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The Role of Basic Fibroblast Growth Factor in Amphetamine-Induced Morphological Alterations of Dopamine Neurons in the Ventral Tegmental Area

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Abstract

The Role of Basic Fibroblast Growth Factor in Amphetamine-Induced Morphological Alterations of Dopamine Neurons in the Ventral Tegmental Area

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Concordia University, 2005

Repeated exposure to psychostimulant drugs leads to changes in the synaptic connectivity and dendritic arborization of neurons in the terminal regions of the mesolimbic dopamine system. The morphology of the dopaminergic neurons themselves, however, has not been characterized following psychostimulant exposure. Thus, the present study was designed to investigate whether repeated amphetamine administration would affect the morphology of dopaminergic neurons in the ventral tegmental area (VTA). In Chapter 1, a novel adaptation of the *in vitro* whole-cell patch-clamp method is introduced, allowing for the identification and morphological analysis of dopamine neurons. In Chapter 2, male Wistar rats were treated with amphetamine (2 mg/kg, s.c.) or saline on postnatal days 10, 12, and 14. Whole cell electrophysiological recordings from dopamine neurons were obtained from acute VTA slices taken from 21 to 42 day-old saline- or amphetamine-treated rats. Subsequent staining and visualization of these neurons revealed that amphetamine treatment induces a large increase in the total dendritic length of dopaminergic neurons in the VTA as compared to saline treatment. In Chapter 3, following amphetamine or saline treatment, the VTA of postnatal day 21 or 30 rats was examined for basic fibroblast growth factor (bFGF, or FGF-2) immunoreactivity.

Amphetamine treatment induced a transient increase in astrocytic bFGF expression in the VTA of postnatal day 21 rats as compared to saline treatment. In Chapter 4, rats received either amphetamine or saline co-administered with a bFGF antibody or control IgG on postnatal days 10, 12, and 14. Amphetamine induced significant dendritic growth in VTA dopamine neurons when co-administered with a control IgG, but neutralization of bFGF prevented amphetamine-induced dendritic growth of VTA dopamine neurons. Early postnatal administration of human recombinant bFGF, however, did not induce changes in dendritic arborization. It can be concluded that early postnatal amphetamine exposure induces marked morphological alterations in VTA dopamine neurons in young rats, an effect that is dependent on the actions of endogenous bFGF. The amphetamine-induced elaboration of the dendritic arbor of dopaminergic neurons is proposed to account for, in part, increased excitability within the mesolimbic dopamine system observed after exposure to psychostimulant drugs.

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Experimentation with drugs of addictive potential is the norm rather than the exception. Approximately 60% of Americans have sampled an illicit drug at least once in their lifetime (Johnston, O'Malley & Bachman, 2001). Of those that have experimented with a powerful psychostimulant like cocaine, however, only a fraction (15–16%) of people become addicted within 10 years of first use (Wagner & Anthony, 2002). As such, drug use does not necessarily result in addiction, but a number of people become addicts. Addiction can be defined specifically as a compulsive pattern of drug-seeking and drug-taking behavior that occurs at the expense of most other activities. The study of addiction, therefore, has focused on why some susceptible individuals undergo a transition from casual drug use to compulsive patterns of drug use, and why addicts find it so difficult to stop using drugs.

Research on addiction has aimed to identify and characterize the neural systems that mediate the rewarding effects of potentially addictive drugs and how these systems are altered by drug use. A generally accepted idea is that addictive drugs usurp the neural circuitry that is normally involved in pleasure, incentive motivation, and learning (Wise, 1989; Berridge & Robinson, 1998; Di Chiara, 1999; Hyman & Malenka, 2001). These neural circuits include the dopamine projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAcc), as well as glutamate inputs from the prefrontal cortex, amygdala and hippocampus. This circuitry did not simply evolve to mediate the effects of drugs, but rather to give important stimuli, such as food, water, and sexual partners,

incentive properties. Thus, these neural circuits are essential for stimulating arousal and motivation to seek stimuli associated with survival. Drugs with addictive potential, however, act on these circuits such that their function is altered.

Sensitization to the Effects of Psychostimulant Drugs

The mesolimbic dopamine system, which arises in the VTA, is an important neural substrate for the acute reinforcing properties of pyschostimulant drugs, including amphetamine and cocaine. A major pharmacological effect of the psychostimulants cocaine and amphetamine is to increase extracellular levels of dopamine by reducing dopamine re-uptake through the dopamine transporter (Reith, Meisler, Sershen, & Lajtha, 1986). In addition, amphetamine enhances the transport of dopamine, norepinephrine, and serotonin from nerve terminals into the synapse through a reversal of their respective plasmalemmal transporters (Seiden, Sabol, & Ricaurte, 1993; Sulzer, Chen, Lau, Kristensen, Rayport, & Ewing, 1995).

Initiation of Sensitization

The repeated administration of psychostimulant drugs is known to induce both behavioral and neurochemical sensitization, wherein the response to amphetamine is augmented long after the cessation of drug injections. The enduring augmentation of the behavioral activating effects of psychostimulant drugs develops gradually (Kolta, Shreve, De Souza, & Uretsky, 1985; Paulson, Camp, & Robinson, 1991; Paulson & Robinson, 1995) and persists after long periods of abstinence from psychostimulant exposure (Paulson et al., 1991; Castner & Goldman-Rakic, 1999). For example, in the rat,

behavioral sensitization has been reported one year after withdrawal from repeated high doses of amphetamine (Paulson et al., 1991) and, in the monkey, at least 28 months after repeated escalating low doses of amphetamine (Castner & Goldman-Rakic, 1999). In addition, repeated systemic or intra-VTA exposure to amphetamine results in the long-term enhancement in the ability of amphetamine to increase extracellular levels of dopamine in the NAcc (Kalivas & Duffy, 1993; Paulson & Robinson, 1995; Pettit, Pan, Parsons, & Justice, 1990; Vezina, 1993). Amphetamine-induced augmentation of dopamine release in the NAcc is accompanied by an up-regulation of the cAMP pathway (Cunningham & Kelley, 1993; Miserendino & Nestler, 1995; Nestler, Terwilliger, Walker, Sevarino, & Duman, 1990). The evidence indicates that the responsiveness of the mesolimbic dopamine system is increased to subsequent psychostimulant exposure.

The development of sensitization is initiated by actions in the cell body region of midbrain dopamine neurons (Cador, Bjijou, & Stinus, 1995; Kalivas & Weber, 1988; Vezina & Stewart, 1990; Vezina, 1993; Vezina, 1996). When microinjected into the VTA, a number of compounds can initiate behavioral sensitization to a subsequent psychostimulant challenge, including amphetamine (Vezina & Stewart, 1990; Kalivas & Weber, 1988; Hooks, Jones, Liem & Justice, 1992; Vezina, 1993), μ-opioid receptor agonists (Kalivas & Duffy, 1990; Kalivas, 1985; Joyce & Iversen, 1979; Vezina, Kalivas, & Stewart, 1987; Vezina & Stewart, 1984), neurotensin (Kalivas & Duffy, 1990; Kalivas & Taylor, 1985; Elliot & Nemeroff, 1986), a calcium-dependent potassium channel antagonist (apamin; Steketee & Kalivas, 1990), and pertussis toxin (Steketee & Kalivas, 1991). This diverse array of molecules acts at different binding sites and on different neuronal elements within the VTA. A common characteristic among these compounds,

however, is that they increase somatodendritic dopamine release (Steketee & Kalivas, 1991; Kalivas, 1993).

Somatodendritic release of dopamine is induced by psychostimulant drugs (Kalivas, Bourdelais, Abhold & Abbot, 1989; Kalivas & Duffy, 1991) and remains increased during the first few days of withdrawal (Kalivas & Duffy, 1993). Increased release of dopamine is sufficient to induce sensitization as pharmacological disinhibition of VTA dopamine neurons by pertussis toxin results in an augmented locomotor response to a subsequent exposure to psychostimulant drugs (Steketee & Kalivas, 1991). Importantly, dopamine acts on D_1 dopamine receptors in the VTA to initiate sensitization; blockade of D_1 dopamine receptors in this region prevents the development of sensitization to both the locomotor- and dopamine-activating effects of amphetamine (Bjijou, Stinus, Le Moal & Cador, 1996; Stewart & Vezina, 1989; Vezina, 1996). Indeed, repeated stimulation of D_1 dopamine receptors by D_1 agonists is sufficient to induce behavioral and neurochemical sensitization to a subsequent psychostimulant exposure (Pierce, Born, Adams & Kalivas, 1996; Vezina, 1996). The development of sensitization, however, is not dependent on the actions of D_2 dopamine receptors in the VTA. Co-administration of intra-VTA D_2 dopamine receptor antagonists with systemic amphetamine has been found to have no effect at high doses (Bjijou et al., 1996) and to enhance dopamine cell firing at low doses by blocking inhibitory D_2 autoreceptors (Tanabe, Suto, Creekmore, Steinmiller & Vezina, 2004). Blockade of D_2 autoreceptors consequently leads to an increase in extracellular dopamine content in the VTA, resulting in sensitization to the locomotor activating effects of amphetamine in a D_1 dopamine receptor-dependent manner (Tanabe et al., 2004). Overall, these findings indicate that

increased somatodendritic release of dopamine, and subsequent activation of D_1 dopamine receptors, is required for the initiation of long-term sensitization.

The fact that dopamine neurons do not express D_1 dopamine receptors (Mansour, Meador-Woodruff, Zhou, Civelli, Akil & Watson, 1992) indicates that the increased somatodendritic release of dopamine in the VTA during the development of sensitization might modulate glutamate release by stimulating pre-synaptic D_1 dopamine receptors. Glutamate release in the VTA is known to be modulated by D_1 dopamine receptors (Cameron & Williams, 1993; Kalivas, 1995; Kalivas & Duffy, 1995; Wolf & Xue, 1998). Glutamate has been proposed to play an essential role in the development of sensitization; co-administration of N-methyl-D-aspartate (NMDA) or α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptor antagonists, given systemically or directly into the VTA, can prevent both the development of psychostimulant-induced behavioral sensitization (Karler, Calder, & Turkanis, 1991a; Karler, Calder, Thai, & Bedingfield, 1994; Stewart & Druhan, 1993; Cador, Bjijou, Cailhol, & Stinus, 1999; Li, Vartanian, White, Xue, & Wolf, 1997) and the enhanced extracellular dopamine release in striatal regions in response to psychostimulant drugs (Jake-Matthews, Jolly, Queen, Brose, & Vezina, 1997). Furthermore, NMDA antagonists prevent alterations seen in the VTA early after drug treatment (Masserano, Baker, Natsukari, & Wyatt, 1996; Wolf, White, & Hu, 1994). The activation of ionotropic glutamate receptors promotes calcium influx into dopamine neurons through AMPA and NMDA receptors, as well as L-type calcium channels (Licata & Pierce, 2003). Administration of L-type calcium channel antagonists in the VTA block psychostimulant-induced sensitization (Karler, Turkanis, Partlow & Calder, 1991b), and

repeated stimulation of L-type calcium channels in the VTA produces locomotor sensitization to psychostimulant drugs (Licata, Freeman, Pierce-Bancroft & Pierce, 2000). Thus, increased calcium influx in VTA dopamine neurons, via glutamate receptors and calcium channels, is necessary for the induction of sensitization.

Expression of Sensitization

Repeated administration of amphetamine into the VTA is sufficient to initiate the development of sensitization, in spite of the fact that acute intra-VTA amphetamine treatment does not, in and of itself, increase locomotor activity (Kalivas & Weber, 1988; Vezina & Stewart, 1990). In contrast, although infusions of amphetamine into the NAcc stimulate locomotor activity and elevate NAcc dopamine concentrations acutely (Pothos, Creese & Hoebel, 1995; Dalia, Uretsky & Wallace, 1998), repeating these infusions does not result in the development of behavioral sensitization (Hooks et al., 1992; Vezina & Stewart, 1990). As such, the VTA has been proposed to be the site of initiation of sensitization, whereas the NAcc has a role in the expression of behavioral sensitization (Cador, Bjijou & Stinus, 1995; Kalivas & Weber, 1988; Perugini & Vezina, 1994; Vezina & Stewart, 1990).

In addition to inducing behavioral sensitization, repeated exposure to psychostimulant drugs enhances the ability of the drug to increase extracellular levels of dopamine in the NAcc (Kalivas & Duffy, 1993; Paulson & Robinson, 1995; Pettit et al., 1990; Vezina, 1993). As with locomotor sensitization, this enhanced dopamine response is produced by repeated administration of amphetamine in the VTA (Vezina, 1993; Vezina, 1996), and is blocked by concurrent treatment with intra-VTA D_1 dopamine

receptor antagonists (Bjijou et al., 1996; Stewart & Vezina, 1989; Vezina, 1996). Following repeated systemic or intra-VTA exposure to psychostimulant drugs, D_1 dopamine receptor super-sensitivity is observed in the NAcc (Hu, Koeltzow, Cooper, Robertson, White & Vezina, 2002; Wolf, White & Hu, 1994). This effect is transient, peaking in the days following the last drug exposure and diminishing with time. Following longer withdrawal periods, however, long-lasting changes in the function of the NAcc have been observed. These changes include increased glutamate overflow (Pierce, Bell, Duffy & Kalivas, 1996; Reid & Berger, 1996), functional up-regulation of AMPA receptors (Bell & Kalivas, 1996; Pierce et al., 1996), decreased synaptic strength at cortico-accumbens glutamate synapses (Thomas, Beurrier, Bonci & Malenka, 2001), and alterations in AMPA and NMDA receptor subunit expression (Lu, Monteggia & Wolf, 1999; Lu & Wolf, 1999). These alterations in the function of the NAcc have been argued to underlie the expression of behavioral and neurochemical sensitization observed following repeated psychostimulant drug exposure (Vezina, 2004).

Psychostimulant-Induced Alterations in the VTA

The development of behavioral and neurochemical sensitization involves a number of functional and structural changes in the mesolimbic dopamine system. In particular, repeated psychostimulant drug exposure produces both transient changes, lasting up to three days, and long-term changes in dopamine neurons of the VTA.

Short-Term Changes

A number of functional short-term changes in the VTA have been observed one hour to three days following the last drug injection. For instance, electrophysiological sub-sensitivity of somatodendritic D_2 dopamine receptors in the VTA has been demonstrated, resulting in an increase in the number of spontaneously active dopamine neurons and an increase in their basal firing rate (Ackerman & White, 1990; White & Wang, 1984). In addition, enhanced AMPA receptor transmission (Giorgetti, Hotsenpiller, Ward, Teppen & Wolf, 2001; Zhang, Hu, White & Wolf, 1997) and an increase in the GLUR1 subunit of the AMPA receptor (Carlezon & Nestler, 2002; but see Lu, Monteggia, & Wolf, 2002) have been observed. These transient changes lead to greater somatodendritic dopamine release and increased calcium influx into dopamine neurons, which could contribute to the development of sensitization to psychostimulant drugs (see White, 1996).

Accompanying the changes in VTA dopamine neuron function, a number of biochemical and molecular changes have been observed in association with chronic exposure to morphine, or repeated exposure to cocaine, nicotine, or alcohol (Ortiz, Fitzgerald, Charlton, Lane, Trevisan, Guitart, Shoemaker, Duman & Nestler, 1995; Nestler, 1992; Bunnemann, Terron, Zantedeschi, Merlo Pich & Chiamulera, 2000). Increased levels of tyrosine hydroxylase and glial fibrillary acidic protein (Beitner-Johnson & Nestler, 1991; Beitner-Johnson, Guitart, & Nestler, 1993), and decreased levels of neurofilament proteins NF200, NF160, and NF68 (Beitner-Johnson, Guitart, & Nestler, 1992; Nestler, Guitart, Ortiz & Trevisan, 1994) have been reported in the VTA 24 hours after the cessation of chronic morphine treatment (via daily subcutaneous

osmotic pump implantation) or repeated exposure to either alcohol or cocaine. These changes were associated with an impairment of axonal transport from the VTA to the NAcc (Beitner-Johnson & Nestler, 1993; Self & Nestler, 1995). In addition, 24 hours following chronic morphine exposure, dopamine neuronal cell body size was decreased (Sklair-Tavron et al., 1996). Whether the decrease in cell body size is persistent remains to be determined. Accordingly, these findings suggest that the administration of drugs of abuse generate short-term changes in dopamine neuron structure and in the expression of proteins involved in the maintenance of the neuronal cytoskeleton.

Long-Term Changes

Long term changes in the terminal regions of VTA dopamine neurons have been reported following repeated, intermittent exposure to amphetamine. There is morphological evidence that psychostimulant drugs can induce neurite extension, remodel synapses and increase the number of dendritic spines in rat medial prefrontal cortex and NAcc (Robinson & Kolb, 1997; Robinson & Kolb, 1999). In addition, repeated amphetamine exposure has been shown to enhance neural sprouting and synaptogenesis in the neocortex after neocortical infarction in rats (Stroemer, Kent, & Hulsebosch, 1998). Interestingly, in cultured rat pheochromocytoma cells (PC12 cells), repeated bath application of amphetamine has been observed to induce neurite outgrowth, as measured by neurite length (Park, Kantor, Wang & Gnegy, 2002).

Such long-term structural changes are dependent on protein synthesis, and inhibition of protein synthesis in the VTA has been shown to block the induction of locomotor sensitization by amphetamine and cocaine (Karler, Finnegan & Calder, 1993;

Sorg & Ulibarri, 1995). Axonal growth and dendritic extension are key morphological features characterizing neuronal development. Furthermore, regulation of neurite outgrowth is an important aspect of neuronal regeneration from injuries or neuropathological conditions. An important set of molecules in the regulation of neurite growth is neurotrophic factors (Kiryushko, Berezin & Bock, 2004). Repeated administration of amphetamine has been shown to elicit long-lasting increases in the expression of basic fibroblast growth factor (bFGF)-immunoreactive cells in the VTA, as well as in terminal regions including the NAcc and prefrontal cortex (Flores, Rodaros, & Stewart, 1998; Flores & Stewart, 2000a). In cultured dopamine neurons, bFGF is known to promote neurite growth (Grothe, Schulze, Semkova, Muller-Ostermeyer, Rege & Wewetzer, 2000), and to promote neurite re-growth following 1-methyl-4-phenylpiridium (MPTP)-induced damage (Mitsumoto, Watanabe, Miyauchi, Jimma & Morizumi, 2001). Moreover, administration of neutralizing antibodies for bFGF has been demonstrated to block neurite growth in cultured neurons (Hatten, Lynch, Ryder, Sanchez, Joseph-Silverstein, Moscattelli & Rifkin, 1988; Le Roux & Esquenazi, 2002). Therefore, the increased expression of bFGF observed in terminal regions of VTA dopamine neurons following repeated exposure to amphetamine (Flores et al., 1998; Flores & Stewart, 2000a) may play a role in amphetamine-induced dendritic growth (Robinson & Kolb, 1997; Robinson & Kolb, 1999). In addition, the amphetamine-induced increase in expression of bFGF in the VTA (Flores et al., 1998) may promote dendritic growth of the dopamine neurons themselves.

Purpose of the Present Experiments

The development of sensitization to psychostimulant drugs is initiated by actions in the cell body region of midbrain dopamine neurons (Cador et al., 1995; Kalivas & Weber, 1988; Vezina & Stewart, 1990; Vezina, 1993; Vezina, 1996). The present series of experiments was designed to examine whether repeated administration of amphetamine induced long-lasting morphological changes in VTA dopamine neurons. Using the *in vitro* whole cell patch-clamp method (Grace & Onn, 1989), dopamine neurons derived from the VTA were identified electrophysiologically and pharmacologically. Staining and visualization of these recorded cells allowed for the subsequent quantification of dendritic morphology. In addition, the role of bFGF in amphetamine-induced dendritic growth was explored.

Chapter 1

Identification and Morphology of Dopamine Neurons in the Ventral Tegmental Area

Midbrain nuclei are the main sources of dopamine in the central nervous system and have been implicated in motivational processes. In particular, the dopamine neurons that originate in the ventral tegmental area (VTA) are an integral part of the natural reward circuitry that has been shown to underlie behavioral sensitization induced by several drugs of abuse (see Di Chiara & Imperato, 1988; Wise, 2004). Accordingly, dopamine seems to be the principal neurotransmitter that mediates the reinforcing effects of drugs of abuse, such as amphetamine, cocaine, and opiates.

The VTA is a heterogeneous structure consisting of at least two neuronal phenotypes, including dopamine and GABA neurons (Steffenson, Svingos, Pickel, & Henriksen, 1998). In studies of VTA neurons, both *in vivo* and *in vitro*, dopamine neurons have been identified by a constellation of properties. Neurons that are immunoreactive for tyrosine hydroxylase, the rate-limiting enzyme in the synthesis of dopamine, have a number of distinct electrophysiological and pharmacological characteristics, including (1) long-duration (2-5 ms), biphasic or triphasic action potentials, (2) a low rate (2-9 impulses/s) of spontaneous activity marked by bursting episodes with a decrement in spike amplitude, (3) relatively slow axonal conduction velocity (approx. 0.5 m/s), (4) inhibition of spontaneous activity by D2-like receptor agonists and subsequent reversal by D2-like receptor antagonists (Wang, 1981a,b; Grace

& Bunney, 1983; Chiodo, 1988), and (5) a marked inward rectification in response to hyperpolarizing current injection (Grace & Onn, 1989; Lacey, Mercuri, & North, 1989).

In the VTA, neurons without detectable TH immunoreactivity lie in close proximity to TH-labeled cells and are presumed to be GABAergic interneurons (Nagai, McGeer & McGeer, 1983; Otterson & Storm-Mathisen, 1984; Mugnaini & Oertel, 1985; Carr & Sesack, 2000). The physiological properties of non-dopamine neurons have been identified in this region, and include (1) relatively high rates of spontaneous activity (>10 impulses/s), (2) action potential durations of less than 1 ms, and (3) absence of the hyperpolarization-induced inward rectification (Grace & Onn, 1989; Lacey et al., 1989).

