 2000; Chao *et al.*, 2002). Studies in cultured NAc neurons suggest that D1 receptor stimulation increases GluR1 surface expression through a similar pathway (Chao *et al.*, 2000). However, these particular mechanisms are unlikely to account for the drug-induced enhancement of AMPA receptor transmission in DA neurons of the VTA (White *et al.*, 1995; Zhang *et al.*, 1997; Giorgetti *et al.*, 2001) because D1 receptors are present at low levels in the VTA and not on DA cells themselves (Morelli *et al.*, 1988; Mansour *et al.*, 1991; Meador-Woodruff *et al.*, 1991).

Narp levels in the PFC are correlated with individual variability in response to a novel environment

While the present experiments failed to demonstrate effects of psychomotor stimulants on Narp expression, a striking observation was the high degree of variability in Narp levels between individual rats, particularly in the VTA, where there was a 3-fold difference between the lowest and highest individual Narp : actin values. This prompted us to consider a possible role for Narp in producing individual differences in sensitivity to drugs of abuse. We examined correlations between Narp expression and the locomotor response to a novel environment, because rats that exhibit a high locomotor response to a novel environment (HRs) show greater propensity to acquire drug-taking behaviour than rats with a low response (LRs)

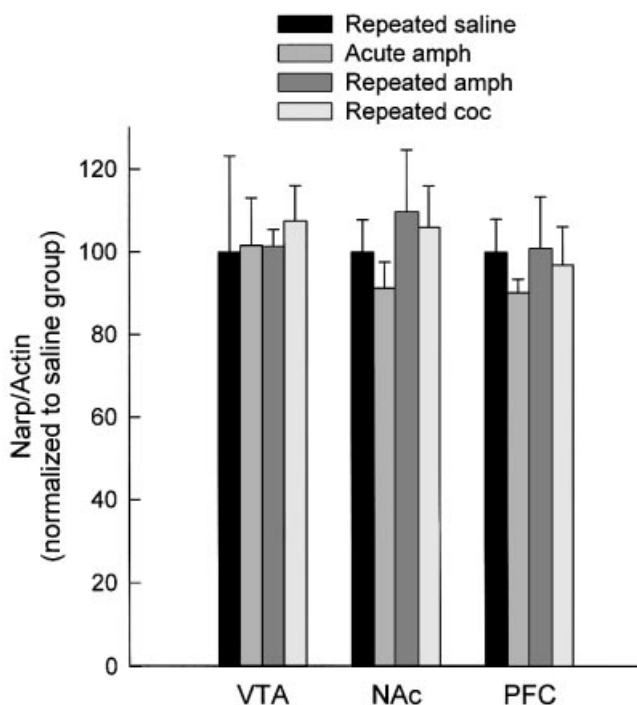


FIG. 5. Western analysis was used to compare Narp expression in the VTA of rats treated with repeated saline ($n = 6$), repeated cocaine ($n = 6$), repeated amphetamine ($n = 6$), or acute amphetamine ($n = 5$). Rats were killed 12 h after the last injection (see Materials and methods for details of drug treatment regimens). The Narp multimer band was scanned and normalized to actin levels. Results are expressed as percent of the saline control group for each brain region. No significant differences in Narp levels were found for VTA (ANOVA, $F_{3,19} = 0.06$, $P = 0.98$), NAc (ANOVA, $F_{3,19} = 0.53$, $P = 0.67$), or PFC (ANOVA, $F_{3,18} = 0.28$, $P = 0.84$).

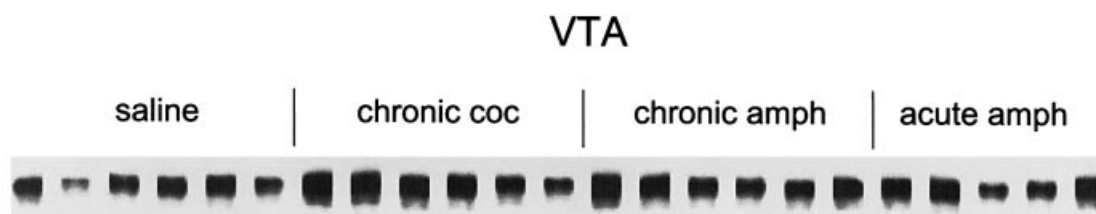


FIG. 6. Narp multimer bands from the VTA of each rat used for the experiment shown in Fig. 5. A high degree of individual variability was evident, independent of drug treatment. This was most pronounced in the VTA but also occurred in the NAc and PFC (data not shown).

