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Short communication

Repeated amphetamine treatment causes a persistent elevation of glial fibrillary acidic protein in the caudate–putamen

Victoria Armstrong[1], Carmela M. Reichel, Jonathan F. Doti, Cynthia A. Crawford, Sanders A. McDougall*

Department of Psychology, California State University, San Bernardino, CA 92407, USA

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Abstract

The ability of repeated D-amphetamine (2 mg/kg) treatment to induce behavioral sensitization in rats and alter glial fibrillary acidic protein (GFAP), dopamine transporter (DAT) and glutamate transporter-1 (GLT-1) immunoreactivities was assessed after a 10-day drug abstinence period. Results showed that a sensitizing regimen of amphetamine caused a persistent increase in the number of GFAP-positive cells in the dorsal and ventral caudate–putamen. DAT and GLT-1 immunoreactivities were unaffected. Although the elevated GFAP expression may be due to a mild neurotoxicity, it is also possible that amphetamine-induced increases in GFAP reflect adaptive changes that may be associated with processes underlying behavioral sensitization.

Keywords: D-Amphetamine; Behavioral sensitization; DAT (dopamine transporter); GFAP (glial fibrillary acidic protein); GLT-1 (glutamate transporter-1)

1. Introduction

Repeated treatment with high doses of amphetamine induces neurotoxicity (Wagner et al., 1980; Ricaurte et al., 1983), while similar treatment regimens using lower doses of amphetamine produce a phenomenon called behavioral sensitization (Kalivas and Stewart, 1991). The sensitized responding of amphetamine-treated rats is typically expressed as a progressive increase in locomotor activity or stereotypy that persists for months after initial psychostimulant exposure (Leith and Kuczenski, 1982; Paulson et al., 1991). It has recently been suggested that psychostimulant-induced neurotoxicity and behavioral sensitization are not completely independent phenomena, and that some of the neural changes underlying behavioral sensitization may involve a mild neurotoxicity (Wolf, 1998; Franke et al., 2003). For example, it is possible that repeated psychostimulant exposure causes a sustained release of glutamate resulting in excitotoxicity and prolonged neuronal hyperexcitability (Wolf, 1998). Consistent with this idea, drugs that attenuate psychostimulant-induced glutamate release also block the induction of behavioral sensitization (Peterson et al., 1997).

The purpose of the present study was to more fully examine the relationship between behavioral sensitization and neurotoxicity. To that end, adult rats were sensitized to a moderate dose of amphetamine (2 mg/kg) and then challenged with an injection of amphetamine (0.5 mg/kg) after a 10-day drug abstinence period. Upon completion of behavioral testing, glial fibrillary acidic protein (GFAP), dopamine transporter (DAT) and glutamate transporter-1 (GLT-1) immunoreactivities were measured in forebrain. GFAP immunoreactivity was examined because this astrocyte-associated protein is a commonly used measure of neuronal damage (Eng et al., 2000) and may be important for some forms of behavioral plasticity (Sirevaag and Greenough, 1991; Shibuki et al., 1996). The purpose of measuring DAT expression was two-fold, as amphetamine-induced reductions in DAT are correlated with damage to dopaminergic terminal fibers (Wagner et al., 1980; Frost and Cadet, 2000) and changes in DAT may be one of the neural adaptations underlying behavioral sensitization (Lu and Wolf, 1997; Shilling et al., 1997). Lastly, GLT-1 immunoreactivity was measured because this transporter, located predominately on glial cells, is the primary mechanism for clearing extracel-

* Corresponding author. Tel.: +1-909-880-5581; fax: +1-909-880-7003.
E-mail address: smcdouga@csusb.edu (S.A. McDougall).
[1] Present address: Department of Psychology, University of Houston, 4800 Calhoun Road, Houston, TX 77204, USA.

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lular glutamate in forebrain (Robinson, 1999) and is responsible for keeping glutamate below neurotoxic levels (Rothstein et al., 1996; Tanaka et al., 1997). Importantly, pharmacological treatments can alter GLT-1 functioning since an antagonist (i.e., clozapine) acting at both dopamine D2-like and 5-HT_2 receptors is capable of down-regulating the expression of GLT-1 in cortex (Melone et al., 2001). Therefore, if glutamate-induced neurotoxicity is a component of behavioral sensitization (see Wolf, 1998), then psychostimulant-induced changes in GLT-1 levels may be a mediating factor.