To characterize the morphological properties of single neurons in the VTA, electrophysiologically identified neurons can be labeled. During intracellular electrophysiological recordings, dyes, such as biocytin (Horikawa & Armstrong, 1988; Chapman & Lacaille, 1999), can be passed through the recording pipette to allow specific neurons to be labeled. Following histochemical processing of the tissue, the single neuron can easily be located under a microscope. Characterization of the morphology of these neurons, however, is subject to a number of methodological problems. In whole tissue slices containing the VTA, high non-specific background staining typically occurs due to the thickness of these slices (200-250 μm). To avoid this problem, fixed slices have traditionally been re-sectioned before processing, a labour-intensive technique that can result in tissue distortion and poor reconstruction of the neuron and its neurites (Horcholle-Bossavit, Gogan, Ivanov, Korogod, & Tyc-Dumont, 2000). More recently, a whole-mount histochemical technique was developed (Hamam & Kennedy, 2003), allowing for the visualization of single neurons in thick slices without the need for re-

sectioning. To date, this technique has not been applied to the study of dopamine neurons.

In the present study, using the *in vitro* whole-cell patch-clamp technique, VTA dopamine neurons were characterized electrophysiologically based on two criteria: (1) the presence of a hyperpolarization-induced sag in the membrane response to negative current injection that is indicative of the inward rectifying current (I_h; Grace & Onn, 1989; Lacey et al., 1989), and (2) a pronounced and prolonged hyperpolarization induced by bath application of the D2/D3 receptor agonist, quinpirole, that acts on autoreceptors on dopamine neurons (Brodie & Bunney, 1996). During the electrophysiological recordings, the cells were filled with biocytin and subsequently processed for visualization using the whole-mount histochemical technique (Hamam & Kennedy, 2003).

Method and Procedures

Animals

Timed-pregnant Wistar rats (Charles River, Quebec) were housed individually with *ad libitum* access to food and water. On the day of birth, litters were cross-fostered and culled to 10–12 pups each (6 males and 4-6 females). Litters were weaned on postnatal day 21, and male offspring were housed in groups of 2-3 per cage. All animals were housed in a normal light/dark cycle (12h lights on/ 12h lights off), with lights on at 8 am.

Tissue slices

Male Wistar rats, 21-42 days old, were anesthetized with isoflurane, and the brains were removed. A block of tissue containing the VTA was sectioned in the horizontal plane (250 μm thick) in an ice-cold (2^0C) high-sucrose, low-Ca^{2+} dissection solution (containing, in mM: 87 NaCl, 2.5 KCl, 7 $MgCl_2$, 1.25 NaH_2PO_4, 0.5 $CaCl_2$, 25 $NaHCO_3$, 75 sucrose, and 10 dextrose saturated with 95% O_2–5% CO_2) and allowed to recover at room temperature for at least 1 h. Slices were transferred to a recording chamber and superfused with artificial cerebral spinal fluid (ACSF, containing, in mM: 124 NaCl, 5 KCl, 2 $MgSO_4$, 1.25 NaH_2PO_4, 2 $CaCl_2$, 26 $NaHCO_3$, and 10 dextrose saturated with 95% O_2–5% CO_2) at a rate of 2-3 ml/min. Individual slices were visualized under an upright microscope (Leica, Germany) using differential interference contrast optics and a long-range water immersion objective (40X).

Whole cell recording

Patch pipettes for whole-cell current-clamp recordings were pulled from borosilicate glass (1.0 mm OD, 4-8 MΩ) using a horizontal puller (Sutter Instruments, P97, Novato, CA). Patch pipettes were filled with a solution containing (in mM): 140 K-gluconate, 10 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), 0.5 ethylene glycol-bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA), 5 NaCl, 2 $MgCl_2$, 2 ATP-Tris, and 0.4 GTP-Tris, and 0.1% biocytin (pH adjusted to 7.2-7.3 with KOH). Biocytin was added to the internal solution in order to fill cells for later visualization. Patch pipettes were placed in contact with soma of putative dopamine neurons under visual guidance, using gentle positive pressure. Tight seals (1-3 GΩ) were

obtained under voltage clamp with the aid of gentle suction, and stronger suction was used to obtain whole cell configuration. Current-clamp recordings were made using an Axopatch 200B amplifier (Axon Instruments, Foster City, CA) and displayed on a digital oscilloscope (Gould 1604, Ilford, UK). Recordings were filtered at 10 kHz and digitized at 20 kHz (Axon Instruments, Digidata 1322A) for storage on computer hard disk. Recordings were accepted if the series resistance was <15 MΩ (mean = 8.6 ± 0.1 MΩ) and if input resistance and mean resting membrane potential were stable.

Identification of DA neurons

Dopamine cells were identified by two criteria: (i) the presence of a large sag in the voltage response to hyperpolarizing current steps, representing activation of the inward rectifying current (I_h), and (ii) a prolonged hyperpolarization of membrane potential induced by bath application of quinpirole. To assess the sag response, cells were held at membrane potentials of approximately -50 mV and membrane responses to current steps ranging from -500 to +150 pA were recorded. Subsequently, 20 μM (-)-quinpirole (Sigma) was added to the bath solution to induce membrane hyperpolarization and membrane potential was recorded for 5 min. Only 2 slices from each animal were tested and only 1 cell from each slice was examined.

Histochemistry.

Immediately after recordings, slices were fixed for 24 to 48 h in 4% paraformaldehyde in 0.1 M phosphate buffer (PB). To visualize the biocytin-filled cells, we adapted a method allowing for whole slice staining in intact slices up to 500 μm thick

(Hamam & Kennedy, 2003). Following fixation, slices were washed three times for 5 min each in 0.1 M PB. This and all subsequent incubations were carried out in a series of wells of a standard 24-well plate (Sarstedt, QC), gently agitated on an orbital shaker at room temperature. Slices were incubated in 1% H_2O_2 for 30 min to eliminate endogenous peroxidases and then rinsed (3 X 5 min) in 0.1 M PB. To block non-specific protein binding and to permeabilize the cells, slices were then incubated in 1% heat-inactivated normal goat serum (Invitrogen, Burlington, ON) and 0.3% triton X-100 (pH 7.5) in 0.1 M PB for 2 h. Slices were then incubated in avidin-biotin complex (ABC kit, Vector Laboratories, Burlingham, CA). The A and B reagents were diluted 1:100 in 0.3% triton X-100, and the tissue slices were incubated overnight at room temperature. After rinsing with Tris-buffered saline (TBS, pH 7.6, 3 X 10 min), the slices were incubated in a solution containing 0.01% H_2O_2, 0.5% 3,3'-diaminobenzidine, and 0.02% $NiSO_4$ in TBS. The development of stain intensity was monitored for 10-12 min and the reaction stopped by washing the slices in TBS (3 x 10 min).

Processed tissue slices were wet-mounted onto gelatin-coated slides and were allowed to dry for at least 1 d before being hydrated in distilled water and gradually dehydrated through a series of graded alcohol solutions. Slides were cleared in citrosolv (Fisher) and coverslipped with Permount.

Camera Lucida Drawings

Following histochemical processing, single stained neurons in slices were identified at low magnification (50X), and the cell was traced using the camera lucida method at a magnification of 200X.

Results

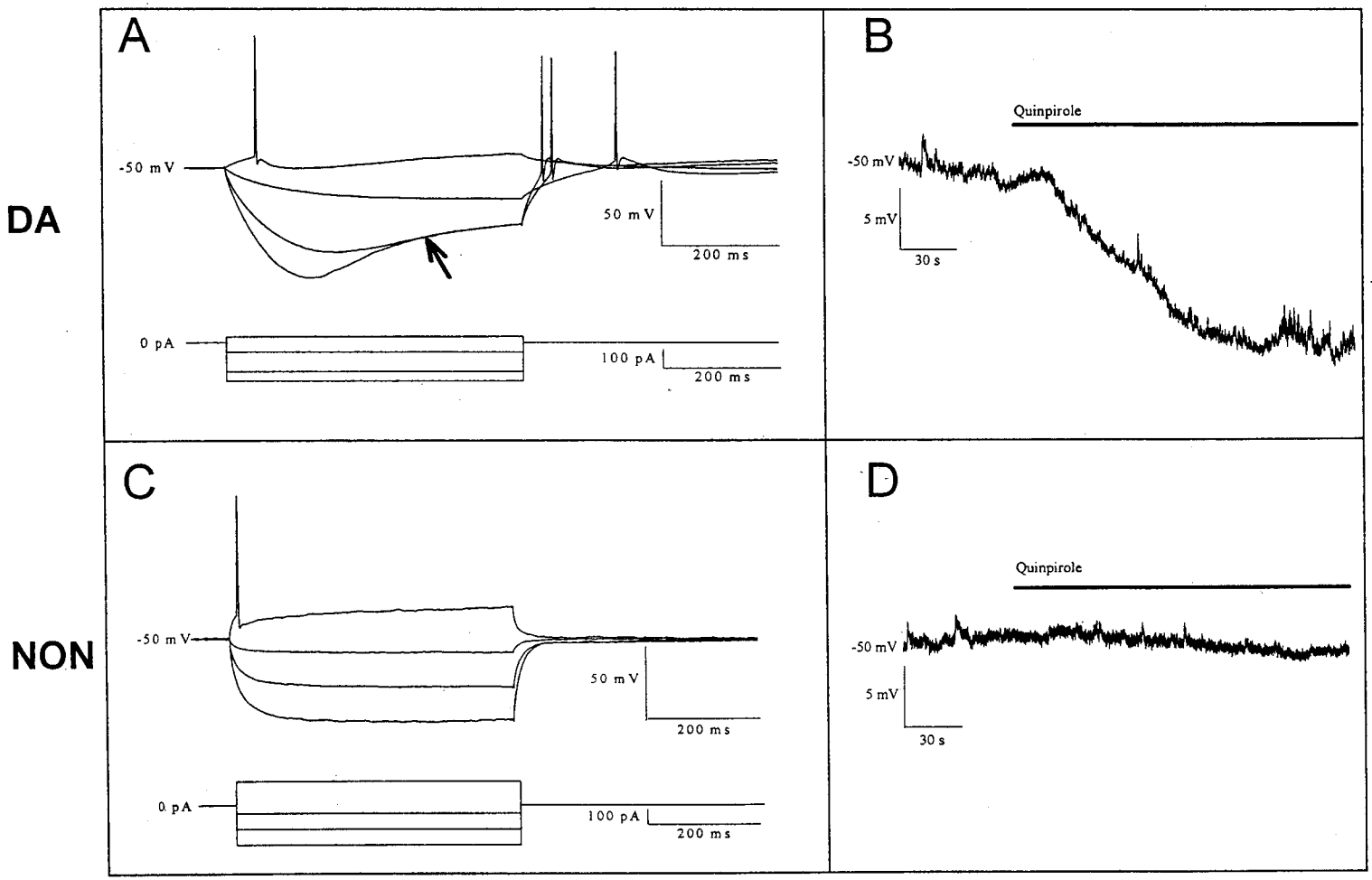
Cell Identification

Neurons recorded within the VTA could readily be classified into two broad subtypes based on their morphological and electrophysiological characteristics. These neuronal subtypes are described as dopamine and non-dopamine (putatively GABA) neurons.

Dopamine neurons. Dopamine neurons recorded in the VTA exhibited distinct characteristics similar to those of identified dopamine neurons recorded *in vivo* and *in vitro* (Wang, 1981a; Grace & Bunney, 1983; Grace & Onn, 1989). A total of 93 dopamine neurons were identified. In those neurons where action potentials were recorded (n=52), the action potentials were long in duration (mean ± SEM: 4.5 ± 0.2 ms), and had fairly depolarized thresholds for activation (-37.0 ± 0.7 mV). The magnitude of the action potentials in these neurons was 78.1 ± 2.0 mV.

Long duration hyperpolarizing current steps revealed the presence of two voltage-dependent conductances: a voltage-dependent sag that occurred during membrane hyperpolarization that is due to the anomalous rectifying current (I_h), and a delay in repolarization of membrane potential following hyperpolarizing current steps. As shown in Figure 1A, the hyperpolarization-induced sag response was most prominent when the membrane potential was decreased below -120 mV. The delayed repolarization was inactive at resting potential but was activated during the transition of the membrane potential from a hyperpolarized state to a depolarized state, i.e., at the offset of membrane hyperpolarization.

Figure 1. Based on their electrophysiological properties, two neuronal types located in the VTA could be distinguished. Current traces are shown below the potential traces. One neuron type (DA) was identified as having dopamine-like characteristics: *(A)* Hyperpolarization of the membrane of this neuron resulted in the activation of the anomalous rectifying current (I_h), as demonstrated by the voltage sag response (black *arrow*), and *(B)* this neuron also showed membrane hyperpolarization in response to bath application of quinpirole. A second neuron type (NON) was identified as non-dopaminergic: *(C)* This neuron did not show a voltage sag in response to hyperpolarizing current steps, and *(D)* this neuron did not show a change in membrane potential in response to bath application of quinpirole.


DA
NON
A
-50 mV
50 mV
200 ms
0 pA
100 pA
200 ms
B
Quinpirole
-50 mV
5 mV
30 s
C
-50 mV
50 mV
200 ms
0 pA
100 pA
200 ms
D
Quinpirole
-50 mV
5 mV
30 s

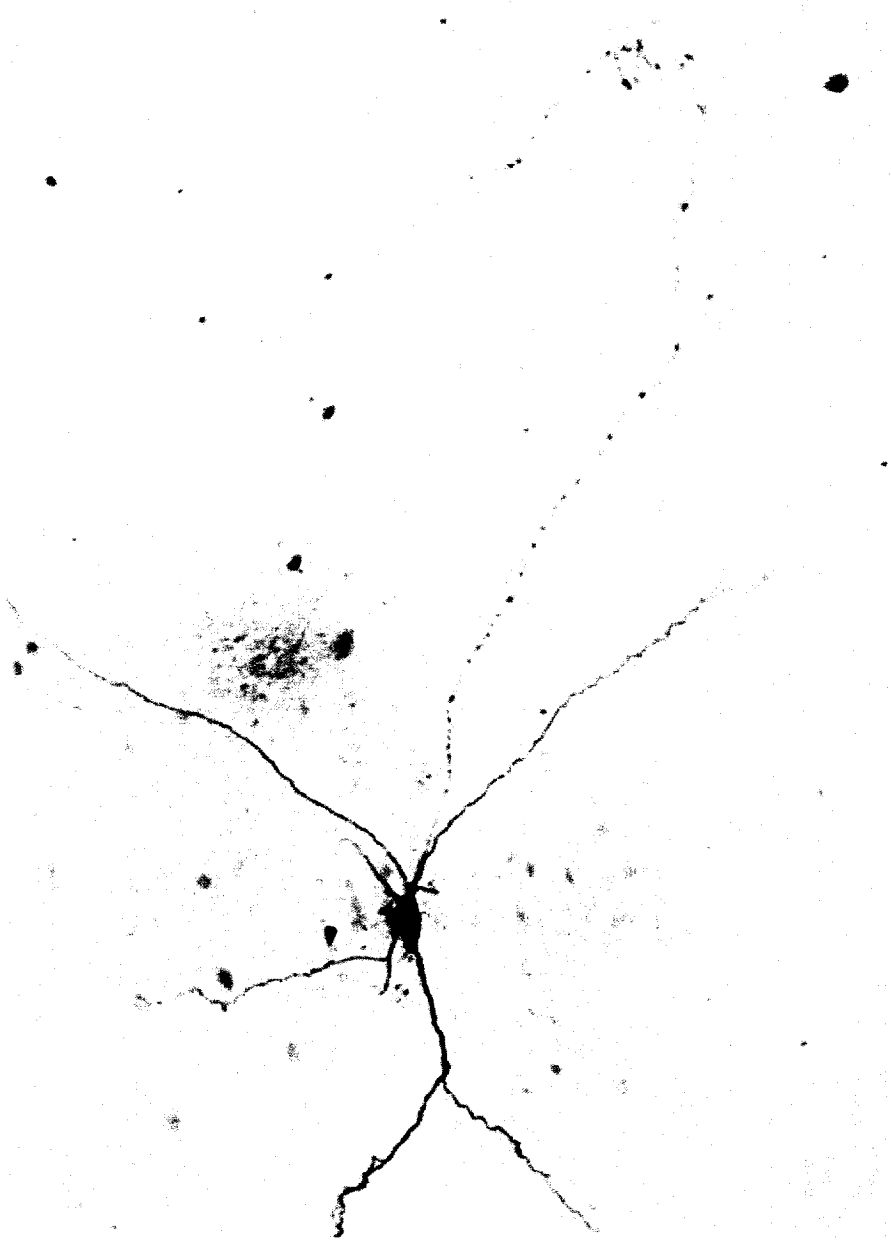
In neurons exhibiting a voltage-dependent sag in membrane potential, bath application of quinpirole induced membrane hyperpolarization (see Figure 1B). As such, these neurons were identified as dopamine neurons. The membrane potential of these dopamine neurons, initially held at approximately -50 mV, showed a prominent hyperpolarization of -12.9 ± 1.1 mV (i.e., approximately -63 mV on average) in response to quinpirole.

Non-DA neurons. One neuronal type recorded within the VTA exhibited physiological properties similar to GABA neurons described previously by Steffenson and colleagues (1998). A total of 32 non-dopamine cells were identified. In those neurons in which action potentials were recorded (n=11), the action potentials were shorter in duration (1.9 ± 0.1 ms) than those in identified dopamine neurons, and had slightly hyperpolarized thresholds for activation (-40.8 ± 1.6 mV). The magnitude of the action potentials was 73.7 ± 5.1 mV. These cells did not show a sag in the membrane potential in response to hyperpolarizing current pulses, and also did not show a substantial delay in repolarization after the offset of hyperpolarizing current pulses (see Figure 1C). Furthermore, as can be seen in Figure 1D, bath application of quinpirole caused no change in the membrane potential of these neurons.

Cell Morphology

The morphological characteristics of dopamine and non-dopamine neurons, characterized by their electrophysiological properties, were studied by histochemical processing of biocytin-filled neurons for imaging cellular morphology. Only those cells that were well-labeled, with clearly definable neurites, were examined. Figure 2 shows a

Figure 2. Digitized image depicting a representative neuron exhibiting dopamine-like characteristics in the VTA. The whole-mount histochemical technique allows for clear staining and visualization of the neuron and its dendritic processes.

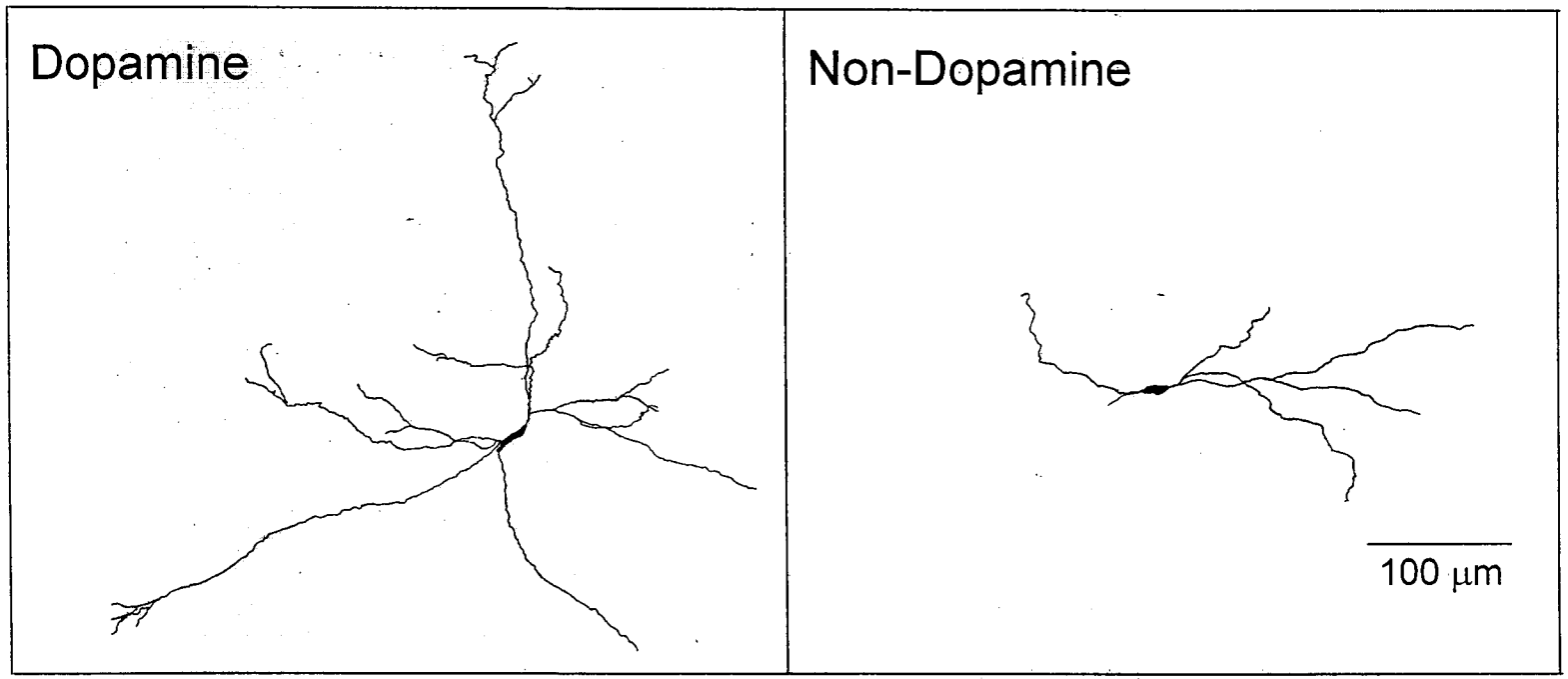


representative dopamine neuron which was visualized using the whole-mount histochemical technique. Camera lucida drawings of cells were achieved due to the clarity of the tissue and the integrity of the neuron and its neurites.