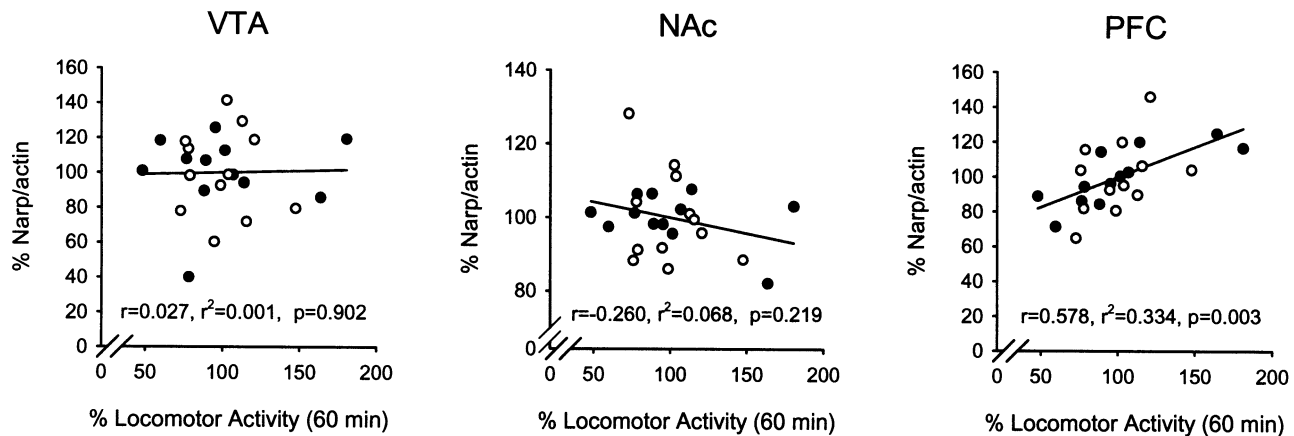


FIG. 7. A significant correlation exists between activity scores in response to a novel environment and Narp levels in the PFC (but not VTA or NAc). Two identical experiments were conducted ($n = 12$ rats per experiment) in which locomotor activity in response to a novel environment was determined and rats were killed on the following day for Western analysis of Narp levels. To combine data from the two experiments, activity levels (60 min cumulative ambulation counts) were normalized to percentage of the mean activity level for each experiment, such that all rats below the mean were $< 100\%$ and all rats above the mean were greater than 100% . Narp : actin ratios were normalized in the same manner. Rats from the first and second experiments are represented by filled and open circles, respectively ($n = 24$ rats total). Pearson correlation coefficients were used to examine correlations between locomotor activity and Narp : actin ratios.

(see Introduction). While no correlation was found between the locomotor response to a novel environment and Narp levels in either VTA or NAc, a significant correlation was found for the PFC.

How could differential Narp expression in the PFC influence the response to a novel environment? Recent work suggests that differences in the activity of midbrain DA neurons may underlie differences between HRs and LR. Thus, Marinelli & White (2000) reported that HRs exhibit higher basal firing rates and bursting activity of DA neurons in the VTA and, to a lesser extent, in the substantia nigra pars compacta. This could explain prior observations that HRs exhibit increased basal and stimulated DA levels in the NAc and striatum (Bradberry *et al.*, 1991; Hooks *et al.*, 1991b, 1992a; Piazza *et al.*, 1991; Rougé-Pont *et al.*, 1993, 1998). One interpretation of the present results is that higher Narp expression in the PFC of HRs results in greater excitatory AMPA transmission to PFC projection neurons, leading to greater excitatory drive in PFC target regions such as the VTA. This would provide a mechanism to account for reports that HRs exhibit faster firing rates of VTA DA cells and increased DA turnover in the NAc (above). It is interesting to consider this hypothesis in light of reports that DA agonists and psychomotor stimulants promote LTP and depress LTD in VTA DA neurons (Jones *et al.*, 2000; Thomas *et al.*, 2001; Ungless *et al.*, 2001), which in turn may produce the transient increase in their activity that is believed to transfer sensitization to forebrain regions innervated by the VTA (Wolf, 2002). Greater excitatory drive to VTA DA neurons in some individuals might predispose their VTA DA neurons to more readily undergo a relative shift towards LTP.