2. Materials and methods

2.1. Animals

Subjects were 40 adult male Sprague–Dawley rats (Harlan, Indianapolis, IN, USA). Rats weighed between 225 and 249 g on arrival and were housed singly for a minimum of 14 days before behavioral testing. The colony room was maintained at 22 – 24 °C and kept under a 12:12-h light/dark cycle. Subjects were treated according to the National Institutes of Health *Guide for Care and Use of Laboratory Animals* (Publication No. 85-23, revised 1985) under a research protocol approved by the Institutional Animal Care and Use Committee of California State University, San Bernardino.

2.2. Apparatus

Behavioral testing was done in activity monitoring chambers (41 × 41 × 41 cm), consisting of Plexiglas walls, a plastic floor and an open top. Each chamber included an X–Y photobeam array, with 16 photocells and detectors, which was used to determine distance traveled (a measure of horizontal locomotor activity).

2.3. Behavioral sensitization procedure

For 7 consecutive days, rats received intraperitoneal (i.p.) injections (1 ml/kg) of saline or 2 mg/kg D-amphetamine (Sigma, St. Louis, MO, USA). Immediately after being injected, rats were placed in activity chambers where distance traveled was measured for 60 min. A single test day occurred after a 10-day drug abstinence period. On the test day, rats were given a challenge injection of either saline or amphetamine (0.5 mg/kg, i.p.). Distance traveled was then measured for 120 min. After testing, rats were deeply anesthetized with phenobarbital and rapidly perfused with 4% paraformaldehyde.

2.4. Immunohistochemistry procedure

Following a postfixation period, 75 μm coronal sections were taken from each brain and incubated for 48–72 h with primary antibodies in goat serum solution (GFAP, 1:2000; DAT, 1:3750; GLT-1, 1:5000; Chemicon, Temecula, CA, USA). Control sections were placed in goat serum solution void of any primary antibody. After incubation, sections were transferred into either biotinylated rabbit antiserum (GFAP and DAT) or biotinylated goat antiserum (GLT-1; Vector Laboratories, Burlingame, CA, USA). GFAP and transporter proteins were then visualized with an avidin–biotin–horseradish peroxidase conjugate solution (Vector Laboratories) and 3,3′ -diaminobenzidine tetrahydrochloride. A total of 10 assays were conducted, with each treatment group being represented once in every assay. At all stages of the immunohistochemistry procedure, sections from the four treatment groups were processed simultaneously to control for differences in antibody penetration (see also Lu et al., 2002).

Similar coronal sections from each rat were selected for quantitative analysis of GFAP, DAT and GLT-1 immunoreactivities. The number of distinguishable immunoreactive cells (GFAP) and processes (DAT and GLT-1) present within the ventral and dorsal caudate–putamen, nucleus accumbens core and shell, and prefrontal cortex were manually counted by observers blind to treatment condition. For each brain region, two to four sample areas were counted with the size of each sample area being 230 μm^2.

2.5. Statistics

To assess for the occurrence of behavioral sensitization, distance traveled data were analyzed using repeated measures (5-min time blocks) analyses of variance. Rats were not habituated to the apparatus on the test day, so time block 1 was analyzed separately from time blocks 2–24. Immunohistochemistry data were analyzed using randomized block analyses of variance, with the 10 assays being treated as separate blocks. This statistical procedure allows for the variance between assays to be partitioned out of the error term. Post-hoc analysis of behavioral and immunohistochemistry data was made using Tukey tests ($P < 0.05$).

3. Results

3.1. Behavioral sensitization and conditioned activity

Amphetamine pretreatment resulted in a sensitized locomotor response on the test day (Fig. 1). More specifically, amphetamine-pretreated rats given a challenge injection of amphetamine (filled triangles) had greater distance traveled scores on time blocks 2–24 than rats given amphetamine for the first time on the test day (filled circles), $F(1,18) = 17.38$, $P < 0.001$. Conditioned activity was also apparent, as rats pretreated with amphetamine and challenged with saline (open triangles) traveled further than saline controls (open circles) on time blocks 2–24, $F(1,18) = 7.70$, $P < 0.05$. The various groups did not differ on time block 1.