Dopamine neurons. Of the 93 dopamine neurons displaying the characteristic electrophysiological properties outlined for dopamine neuron identification, 70 were well-labeled. As seen in Figure 3A, these neurons showed a distinct morphology, consisting of medium-sized somata that gave rise to 2 to 6 thick major dendrites. Typically, the dendrites extended for distances of up to 1000 μm from the soma. When the dendrites of dopamine neurons could be followed to their termination, they were observed to end in a tuft of 2 to 4 short dendritic protrusions, ranging in length from 5 to 50 μm. As described previously, this morphology is predominant in VTA dopamine neurons rather than in nigral dopamine neurons (Grace & Onn, 1989).

Non-dopamine neurons. In order to describe the morphology of the non-dopamine neurons recorded in this study, 9 non-dopamine neurons that were well-filled with biocytin and labeled were examined. In Figure 3B, it can be seen that non-dopamine neurons were smaller in size than the dopamine neurons described previously. These neurons were typically multipolar in shape with 2-6 dendritic processes branching from their somata. The dendrites could frequently be seen to branch within 20 μm of the soma.

Figure 3. Camera lucida drawings of representative neurons identified as dopaminergic or non-dopaminergic. Morphological characteristics of these neurons are described in the text.


Dopamine
Non-Dopamine
100 μm

Discussion

Based on the electrophysiological and pharmacological properties exhibited by neurons in the VTA, two subtypes were readily identifiable. Dopamine neurons showed a characteristic sag in membrane potential in response to hyperpolarizing current pulses and showed a prominent hyperpolarization in response to the D2/D3 agonist quinpirole, whereas non-dopamine neurons did not. Furthermore, use of the whole-mount histochemical technique resulted in well-stained cells such that camera lucida drawings could be made to examine the morphology of these neurons. Thus, this preparation provides a means to identify dopamine neurons in the heterogeneous cell population of the VTA and to answer questions concerning morphological alterations.

Hyperpolarization of dopamine neurons to -80 mV or more was accompanied by the development of a prominent sag in the membrane potential (Grace & Bunney, 1983; Pinnock, 1985; Kita et al., 1986), thereby decreasing the amount of hyperpolarization produced. The change in conductance underlying this sag had properties similar to those described for the anomalous rectifier in cortical neurons (Schwindt et al., 1988) and appeared to be larger in amplitude than that observed in dopamine neurons recorded *in vivo* (Grace & Bunney, 1983). The anomalous rectifier is thought to play a role in maintaining pacemaker firing patterns of neurons (Crepel & Penit-Soria, 1986).

Action potentials were longer in duration than those reported in previous studies. This difference may be attributed to a number of factors. First, during slice preparation, the slices were incubated in a high sucrose dissection saline during recovery to room temperature. Second, neurons in the VTA were recorded at room temperature rather than at physiologically-relevant temperatures. Interestingly, previous studies have shown that

very few spontaneously firing dopamine neurons could be recorded from when the temperature of the preparation fell below 35^0C (Grace & Onn, 1989). Thus, the lower temperature during recordings from VTA neurons in this study, and the use of a high sucrose dissection saline bath, may have resulted in slower action potentials. Although recording from neurons at room temperature may have affected the duration of action potentials, the lack of spontaneous firing allowed for clear visualization of the quinpirole-induced hyperpolarization of dopamine neurons.

In previous studies, the presence of an I_h current alone has been deemed sufficient to identify primary dopamine neurons (Borgland, Malenka, & Bonci, 2004). This criterion alone, however, has been criticized on the grounds that neurons exhibiting an I_h current do not always stain for TH-immunoreactivity (Grace & Onn, 1989). Therefore, in this study, dopamine neurons were positively identified using a second criterion: a prominent hyperpolarization upon bath application of quinpirole. Quinpirole, a D2/D3 dopamine receptor agonist, acts at the somatodendritic autoreceptors of dopaminergic neurons in much the same manner as dopamine itself. As such, quinpirole acts to hyperpolarize the dopamine neurons, reducing cell firing. D2-like receptors involve the G_i and G_o type G proteins. These inhibitory G proteins activate an inward rectifying current by way of K^+ channels and possibly inhibit voltage-dependent Ca^{2+} channels. Thus, dopamine neurons were identified by the presence of both an I_h current and a quinpirole-induced hyperpolarization.

The typical method used to reveal the morphological structure of individual neurons has been the Golgi impregnation technique (Golgi, 1886). This method is limited by the fact that the cell staining is stochastic, making it impossible to predict

exactly which cells will be stained. The VTA is a heterogeneous structure comprised of both dopamine and GABA neurons, and is densely packed with fibers of passage. While several studies have used the Golgi method to study the morphology of VTA neurons (Phillipson, 1979; Kline & Felton, 1989), differentiating between dopamine and non-dopamine neurons is difficult (B. Kolb, personal communication).

Using *in vitro* whole cell patch-clamp method, dopamine neurons were identified electrophysiologically and pharmacologically. Within a tissue slice containing the VTA, single dopamine cells were then filled with biocytin and later visualized. This method allows for the identification of neuronal subtypes within the VTA, and allows for the subsequent study of neuronal morphology. Thus, morphological alterations in response to stimuli, such as drugs of abuse, can be studied. A limitation for the study of morphology using this method is that very young rats are required to obtain viable cells. Thus, morphological studies are limited to factors affecting morphology during early postnatal development.

Chapter 2

Effects of Early Postnatal Exposure to Amphetamine on the Morphology of Dopamine Neurons in the Ventral Tegmental Area

In Chapter 1, a novel adaptation of the *in vitro* whole-cell patch clamp recording procedure combined with whole slice staining was developed. Use of this methodology allows for the identification of dopamine neurons by their electrophysiological and pharmacological properties and subsequent visualization in the intact slice. In the present chapter, this methodology was applied to determine whether repeated exposure to amphetamine during the early postnatal period in rats would alter the morphology of dopamine neurons in the ventral tegmental area (VTA; see Experiment 1). Experiment 2 was designed to assess whether treatment with amphetamine during this early postnatal period would result in a change in the locomotor response to an acute injection of amphetamine in adolescence and adulthood. In addition, rats tested in adulthood received a second series of amphetamine injections and were subsequently tested for locomotor responsiveness to a challenge injection of amphetamine two weeks following the previous injection.

Experiment 1: Morphology

Repeated administration of psychostimulants results in a progressive augmentation of the behavioral and neurochemical responses upon re-exposure to the drug (Kalivas & Stewart, 1991; Robinson & Becker, 1986). This phenomenon is termed

sensitization, and is initiated through a series of transient events leading to enduring plastic changes that mediate the expression of the sensitized response (Kalivas & Stewart, 1991).

Psychostimulant drugs, such as amphetamine and cocaine, increase extracellular levels of dopamine by reducing dopamine re-uptake through the dopamine transporter (Reith et al., 1986). In addition, amphetamine acts to enhance the transport of dopamine from nerve terminals into the synapse through a reversal of the plasmalemmal transporters (Seiden et al., 1993; Sulzer et al., 1995). The mesolimbic dopamine system that projects from the VTA to the nucleus accumbens (NAcc; Fallon, Koziell, & Moore, 1978) has been described as a primary target in the initiation of sensitization to psychostimulant drugs (Vezina, 1993). Moreover, it has been proposed that psychostimulant-induced changes in the VTA play an important role in initiating the sensitization process, while the nucleus accumbens has a role in the expression of behavioral sensitization (Kalivas & Stewart, 1991; White & Kalivas, 1998). Thus, the administration of D_1 dopamine receptor antagonists into the VTA disrupts the development of sensitization to amphetamine, and repeated administration of amphetamine or a D_1 receptor agonist into the VTA elicits a sensitized motor response to systemically administered amphetamine or cocaine (Bjijou et al., 1996; Stewart & Vezina, 1989; Vezina, 1996). In addition, a number of pharmacological manipulations, including intra-VTA infusions of glutamate receptor antagonists (Kalivas & Alesdatter, 1993; Vezina & Queen, 2000) and L-type calcium channel blockers (Karler et al., 1991b), have been shown to prevent the development of sensitization. Thus, the VTA is a critical site for the initiation and development of sensitization.

Following repeated exposure to psychostimulants, dopamine neurons show enhanced responding to their excitatory synaptic inputs. In the VTA, previous exposure to psychostimulants results in increased extracellular glutamate levels (Kalivas & Duffy, 1995, 1998), and reduced inhibition by $GABA_B$ receptor-mediated inhibitory postsynaptic potentials (Bonci & Williams, 1996). These findings suggest that drug exposure alters the function of the VTA dopamine neurons such that the dopamine neurons are under less inhibitory control and favor excitation to glutamate. The enhanced responding of dopamine neurons to excitatory inputs results in augmented dopamine overflow in the NAcc (Robinson, Jurson, Bennett, & Bentgen, 1988; Kalivas & Duffy, 1993), which subsequently affects their synaptic targets. In the terminal regions of the VTA, repeated exposure to psychostimulant drugs has been shown to alter the synaptic connectivity of neurons (Robinson & Kolb, 1997; Robinson & Kolb, 1999; Heijtz, Kolb, & Forssberg, 2003). In those experiments, one month following an escalating dose regimen of amphetamine (Robinson & Kolb, 1997) or 30 days of cocaine self-administration (Robinson, Gorny, Mitton & Kolb, 2001), overall dendritic length of postsynaptic neurons in the NAcc and prefrontal cortex was increased. The morphology of the dopaminergic neurons that project to these regions from the VTA, however, has not been characterized following psychostimulant exposure. Interestingly, in cultured pheochromocytoma (PC12) cells, intermittent exposure to amphetamine results in neurite outgrowth (Park et al., 2002; Park, Kantor, Guptaroy, Zhang, Wang & Gnegy, 2003). Thus, the present study was designed to investigate whether repeated administration of amphetamine would affect the morphology of dopaminergic neurons in the VTA.

Dendritic length was assessed in VTA dopamine neurons identified using *in vitro* electrophysiology as described in Chapter 1. Importantly, *in vitro* slices containing the VTA were derived from rats 3 to 6 weeks of age, as the viability of dopamine neurons diminishes into adulthood (P. Congar, personal communication; unpublished observations). This age range, however, is typically used in the study of dopamine neurons *in vitro* (Bonci & Malenka, 1999; Borgland, Malenka, & Bonci, 2004).

Method and Procedures

Treatment

Male Wistar rats were obtained from cross-fostered litters as described in Chapter 1. Individual litters were treated with subcutaneous injections of either d-amphetamine sulphate (2 mg/kg, s.c.; SmithKline Beecham Pharma, Oakville, ON) or 0.9% saline (2 ml/kg, s.c.) on postnatal days 10, 12, and 14.

Intracellular Recordings and Histochemistry

Following treatment, tissue slices containing the VTA were derived from male Wistar rats, 21–42 days old. Intracellular recordings and histochemistry were carried out as described in Chapter 1. The morphology of electrophysiologically identified dopamine neurons was analyzed by drawing the cells via camera lucida and quantifying dendritic length using the concentric ring method of Sholl (1956). The number of intersections of dendrites with a series of concentric circles at 20 μm intervals from the center of the cell body was counted for each cell. An estimate of the mean total dendritic length (in μm) was made by multiplying the mean total number of intersections by 20.

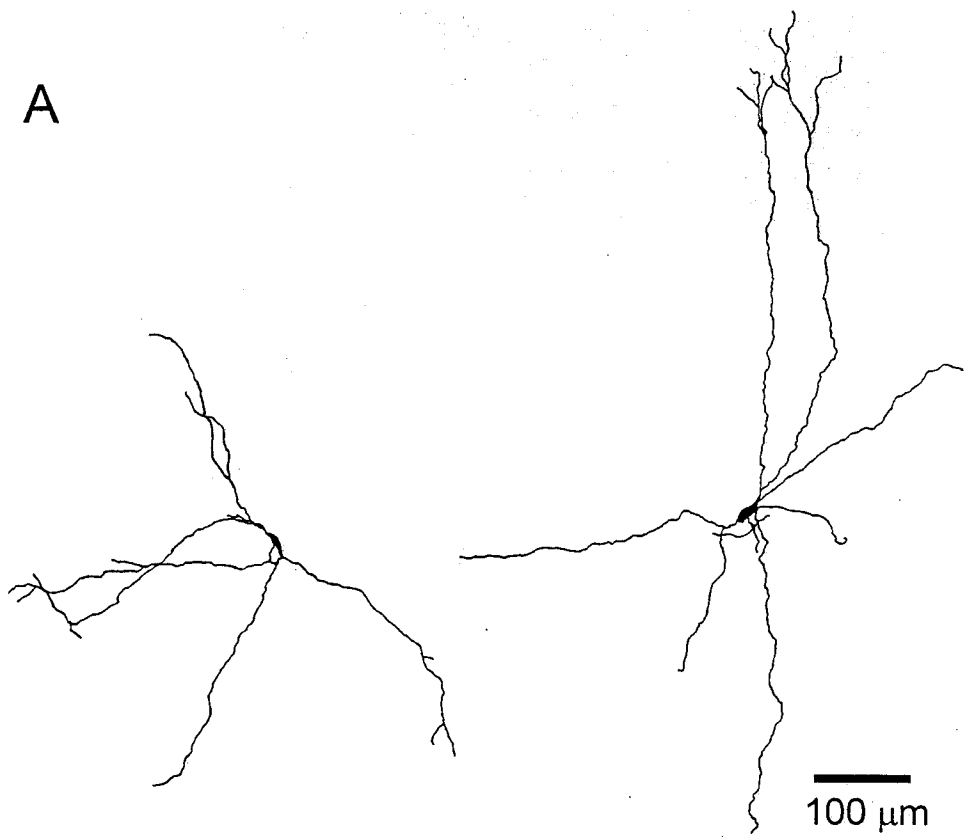
The total estimated dendritic length of neurons recorded in slices derived from saline- and amphetamine-treated rats were compared using an independent-samples t-test.

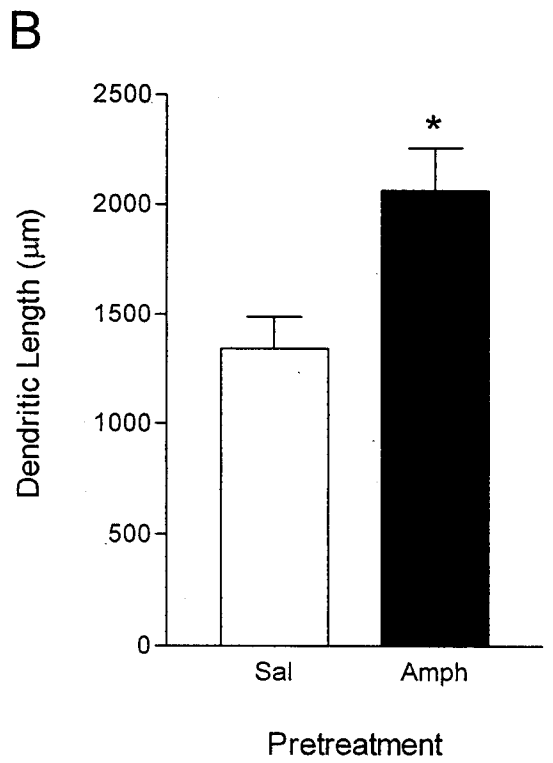
Results

Dendritic Length

A total of 19 dopamine neurons were identified electrophysiologically, and were subsequently labeled histochemically. The mean age of the neurons derived from saline- (n=9; 31.33 ± 1.72 d) and amphetamine-treated rats (n=10; 33.6 ± 1.93 d) was not significantly different ($t_{(17)} = 0.87$, n.s.). Figure 4A shows camera lucida drawings of representative dopamine neurons in the VTA from saline- and amphetamine-treated rats. It can be seen that the dendritic arbor was more elaborate in cells derived from amphetamine-treated rats than in those derived from saline-treated rats. The total estimated dendritic length, as assessed by Sholl analysis, is shown in Figure 4B. Amphetamine treatment significantly increased the dendritic length of VTA dopamine neurons ($t_{(17)} = 2.91$, $p<.01$; Figure 4B).

Figure 4. Morphological changes in VTA dopamine neurons after early postnatal exposure to amphetamine. *(A)* Camera lucida drawings of representative dopamine neurons in the VTA of saline- or amphetamine-treated rats. *(B)* Quantitative analysis of total dendritic length. *Asterisks* indicate a significant increase in dendritic length as compared to saline-treated rats, $p<.01$.


A
100 µm
Saline
Amphetamine


B
2500
2000
1500
1000
500
0
*
Dendritic Length (µm)
Sal
Amph
Pretreatment

Discussion

Repeated administration of amphetamine resulted in the elaboration of the dendritic arbor of VTA dopamine neurons; the total estimated dendritic length increased by approximately 54%. This dendritic elongation was induced using a moderate regimen of amphetamine administration as compared to studies of neurons in the terminal regions of the VTA. In those studies, changes in the dendritic morphology of neurons in the prefrontal cortex and NAcc were induced by either an escalating dose regimen of amphetamine over 5 weeks (Robinson & Kolb, 1997), or by allowing animals to self-administer cocaine 3 hours daily over 30 days (Robinson et al., 2001). In the present study, the magnitude of the morphological alterations seen in VTA dopamine neurons after three injections of amphetamine suggests that the VTA is a major site of neuronal plasticity.

The VTA has been implicated as the site of initiation for the development of behavioral and neurochemical sensitization (Kalivas & Stewart, 1991; Vezina, 2004). Repeated intra-VTA amphetamine administration, but not intra-NAcc administration, is sufficient to induce behavioral sensitization and to increase dopamine release in striatal regions in response to systemic injections of stimulant drugs and intra-NAcc amphetamine (Vezina, 1993: Perugini & Vezina, 1994). Importantly, the long-lasting changes in the responsiveness to psychostimulant drugs takes time to develop following the cessation of repeated drug exposure and appears 1 to 3 weeks after the last drug injection (Kolta, Shreve, De Souza & Uretsky, 1985; Vezina, 1993; Paulson & Robinson, 1995). These long-lasting changes in dopamine function coincide with the morphological changes in VTA dopamine neurons demonstrated in this study.

Changes in the dendritic morphology of dopamine neurons in the VTA likely reflect changes in synaptic connectivity and efficacy. As the length of the dendritic branches of VTA dopamine neurons increases, so does the total dendritic surface area. Importantly, it has been shown that the number of synaptic contacts increases in proportion to increases in dendritic surface (Harris & Kater, 1994). Furthermore, previous experiments have shown that rats given learning and memory tasks or raised in complex environments had increased dendritic surface area in cortical neurons which was accompanied by an increase in the number of synapses per neuron, as assessed by electron microscopy (Greenough & Bailey, 1988; Greenough, Withers & Wallace, 1990). Thus, early postnatal amphetamine exposure is proposed to increase the number of synaptic connections within the VTA.

Changes in synaptic connectivity in the VTA may underlie changes in the efficacy of the synaptic connections in this region. Recent *in vitro* studies have shown that afferent excitatory inputs to dopamine neurons undergo an NMDA-dependent long-term potentiation (LTP) following sufficient depolarization (Bonci & Malenka, 1999; Overton, Richards, Berry & Clark, 1999) and a long-term depression (LTD) following low-frequency stimulation (Thomas, Malenka & Bonci, 2000). In addition, it has been reported that a single *in vivo* exposure to cocaine or amphetamine increases the ratio of AMPA to NMDA receptor-mediated currents, an index of membrane excitability, and induces LTP of AMPA-mediated excitatory transmission in dopamine neurons (Ungless, Whistler, Malenka & Bonci, 2001; Saal, Dong, Bonci & Malenka, 2003). Amphetamine has also been shown to block LTD, an effect mediated by the actions of dopamine on D_2 receptors (Jones, Kornblum & Kauer, 2000). The resulting increase in excitatory

synaptic drive might enhance glutamate-driven activity in dopamine neurons (Paladini, Fiorillo, Morikawa & Williams, 2001), favoring dopamine release in synaptic terminals (Garris, Ciolkowski, Pastor & Wightman, 1994). In fact, extracellular glutamate has been shown to be increased in the VTA during (Kalivas & Duffy, 1995, 1998) and after (Xue, Ng, Li, & Wolf, 1996; Wolf & Xue, 1998) amphetamine or D_1 dopamine receptor agonist administration. As such, the long-lasting synaptic effects of psychostimulant drugs that promote cellular excitation are considered an important substrate for the development of drug-related learning that contributes to persistent behavioral changes (Cooper, 2002; Hyman & Malenka, 2001; Wolf, 1998; Jones & Gutlerrner, 2002). These synaptic changes are accompanied by dendritic elongation induced by amphetamine, suggesting greater responsiveness to afferent inputs.

There are at least two possible explanations for the amphetamine-induced elaboration of the dendritic arbor in VTA dopamine neurons. The remarkable dendritic growth observed in this study suggests a role for neurotrophic factors. Recent studies have demonstrated that repeated intermittent amphetamine treatment in the adult rat induces an increase in the expression of astrocytic basic fibroblast growth factor (bFGF or FGF-2) in the VTA (Flores, Rodaros, & Stewart, 1998; Flores, Samaha, & Stewart, 2000). In addition, intra-VTA infusion of an antibody for bFGF administered concurrently with amphetamine has been shown to block the development of behavioral sensitization (Flores et al., 2000). bFGF promotes growth and survival of dopamine neurons in the VTA (Chadi et al., 1993; Bouvier & Mytilineou, 1995; Takayama et al., 1995; Hou, Cohen & Mytilineou, 1997) and bFGF-immunoreactivity has been observed to increase in the VTA and substantia nigra pars compacta after 6-hydroxydopamine

lesions (Chadi, Cao, Pettersson & Fuxe, 1994). Thus, the growth of the dendritic branches of dopamine neurons in the VTA observed here may be mediated by bFGF.

