Amphetamine increases tyrosine kinase-B receptor expression in the dorsal striatum

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Sponsorship: This work was supported in part by a USPHS grant from the National Institute on Drug Abuse, DA 016662 to G.E.M.

Received 1 October 2005; revised 7 November 2005; accepted 8 November 2005

Neurotrophic signaling is thought to be important for neuroplasticity in certain forebrain regions following psychostimulant exposure. In this study, we found that repeated administration of amphetamine (5 mg/kg, once daily, 5 days) to rats significantly increased tyrosine kinase-B receptor mRNA levels in the striatum, ventral bed nucleus, and piriform cortex. The most robust increase in tyrosine kinase-B expression occurred in dorsal aspects of the striatum, which also showed elevated levels after a single amphetamine injection. These findings indicate that changes in striatal tyrosine kinase-B signaling could play a role in neuroadaptations and behavioral changes induced by amphetamine treatment.

**NeuroReport** 17:75–78 © 2006 Lippincott Williams & Wilkins.

**Keywords**: amphetamine, bed nucleus of the stria terminalis, brain-derived neurotrophic factor, nucleus accumbens, piriform cortex, striatum, tyrosine kinase-B

Introduction

A growing body of evidence indicates that the brain-derived neurotrophic factor (BDNF) is important for activity-dependent synaptic plasticity and that such effects are mediated by the tyrosine kinase-B (trkB) receptor [1,2]. trkB is expressed widely in the brain and is present in moderate to high levels in forebrain regions [3], many of which are important for the expression of psychostimulant-induced behavioral changes [4]. The trkB receptor containing the catalytic domain forms a homodimer in the presence of its ligand, BDNF or neurotrophic factor (NT)-4/5, and becomes phosphorylated [5]; the phosphorylated form in turn regulates downstream signaling cascades that are known to participate in psycho-stimulant-induced neuroadaptations [6,7].

Previous studies reported that BDNF has behavioral effects. For example, infused into the ventral midbrain or nucleus accumbens, BDNF enhanced various spontaneous behaviors [8,9], and facilitated the development of behavioral sensitization by repeated psychostimulant treatment [10]. It is thus of interest to determine whether neuroplasticity associated with repeated exposure to psychostimulants involves changes in the endogenous expression of BDNF and/or its receptor [11,12]. We have previously reported that BDNF mRNA levels are significantly elevated in the basolateral nucleus of the amygdala following repeated amphetamine administration [13]. Neurons in the basolateral nucleus of the amygdala project densely to many forebrain areas activated by psychostimulants, including the nucleus accumbens, dorsal striatum, ventral pallidum, bed nucleus of the stria terminalis, and the central nucleus of the amygdala, and neurons in these areas express trkB [3]. We therefore investigated whether these regions showed altered trkB expression after repeated amphetamine treatment.

Materials and methods

**Animals**

Twenty-four male Sprague–Dawley rats (260–350 g; Charles River, Wilmington, Massachusetts, USA) were housed two to a cage and provided with food and water ad libitum. All animal protocols were conducted in accordance with the National Institutes of Health (NIH) Guidelines (NIH Publications No. 80–23) and approved by the Institutional Animal Care and Use Committee.