Our hypothesis is consistent with considerable evidence that PFC neurons exert an excitatory influence on VTA DA cell activity (Clark & Overton, 1998). However, we are not proposing a direct excitatory link between PFC neurons and mesoaccumbens DA neurons, as PFC neurons innervate VTA GABA neurons projecting to the NAc but do not innervate VTA DA neurons projecting to the NAc (Carr & Sesack, 2000). A more likely possibility is that such effects are mediated indirectly, perhaps via PFC projections to mesopontine regions that influence the activity of midbrain DA neurons (see Clark & Overton, 1998; Forster & Blaha, 2000).

Because HRs and LR did not differ in Narp expression within the VTA itself, it is unlikely that local effects of Narp are important in producing differential excitation of VTA DA neurons. However, Narp protein expressed in the VTA may nevertheless play a role in regulating VTA AMPA receptors, even though it does not appear to contribute to individual differences or to drug-induced alterations in AMPA receptor transmission.

Conclusions

We had initially hypothesized that cocaine or amphetamine administration leads to increased expression of Narp protein in mesocorticolimbic brain regions, providing a mechanism for coupling changes in synaptic transmission in addiction-related pathways to adaptations in AMPA receptor function. Our results do not support this hypothesis. Instead, they suggest that pre-existing individual differences in Narp levels in the PFC play a role in producing individual differences in vulnerability to drugs of abuse, perhaps by altering excitatory drive to PFC neurons and hence to their target brain regions.

Acknowledgements

Supported by: DA09621 and DA00453 (MEW); NS39156, MH001152 and the Howard Hughes Medical Institute (PFW). We thank Drs Irving Reti, Jay Baraban, and Susan Sesack for helpful discussions.

Abbreviations

AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; DA, dopamine; IEG, immediate early gene; NAc, nucleus accumbens; PFC, prefrontal cortex; VTA, ventral tegmental area.

References

Berke, J.D., Paletzki, R.F., Aronson, G.J., Hyman, S.E. & Gerfen, C.R. (1998)