A second possibility is that amphetamine itself may have neurotrophic effects and may have directly induced dendritic growth. In cultured PC12 cells, amphetamine is known to induce growth independent of nerve growth factor (NGF), because the presence of a NGF antibody in the bath did not block amphetamine-induced neurite outgrowth (Park et al., 2002). Furthermore, reverse transcription polymerase chain reaction (RT-PCR) has failed to detect mRNA for bFGF in PC12 cells (Zaheer, Zhong, & Lim, 1995). In fact, in cultured PC12 cells, amphetamine-induced neurite outgrowth has been shown to be mediated by protein kinase C (PKC) and mitogen activated protein kinase (MAPK, Park et al., 2003). Although amphetamine may influence dendritic elongation independent of growth factors, it is interesting to note that activation of the primary receptor for bFGF, FGFR1, in turn, activates an intracellular signaling pathway that includes the activation of both PKC and MAPK (Kiryushko, Berezin & Bock, 2004). Thus, amphetamine and bFGF may act synergistically to induce dendritic growth.

In order to explore these possibilities, further experiments were carried out to investigate whether repeated exposure to amphetamine increased bFGF expression in the VTA of young rats (Chapter 3) and whether the amphetamine-induced dendritic growth observed in the present study was dependent on the actions of bFGF (Chapter 4). The remainder of this chapter is concerned with whether early postnatal exposure to amphetamine induces sensitization of locomotor activity.

Experiment 2: Locomotor Activity

In the adult animal, the long-lasting changes induced by repeated exposure to psychostimulants are accompanied by an augmentation in the behavioral activating effects of these drugs (Kalivas & Stewart, 1991; Robinson & Becker, 1986). This is termed behavioral sensitization.

The ontogeny of behavioral sensitization to psychostimulant drugs in developing rats has been examined (Tirelli, Laviola, & Adriana, 2003). Repeated psychostimulant exposure for 4 consecutive days, starting at postnatal day 11 or 17, results in behavioral sensitization when animals are tested 48 h after the last drug injection, but not when tested one week later (McDougall, Duke, Bolanos, & Crawford, 1994; McDougall, Collins, Karper, Watson, & Crawford, 1999). These experiments indicate that psychostimulant drugs are able to induce a short-term behavioral sensitization in pre-weanling rats, an effect that disappears after longer retention periods. Short-term sensitization in developing rats has, however, been shown to be context-dependent (Wood, Tirelli, Snyder, Heyser, LaRocca, & Spear, 1998; Tirelli, 2001). When preweanling pups are exposed to psychostimulant drugs in their home cages, no evidence of short-term sensitization has been demonstrated (Meyer & Yacht, 1993; Bowman & Kuhn, 1996).

The expression of long-lasting behavioral sensitization to the effects of amphetamine does not appear until the fourth week of life. When tested 10 days or 3 weeks following repeated psychostimulant administration, rats did not show behavioral or neurochemical sensitization if treated during the first 3 postnatal weeks, whereas they did show it if treated during the fourth week (Tsuchida, Ujike, Kanzaki, Fujiwara &

Akiyama, 1994; Ujike, Tsuchida, Akiyama, Fujiwara & Kuroda, 1995; Fujiwara, Kazahaya, Nakashima, Sato & Otsuki, 1987; Scalzo & Holson, 1992).

Although the literature suggests that behavioral sensitization is difficult to induce in pre-weanling rats, the purpose of this experiment was to determine whether the amphetamine-induced dendritic growth seen in the first experiment was associated with changes in amphetamine-induced behavioral responding during adolescence and adulthood. In addition, rats tested in adulthood received a second regimen of amphetamine injections and were retested.

Method and Procedures

Locomotor Activity

Separate groups of rats, treated with saline or amphetamine as described in experiment 1, were tested for locomotor activity on postnatal days 21, 30, or 60. All animals tested on postnatal days 21 or 30 were injected with 1 mg/kg amphetamine (s.c.) prior to being placed in locomotor activity boxes for measurement of locomotor activity. For all tests, locomotor activity was recorded over 2 h. Animals tested on postnatal day 60 were divided into 4 separate groups according to treatment (saline/amphetamine) and treatment at test (saline/amphetamine). Animals tested on postnatal day 60 were subsequently administered saline (1 ml/kg, s.c.) or amphetamine (2 mg/kg, s.c.) on postnatal days 62, 64, and 66, and retested 2 weeks later. For the final test, all animals were administered amphetamine (1 mg/kg, s.c.).

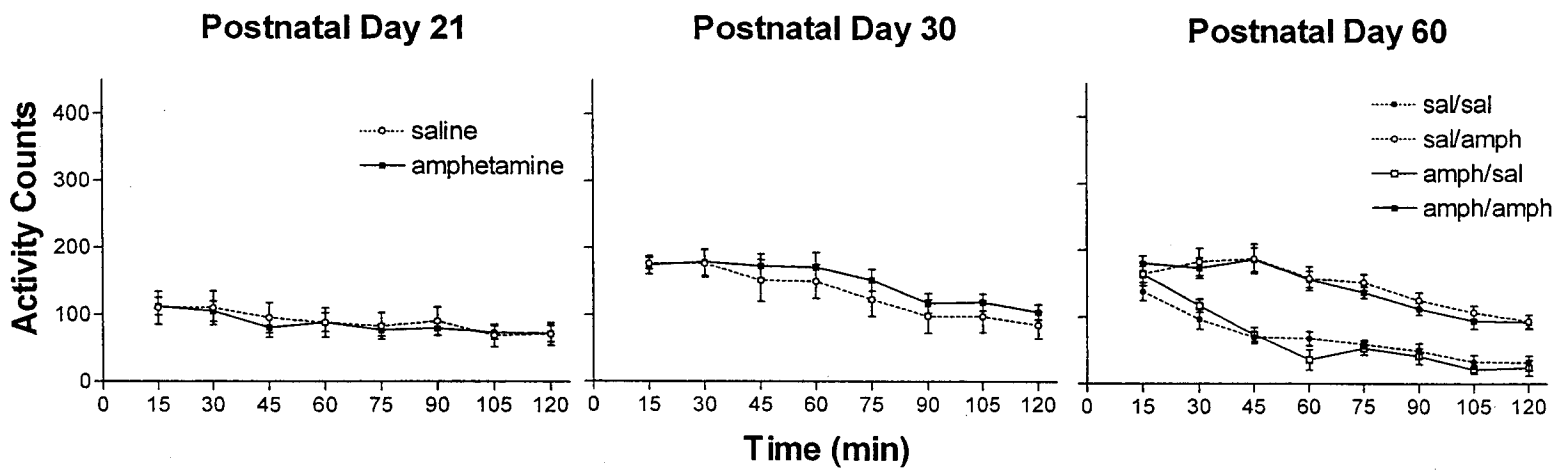
Locomotor activity was measured in a set of 12 activity boxes. Each box measured 20 cm (width) by 40 cm (length) by 25 cm (height) and was constructed with

sides of pressed wood, a hinged Plexiglas front wall, a floor of stainless steel rods, and a wire-mesh top. Four photocells were located around the perimeter of the boxes to monitor vertical and horizontal movements. Each time the animal blocked a photocell beam an activity count was recorded. At each time point tested, total activity counts were analyzed using a treatment by time (in 15 min bins) mixed ANOVA.

Results

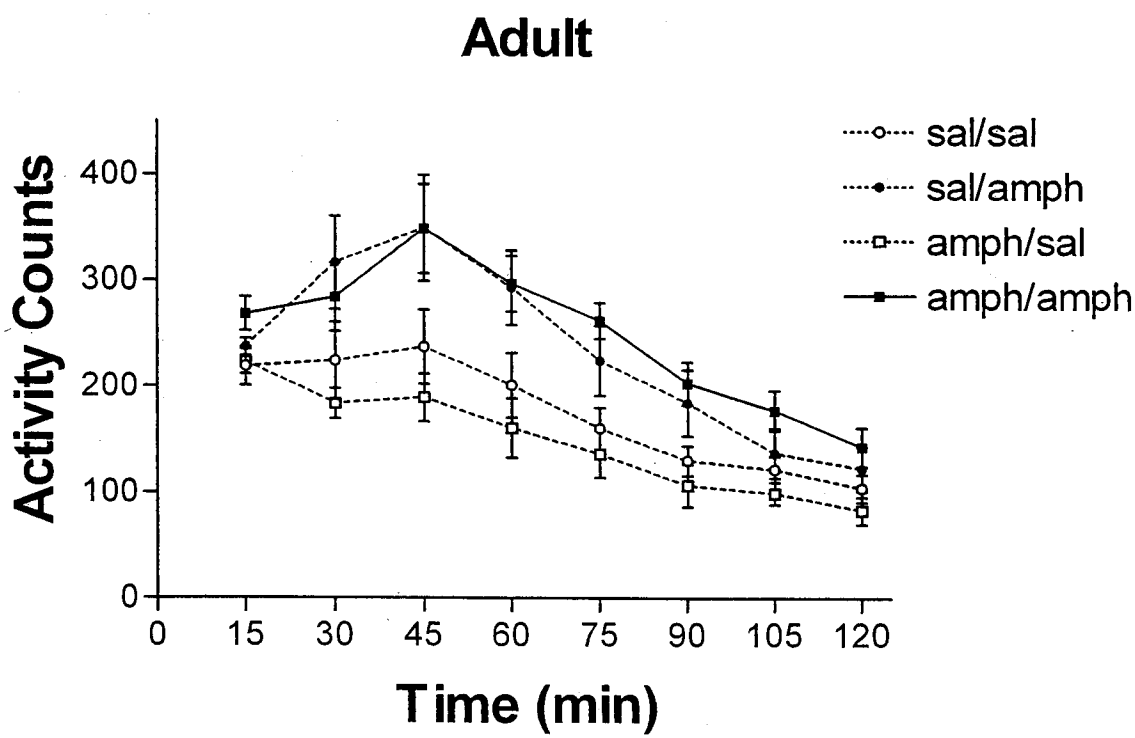
Rats exposed to amphetamine on postnatal days 10, 12, and 14 showed similar stimulatory effects to a 1 mg/kg dose of amphetamine as those exposed to vehicle at test on postnatal day 21, 30 or 60. As seen in Figure 5A, the locomotor activity of amphetamine-treated rats (n=9) on postnatal day 21 was not significantly different from saline-treated rats (n=8). A treatment by time (2 X 8) mixed ANOVA confirmed this observation, as there was no main effect of treatment ($F_{(1,15)} = 0.02$, n.s.) nor a treatment by time interaction ($F_{(7,105)} = 0.54$, n.s.). A similar lack of difference among groups was observed for rats tested at postnatal day 30 (see Figure 5B). There was no main effect of treatment ($F_{(1,14)} = 0.41$, n.s.) and no treatment by time interaction ($F_{(7,98)} = 0.69$, n.s.). Rats tested at postnatal day 60 were divided into 4 groups according to treatment (saline/amphetamine) and treatment at test (saline/amphetamine). Figure 5C shows that, regardless of treatment, rats that received amphetamine at test were more active than rats that received saline at test. The ANOVA revealed a main effect of the drug given at test ($F_{(1,25)} = 83.3$, $p<.001$), but no effect of early postnatal treatment ($F_{(1,25)} = 0.15$, n.s.). Thus, early exposure to amphetamine did not alter sensitivity to amphetamine in rats tested after weaning or in adulthood.

Figure 5. Time course of the locomotor effects of a single injection of amphetamine (1 mg/kg, s.c.) after early postnatal exposure to saline or amphetamine. Following treatment, groups of rats (n=8-9) were tested on either *(A)* postnatal day 21, *(B)* 30, or *(C)* 60.


Postnatal Day 21
Postnatal Day 30
Postnatal Day 60
Activity Counts
400
300
200
100
0
saline
amphetamine
sal/sal
sal/amph
amph/sal
amph/amph
0 15 30 45 60 75 90 105 120
Time (min)

The four groups of rats tested on postnatal day 60 subsequently received repeated injections of amphetamine (2 mg/kg, s.c.) or saline (1 ml/kg, s.c.) on postnatal days 62, 64, and 66. All groups were given a challenge injection of amphetamine (1 mg/kg, s.c.) and tested again on postnatal day 80. In Figure 6, it can be seen that a challenge injection elicited hyper-locomotion in saline-treated rats, and further increase in locomotor response in amphetamine-treated rats. The ANOVA revealed an effect of drug treatment in adulthood ($F_{(1,25)} = 15.5$, $p<.001$), a time by drug treatment interaction ($F_{(7,175)} = 4.6$, $p<.001$), but no effect of early postnatal treatment ($F_{(1,25)} = 0.9$, n.s.). Thus, repeated exposure to amphetamine in adulthood resulted in an augmented locomotor response to the challenge injection of amphetamine. As such, it can be concluded that early postnatal exposure to amphetamine does not alter the responsiveness of adult rats to amphetamine, whereas repeated exposure to amphetamine in the adult leads to an augmented behavioral response.

Figure 6. Time course of the locomotor effects of a single injection of amphetamine after repeated exposure to saline or amphetamine (2 mg/kg, s.c, on three occasions, once every other day) in adulthood. *Asterisks* indicate a significant increase in locomotor activity as compared to saline-treated rats, $p<.05$.


Adult
Activity Counts
400
300
200
100
0
0
15
30
45
60
75
90
105
120
Time (min)
sal/sal
sal/amph
amph/sal
amph/amph

Discussion

It was found here that repeated administration of amphetamine in pre-weanling rats does not induce long-lasting behavioral sensitization. When tested on postnatal days 21, 30, or 60 (7, 16, or 46 days after the last injection), saline- and amphetamine-treated rats showed similar locomotor responses to a challenge injection of amphetamine. In rats tested in adulthood, a subsequent regimen of amphetamine administration induced behavioral sensitization relative to saline controls. Thus, behavioral sensitization was observed only in rats that received repeated exposure to amphetamine in adulthood (after postnatal day 60).

There is conflicting evidence in the literature concerning the question of whether young animals express locomotor sensitization after repeated exposure to psychostimulant drugs. When these studies are considered together, however, it appears that young rats are able to show a sensitized response after prolonged abstinence (7 days or more) if a substantial number of drug exposures are given in a novel context (Snyder, Katovic, & Spear, 1998; Zavala, Nazarian, Crawford, & McDougall, 2000). A persistent sensitized response is typically not observed either when the drug is administered a few times (McDougall, Duke, Bolanos, & Crawford, 1994) or when the drug is not administered in a novel test environment (Fujiwara, Kazahaya, Nakashima, Sato, & Otsuki, 1987; Kolta, Scalzo, Ali, & Holson, 1990; Snyder et al., 1998; Ujike, Tsuchida, Akiyama, Fujiwara, & Kuroda, 1995; Zavala et al., 2000). Furthermore, the evidence suggests that behavioral sensitization is more likely to be expressed in cocaine- rather than amphetamine-treated young rats. Thus, the neurobiological mechanisms that are critical for the long-term expression of either contextual or pharmacological sensitization

are not functionally mature in pre-weanling rats. These factors could have been responsible for the lack of sensitized locomotor responding seen in rats treated prior to weaning.

In pre-weanling rats, the mesolimbic dopamine system is not fully developed. Although dopaminergic neurons and their receptors have been shown to be present in the middle embryonic period (Bruinink, Lichtensteiger & Schlumpf, 1983; Kalsbeek, Voorn & Buijs, 1992; Reisert, Schuster, Zienecker & Pilgrim, 1990; Sales, Martres, Bouthenet & Schwartz, 1989), specific components of the dopaminergic system have been shown to mature at different time points during development. Dopamine content and activity of tyrosine hydroxylase, the rate-limiting enzyme in dopamine synthesis, in the rat striatum was shown to increase linearly up to at least postnatal day 30 (Coyle & Axelrod, 1972; Coyle & Campochiaro, 1976; Giorgi, De Montis, Porceddu, Mele, Calderini, Toffano & Biggio, 1987; Keller, Bartholini & Pletscher, 1973; Nomura, Naitoh & Segawa, 1976; Porcher & Heller, 1972). In addition, studies of [^{3}H]-dopamine uptake (Kirksey & Slotkin, 1979) and [^{3}H]-GBR-12783 or [^{3}H]-mazidol binding (Bonnet & Costentin, 1989; Rao, Molinoff & Joyce, 1991) have shown that the dopamine reuptake transporter becomes fully mature 40 to 60 days after birth. Whereas somatodendritic dopamine D_2 autoreceptors are functionally mature at birth (Noisin & Thomas, 1988; Trent, Nakamura & Tepper, 1991), postsynaptic dopamine receptors in the striatum seem to mature around the time at which rats become susceptible to psychostimulant sensitization. Specifically, the density of striatal D_2 dopamine receptors, labeled by [^{3}H]-spiperone and [^{125}I]-iodobenzamide, reached the adult level 3 weeks after birth in rats (Murrin, 1982; Rao et al., 1991) and were accompanied by an increase in striatal D_2 receptor mRNA level

(Srivastava, Morency & Mishra, 1992). Striatal D_1 receptors, which are located at postsynaptic sites, also reach the adult level at this age (Giorgi et al., 1987; Zeng, Hyttel & Murrin, 1988), and striatal adenylate cyclase activity, which is mediated by activation of D_1 receptors, was shown to reach maturity during this postnatal period (Coyle & Campochiaro, 1976; Pardo, Creese, Burt & Snyder, 1977). As such, the time period at which susceptibility to long-term sensitization begins coincides with the functional maturity of postsynaptic D_1 and D_2 dopamine receptors.

The time course of development of different aspects of the dopaminergic system may explain why amphetamine-induced morphological changes in VTA dopamine neurons, as seen in the first experiment, do not correlate with behavioral sensitization to amphetamine. Although dopamine neurons are present at the time of treatment (Bruinink et al., 1983; Kalsbeek et al., 1992; Reisert et al., 1990; Sales et al., 1989), the number of postsynaptic dopamine receptors are not at adult levels and the maturation of these receptors is not yet complete (Giorgi et al., 1987; Murrin, 1982; Rao et al., 1991; Zeng et al., 1988). The developing brain may adapt to the altered morphology of the dopamine neurons induced by amphetamine such that, upon reaching maturity, the postsynaptic neurons have functionally compensated for any alteration in dopamine neuron function. Importantly, the mesolimbic dopamine system remains plastic as repeated exposure to amphetamine in adulthood, in rats that were pre-exposed to amphetamine during development, induces behavioral sensitization to the locomotor-activating effects of the drug.

In the present study, repeated exposure to 2 mg/kg amphetamine beginning at postnatal day 62 induced long-lasting behavioral sensitization as demonstrated by the

augmented locomotor response to amphetamine observed two weeks after the last drug injection, as compared to animals that had never before received amphetamine. The sensitized locomotor response to amphetamine was independent of the drug treatment given during early development. It is important to note that the doses used to induce behavioral sensitization in the present study were the same as those used to induce morphological changes in the previous one (Experiment 1). Given that the same dose was used in these two studies, and that this dose induces behavioral sensitization in the adult, it is proposed that the dendritic elongation observed in young animals may also occur in the adult. It remains to be tested, however, whether repeated exposure to amphetamine in the adult affects the morphology of VTA dopamine neurons.

Chapter 3

Effects of Early Exposure to Amphetamine on the Expression of Basic Fibroblast Growth Factor in the Ventral Tegmental Area

As demonstrated in Chapter 2, early postnatal exposure to amphetamine was found to induce marked morphological alterations in dopamine neurons of the ventral tegmental area (VTA) in young rats. These morphological changes suggest the operation of neurotrophic factors. The neurotrophic factor, basic fibroblast growth factor (bFGF or FGF-2), is a likely candidate. In a recent study, a series of injections of amphetamine was found to induce long-lasting expression of bFGF in the VTA of adult rats (Flores et al., 1998). bFGF is known to promote the growth of dopamine neurons *in vitro* (Rydel & Greene, 1987) and is known to be expressed in the cell body region of dopamine neurons early in development (Riva & Mocchetti, 1991). Thus, bFGF could play a role in the dendritic elongation of VTA dopamine neurons observed previously (Chapter 2). The following experiment was designed to examine the expression of bFGF in the VTA following repeated exposure to amphetamine during the early postnatal period.

Experiment: bFGF Expression

Until recently, the role of astrocytes in the central nervous system was thought to be one of physical support. This belief has changed in light of several lines of evidence showing that astrocytes play a major role in neural development, neuroprotection, and neural adaptation (i.e., plasticity). These cells have functional receptors for monoamines,

purines, amino acids, prostanoids and peptides; indeed, they have receptors to just about all neurotransmitters found in the central nervous system (see Deschepper, 1998).