**Drugs and behavior**

All injections were administered between 10:00 and 12:00 h in the home cage. Two groups of rats were given daily injections of d-amphetamine sulfate (AMPH; Sigma, St Louis, USA, 5 mg/kg, intraperitoneal) or vehicle (0.9% sterile, isotonic saline) for 5 days. In a separate set of experiments, two groups of rats received a single, acute amphetamine injection (5 mg/kg) or vehicle. The motor behavior of each rat was assessed using a rating paradigm, and the results have been reported before [13]. In short, rats treated repeatedly with AMPH displayed more pronounced behavioral activation than those in the acute group, and both groups scored significantly higher than controls [13]. In addition, animals treated with the repeated paradigm showed a shorter onset latency for stereotypical movements.
In-situ hybridization
At 2 h after the final injection, rats were anesthetized with sodium pentobarbital (75 mg/kg) and immediately killed by decapitation. Their brains were removed, frozen in isopentane cooled on dry ice, and the forebrains cut on a cryostat. Coronal sections (12 μm) were thaw-mounted onto poly-L-lysine-coated slides, fixed (4% paraformaldehyde/0.9% saline) for 10 min, acetylated, and stored at −70 °C before being hybridized 2 weeks later. An oligonucleotide probe (34-mer, complementary to bases 2214–2247, GenBank accession number M55291; Life Technologies, Rockville, Maryland, USA) was used to detect the full-length trkB transcript. The trkB probe was radiolabeled at the 3′ end using [α-35S]deoxyadenosine triphosphate (1250 Ci/mmol, Perkin Elmer, Boston, Massachusetts, USA) and terminal deoxynucleotidyl transferase to a specific activity of 8–10 × 106 cpm/μl [13]. Slides were incubated overnight at 37 °C in a humidified chamber and, after washing, apposed to Kodak BioMax film (Fisher Scientific International Inc., Houston, Texas, USA) for 10 days at 4 °C.

Gene expression in the forebrain was assessed in sections from three different levels from bregma (rostral, at +1.6 mm; middle, at −0.3; and caudal, at −1.32): the striatum (rostral and caudal levels), nucleus accumbens shell and core (rostral), piriform cortex (rostral), medial, lateral and ventral bed nucleus of the stria terminalis (middle), dorsomedial ventral pallidum (middle), basolateral nucleus of the amygdala and central nucleus of the amygdala (caudal). All measurements were carried out with NIH image (Scion Corp, Rockville, Maryland, USA). The films were captured using a light table (Northern Light: Imaging Research, Ontario, Canada) and a video camera attached to a photographic stand. For each animal and region of interest, optical density measurements from two to three sections were obtained and averaged. A white matter reading was subtracted from gray matter values as a background correction. Acute and repeated treatments were performed as two independent studies. Therefore, the density measurements from these investigations could not be compared statistically. For each study, treatment effects were assessed by comparing the two groups with a Student’s t-test, and values are expressed as percentage of controls (Fig. 1, Table 1).

Results
The regional patterns of trkB expression observed in our study matched those described by others [3,14]. Repeated injections of AMPH significantly increased trkB mRNA levels in the striatum, ventral bed nucleus of the stria terminalis, and piriform cortex (Fig. 1, Table 1). In the striatum, this increase was most pronounced in dorsal aspects, especially at more caudal levels (Fig. 1a). At rostral but not caudal striatal levels, increased trkB expression appeared heterogeneous (patchy). In contrast, no statistically significant changes in trkB mRNA levels were found in the core or shell of the nucleus accumbens (Fig. 1), or in any of the other regions examined (Table 1).

Table 1  Effects of acute and repeated amphetamine treatment on tyrosine kinase-B expression in various forebrain regions

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Increase (percentage of saline control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventral pallidum</td>
<td>Acute amphetamine: 96.0 ± 4.2% (100 ± 10.6)</td>
</tr>
<tr>
<td>Piriform cortex</td>
<td>96.8 ± 2.6% (100 ± 8.4)</td>
</tr>
<tr>
<td>Basolateral amygdala</td>
<td>113.1 ± 4.1% (100 ± 5.4)</td>
</tr>
<tr>
<td>Central amygdala</td>
<td>104.6 ± 11.8% (100 ± 4.0)</td>
</tr>
<tr>
<td>Medial bed nucleus</td>
<td>93.2 ± 9.0% (100 ± 3.5)</td>
</tr>
<tr>
<td>Lateral bed nucleus</td>
<td>93.1 ± 5.0% (100 ± 2.7)</td>
</tr>
<tr>
<td>Ventral bed nucleus</td>
<td>111.0 ± 14.1% (100 ± 5.0)</td>
</tr>
</tbody>
</table>

*p < 0.05 vs. saline control. Animals were killed 2 h after the final drug injection. Hybridization signals (mean density values, mean ± SEM) in amphetamine-treated animals are expressed in percentage of saline controls (set at 100%, in brackets).
Given the importance of establishing whether repeated AMPH treatment was required for increased trkB expression, we also examined rats after an acute AMPH injection. Only the striatum showed a minor, but statistically significant, increase in trkB expression after a single injection of AMPH (Fig. 1b, Table 1).