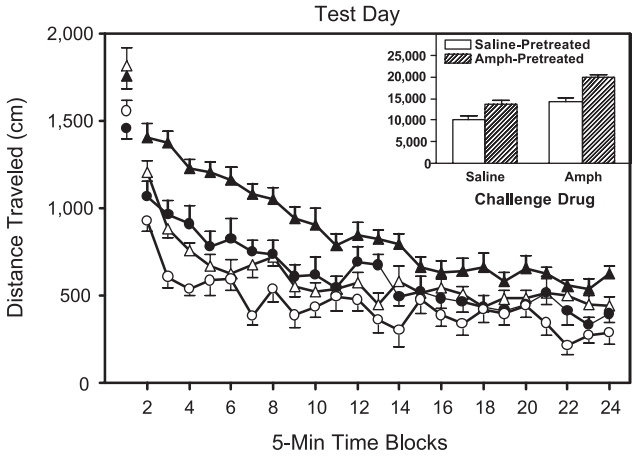

Fig. 1. Mean (± S.E.M.) distance traveled of rats ($n = 10$ per group) receiving a challenge injection of saline or 0.5 mg/kg amphetamine after 10 drug abstinence days. Rats had previously received daily injections of saline or 2 mg/kg amphetamine for 7 consecutive days. The inset shows mean distance traveled collapsed across time blocks 2–24. Group designations (pretreatment–challenge): ○: saline–saline, △: amphetamine–saline, ●: saline–amphetamine, ▲: amphetamine–amphetamine.

3.2. Immunohistochemistry

Pretreating rats with 2 mg/kg amphetamine caused a significant increase in the number of GFAP-positive cells in the ventral caudate–putamen (Table 1), $F(1,9) = 5.21$, $P < 0.05$. Amphetamine pretreatment also increased GFAP expression in the dorsal caudate–putamen, $F(1,9) = 29.49$, $P < 0.001$. GFAP immunoreactivity in the nucleus accumbens core or shell and the prefrontal cortex were unaffected by pretreatment injections of amphetamine. Separate statistical analyses showed that a challenge injection of 0.5 mg/kg amphetamine or saline did not differentially affect GFAP expression (data not shown).

Amphetamine pretreatment did not alter the number of DAT or GLT-1 immunoreactive processes in any of the brain areas examined (Table 1). Thus, repeated amphetamine treatment only affected GFAP immunoreactivity, and not DAT or GLT-1 expression, after a 10-day drug abstinence period.

4. Discussion

Wolf (1998) has hypothesized that psychostimulant-induced behavioral sensitization may involve a mild neurotoxicity. To further examine this issue, we sensitized rats to amphetamine and then measured GFAP, DAT and GLT-1 immunoreactivities. After a 10-day drug abstinence period, amphetamine-pretreated rats exhibited increased GFAP immunoreactivity in the dorsal and ventral caudate–putamen. There was no evidence of increased GFAP expression in other forebrain areas known to mediate behavioral sensitization (i.e., the nucleus accumbens core and shell or the prefrontal cortex). The latter finding was unexpected since it has recently been reported that repeated treatment with both cocaine (Bowers and Kalivas, 2003) and amphetamine (Franke et al., 2003) increases GFAP immunoreactivity in the nucleus accumbens. In any event, the hypothesis that a mild neurotoxicity may underlie behavioral sensitization is supported by the finding that GFAP immunoreactivity is elevated after a sensitizing regimen of amphetamine. However, since the changes in GFAP occurred in the absence of any DAT or GLT-1 effects, it is possible that amphetamine-induced increases in GFAP may reflect adaptive changes that do not involve neurotoxicity. More specifically, it has been established that astrocytes are not only important for neuronal communication, but they also play a role in synaptic plasticity (Vernadakis, 1996; Haydon, 2001). Astrocyte–neuron communication takes many forms, but in terms of behavioral sensitization it is perhaps most interesting that astrocytes regulate neuronal Ca^{2+} levels and facilitate glutamate transmission (for a review, see Vernadakis, 1996). GFAP expression appears to be a component of astrocyte–neuron interactions, since GFAP supports astrocyte arborization (Weinstein et al., 1991) and changes in astrocyte surface area are associated with synaptic plasticity (Sirevaag and Greenough, 1991). Notably, GFAP-knockout mice perform poorly on an eye blink conditioning task and do not show normal synaptic plasticity (i.e., long-term depression) (Shibuki et al., 1996). Thus, the finding that repeated amphetamine treatment caused enduring changes in GFAP immunoreactivity in the caudate–putamen suggests that this brain structure is capable of supporting