Astrocytes have the ability to release neurotrophic factors in response to neuronal injury and stress. One such neurotrophic factor, bFGF, is synthesized and stored within astrocytes (Flott-Rahmel, Gerdes, Pechan, Brysch, Schlingensiepen, & Seifert, 1992; Woodward, Nishi, Meshul, Williams, Coulombe, & Eckenstein, 1992). bFGF is a member of the family of fibroblast growth factors (FGFs) which consists of at least 23 structurally related polypeptides (FGF1-23; see Plotnikov, Schlessinger, Hubbard, & Mohammadi, 1999). bFGF is a potent protein mitogen capable of acting on multiple cells such as fibroblasts, nerve cells, and glial cells (see Baird & Klagsbrun, 1991). This growth factor has been found in relatively high concentrations throughout the brain (Gospodarowics, Cheng, Lui, Baird, & Bohlent, 1984) and bFGF messenger RNA (mRNA) is expressed in a number of areas, including the substantia nigra and the striatum of various mammals (Bean, Elde, Cao, Oellig, Tamminga, Goldstein, Petterson, & Hokfelt, 1991; Cintra, Cao, Oellig, Tinner, Bortolotti, Goldstein, Petterson, & Fuxe, 1991; Ernfors, Lonnerberg, Ayer-LeLievre, & Persson, 1990). bFGF is involved in the differentiation and growth of neurons, and has neurotrophic effects on a number of neuronal populations (Walicke, 1988; Wanaka, Johnson, & Milbrandt, 1991). For example, bFGF has been shown to protect cultured hippocampal and cortical neurons against glutamate toxicity and glucose deprivation (Cheng & Mattson, 1991; Mattson, Murrain, Guthrie, & Kater, 1989).

bFGF has been shown to promote the growth and survival of dopamine cells *in vitro* (Engele & Bohn, 1991; Ferrari, Minozzi, Toffano, Leon, & Skaper, 1989), as well

as *in vivo* (Chadi, Moller, Rosen, Janson, Agnati, Goldstein, Ogren, Petterson, & Fuxe, 1993; Hou, Cohen, & Mytilineou, 1997; Takayama, Ray, Raymon, Baird, Hogg, Fisher, & Gage, 1995). Importantly, this effect is mediated by astrocytes (Engele & Bohn, 1991). In adult animals, bFGF expression is increased after injury to the midbrain dopamine neurons (Chadi, Cao, Petterson, & Fuxe, 1994) and appears to participate in the survival and sprouting of injured neurons (Chadi et al., 1993; Kawamata, Dietrich, Schallert, Gotts, Cocke, Benowitz, & Finklestein, 1997). bFGF protects dopamine neurons from 6-hydroxydopamine (6-OHDA) toxicity (Hou et al., 1997) and protects cultured dopamine neurons against damage induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 1-methyl-4-phenylpyridinium (MPP^+; Otto & Unsicker, 1993). As such, astrocytic bFGF is a potent neurotrophic factor for dopamine neurons, and is well-situated to play a significant role in the survival and plasticity of dopamine neurons.

As demonstrated in Chapter 2, repeated exposure to amphetamine induced dendritic elongation of dopamine neurons in the VTA. Dendritic growth, but not axonal growth, is supported by mature or reactive astrocytes (Le Roux & Reh, 1995; Le Roux & Reh, 1996). Importantly, astrocytes support neurite growth, in part, by release of diffusible factors (Le Roux & Esquenazi, 2002). Thus, morphological alterations in the dendritic arbor of VTA dopamine neurons may be a consequence of the actions of astrocyte-derived bFGF. bFGF induces neurite growth in cultured neurons (Walicke, Cowan, Ueno, Baird & Guillemin, 1986; Rydel & Greene, 1987; Mattson et al., 1989; Katsuki, Itsukaichi & Matsuki, 2000) and promotes neurite re-growth in dopamine neurons following MPTP-induced damage (Mitsumoto, Watanabe, Miyauchi, Jimma &

Morizumi, 2001). Importantly, the presence of neutralizing antibodies for bFGF has been shown to significantly reduce astrocyte-mediated neurite growth of cerebral cortical neurons and cerebellar granule neurons in culture (Hatten et al., 1988; Le Roux & Esquenazi, 2002). Furthermore, in the adult rat, recent studies have demonstrated that repeated intermittent amphetamine administration increased the expression of astrocytic bFGF in the VTA (Flores et al., 1998), and that bFGF was necessary for the development of behavioral sensitization (Flores et al., 2000). Thus, astrocytic bFGF is well situated to play a significant role in the growth and plasticity of dopamine neurons, and may contribute to the dendritic elongation of VTA dopamine neurons observed following repeated amphetamine exposure (Chapter 2).

If astrocytic bFGF plays a pivotal role in amphetamine-induced dendritic growth (Chapter 2), then expression of bFGF in the VTA would be expected to be greater following repeated exposure to amphetamine. Although repeated administration of amphetamine in the adult rat has been shown to increase the expression of astrocytic bFGF in the VTA (Flores et al., 1998), it remains unknown whether a similar increase in bFGF would be seen following early postnatal exposure to amphetamine. In the present experiment, the expression of bFGF in the VTA was examined following repeated exposure to amphetamine during the early postnatal period. In particular, bFGF expression in the VTA was examined 1 week (postnatal day 21) and 16 days (postnatal day 30) after the last of three amphetamine injections.

Method and Procedures

Treatment

Male Wistar rats were obtained from cross-fostered litters as described in Chapter 1. Individual litters were treated with subcutaneous injections of either d-amphetamine sulphate (2 mg/kg, s.c.; SmithKline Beecham Pharma, Oakville, ON) or 0.9% saline (2 ml/kg, s.c.) on postnatal days 10, 12, and 14. Separate groups of amphetamine-treated (n=7) or saline-treated (n=7) rats were killed and perfused on postnatal days 21 and 30, and their brains processed for bFGF and tyrosine hydroxylase (TH) immunohistochemistry.

Immunohistochemistry

bFGF immunoreactivity was detected using a mouse monoclonal antibody purchased from Upstate Biotechnology (Lake Placid, NY). This antibody recognizes the biologically active isoform of bFGF (Matsuzaki, Yoshitake, Matuo, Sasaki & Nishikawa, 1989). For TH immunoreactivity, a rabbit polyclonal antibody obtained from Eugene Tech (Ramsay, NJ) was used. Animals received an overdose of sodium pentobarbital (120 mg/kg) and were perfused transcardially with 75 ml of ice-cold PBS followed by 50 ml of an ice-cold solution of 4% paraformaldehyde (w/v) in 0.1 M phosphate buffer (PB, pH 6.9). When the perfusion was completed, the brains were removed and placed overnight in the fixative solution at 4°C. Coronal sections, 50-μm-thick, were cut on a microtome, collected in ice-cold PB, and stored overnight at 4°C. Tissue sections were processed for bFGF immunohistochemistry according to the avidin-biotin method (Hsu et al., 1981). Briefly, free-floating tissue sections were incubated for 24 h at 4°C with the

anti-bFGF antibody diluted to 1:500 with 0.3% Triton X-100 (Sigma) in PB and 1% normal horse serum (Vector Laboratories, Burlingame, CA). After incubation in the primary antibody, sections were rinsed three times in cold PB and incubated for 1 h at room temperature in a solution of rat adsorbed biotinylated anti-mouse antibody (Vector) diluted 1:200 with PB and 1% normal horse serum. After three 5 min washes in cold PB, sections were then incubated in an avidin–horseradish peroxidase complex (Vectastain Elite ABC Kit, Vector) for 30 min at room temperature, and rinsed again three times (5 min each) in cold PB. Sections were then incubated for 10 min at room temperature and under constant agitation in a solution of 0.05% 3,3'-diaminobenzidine (Sigma) in PB. Without washing, the sections were then transferred to a 3,3'-diaminobenzidene/PB solution, pH 7.8, containing 0.01% H_2O_2 , which catalyzed the reaction, and 8% $NiCl_2$, which darkened the reaction product. Sections were incubated in this solution at room temperature and under constant agitation for 8 min. Special care was taken to maintain this time rigorously constant for all sections processed within one single experiment and throughout the entire study. Three 10 min washes with cold PB terminated this final incubation. Double-labeling for bFGF/TH was performed by processing the sections first for bFGF immunohistochemistry and then for TH immunohistochemistry. The TH antibody was used at a concentration of 1:2000. For TH immunohistochemistry, sections were first incubated in 0.3% Triton X-100 PB and 1% normal goat serum for 1 h at room temperature. Sections were then processed with the avidin-biotin method, as outlined above, with the sole difference being that no $NiCl_2$ was added to the 3,3'-diaminobenzidene/PB/H_2O_2 solution in order to obtain a lighter reaction product.

Histology

Processed tissue slices were wet-mounted onto gelatin-coated slides and were allowed to dry for at least 1 d before being hydrated in distilled water and gradually dehydrated throughout a series of graded alcohol solutions. Slides were cleared in citrosolv (Fisher Scientific) and coverslipped with Permount.

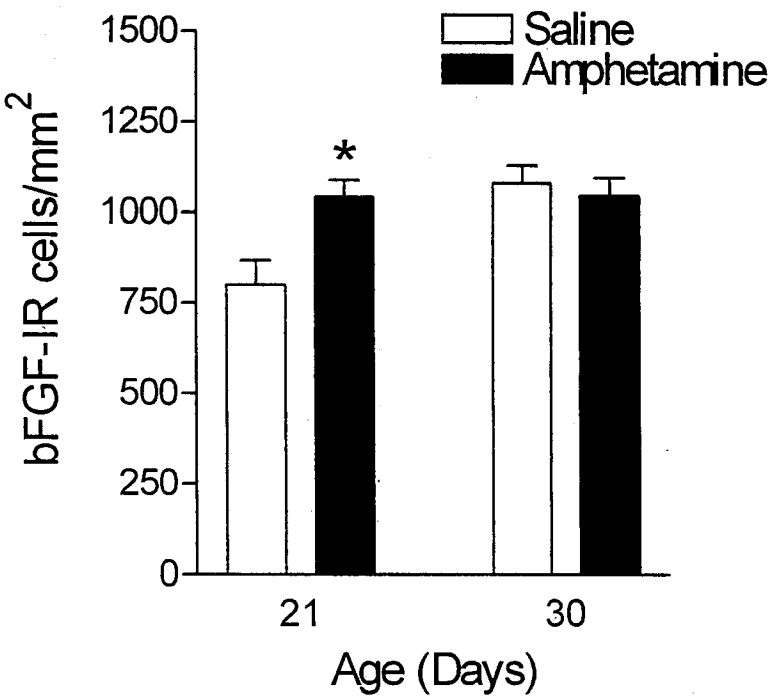
Image Analysis

bFGF immunostained sections were observed under a Leica microscope. Quantitative analysis was conducted using an image analysis system (NIH Image 6.1) on digitized images of sampling areas of the VTA. VTA sampling areas were taken from sections corresponding to plates 38 and 39 (Paxinos & Watson, 1997). Images of the VTA were taken from three sections from each brain. The total number of bFGF immunoreactive cells in the VTA, structurally defined by regional expression of TH immunoreactivity, was quantified per square millimeter. The data were analyzed using a 2 x 2 (age x treatment) between-subjects factorial ANOVA.

Results

Repeated exposure to amphetamine in pre-weanling rats induced a temporal increase in bFGF immunoreactivity in the VTA of postnatal day 21 rats, but not of postnatal day 30 rats. In Figure 7, it can be seen that the number of cells expressing bFGF immunoreactivity was increased in postnatal day 21 rats, but this increase was not maintained in postnatal day 30 rats. The ANOVA revealed a significant effect of age ($F_{(1,24)} = 7.1$, $p<.05$) and an age by treatment interaction ($F_{(1,24)} = 6.7$, $p<.05$). Overall,

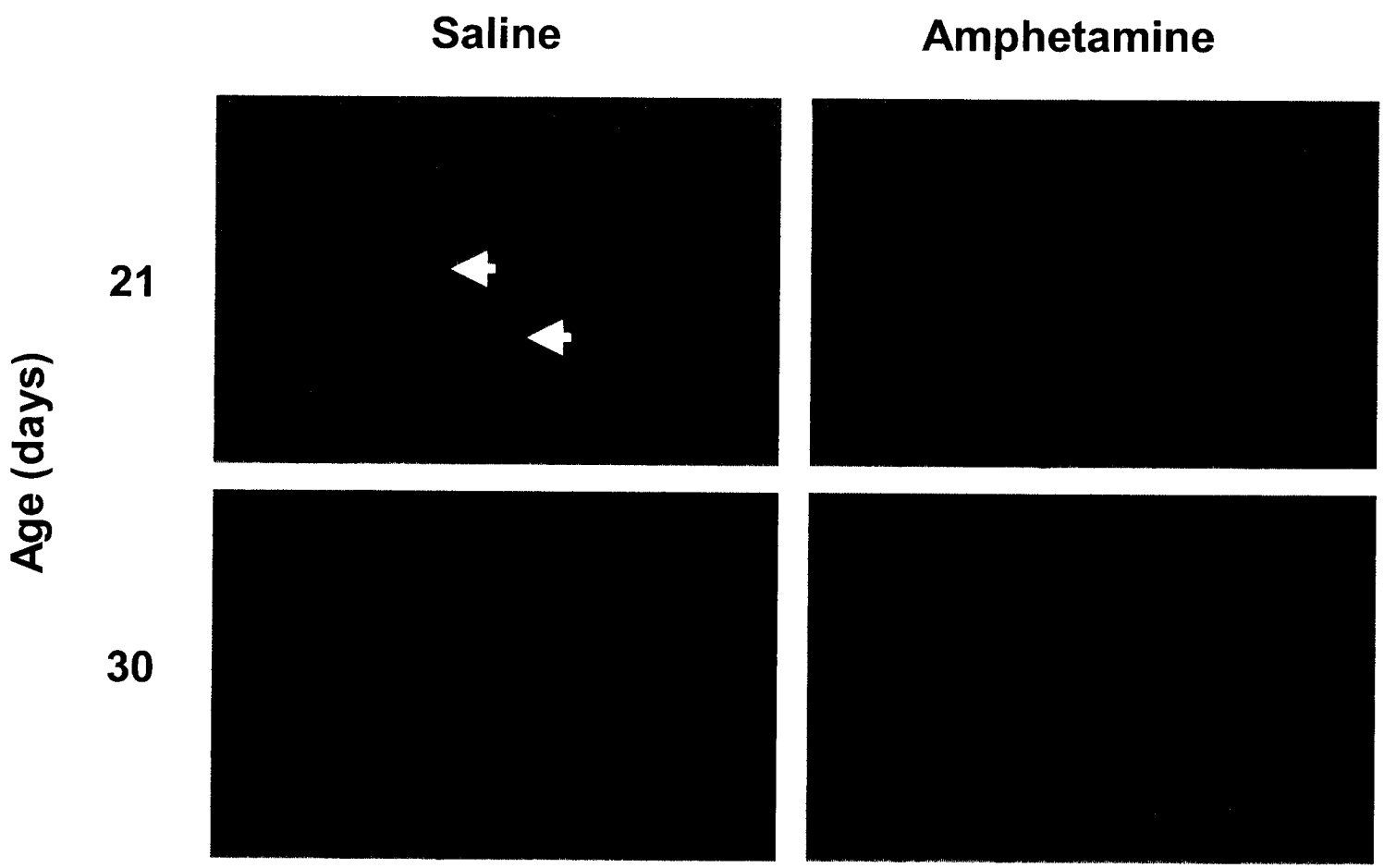
Figure 7. Time course of bFGF expression in the VTA. Mean (±SEM) bFGF-immunoreactive cells (per mm^2) in the VTA of groups (n=7) of saline- or amphetamine-treated animals (2 mg/kg, s.c., on three occasions, once every other day) killed on postnatal day 21 or 30. *Asterisks* indicate significant differences from the saline group, $p<.05$.


Saline
Amphetamine
1500
1250
1000
750
500
250
0
bFGF-IR cells/mm2
*
21
30
Age (Days)

postnatal day 30 rats had a greater number of cells expressing bFGF immunoreactivity than postnatal day 21 rats in the VTA. Only in postnatal day 21 rats, however, was there a significant amphetamine-induced increase in the number of bFGF immunoreactive cells.

Figure 8 shows representative sections of the VTA from rats killed on postnatal day 21 or postnatal day 30, 1 week or 16 days after the last injection of either amphetamine or saline. It can be seen that there was a greater number of darkly-stained cells expressing bFGF immunoreactivity in amphetamine-treated rats on postnatal day 21 than in saline-treated rats. No difference in the number of bFGF immunoreactive cells was found in postnatal day 30 rats regardless of treatment. In both amphetamine- and saline-treated rats, double-labeling for bFGF/TH revealed that cells immunoreactive for TH were not immunoreactive for bFGF.

Figure 8. Digitized images showing darkly labeled bFGF-immunoreactive cells. Images were taken from VTA tissue of representative animals that were injected with either saline or amphetamine (2 mg/kg, s.c.), on three occasions, once every other day, and killed on postnatal day 21 or 30. As shown in the top left panel, bFGF-immunoreactivity (indicated by the *black arrows*) was not found in the large TH-positive cells (see *white arrows*).


Saline
Amphetamine
Age (days)
21
30

Discussion

Repeated administration of amphetamine in pre-weanling rats induced a transient increase in bFGF immunoreactivity in the VTA on postnatal day 21 that was no longer evident on postnatal day 30, as compared to saline-treated controls. The increased expression of bFGF was not associated with TH-immunoreactive cells. Consistent with this finding, bFGF in the VTA is expressed in astrocytes rather than in dopamine neurons (Flores et al., 1998; Flores, Stewart, Salmaso, Zhang & Boksa, 2002), and the effects of bFGF are mediated by astrocytes (Engele & Bohn, 1991).

Increased expression of bFGF in the somatodendritic region of midbrain dopamine neurons is related to the development of sensitization (Flores et al., 1998; Flores & Stewart, 2000b). Sensitization takes time to develop such that the augmented release of dopamine in terminal regions of the VTA, or enhanced locomotor activity, in response to psychostimulant drugs appears 1-3 weeks after the last drug injection (Kolta, Shreve, De Souza & Uretsky, 1985; Vezina, 1993, Wolf, White, Nassar, Brooderson & Khansa, 1993; Paulson & Robinson, 1995). In addition, the development of sensitization is prevented by concurrent treatment with an antibody for bFGF in the VTA with repeated exposure to amphetamine (Flores et al., 2000). As such, the increase in expression of bFGF in the VTA may be part of the cascade of extracellular and intracellular events that lead to enhanced functioning of the mesolimbic dopamine system.

The transient amphetamine-induced increase in bFGF expression coincides with the morphological changes observed in VTA dopamine neurons (Chapter 2). The implication is that bFGF is a potential mediator of the amphetamine-induced dendritic

elongation observed in VTA dopamine neurons (see Chapter 2). As such, the neurotrophic effects of bFGF in the VTA may underlie changes in synaptic efficacy and connectivity, as implicated by amphetamine-induced dendritic elongation. bFGF-induced functional enhancement of synaptic strength has been observed in hippocampal neurons such that bFGF promotes the development of LTP *in vivo* and *in vitro* (Terlau & Seifert, 1990; Ishiyama, Saito & Abe, 1991). In addition, bFGF increases the expression of the AMPA-receptor subunit GluR1 (Cheng et al., 1995). Given that activation of postsynaptic AMPA receptors is involved in the expression of LTP (Manabe, Renner & Nicoll, 1992; Soderling, Tan, McGlade-McCulloh, Yamamoto & Fukunaga, 1994), it is reasonable to consider that bFGF (by increasing levels of GluR1) would potentiate such synaptic processes. Exposure to amphetamine increases bFGF expression in the VTA (Flores et al., 1998; present study), and also enhances synaptic strength at excitatory synapses on VTA dopamine neurons (Saal, Dong, Bonci & Malenka, 2003). Therefore, amphetamine-induced expression of bFGF may modulate the structural alterations associated with synaptic strengthening.

In the developing rat, bFGF plays an important role in the maturation and differentiation of dopamine neurons. In the central nervous system, bFGF immunoreactivity and mRNA levels were found to be low at birth, increasing to adult levels over the first three to four weeks of life (Caday, Klagsbrun, Fanning, Mirzabegian & Finklestein, 1990; Riva & Mocchetti, 1991; Eckenstein, Andersson, Kuzis & Woodward, 1994; Kuzis, Reed, Cherry, Woodward & Eckenstein, 1995). In the present study, a similar pattern of increasing bFGF expression in the VTA was found in saline-treated rats from postnatal day 21 to 30. The developmental expression of bFGF suggests

that the role of bFGF is to regulate the maturation and maintenance of neurons. In concordance with this notion, bFGF has been shown to promote the growth and survival of dopamine neurons (Otto & Unsicker, 1993; Bouvier & Mytilineou, 1995; Takayama et al., 1995; Reuss & Unsicker, 2000), and is neuroprotective against damage induced by 6-OHDA and MPTP (Chadi et al., 1993; Hou et al., 1997). In addition, bFGF mediates neurite-promoting activities on cultured embryonic dopaminergic neurons (Grothe, Schulze, Semkova, Muller-Ostermeyer, Rege & Wewetzer, 2000), a finding in agreement with the suggestion that bFGF mediates dendritic elongation induced by amphetamine (Chapter 2). Several lines of evidence, both *in vivo* and *in vitro*, demonstrate that astrocytes provide a preferred substrate for neuronal process growth (Noble, Fok-Seang & Cohen, 1984; Norris & Kalil, 1991; Smith, Rutishauser, Silver & Miller, 1990). In particular, astrocytes support axon and dendrite growth from embryonic mouse dopaminergic neurons *in vitro* (Rousselet, Fetler, Chamak & Prochiantz, 1988; Rousselet, Autillo-Touati, Araus & Prochiantz, 1990). In addition, a recent report demonstrated that astrocyte-released bFGF is necessary for astrocyte-mediated neurite growth from embryonic cerebral cortical neurons *in vitro* (Le Roux & Esquenazi, 2002). Thus, astrocytic bFGF promotes neurite growth and therefore may underlie amphetamine-induced dendritic elongation of VTA dopamine neurons.

Chapter 4

The Role of Endogenous Basic Fibroblast Growth Factor in Amphetamine-Induced Morphological Alterations of Dopamine Neurons in the Ventral Tegmental Area

As was shown in the experiment described in Chapter 2, repeated exposure to amphetamine in pre-weanling rats induced dendritic elongation in dopamine neurons of the ventral tegmental area (VTA). In addition, it was found that repeated amphetamine injections induced a transient increase in the expression of basic fibroblast growth factor (bFGF or FGF-2) in the VTA 1 week, but not 16 days, after the last injection (Chapter 3). The present set of experiments is concerned with determining whether bFGF mediates the dendritic elongation induced by repeated exposure to amphetamine in pre-weanling rats, and whether bFGF alone is sufficient to induce similar morphological changes in the VTA. As well, the effects of early postnatal exposure to bFGF on the behavioral response to amphetamine in adolescence and adulthood were explored.