- A complex program of striatal gene expression induced by dopaminergic stimulation. *J. Neurosci.*, **18**, 5301–5310.
- Bradberry, C.W., Gruen, R.J., Berridge, C.W. & Roth, R.H. (1991) Individual differences in behavioral measures: correlations with nucleus accumbens dopamine measured by microdialysis. *Pharmacol. Biochem. Behav.*, **39**, 877–882.
- Carr, D.B. & Sesack, S.R. (2000) Projections from the rat prefrontal cortex to the ventral tegmental area: Target specificity in the synaptic associations with mesoaccumbens and mesocortical neurons. *J. Neurosci.*, **20**, 3864–3873.
- Carroll, R.C., Beattie, E.C., von Zastrow, M. & Malenka, R.C. (2001) Role of AMPA receptor endocytosis in synaptic plasticity. *Nature Rev. Neurosci.*, **2**, 315–324.
- Chao, S.Z., Lu, W.X., Lee, H.-K., Haganir, R.L. & Wolf, M.E. (2002) D1 dopamine receptor stimulation increases GluR1 phosphorylation in postnatal nucleus accumbens cultures. *J. Neurochem.*, **81**, 984–992.
- Chao, S.Z., Peterson, D.A. & Wolf, M.E. (2000) Dopamine and glutamate receptors regulate surface expression of GluR1 in postnatal nucleus accumbens cultures. *Soc. Neurosci. Abstr.*, **26**, 789.
- Churchill, L., Swanson, C.J., Urbina, M. & Kalivas, P.W. (1999) Repeated cocaine alters glutamate receptor subunit levels in the nucleus accumbens and ventral tegmental area of rats that develop behavioral sensitization. *J. Neurochem.*, **72**, 2397–2403.
- Clark, D. & Overton, P.G. (1998) Alterations in excitatory amino acid-mediated regulation of midbrain dopaminergic neurons induced by chronic psychostimulant administration and stress: Relevance to behavioural sensitisation and drug addiction. *Addiction Biol.*, **3**, 109–135.
- Forster, G.L. & Blaha, C.D. (2000) Laterodorsal tegmental stimulation elicits dopamine efflux in the rat nucleus accumbens by activation of acetylcholine and glutamate receptors in the ventral tegmental area. *Eur. J. Neurosci.*, **12**, 3596–3604.
- Ghasemzadeh, M.B., Nelson, L.C., Lu, X.-Y. & Kalivas, P.W. (1999) Neuroadaptations in ionotropic and metabotropic glutamate receptor mRNA produced by cocaine treatment. *J. Neurochem.*, **72**, 157–165.
- Giorgetti, M., Hotsenpiller, G., Ward, P., Teppen, T. & Wolf, M.E. (2001) Amphetamine-induced plasticity of AMPA receptors in the ventral tegmental area: Effects on extracellular levels of dopamine and glutamate in freely moving rats. *J. Neurosci.*, **21**, 6362–6369.
- Grimm, J.W. & See, R.E. (1997) Cocaine self-administration in ovariectomized rats is predicted by response to novelty, attenuated by 17- α estradiol, and associated with abnormal vaginal cytology. *Physiol. Behav.*, **61**, 755–761.
- Harlan, R.E. & Garcia, M.M. (1998) Drugs of abuse and immediate-early genes in the forebrain. *Mol. Neurobiol.*, **16**, 221–267.
- Hooks, M.S., Colvin, A.C., Juncos, J.L. & Justice, J.B. Jr (1992a) Individual differences in basal and cocaine-stimulated extracellular dopamine in the nucleus accumbens using quantitative microdialysis. *Brain Res.*, **587**, 306–312.
- Hooks, M.S., Jones, G.H., Neill, D.B. & Justice, J.B. Jr (1992b) Individual differences in amphetamine sensitization: dose-dependent effects. *Pharmacol. Biochem. Behav.*, **41**, 203–210.
- Hooks, M.S., Jones, G.H., Smith, A.D., Neill, D.B. & Justice, J.B. Jr (1991a) Individual differences in locomotor activity and sensitization. *Pharmacol. Biochem. Behav.*, **38**, 467–470.
- Hooks, M.S., Jones, G.H., Smith, A.D., Neill, D.B. & Justice, J.B. Jr (1991b) Response to novelty predicts the locomotor and nucleus accumbens dopamine response to cocaine. *Synapse*, **9**, 121–128.
- Jackson, A. & Crossman, A.R. (1983) Nucleus tegmenti pedunculopontinus: Efferent connections with special reference to the basal ganglia, studied in the rat by anterograde and retrograde transport of horseradish peroxidase. *Neuroscience*, **10**, 725–765.
- Jones, S., Kornblum, J.L. & Kauer, J.A. (2000) Amphetamine blocks long-term synaptic depression in the ventral tegmental area. *J. Neurosci.*, **20**, 5575–5580.
- Kalivas, P.W. & Duffy, P. (1993) Time course of extracellular dopamine and behavioral sensitization to cocaine. II. Dopamine perikarya. *J. Neurosci.*, **13**, 276–284.
- Lu, W., Chen, H., Xue, C.-J. & Wolf, M.E. (1997) Repeated amphetamine administration alters the expression of mRNA for AMPA receptor subunits in rat nucleus accumbens and medial prefrontal cortex. *Synapse*, **26**, 269–280.
- Lu, W., Monteggia, L.M. & Wolf, M.E. (2002) Repeated administration of amphetamine or cocaine does not alter AMPA receptor subunit expression in the rat midbrain. *Neuropsychopharmacology*, **26**, 1–13.
- Lu, W. & Wolf, M.E. (1999) Repeated amphetamine administration alters immunoreactivity for AMPA receptor subunits in rat nucleus accumbens and medial prefrontal cortex. *Synapse*, **32**, 119–131.