Table 1
Mean (± S.E.M.) number of GFAP immunoreactive cells and DAT and GLT-1 immunoreactive processes found in various brain regions of rats ($n = 10$ per group) pretreated with saline or amphetamine (2 mg/kg) for 7 consecutive days and challenged with saline on the test day (amphetamine-challenged rats are not included)

Brain region	GFAP		DAT		GLT-1	
	Saline	Amph	Saline	Amph	Saline	Amph
Dorsal caudate–putamen	74.8 ± 4	100.3 ± 9[a]	78.4 ± 2	85.4 ± 9	156.8 ± 5	155.5 ± 9
Ventral caudate–putamen	66.8 ± 3	96.6 ± 5[a]	89.7 ± 3	78.0 ± 4	148.1 ± 5	146.0 ± 6
Nucleus accumbens core	43.6 ± 2	44.6 ± 2	55.0 ± 2	55.8 ± 4	49.8 ± 3	50.0 ± 3
Nucleus accumbens shell	44.0 ± 4	41.6 ± 4	55.8 ± 3	52.4 ± 3	51.8 ± 2	53.5 ± 3
Prefrontal cortex	52.6 ± 5	49.2 ± 8	74.2 ± 7	74.0 ± 7	51.8 ± 4	60.8 ± 4

[a] Significantly different from saline-pretreated rats ($P < 0.05$).

amphetamine-induced plasticity long after the acute effects of the drug have dissipated.

High doses of amphetamine and methamphetamine are toxic to both dopamine neurons and postsynaptic elements (Wagner et al., 1980; Cadet et al., 1998; Frost and Cadet, 2000). Even so, there was no evidence that a drug regimen sufficient to induce behavioral sensitization (seven daily injections of 2 mg/kg amphetamine) damaged dopaminergic terminal fibers (i.e., caused a reduction in DAT content). This result is not surprising since previous studies have shown that repeated treatment with sensitizing doses of amphetamine (3–5 mg/kg) did not affect DAT numbers or function in the caudate–putamen (Kula and Baldessarini, 1991; Mintz et al., 1994), while repeated treatment with cocaine caused a transient increase in striatal DAT binding that disappeared after a 7-day drug abstinence period (Koff et al., 1994). Thus, the finding that repeated amphetamine treatment produced behavioral sensitization without causing a concomitant decline in DAT is consistent with an interpretation that amphetamine-induced neurotoxicity and behavioral sensitization are independent phenomena (see also Wallace et al., 2001). It remains possible, however, that repeated amphetamine treatment may have caused a neurotoxic response in postsynaptic fibers (Cadet et al., 1998; Frost and Cadet, 2000) or produced subtle damage to dopamine terminals that was undetectable by measuring DAT immunoreactivity (see Wolf, 1998).

There is increasing evidence that a sustained increase in glutamate efflux is a necessary neural adaptation underlying behavioral sensitization (Kalivas and Duffy, 1998; Wolf, 1998). Because the GLT-1 transporter is the primary mechanism for removing extracellular glutamate and, consequently, preventing excitotoxicity (Rothstein et al., 1996; Robinson, 1999), we assessed whether repeated amphetamine treatment would impact this transporter. Regardless of brain area examined, there was no indication that chronic amphetamine treatment affected GLT-1 expression (see also Sidiropoulou et al., 2001). Thus, there is no evidence that alterations in the number of GLT-1 transporters is either a cause or a consequence of the increased glutamate levels characteristic of psychostimulant-induced behavioral sensitization.

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