Experiment 1: Morphology

Neurotrophic factors are proteins that control the survival, growth, and differentiation of neurons during development. In particular, neurotrophic factors are known to control the growth and retraction of neuronal processes, and are well poised to affect plasticity in the adult nervous system associated with rearrangement of neuronal processes (McAllister, Katz & Lo, 1999; Smith, 1999). Neurotrophic factors can also exert direct, acute effects on the physiological activity of neurons, eliciting changes in

phosphorylation of their receptors and altering many signal transduction cascades that can affect synaptic function and plasticity (Bonhoeffer, 1996; Patterson & Nawa, 1993; Stoop & Poo, 1996).

One such neurotrophic factor that is known to modify synaptic plasticity and morphology of neurons is bFGF. bFGF and its high-affinity receptors are abundant in the embryonic and adult central nervous system (Caday, Klagsbrun, Fanning, Mirzabegian & Finklestein, 1990; Riva & Mocchetti, 1991; Eckenstein, Andersson, Kuzis & Woodward, 1994; Kuzis, Reed, Cherry, Woodward & Eckenstein, 1995; Gomez-Pinilla, Lee & Cotman, 1994; Yazaki, Hosoi, Kawabata, Miyake, Minami, Satoh, Ohta, Kawasaki & Itoh, 1994), and play a pivotal role in neuronal development and function (Dai & Peng, 1995; Desire, Head, Fayein, Courtois & Jeanny, 1998; Unsicker, Grothe, Ludecke, Dorte & Westermann, 1993). bFGF has been shown to exert various biological effects on neurons, such as promotion of progenitor cell proliferation (Ray, Peterson, Schinstine & Gage, 1993), support for cell survival (Walicke, Cowan, Ueno, Baird & Guillemin, 1986), enhancement of neurite extension (Williams, Furness, Walsh & Doherty, 1994) and acceleration of neuritic branching (Aoyagi, Nishikawa, Saito & Abe, 1994). Furthermore, bFGF protects dopamine neurons against damage induced by 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenylpiridium (MPTP; Chadi et al., 1993; Hou et al., 1997). In cultured embryonic dopaminergic neurons, bFGF mediates neurite-promoting activities (Grothe et al., 2000), and promotes neurite re-growth following MPTP-induced damage (Mitsumoto et al., 2001). Furthermore, administration of neutralizing antibodies for bFGF significantly reduced process growth (both dendrite and axon) of cerebral cortical neurons and cerebellar granule neurons (Hatten et al., 1988; Le

Roux & Esquenazi, 2002). The neurotrophic effects of bFGF on dopamine neurons are known to be mediated by astrocytes (Engle & Bohn, 1991), and endogenous astrocytic bFGF is expressed in dopamine cell body regions (Flores et al., 1998; Flores et al., 2002). Therefore, bFGF is well situated as a neurotrophic factor to modify the morphology of dopamine neurons in response to injury or stress.

In the experiment reported in Chapter 2, it was shown that repeated exposure to amphetamine induced structural changes in dopamine neurons of the VTA that were seen 7 to 21 days after drug exposure. This growth in the dendritic arbor of dopamine neurons coincided with an increase in the expression of astrocytic bFGF in the VTA of young rats one week after the cessation of repeated amphetamine injections (Chapter 3). In the adult rat, it was found that repeated injections of amphetamine led to an increase in bFGF expression in the VTA 24 hours, 1 week, and 1 month after the last injection of amphetamine (Flores et al., 1998). These findings suggest that astrocytic bFGF may, in fact, mediate the dendritic growth induced by amphetamine in VTA dopamine neurons.

The purpose of the present study was to determine whether blockade of endogenous bFGF by intracranial infusions of a bFGF antibody would prevent the dendritic elongation induced by repeated exposure to amphetamine in pre-weanling rats. In addition, the effect of early postnatal injections of bFGF on VTA dopamine neuron morphology was examined to determine whether bFGF could induce dendritic elongation in the absence of amphetamine.

Method and Procedures

Early Postnatal Treatments

Male Wistar rats were obtained from cross-fostered litters as described in Chapter 1. In Experiment 1A, individual litters were treated under separate conditions: saline/IgG (S_{igg}), amphetamine/IgG (A_{igg}), saline/anti-bFGF (S_x), or amphetamine/anti-bFGF (A_x). The anti-bFGF antibody used was a mouse monoclonal antibody that recognizes the biologically active conformation of bFGF (Upstate Biotechnology, Lake Placid, NY). This antibody was shown previously in this laboratory to block the effects of induction of bFGF by injections of amphetamine (Flores et al., 2000). Mouse IgG (Upstate Biotechnology) was used as a control. Antibodies were administered intracisternally in isoflurane-anaesthetized rat pups at a concentration of 10 μg/10 μl over 60 s. Following recovery from the anesthetic (5 min), rats were given subcutaneous injections of either 0.9% saline (2 ml/kg, s.c.) or d-amphetamine sulphate (2 mg/kg, s.c.; SmithKline Beecham Pharma, Oakville, ON). Thus, on postnatal days 10, 12, and 14, each litter received intracisternal injections of either anti-bFGF or mouse IgG, followed by subcutaneous injections of either d-amphetamine sulphate or 0.9% saline.

In Experiment 1B, individual litters were injected subcutaneously on postnatal days 10, 12, and 14 with one of three doses (0 ng/g, 1 ng/g, or 3 ng/g) of human recombinant bFGF (R&D Systems). These doses were chosen on the basis that a single systemic injection of 5 ng/g bFGF enhanced neurogenesis (Wagner, Black & DiCicco-Bloom, 1999) and repeated systemic injections of 1 ng/g bFGF enhanced recovery of function following cortical lesions (Waite & Kolb, 2003) in early postnatal rats. The

bFGF protein was dissolved in a vehicle solution consisting of 0.1 M phosphate buffer (PB) with 1% bovine serum albumin (BSA) and 1 mM DTT.

Intracellular Recordings and Histochemistry

Following these postnatal treatments, tissue slices containing the VTA were taken at 21 to 42 days of age. Intracellular recordings and histochemistry were carried out as described in Chapter 1. The morphology of the dopamine neurons was analyzed by drawing the cells via camera lucida and quantifying dendritic length using the concentric ring method of Sholl (1956). The number of intersections of dendrites with a series of concentric circles at 20 μm intervals from the center of the cell body was counted for each cell. An estimate of the mean total dendritic length (in μm) was made by multiplying the mean total number of intersections by 20. The total estimated dendritic length of neurons recorded in slices derived from the different groups of rats was compared using between-subjects ANOVAs. For Experiment 1A, a 2 x 2 (drug x antibody) ANOVA was performed, whereas for Experiment 1B a one-way ANOVA was carried out on the data from the three bFGF dose groups. All significant effects were further analyzed by post hoc mean comparisons using Fisher's least significant differences test.

Results

Exp. 1A: anti-bFGF and amphetamine-induced dendritic elongation

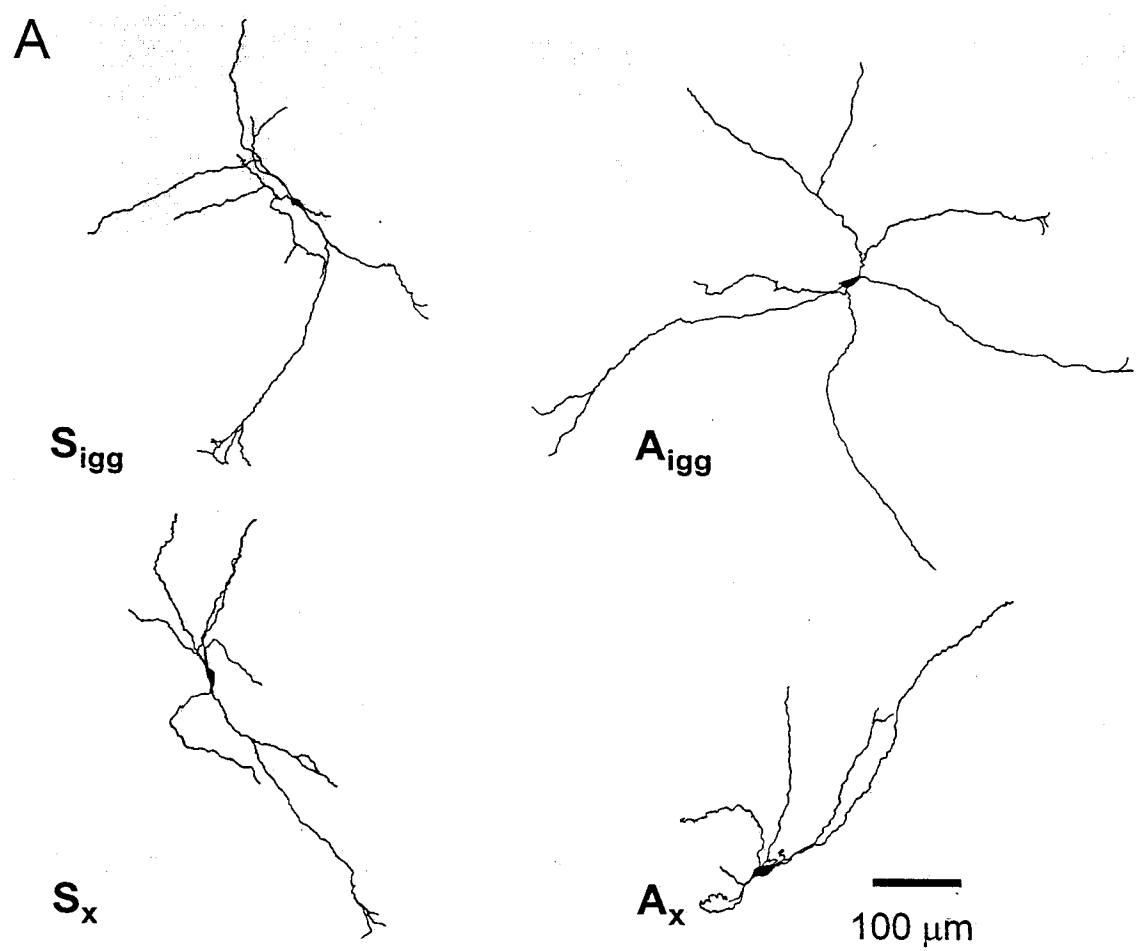
In slices containing the VTA, 30 dopamine neurons were identified by their electrophysiological and pharmacological properties as described previously (Chapter 1).

The mean age of the neurons derived from the four groups did not differ significantly ($F_{(3,26)} = 1.66$, n.s.): S_{igg}, 31.7 ± 1.7 d (n=6), A_{igg}, 27.1 ± 1.6 d (n=9), S_x, 27.3 ± 1.6 d (n=8), and A_x, 27.0 ± 1.7 d (n=7). Figure 9A shows camera lucida drawings of representative dopamine neurons in the VTA from the four different groups of rats. In rats treated with mouse IgG, it can be seen that repeated amphetamine injections resulted in the elaboration of the dendritic arbor of dopamine neurons in the VTA, as compared to repeated saline injections. In rats treated with a neutralizing anti-bFGF antibody, however, no change in the morphology of the dendritic arbor was observed following either repeated injections of amphetamine or saline. The overall dendritic length of dopamine neurons from the 4 different treatment groups are summarized in Figure 9B. ANOVA revealed a significant effect of drug ($F_{(1,26)} = 5.77$, $p<.05$) and antibody ($F_{(1,26)} = 8.06$, $p<.01$). Post hoc analyses revealed that the dendritic length of neurons from A_{igg} rats was greater than neurons derived from rats in any other group ($p<.05$).

Exp. 1B: bfgf and dendritic length

In rats that received postnatal injections of bFGF, a total of 21 VTA dopamine neurons were collected. These neurons came from rats of similar ages, as evidenced by a lack of an effect of age ($F_{(2,18)} = .29$, n.s.). The mean age of the neurons derived from animals treated with one of three doses of bFGF are as follows: 0 ng/g, 26.7 ± 1.9 d (n=7), 1 ng/g, 28.9 ± 1.9 d (n=8), and 3 ng/g, 27.8 ± 2.4 d (n=6). Representative dopamine neurons derived from bFGF-treated rats are shown in Figure 10A. It can be seen that there were no observable differences in the size and complexity of these

Figure 9. Effects of bFGF blockade on early postnatal exposure to amphetamine. *(A)* Camera lucida drawings of representative dopamine neurons in the VTA of S_{igg}, A_{igg}, S_x or A_x treated rats. *(B)* Quantitative analysis of total dendritic length. *Asterisks* indicate a significant increase in dendritic length as compared to S_{igg} rats, $p<.05$.


A
S_{igg}
A_{igg}
S_x
A_x
100 µm

B

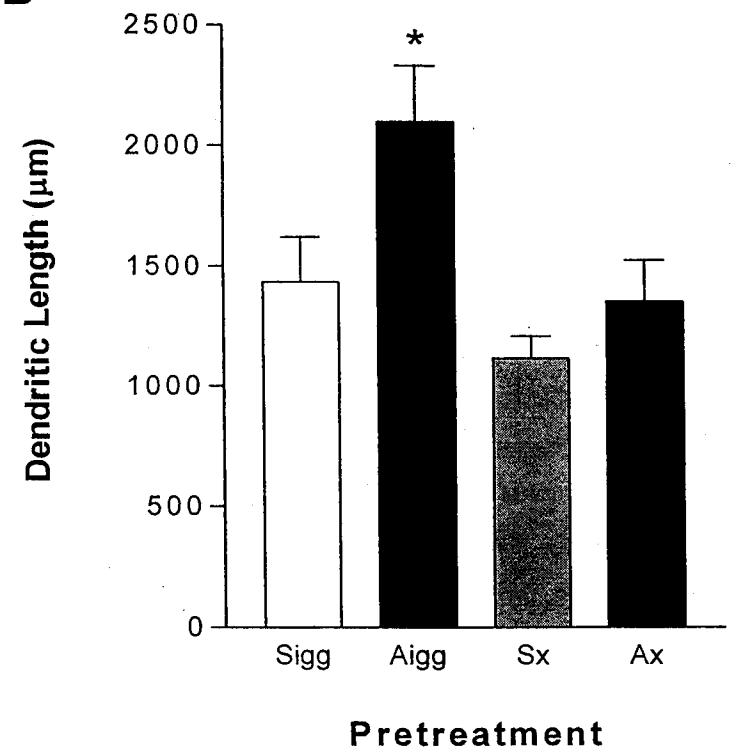
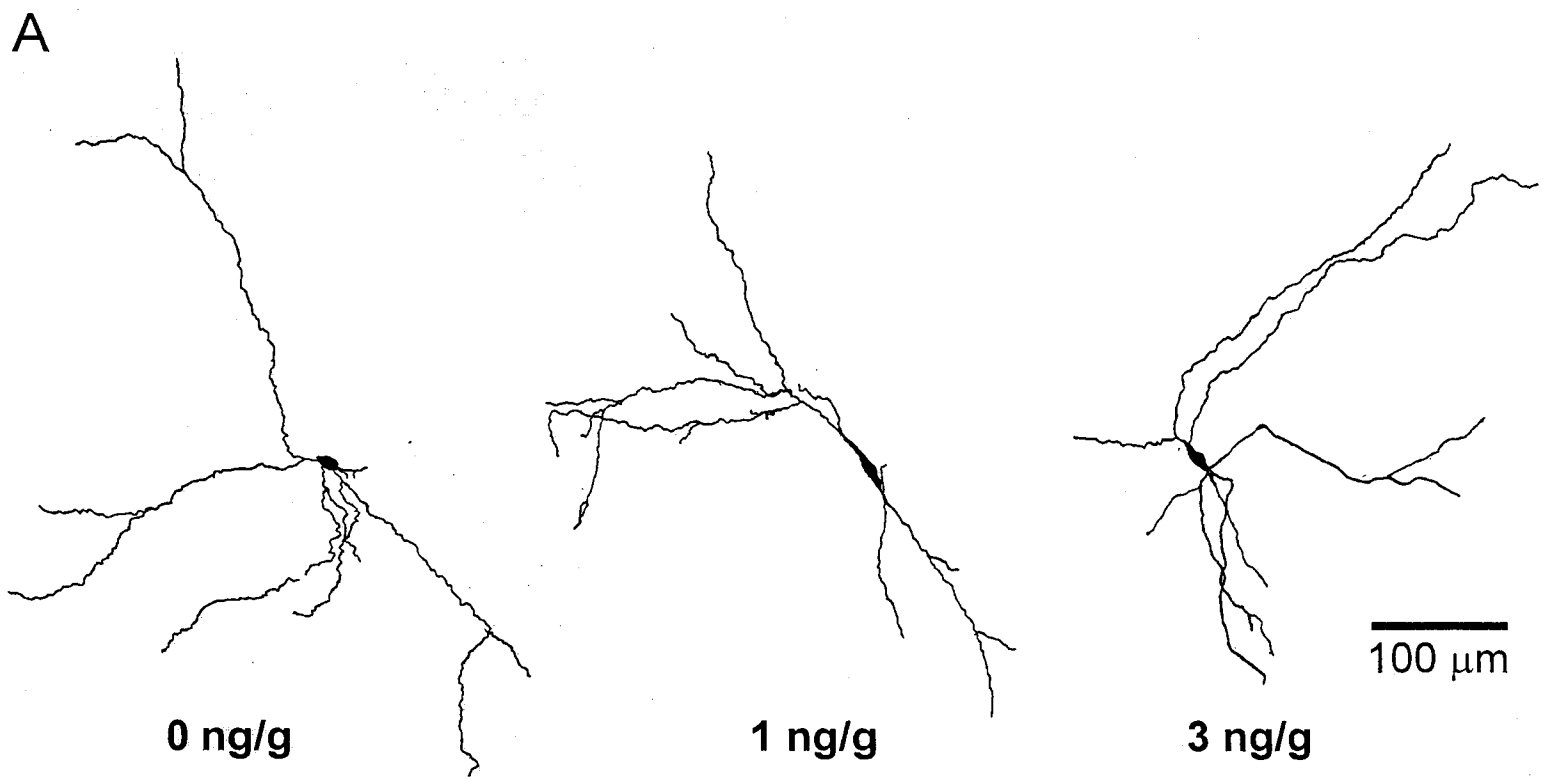
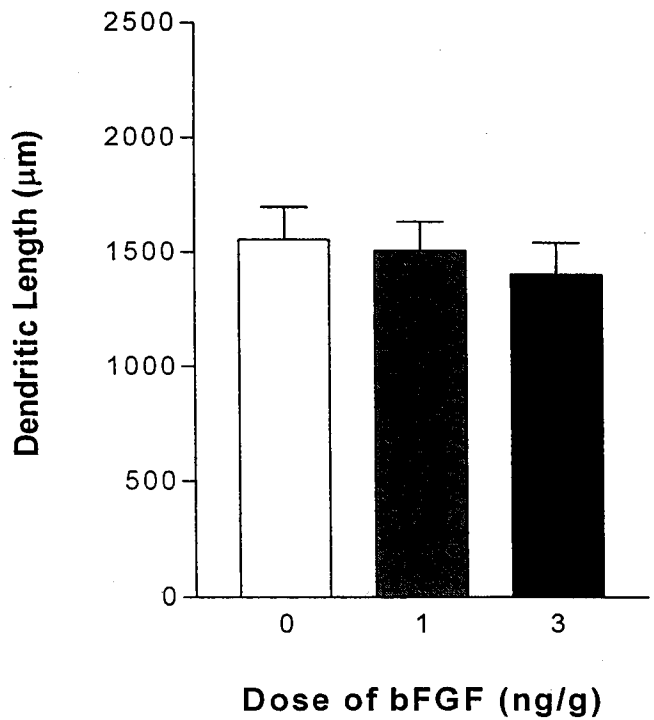

2500
2000
1500
1000
500
0
*
Dendritic Length (µm)
Sigg
Aigg
Sx
Ax
Pretreatment

Figure 10. Lack of morphological changes in VTA dopamine neurons after early postnatal exposure to bFGF. *(A)* Camera lucida drawings of representative dopamine neurons in the VTA of bFGF-treated rats (0, 1, or 3 ng/g, s.c, on three occasions, every other day). *(B)* Quantitative analysis of total dendritic length.


A
100 μm
0 ng/g
1 ng/g
3 ng/g

B


2500
2000
1500
1000
500
0
Dendritic Length (μm)
0
1
3
Dose of bFGF (ng/g)

neurons regardless of the treatment dose of bFGF administered. The overall dendritic length of dopamine neurons is summarized in Figure 10B according to treatment dose of bFGF. There was no significant effect of bFGF dose on dendritic length ($F_{(2,18)} = .78$, n.s.).

Discussion

Consistent with the results from Chapter 2, repeated amphetamine administration in young rats induced dendritic elongation of VTA dopamine neurons. Most importantly, this effect was blocked completely by concurrent treatment with intracisternally administered neutralizing antibodies for bFGF. Thus it appears that amphetamine-induced dendritic elongation in the VTA requires the actions of endogenous bFGF. Exogenously administered bFGF during the early postnatal period, however, did not affect dopamine neuronal morphology. Collectively, these findings indicate that endogenous bFGF is necessary for amphetamine-induced dendritic elongation in the VTA, whereas artificially increasing levels of bFGF by systemically administering bFGF is not sufficient to induce similar morphological changes.