- Lüscher, C., Nicoll, R.A., Malenka, R.C. & Muller, D. (2000) Synaptic plasticity and dynamic modulation of the postsynaptic membrane. *Nature Neurosci.*, **3**, 545–550.
- Malinow, R., Mainen, Z.F. & Hayashi, Y. (2000) LTP mechanisms: from silence to four-lane traffic. *Curr. Opin. Neurobiol.*, **10**, 352–357.
- Mansour, A., Meador-Woodruff, J.H., Zhou, Q.-Y., Civelli, O., Akil, H. & Watson, S.J. (1991) A comparison of D₁ receptor binding and mRNA in rat brain using receptor autoradiographic and *in situ* hybridization techniques. *Neuroscience*, **45**, 359–371.
- Marinelli, M. & White, F.J. (2000) Enhanced vulnerability to cocaine self-administration is associated with elevated impulse activity of midbrain dopamine neurons. *J. Neurosci.*, **20**, 8876–8885.
- Meador-Woodruff, J.H., Mansour, A., Healy, D.J., Kuehn, R., Zhou, Q.-Y., Bunzow, J.R., Akil, H., Civelli, O. & Watson, S.J. (1991) Comparison of the distributions of D1 and D2 dopamine receptor mRNAs in rat brain. *Neuropsychopharmacology*, **5**, 231–242.
- Morelli, M., Mennini, T. & Di Chiara, G. (1988) Nigral dopamine autoreceptors are exclusively of the D₂ type: quantitative autoradiography of [¹²⁵I]iodosulpiride and [¹²⁵I]SCH 23982 in adjacent brain sections. *Neuroscience*, **27**, 865–870.
- O'Brien, C.P., Ehrman, R.N. & Terns, J.N. (1986) Classical conditioning in human opioid dependence. In Goldberg, S.R. & Stolerman, I.P. (eds), *Behavioral Analysis of Drug Dependence*. Academic Press, London, pp. 329–365.
- O'Brien, R.J., Xu, D., Petralia, R.S., Stewart, O., Haganir, R.L. & Worley, P. (1999) Synaptic clustering of AMPA receptors by the extracellular immediate early-gene product Narp. *Neuron*, **23**, 309–323.
- Oades, R.D. & Halliday, G.M. (1987) Ventral tegmental (A10) system: neurobiology. 1. Anatomy and connectivity. *Brain Res. Rev.*, **12**, 117–165.
- Paxinos, G. & Watson, C. (1986) The rat in stereotaxic coordinates. Academic Press, New York.
- Peterson, J.D., Wolf, M.E. & White, F.J. (2000) Altered responsiveness of medial prefrontal cortex neurons to glutamate and dopamine after withdrawal from repeated amphetamine treatment. *Synapse*, **36**, 342–344.
- Phillipson, O.T. (1979) Afferent projections to the ventral tegmental area of Tsai and interfascicular nucleus: a horseradish peroxidase study in the rat. *J. Comp. Neurol.*, **187**, 117–144.
- Piazza, P.V., Deminière, J.M., Le Moal, M. & Simon, H. (1989) Factors that predict individual vulnerability to amphetamine self-administration. *Science*, **245**, 1511–1513.
- Piazza, P.V., Deroche, V., Rougé-Pont, F. & Le Moal, M. (1998) Behavioral and biological factors associated with individual vulnerability to psychostimulant abuse. *NIDA Res. Monogr.*, **169**, 105–133.
- Piazza, P.V., Deroche-Gamonet, V., Rougé-Pont, F. & Le Moal, M. (2000) Vertical shifts in self-administration dose–response functions predict a drug-vulnerable phenotype predisposed to addiction. *J. Neurosci.*, **20**, 4226–4232.
- Piazza, P.V., Rougé-Pont, F., Deminière, J.M., Kharouby, M., Le Moal, M. & Simon, H. (1991) Dopaminergic activity is reduced in the prefrontal cortex and increased in the nucleus accumbens of rats predisposed to develop amphetamine self-administration. *Brain Res.*, **567**, 169–174.
- Pierre, P.J. & Vezina, P. (1997) Predisposition to self-administer amphetamine: the contribution of response to novelty and prior exposure to the drug. *Psychopharmacology*, **129**, 277–284.
- Price, C.J., Kim, P. & Raymond, L.A. (1999) D1 dopamine receptor-induced cyclic AMP-dependent protein kinase phosphorylation and potentiation of striatal glutamate receptors. *J. Neurochem.*, **73**, 2441–2446.
- Reti, I.M. & Baraban, J.M. (2000) Sustained increase in Narp protein expression following repeated electroconvulsive seizure. *Neuropsychopharmacology*, **23**, 439–443.
- Rougé-Pont, F., Deroche, V., Le Moal, M. & Piazza, P.V. (1998) Individual differences in stress-induced dopamine release in the nucleus accumbens are influenced by corticosterone. *Eur. J. Neurosci.*, **10**, 3903–3907.
- Rougé-Pont, F., Piazza, P.V., Kharouby, M., Le Moal, M. & Simon, H. (1993) Higher and longer stress-induced increase in dopamine concentrations in the nucleus accumbens of animals predisposed to amphetamine self-administration. A microdialysis study. *Brain Res.*, **602**, 169–174.
- Scannevin, R.H. & Haganir, R.L. (2000) Postsynaptic organization and regulation of excitatory synapses. *Nature Rev. Neurosci.*, **1**, 133–141.
- Sesack, S.R. & Pickel, V.M. (1992) Prefrontal cortical efferents in the rat synapse on unlabeled neuronal targets of catecholamine terminals in the nucleus accumbens and on dopamine neurons in the ventral tegmental area. *J. Comp. Neurol.*, **320**, 145–160.