**Discussion**

Our results demonstrate that a region-specific upregulation of trkB expression occurs during repeated amphetamine treatment. This effect was present in several forebrain areas, but was most robust in dorsal aspects of the striatum. Indeed, a single amphetamine injection was sufficient to elevate trkB mRNA levels in this area. These findings suggest that BDNF and/or NT-4/5 signaling via the trkB receptor is increased after amphetamine treatment, an effect that may contribute to psychostimulant-induced neuroplasticity.

The increase in striatal trkB expression after amphetamine treatment displayed distinct regional variations, which may be related to the neurochemical effects of this psychostimulant. Amphetamine enhances extracellular dopamine levels and multunit activity to a greater extent in dorsal and caudal than ventral parts of the striatum [15,16]. Consistently, while present across dorsal and ventral parts of the striatum (caudate/putamen), the increase in trkB mRNA levels was most robust in dorsal aspects, especially at more caudal levels (sensorimotor striatum). This overall topography is similar to that of changes in gene regulation induced by psychostimulants such as cocaine and methylphenidate [17–19].

Repeated amphetamine treatment did not produce a statistically significant increase in trkB expression in the nucleus accumbens, also similar to the relatively modest gene regulation effects of other psychostimulants in this region [17–19]. While there were tendencies in the nucleus accumbens shell and core, it is possible that longer treatment and/or withdrawal durations are required for psychostimulant-induced changes in BDNF/trkB levels in (parts of) the mesolimbic system [11,12] than that in the dorsal striatum. Consistent with this notion, our present findings also showed increased trkB expression in the ventral bed nucleus of the stria terminalis (and piriform cortex) after repeated, but not acute, amphetamine treatment.

Psychostimulant-induced changes in gene regulation in the striatum are principally driven by dopamine and glutamate inputs [20]. Evidence indicates that some of the involved second messenger pathways are also under the influence of neurotrophins (see Introduction). Our finding of elevated trkB expression in the striatum thus indicates that enhanced trkB signaling may contribute to molecular changes after amphetamine treatment. While the trkB ligand BDNF is only marginally expressed in the striatum (and NT-4/5 levels are very low in the central nervous system), high BDNF levels are present in the cortex (especially in prefrontal regions) as well as in midbrain dopamine neurons [21,22]. Indeed, BDNF released from corticostriatal and/or mesostriatal afferents has been implicated in the regulation of some striatal genes (see [23]). These inputs may thus provide the necessary BDNF tone. In the rostral striatum, however, increased trkB expression appeared patchy, reminiscent of the pattern of amphetamine-induced immediate-early gene expression, which occurs preferentially in striosomes [24]. Findings in the cat indicate that amygdalo-striatal neurons from the basolateral nucleus of the amygdala project selectively to the striosome compartment of the rostral striatum (caudate nucleus) [25]. It is in the basolateral nucleus of the amygdala that our previous study showed increased BDNF expression after repeated amphetamine treatment [13]. Therefore, increased amygdalar BDNF may play a role in psychostimulant-induced neuroplasticity in the dorsal striatum.

**Conclusions**

Increased expression of trkB after repeated amphetamine treatment indicates that this receptor and its ligand, BDNF, participate in psychostimulant-induced neuroplasticity in the brain. The finding that this receptor is preferentially upregulated in dorsal aspects of the striatum suggests a special role for trkB signaling in neuroadaptations in associative and sensorimotor basal ganglia–cortical circuits.

**Acknowledgement**

We gratefully acknowledge the technical assistance of Shannon Callen and Heather Milligan.

**References**