Previous studies in cultured neurons have demonstrated that bFGF induces neurite outgrowth (Walicke et al., 1986; Rydel & Greene, 1987; Mattson, Murrain, Guthrie & Kater, 1989; Katsuki, Itsukaichi & Matsuki, 2000), and that this effect is mediated by astrocytes (Le Roux & Esquanazi, 2002). bFGF has been shown to stimulate neuronal sprouting following diverse types of neuronal injury (Chadi et al., 1993; Date et al., 1993; Kawamata, Dietrich, Schallert, Gotts, Cocke, Benowitz & Finklestein, 1997; Otto, Frotscher & Unsicker, 1990; Ramirez, Finklestein, Keller, Abrams, George & Parakh,

1999), an effect that is prevented by bFGF blockade (Fagan, Suhr, Lucidi-Phillipi, Peterson, Holtzman & Gage, 1997; Rowntree & Kolb, 1997). For example, following damage induced by 6-OHDA and MPTP, cultured dopamine neurons showed increased neurite sprouting and elongation when bFGF was added to the bath (Mitsumoto et al., 2001). As well, following motor cortex injury, blockade of endogenous bFGF by neutralizing antibodies resulted in the retardation of recovery of function (Rowntree & Kolb, 1997). Importantly, bFGF expression in midbrain dopamine neurons is induced following injury-like conditions, such as perinatal anoxia (Flores et al., 2002) or 6-OHDA lesions (Chadi et al., 1994). Overall, it appears that damage to or sufficiently high activation of dopamine neurons results in the increased expression and recruitment of astrocytic bFGF.

In the present study, the trophic actions of endogenous bFGF were necessary for dendritic elongation, but exogenous administration of bFGF was not sufficient to induce changes. Thus, amphetamine must act to facilitate the neurotrophic activity of bFGF directly, or recruit other factors to work synergistically with bFGF. In cultured PC12 cells, which do not express mRNA for bFGF (Zaheer et al., 1995), amphetamine has been shown to induce neurite outgrowth independent of neurotrophic factors (Park et al., 2002). Furthermore, amphetamine-induced neurite outgrowth in cultured PC12 cells has been shown to be mediated by protein kinase C (PKC) and mitogen activated protein kinase (MAPK; Park et al., 2003). Interestingly, in cultured neurons, the activation of the high-affinity bFGF receptor, FGFR1, results in the activation of both PKC and MAPK (Perron & Bixby, 1999; Kiryushko, Berezin & Bock, 2004). Thus, it is possible that

amphetamine and bFGF may act synergistically through a common mechanism to promote dendritic growth.

Both bFGF and amphetamine can act to enhance calcium signaling pathways. Calcium is a key second messenger that mediates neurite outgrowth and elongation (Lankford & Letourneau, 1989; Mattson & Kater, 1987). In neurons, bFGF is known to enhance intracellular calcium concentrations (Abe & Saito, 1992; Tanaka et al., 1996). When bFGF is bound to its high affinity receptor, it activates an intracellular cascade of events that promotes calcium influx leading to neurite outgrowth (Williams et al., 1994; see General Discussion for details). Amphetamine, on the other hand, enhances calcium signaling through glutamate transmission. Amphetamine induces glutamate release in the VTA (Wolf & Xue, 1999; Xue et al., 1996) which, in turn, activates NMDA and AMPA receptors on dopamine neurons. Interestingly, dendritic growth in cultured embryonic hippocampal neurons has been shown to be dependent on the actions of glutamate, as chronic bath application of glutamate receptor antagonists arrested the growth of dendrites (Nuijtinck, Baker, Ter Gast, Struik, & Mud, 1997). NMDA receptors, and to some extent AMPA receptors, are conduits through which calcium transmission and signaling can be amplified. Stimulation of all AMPA receptor subtypes results in membrane depolarization via sodium influx; only AMPA receptors lacking the GluR2 subunit are calcium permeable (Tanaka et al., 2000). Membrane depolarization is necessary to remove the tonic block of the NMDA receptor channels by magnesium. When activated, NMDA receptors further depolarize the membrane through calcium and sodium influx. The combined membrane depolarization mediated by AMPA and NMDA receptors results in the activation of voltage-dependent channels, including L-type

calcium channels. L-type calcium channels inactivate slowly and produce a more sustained influx of calcium than other voltage-dependent calcium channels or ionotropic glutamate receptors (Jones, 1998).

The activation of the high affinity receptor for bFGF leads to a chemical cascade that modulates the function of L-type calcium channels, allowing greater calcium influx (Williams et al., 1994; Kiryushko et al., 2004). Furthermore, L-type calcium channel blockers prevent bFGF-induced neurite growth (Williams et al., 1994; Shitaka, Matsuki, Saito & Katsuki, 1996; Katsuki et al., 2000). bFGF has been shown to increase the number of functional L-type calcium channels (Shitaka et al., 1996), which could lead to long-term changes in calcium signaling. Thus, both amphetamine and bFGF may work synergistically to enhance calcium signaling which, in turn, is required for neurite growth (Lankford & Letourneau, 1989; Mattson & Kater, 1987).

Changes in the VTA, as a result of repeated amphetamine exposure, lead to the development of behavioral sensitization (Vezina, 2004). These changes include dendritic elongation (Chapter 2), which may serve to enhance the responsiveness of VTA dopamine neurons to afferent inputs. Both L-type calcium channels (Karler et al., 1991b) and bFGF (Flores et al., 2000) have been shown to be necessary for the development of sensitization. Given that bFGF can modify the function of L-type calcium channels to increase calcium influx (Williams et al., 1994; Kiryushko et al., 2004), and that calcium is necessary for dendritic growth (Lankford & Letourneau, 1989; Mattson & Kater, 1987), it is reasonable to expect that bFGF mediates amphetamine-induced dendritic elongation via enhancement of calcium signaling.

As discussed previously, dendritic elongation may require the synergistic actions of amphetamine and bFGF, such that bFGF alone may not be sufficient to induce dendritic growth. In the present study, repeated systemic administration of bFGF did not alter the morphology of VTA dopamine neurons. It is possible, however, that the doses of bFGF were not sufficiently high enough to stimulate dendritic growth. The doses of bFGF used in the present study were based on previous studies. For example, a single subcutaneous infusion of 5 ng/g bFGF was sufficient to induce a 25-30 % increase in the number of neural precursor cells engaged in mitosis in the dentate gyrus and subventricular zone of postnatal day 7, 14, and 21 rats (Wagner et al., 1999). Furthermore, following cortical lesions after birth, rats that received subcutaneous infusions of 1 ng/g bFGF for 7 days showed fewer behavioral deficits in adulthood than in untreated rats (Waite & Kolb, 2003). Golgi analysis on the postnatal brains showed an increase in length of the distal portions of cortical pyramidal cells, suggesting that exogenously administered bFGF promoted dendritic elongation. Other studies, however, indicate that higher doses of bFGF may be required. For example, suppression of locomotor behaviour induced by systemic bFGF administration was seen at much higher doses of bFGF, ranging from 1 to 100 μg/g (Guaza, Garcia-Andres, Sandi, Munoz-Willery, Cuevas, & Gimenez-Gallego, 1996). Although systemic injections of bFGF have been shown to enhance recovery of function and growth, and bFGF has been shown to cross the blood-brain barrier during early postnatal development (Wagner et al., 1999), central administration, via intracisternal injections, may have produced different results. For example, following focal cerebral infarction in rats, intracisternally administered bFGF promoted recovery of function and upregulated a marker for neuronal sprouting

(Kawamata et al., 1997). Thus, central administration of bFGF may have influenced dendritic growth, whereas the doses of bFGF administered subcutaneously in the present study may not have been sufficiently high to induce dendritic growth. Another possibility, as discussed above, is that injury or excessive activation of neurons may be required for bFGF to exert its neurotrophic effects.

In summary, repeated exposure to amphetamine in the early postnatal period resulted in dendritic elongation, an effect that was blocked by co-administration of an anti-bFGF antibody. Exogenous administration of bFGF did not alter the morphology of VTA dopamine neurons, indicating that bFGF alone is not sufficient to induce dendritic elongation. Clearly, amphetamine-induced dendritic elongation in VTA dopamine neurons is dependent on the actions of endogenous bFGF, but additional factors stimulated by amphetamine also appear to be required to facilitate growth.

Experiment 2: Locomotor Activity

Basic fibroblast growth factor (bFGF or FGF-2) is present throughout the central nervous system (Caday et al., 1990; Kuzis et al., 1995; Gomez-Pinilla et al., 1994), and is known to affect the growth, function, and survival of neural cells *in vitro* and *in vivo* (Ferrari et al., 1989; Chadi et al., 1993; Bouvier & Mytilineou, 1995; Takayama et al., 1995; Hou et al., 1997; Reuss & Unsicker, 2000; Walicke et al., 1986). In addition to having a regulatory role in the maintenance and survival of neurons, bFGF can also exert acute effects on the physiological activity of neurons (Stoop & Poo, 1996), and subsequently on behavior.

Few studies have examined the effects of exogenously administered bFGF on behavior. Acutely, bFGF has been reported to decrease locomotor activity in a novel environment whether injected systemically (Guaza, Garcia-Andres, Sandi, Munoz-Willery, Cuevas, & Gimenez-Gallego, 1996) or centrally (Hotta, Kuriyama, Arai, Takano & Shibasaki, 2001). Interestingly, transgenic mice expressing a mutant form of the FGFR1 receptor, in which the tyrosine kinase domain is deficient, show increased locomotor activity that is exacerbated by moderate doses of amphetamine (Shin, Korada, Raballo, Shashikant, Simeone, Taylor & Vaccarino, 2004). Thus, it appears that acute administration of bFGF suppresses locomotor behavior.

To date, the long-term consequences of repeated administration of bFGF on locomotor activity have not been examined. Thus, the purpose of the present study was to examine the effects of repeated exposure to bFGF during the early postnatal period on locomotor activity 7, 16, and 46 days after the last bFGF infusion. In addition, rats tested in adulthood received repeated amphetamine injections and were subsequently tested for locomotor responsiveness to a challenge injection of amphetamine 2 weeks later.

Method and Procedures

Treatment

As previously described in Chapter 1, male Wistar rats were obtained from cross-fostered litters. Individual litters were injected subcutaneously on postnatal days 10, 12, and 14 with 0, 1, or 3 ng/g of human recombinant bFGF (R&D Systems). The bFGF protein was dissolved in a vehicle solution consisting of 0.1 M phosphate buffer (PB) with 1% bovine serum albumin (BSA) and 1 mM DTT.

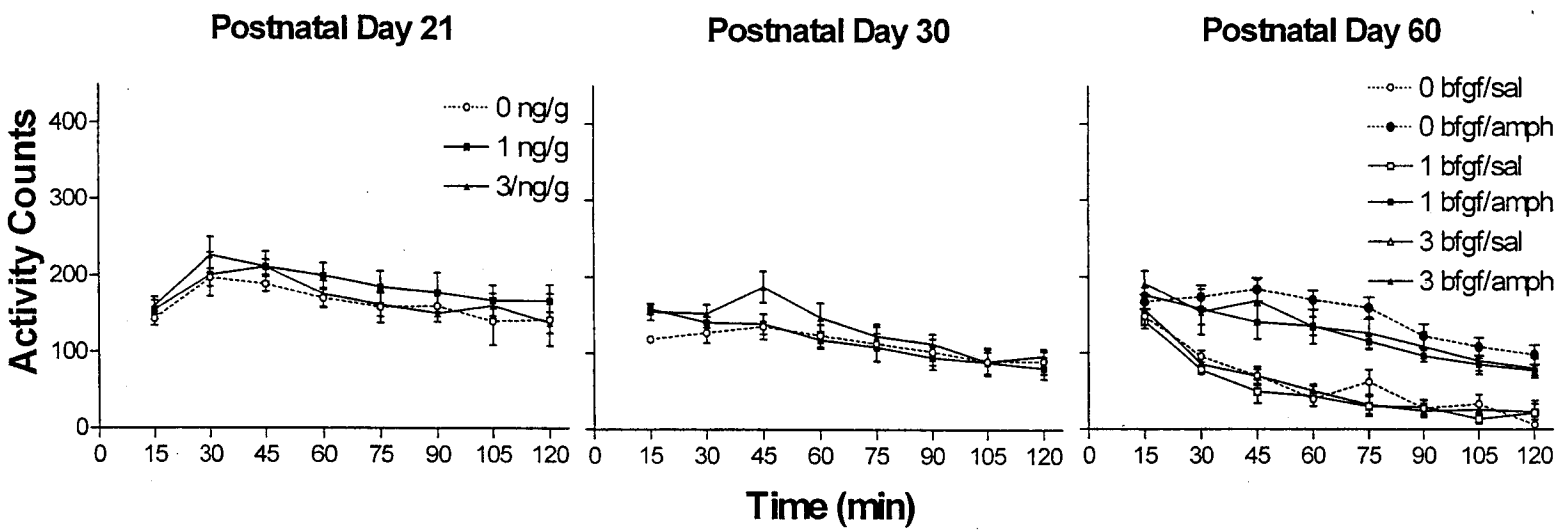
Locomotor Activity

The locomotor activity boxes used in these experiments have been described previously (see Chapter 2). Separate groups of rats, treated with one of three doses of bFGF (0, 1, and 3 ng/g) were tested for locomotor activity on postnatal days 21, 30, or 60. All animals tested on postnatal days 21 or 30 were injected with 1 mg/kg amphetamine (s.c.) prior to being placed in locomotor activity boxes for measurement of locomotor activity. Animals tested on postnatal day 60 were divided into six separate groups according to treatment (0, 1, or 3 ng/g bFGF) and drug at test (saline/amphetamine). Animals tested on postnatal day 60 were subsequently administered saline (1 ml/kg, s.c.) or amphetamine (2 mg/kg, s.c.) on postnatal days 69, 71, and 73, and retested 2 weeks later. For the final test, all animals were administered amphetamine (1 mg/kg, s.c.). For all tests, locomotor activity was recorded over 2 h. At each time point tested, total activity counts were analyzed using a treatment by time (in 15 min bins) mixed ANOVA.

Results

Rats exposed to 0, 1, or 3ng/g bFGF on postnatal days 10, 12, and 14, regardless of dose, showed a similar degree of behavioral activation following a 1.0 mg/kg dose of amphetamine at test on postnatal day 21, 30 or 60 (see Figure 11). There was no significant effect of treatment in rats tested on either postnatal day 21 ($F_{(2,18)} = 0.46$, n.s.) or postnatal day 30 ($F_{(2,18)} = 0.89$, n.s.). Rats tested first on postnatal day 60 were divided into 6 groups according to bFGF treatment (0, 1, and 3 ng/g) and drug at test

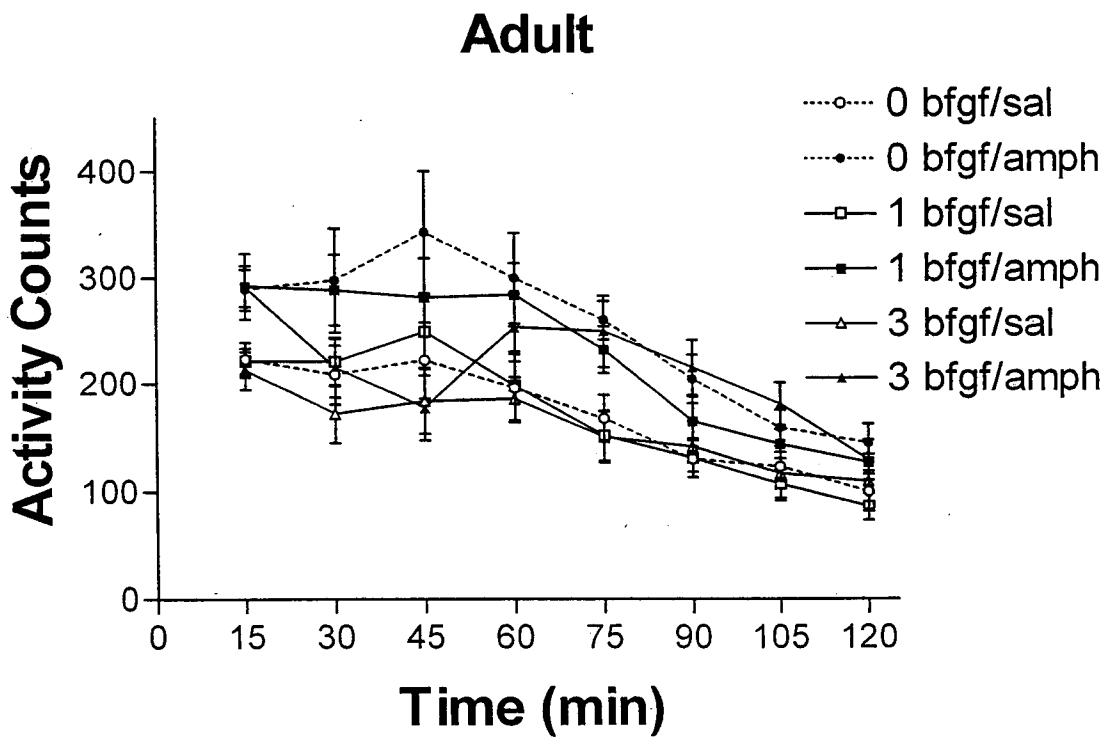
Figure 11. Time course of the locomotor effects of a single injection of amphetamine (1 mg/kg, s.c.) after early postnatal exposure to bFGF (0, 1, or 3 ng/g, s.c., on three occasions, once per day). Following treatment, groups of rats (n=8) were tested on either *(A)* postnatal day 21, *(B)* 30, or *(C)* 60.


Postnatal Day 21
Postnatal Day 30
Postnatal Day 60
Activity Counts
0
100
200
300
400
0 15 30 45 60 75 90 105 120
0 ng/g
1 ng/g
3/ng/g
0 bfgf/sal
0 bfgf/amph
1 bfgf/sal
1 bfgf/amph
3 bfgf/sal
3 bfgf/amph
Time (min)

(saline/amphetamine). Figure 11C shows that, regardless of treatment, rats that received amphetamine at test were more active than rats that received saline at test. The ANOVA revealed a main effect of the drug given at test ($F_{(1,36)} = 91.44$, $p<.0001$), but no effect of treatment ($F_{(2,36)} = 1.48$, n.s.). Thus, early exposure to bFGF did not alter sensitivity to amphetamine in rats tested after weaning or in adulthood.

The 6 groups of rats tested on postnatal day 60 subsequently received repeated injections of amphetamine (2 mg/kg, s.c.) or saline (1 ml/kg, s.c.) on postnatal days 69, 71, and 73. All groups were given a challenge injection of amphetamine (1 mg/kg, s.c.) and tested again on postnatal day 87. The challenge injection elicited hyper-locomotion in all rats, a response that was further augmented in rats that had previously received repeated administration of amphetamine (Figure 12). The ANOVA revealed no effect of early postnatal treatment with bFGF ($F_{(2,36)} = 0.68$, n.s.), but did reveal an effect of drug treatment in adulthood ($F_{(1,36)} = 14.22$, $p<.001$), and a time by bFGF treatment interaction ($F_{(14,252)} = 3.41$, $p<.001$). Thus, repeated exposure to amphetamine in adulthood resulted in an augmented locomotor response to the challenge injection of amphetamine as compared to rats exposed to saline. Although bFGF treatment did not have an effect on locomotor activity in response to amphetamine, rats treated with 3ng/g bFGF did show a significant transient decrease in activity that peaked at 45 min ($p<.05$). This finding may be explained by an increase in stereotypy induced by amphetamine, such that the locomotor behavior was suppressed. It is possible that early postnatal exposure to bFGF may alter the stereotypical responses of rats to amphetamine in adulthood. Overall, repeated exposure to amphetamine in the adult leads to an augmented behavioral response to an acute injection of amphetamine.

Figure 12. Time course of the locomotor effects of a single injection of amphetamine after repeated exposure to saline or amphetamine (2 mg/kg, s.c, on three occasions, once every other day) in adulthood in rats previously exposed to bFGF.


Adult
Activity Counts
400
300
200
100
0
0
15
30
45
60
75
90
105
120
Time (min)
0 bfgf/sal
0 bfgf/amph
1 bfgf/sal
1 bfgf/amph
3 bfgf/sal
3 bfgf/amph

Discussion

Repeated systemic injections of bFGF during development did not affect subsequent responsiveness to an acute injection of amphetamine in adolescence and adulthood. There are a number of possible explanations for the lack of effects on amphetamine-induced locomotor activity. Although circulating levels of bFGF were increased at the time of the injections, those levels likely normalized long before the rats were tested for locomotor activity. Furthermore, the doses of bFGF that were administered may not have been sufficiently high enough to induce plastic changes in the cytoarchitecture of the developing rat brain, changes that would be necessary for an increased sensitivity to amphetamine. As demonstrated in Experiment 1B above, the same regimen of bFGF injections did not alter the morphology of VTA dopamine neurons. In addition, the acute suppressive effects of systemically administered bFGF on locomotor activity were elicited by much higher doses (1 to 100 μg/kg; Guaza et al., 1996). Thus, early postnatal treatment with bFGF does not appear to alter subsequent sensitivity to the behavioral activating effects of amphetamine.

As expected, behavioral sensitization was induced following repeated amphetamine injections in adulthood. In the adult animal, repeated exposure to psychostimulants results in an augmentation of the behavioral activating effects of these drugs (Kalivas & Stewart, 1991; Robinson & Becker, 1986). Rats that received the highest dose of bFGF (3 ng/g) during the early postnatal period, and that were subsequently treated with repeated amphetamine exposure in adulthood, showed a pattern of locomotor activity that suggested an increase in stereotypic behaviors in response to a challenge injection of amphetamine. Interestingly, FGFR1 receptor deficient mice show

hyperlocomotion and stereotypic behavior that is worsened by the effects of amphetamine (Shin et al., 2004). Perhaps early postnatal bFGF treatment alters the developmental expression of FGFR1 that is later revealed by the stereotypy induced by repeated exposure to amphetamine. Thus, early postnatal exposure to bFGF may alter the responsiveness of adult rats to repeated administration of amphetamine such that the effects of the drug on stereotypic behaviors are enhanced.

General Discussion

The present set of experiments demonstrates that repeated exposure to amphetamine during the early postnatal period induces dendritic growth in dopamine neurons of the ventral tegmental area (VTA). This is the first demonstration of long-term amphetamine-induced changes in the cytoarchitecture of VTA dopamine neurons. Previous research has shown that repeated exposure to amphetamine in juvenile or adult rats increases dendritic length in the postsynaptic targets of VTA dopamine neurons, including neurons in the nucleus accumbens (NAcc) and prefrontal cortex (Robinson & Kolb, 1997; Heijtz et al., 2003). Taken together, these findings demonstrate that the neurons of the mesolimbic dopamine system, including the VTA and its targets, undergo significant alterations in morphology as a consequence of repeated exposure to psychostimulant drugs.