- Sheng, M. & Lee, S.H. (2001) AMPA receptor trafficking and the control of synaptic transmission. *Cell*, **105**, 825–828.
- Snyder, G.L., Allen, P.B., Fienberg, A.A., Valle, C.G., Haganir, R.L., Nairn, A.C. & Greengard, P. (2000) Regulation of phosphorylation of the GluR1 AMPA receptor in the neostriatum by dopamine and psychostimulants *in vivo*. *J. Neurosci.*, **20**, 4480–4488.
- Thomas, M.J., Malenka, R.C. & Bonci, A. (2000) Modulation of long-term depression by dopamine in the mesolimbic system. *J. Neurosci.*, **20**, 5581–5586.
- Tsui, C.C., Copeland, N.G., Gilbert, D.J., Jenkins, N.A., Barnes, C. & Worley, P.F. (1996) Narp, a novel member of the pentraxin family, promotes neurite outgrowth and is dynamically regulated by neuronal activity. *J. Neurosci.*, **16**, 2463–2478.
- Ungless, M.A., Whistler, J.L., Malenka, R.C. & Bonci, A. (2001) Single cocaine exposure *in vivo* induces long-term potentiation in dopamine neurons. *Nature*, **411**, 583–587.
- Vanderschuren, L.J.M.J. & Kalivas, P.W. (2000) Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies. *Psychopharmacology*, **151**, 99–120.
- White, F.J., Hu, X.-T., Zhang, X.-F. & Wolf, M.E. (1995) Repeated administration of cocaine or amphetamine alters neuronal responses to glutamate in the mesoaccumbens dopamine system. *J. Pharmacol. Exp. Ther.*, **273**, 445–454.
- de Wit, H., Uhlhuth, E.H. & Johanson, C.E. (1986) Individual differences in the reinforcing and subjective effects of amphetamine and diazepam. *Drug Alcohol Depend.*, **16**, 341–360.
- Wolf, M.E. (1998) The role of excitatory amino acids in behavioral sensitization to psychomotor stimulants. *Prog. Neurobiol.*, **54**, 679–720.
- Wolf, M.E. (2002) Addiction and glutamate-dependent plasticity. In Herman, B.H., Frankenheim, J., Litten, R., Sheridan, P.H., Weight, F.F. & Zuckin, S.R. (eds), *Glutamate and Addiction*. The Humana Press, Totowa, NJ, USA, in press.
- Wolf, M.E. & Jezierski, M. (1993) Coadministration of MK-801 with amphetamine, cocaine or morphine prevents rather than transiently masks the development of behavioral sensitization. *Brain Res.*, **613**, 291–294.
- Yan, Z., Hsieh-Wilson, L., Feng, J., Tomizawa, K., Allen, P.B., Fienberg, A.A., Nairn, A.C. & Greengard, P. (1999) Protein phosphatase 1 modulation of neostriatal AMPA channels: regulation by DARPP-32 and spinophilin. *Nature Neurosci.*, **2**, 13–17.
- Zhang, X.-F., Hu, X.-T., White, F.J. & Wolf, M.E. (1997) Increased responsiveness of ventral tegmental area dopamine neurons to glutamate after repeated administration of cocaine or amphetamine is transient and selectively involves AMPA receptors. *J. Pharmacol. Exp. Ther.*, **281**, 699–706.