The amphetamine-induced dendritic elongation of VTA dopamine neurons corresponded with a transient increase in the expression of astrocytic basic fibroblast growth factor (bFGF or FGF-2) in the VTA 1 week, but not 16 days, after the last of three injections of 2 mg/kg amphetamine. Similarly, recent studies have demonstrated that repeated intermittent amphetamine administration increased the expression of astrocytic bFGF in the VTA of adult rats (Flores et al., 1998), and that bFGF was necessary for the development of behavioral sensitization (Flores et al., 2000). bFGF is neurotrophic for dopamine neurons, and has been shown to promote their growth and survival (Chadi et al., 1993; Hou et al., 1997; Takayama et al., 1995). In addition, bFGF promotes neurite growth (Walicke et al., 1986; Rydel & Greene, 1987; Mattson et al., 1989; Katsuki et al.,

2000) and neurite re-growth in dopamine neurons following MPTP-induced damage (Mitsumoto et al., 2001). The presence of neutralizing antibodies for bFGF has been shown to significantly reduce astrocyte-mediated neurite growth of cerebral cortical neurons and cerebellar granule neurons in culture (Hatten et al., 1988; Le Roux & Esquenazi, 2002). Thus, astrocytic bFGF is well poised to play a significant role in the growth and plasticity of dopamine neurons

A second major finding from these experiments is that the dendritic elongation of VTA dopamine neurons observed following repeated amphetamine exposure is dependent on the actions of endogenous bFGF, inasmuch as co-administration of a neutralizing anti-bFGF antibody prevented the morphological changes. Furthermore, although endogenous bFGF appears to play a pivotal role in amphetamine-induced dendritic elongation, exogenously administered bFGF did not induce similar growth. These findings indicate that endogenous bFGF is necessary for amphetamine-induced dendritic elongation in the VTA, whereas artificially increasing levels of bFGF by systemically administering bFGF is not sufficient to induce similar morphological changes.

Alterations in the structure of VTA dopamine neurons were not associated with changes in sensitivity to the locomotor activating effects of amphetamine. Repeated amphetamine exposure during the early postnatal period did not alter locomotor behavior in response to an acute administration of amphetamine in adolescence or adulthood. Interestingly, a recent study demonstrated that although repeated administration of amphetamine induced dendritic growth in neurons of the prefrontal cortex, this treatment was not associated with alterations in the subsequent motor response to amphetamine

(Heijtz et al., 2003). The results of these studies are consistent with a large body of literature demonstrating that repeated administration of psychostimulant drugs during the first three weeks of life does not induce behavioral sensitization to a challenge injection given ten days to three weeks following the last psychostimulant exposure (Tsuchida et al, 1994; Ujike et al., 1995; Fujiwara et al, 1987; Scalzo & Holson, 1992). As discussed in Chapter 2, the inability of amphetamine exposure in the early postnatal period to induce behavioral sensitization may be a consequence of an underdeveloped dopamine system and its connections. Although dopamine neurons are present at the time of treatment (Bruinink et al., 1983; Kalsbeek et al., 1992; Reisert et al., 1990; Sales et al., 1989), the number of postsynaptic dopamine receptors are not at adult levels and the maturation of these receptors is not yet complete (Giorgi et al., 1987; Murrin, 1982; Rao et al., 1991; Zeng et al., 1988). Importantly, it was observed that repeated administration of amphetamine in adulthood, at the same dose (2 mg/kg) as was used in the present studies, induced behavioral sensitization to an acute administration of amphetamine two weeks following the last injection. Thus, there is reason to think that the changes observed in the dendritic arbor of the dopamine neurons of young animals may also occur in adult animals. We know, for example, that repeated exposure to amphetamine enhances the release of dopamine from dopamine neurons in response to a subsequent injection of amphetamine (Kalivas, 1993), an effect that could be due, in part, to greater sensitivity of the somatodendritic field of these neurons to afferent inputs (see below). Furthermore, amphetamine-induced increases to the dendritic arbor of prefrontal cortical neurons has been shown in both juvenile (Heijtz et al., 2003) and adult rats (Robinson &

Kolb, 1997). Whether repeated exposure to amphetamine in the adult affects the morphology of VTA dopamine neurons, however, remains to be determined.

The remainder of the discussion is concerned with exploring the consequences of dendritic elongation in VTA dopamine neurons, the role of bFGF in psychostimulant-induced sensitization, and the possible mechanisms underlying bFGF-mediated dendritic growth. The implications of changes in neural circuitry for psychostimulant abuse and possibilities for future research will be raised.

Structural Changes in Mesolimbic Dopamine Circuitry

Changes in the VTA following repeated administration of amphetamine increase the sensitivity of dopamine neurons to synaptic inputs. Initially, a number of transient changes occur in the VTA that result in an increase in basal firing rate and in total number of spontaneously active dopamine neurons. These changes include electrophysiological sub-sensitivity of somatodendritic dopamine autoreceptors in the VTA (Ackerman & White, 1990; White & Wang, 1984), enhanced AMPA receptor transmission (Giorgetti et al., 2001; Zhang et al., 1997) and an increase in the GLUR1 subunit of the AMPA receptor (Carlezon & Nestler, 2002; but see Lu, Monteggia & Wolf, 2002). These transient changes promote greater somatodendritic dopamine release and increased responsiveness to excitatory glutamatergic inputs. Accompanying the increased sensitivity of VTA dopamine neurons to glutamate is an increase in extracellular glutamate levels during (Kalivas & Duffy, 1995; Kalivas & Duffy, 1998) or after (Xue, Ng, Li & Wolf, 1996: Wolf & Xue, 1998) amphetamine administration. The long-term maintenance of the increased sensitivity to synaptic inputs in VTA dopamine

neurons can be achieved by increasing the number of synaptic contacts via the long-lasting dendritic elongation of VTA dopamine neurons. As the length of the dendritic branches of VTA dopamine neurons increases, so does the total dendritic surface area. Importantly, it has been shown that the number of synaptic contacts increases in proportion to increases in dendritic surface (Harris & Kater, 1994). As such, dendritic growth could account for an increase in VTA dopamine neuron sensitivity to synaptic inputs.

Changes in the responsiveness of VTA dopamine neurons to synaptic inputs have been observed following psychostimulant exposure, including increases in synchronous burst firing in response to stimulation (Overton & Clark, 1992; Tong, Overton & Clark, 1995). The enhanced responding of dopamine neurons to excitatory inputs consequently results in augmented dopamine overflow in the NAcc (Robinson et al., 1988; Kalivas & Duffy, 1993), which may place excessive demands on neurons in terminal regions. In fact, repeated psychostimulant administration is also associated with persistent changes in the physical structure of these neurons (Robinson & Kolb, 1997; Robinson & Kolb, 1999). For example, cells in the NAcc and prefrontal cortex show changes in the length of dendrites and the extent to which dendrites are branched. Changes also occur in the density and types of dendritic spines, the primary site of excitatory glutamate synapses. These sensitization-related changes in dendritic structure may reflect changes in patterns of synaptic connectivity within these brain regions and therefore may alter information processing within the mesolimbic dopamine system. In general, it is now well accepted that sensitization is accompanied by a major reorganization of brain reward systems

(Pierce & Kalivas, 1997; Wolf, 1998; Robinson & Berridge, 2000; Hyman & Malenka, 2001; Everitt & Wolf, 2002; Vanderschuren & Kalivas, 2000).

The structural and functional changes induced by psychostimulant exposure in the mesolimbic dopamine system have been proposed to underlie compulsive drug seeking, drug taking, and relapse (Robinson & Berridge, 1993; Robinson & Berridge, 2000). The central idea is that exposure to addictive drugs results in enduring changes in NAcc-related brain systems that mediate a basic incentive-motivational function, the attribution of incentive salience. As a consequence, these neural circuits become hypersensitive to specific drug effects and to drug-associated stimuli. Robinson and Berridge (1993) proposed that this results in excessive attribution of incentive salience to drug-related representations, causing pathological "wanting" to take drugs. These authors suggest that addiction is a disorder of aberrant incentive motivation due to drug-induced sensitization of neural systems that attribute salience to particular stimuli (Robinson & Berridge, 2003). The morphological changes in VTA dopamine neurons may contribute by making the neurons more responsive to stimuli associated with drugs.

bFGF and Functional Enhancement of the Mesolimbic Dopamine System

The dendritic growth observed in VTA dopamine neurons following repeated amphetamine exposure was demonstrated to be dependent on the actions of endogenous bFGF. Neutralization of bFGF prevented the amphetamine-induced structural modifications. Thus, bFGF is involved in the induction of dendritic growth and, by implication, changes in neural connectivity. Neuronal sprouting observed after different types of injury, including lesions of the dopaminergic neurons, is stimulated by bFGF

(Otto et al., 1990; Chadi et al., 1993; Date et al., 1993; Kawamata et al., 1997; Ramirez et al., 1999). Not only does neutralization of bFGF prevent dendritic growth in dopamine neurons, but neurite growth is prevented by blocking the effects of bFGF in other types of neurons both *in vitro* and *in vivo* (Fagan et al., 1997; Rowntree & Kolb 1997; Hatten et al., 1988; Le Roux & Esquenazi, 2002). In addition to the actions of amphetamine on dendritic length of dopamine neurons, repeated exposure to amphetamine and cocaine has been observed to induce structural changes in neurons of the terminal regions of the VTA (Robinson & Kolb, 1997; Robinson & Kolb, 1999). Increased expression of bFGF, not only in the VTA, but also in the NAcc and prefrontal cortex, is seen one week after the last injection of escalating doses (1-4 mg/kg) of amphetamine over two weeks (Flores & Stewart, 2000). Corresponding to the sites of greater bFGF expression following amphetamine treatment, increased dendritic length was observed in VTA dopamine neurons (Chapter 2), and in neurons in the terminal regions, including the NAcc and prefrontal cortex (Robinson & Kolb, 1997). Interestingly, bFGF has neurotrophic actions on both striatal medium spiny neurons and cortical pyramidal cells (Zhou & Difiglia, 1993; Rowntree & Kolb, 1997).

In addition to promoting dendritic growth, bFGF may alter glutamate receptors on dopamine neurons. bFGF increases the expression of the AMPA-receptor subunit GluR1 (Cheng et al., 1995) and GluR1 immunoreactivity in the VTA is increased following repeated exposure to stimulant drugs (Fitzgerald, Ortiz, Hamedani & Nestler, 1996; but see Lu et al., 2002). Moreover, dopamine neurons are more responsive to AMPA receptor activation following treatment with amphetamine or cocaine (Zhang et al., 1997). Up-regulation of the GluR1 subunit by viral-mediated gene transfer has been

shown to increase sensitivity to the locomotor stimulant effects of morphine (Carlezon, Boundy, Haile, Lane, Kalb, Neve & Nestler, 1997). As well, both psychostimulant drugs and bFGF appear to increase GluR1 via post-transcriptional mechanisms (Mattson et al., 1993; Wolf, 1998; Ghasemzadeh, Nelson, Lu & Kalivas, 1999).

Increased GluR1 expression is associated with the elevated responsiveness of post-synaptic AMPA receptors following the induction of long-term potentiation (LTP; Malenka & Nicoll, 1999; Nicoll & Malenka, 1999). bFGF-induced functional enhancement of synaptic strength, presumably through up-regulation of the AMPA-receptor GluR1 subunit (Cheng et al., 1995), has been observed in hippocampal neurons such that bFGF promotes the development of LTP (Terlau & Seifert, 1990; Ishiyama et al., 1991). Recent *in vitro* studies have shown that afferent excitatory inputs to DA neurons undergo a NMDA-dependent LTP (Bonci & Malenka, 1999; Overton et al., 1999). In addition, a single *in vivo* exposure to cocaine or amphetamine induces LTP of AMPA-mediated excitatory transmission in DA neurons (Ungless et al., 2001; Saal et al., 2003). Furthermore, repeated cocaine administration has been shown to induce LTP-like potentiation of synaptic strength in dopamine neurons of the VTA (Borgland et al., 2004). Exposure to amphetamine increases bFGF expression in the VTA (Flores et al., 1998; Chapter 3), and also enhances synaptic strength at excitatory synapses on VTA dopamine neurons (Saal et al., 2003). Therefore, amphetamine-induced expression of bFGF can modulate both structural and functional changes associated with synaptic strengthening and membrane excitability. The implication is that the development of behavioral sensitization to psychostimulant drugs involves a LTP-like process in the VTA (Karler et al., 1991; Wolf, 1998; Wolf, 2003) that could involve bFGF-dependent actions.

Recruitment of bFGF

The increase in bFGF expression in response to repeated amphetamine exposure may be initiated by the actions of glutamate (see Flores & Stewart, 2000b). In the VTA, previous exposure to psychostimulants results in enhanced extracellular glutamate levels during (Kalivas & Duffy, 1995; Kalivas & Duffy, 1998) or after (Xue et al., 1996; Wolf & Xue, 1998) amphetamine administration. The drug-induced increase in the extracellular concentration of glutamate may place excessive demands on the VTA dopamine neurons. *In vitro*, the induction of bFGF mRNA has been demonstrated in response to glutamate (Pechan, Chowdhury, Gerdes & Seifert, 1993). bFGF has been demonstrated to be neuroprotective against NMDA neurotoxicity in cultured hippocampal neurons (Mattson et al., 1993). Moreover, the expression of the 71 kDa NMDA receptor protein associated with glutamate neurotoxicity is attenuated by bFGF (Mattson, Wang & Michaelis, 1991). Thus, bFGF may be recruited in the VTA in response to excessive stimulation by glutamate. bFGF is neuroprotective for dopamine neurons, promoting survival following 6-OHDA toxicity (Hou et al., 1997; Shults, Ray, Tsuboi & Gage, 2000), as well as MPTP and MPP^+ (Otto & Unsicker, 1993). In fact, bFGF promotes the regeneration of damaged dopamine neurons as cultured dopamine neurons showed increased neurite sprouting and elongation in response to bFGF following 6-OHDA or MPTP treatment (Mitsumoto et al., 2001). As such, bFGF may be recruited initially as a neuroprotective agent in response to excessive glutamate in order to protect dopamine neurons from glutamate toxicity and damage.

Mechanisms Mediating the Effects of bFGF on Dendritic Elongation

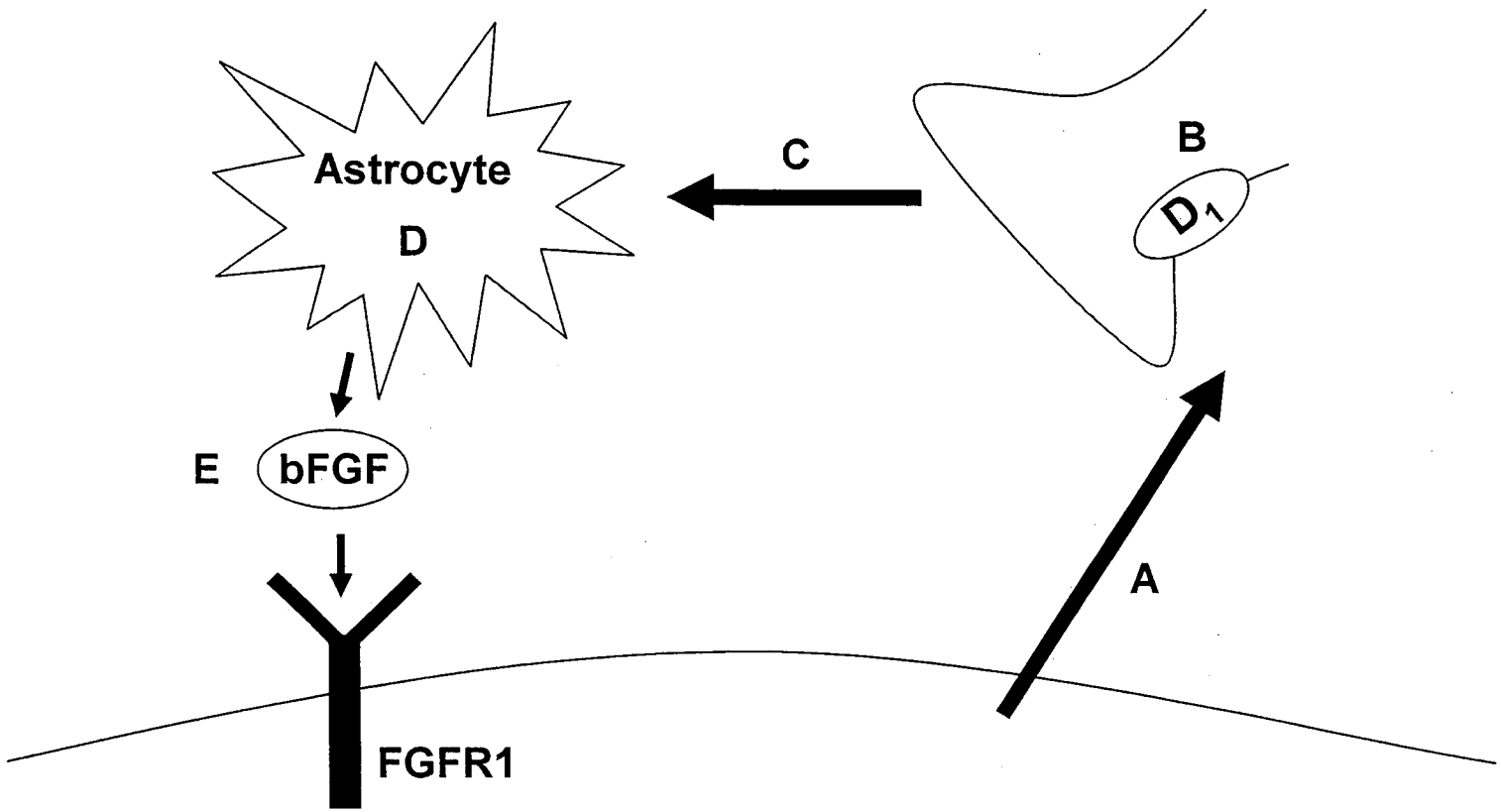
The observation that repeated exposure to amphetamine induces dendritic elongation of VTA dopamine neurons, and that this effect is the dependent on the actions of astrocytic bFGF, indicates that the dendritic growth is a product of the interaction between neurons and astrocytes. Astrocytes are known to affect neurogenesis, neuronal survival, process outgrowth, and synapse formation of neurons (Le Roux & Reh, 1994; Ohgoh, Kimura, Ogura, Katayama & Nishizawa, 1998; Hartley, Margulis, Fishman, Lee & Tang, 1999). In particular, evidence exists that neurite outgrowth and branching of cultured neurons are co-regulated through neuro-astroglial interactions (Chamak, Fellous, Glowinski & Prochiantz, 1987). Astrocytes have been demonstrated to support axon and dendrite growth in embryonic mouse dopaminergic neurons *in vitro* (Rousselet et al., 1988; Rousselet et al., 1990). Importantly, dendritic growth, but not axonal growth, is supported by mature or reactive astrocytes (Le Roux & Reh, 1995; Le Roux & Reh, 1996). Following repeated drug exposure, increased levels of glial fibrillary acidic protein (GFAP), indicative of reactive astrocytes, have been observed in the VTA (Beitner-Johnson et al., 1993; Ortiz et al., 1995; Haile, Hiroi, Nestler & Kosten, 2001). The increase in reactive astrocytes is accompanied by a parallel increase in bFGF immunoreactivity in the VTA (Flores et al., 1998). The neurotrophic effects of bFGF depend on the stimulation of glial cell division, and these effects are eliminated by inhibition of cell proliferation (Engele & Bohn, 1991; Knusel, Michel, Schwaber & Hefti, 1990). One possibility is that bFGF may promote the production of more astrocytes. Interestingly, in culture, bFGF can induce gliogenesis and the aggregation of astrocytes to neuronal bodies (Zamburlin, Gilardino, Ariano, Lovisolo & Distasi, 2003), resulting in

greater cell surface contact. Cell surface contact between astrocytes and neurons provides optimal conditions for neurite growth (Piontek, Régnier-Vigouroux & Brandt, 2002). Thus, the increased number of reactive astrocytes, and increased cell surface contact, provide an appropriate substrate for dendritic growth.

Relatively little exposure to amphetamine was needed to induce increased bFGF expression in the VTA (Chapter 3). In astrocytic cultures, bFGF has been shown to induce the expression of its own gene and protein (Flott-Rahmel et al., 1992; Moffett, Kratz, Florkiewicz & Stachowiak, 1996; Moffett, Kratz, Myers, Stachowiak, Florkiewicz & Stachowiak, 1998). bFGF mRNA is increased in low density cell cultures, but is decreased in high density cell cultures. As such, increased cell contact may inhibit the bFGF gene promoter (Moffett et al., 1996). In addition, cell contact can prevent bFGF induction by either bFGF, other neurotrophic factors, or by stimulation of cAMP or protein kinase C (PKC) signaling pathways (Moffett et al., 1998). The fact that amphetamine-induced increases in bFGF expression was transient, observed on postnatal day 21 but not on postnatal day 30, suggests that increases in astrocytic processes and neuronal sprouting were complete. The additional cell contact may have inhibited the transcription of the bFGF gene.

Astrocytic bFGF, released in response to repeated amphetamine administration, could promote dendritic growth. As shown in Figure 13, amphetamine exposure stimulates somatodendritic release of dopamine from neurons of the VTA (Kalivas & Duffy, 1993), and attenuates the sensitivity of D_2 dopamine autoreceptors (Ackerman & White, 1990; White & Wang, 1984). The resulting increase in extracellular dopamine may stimulate D_1 dopamine receptors, necessary for the induction of behavioral

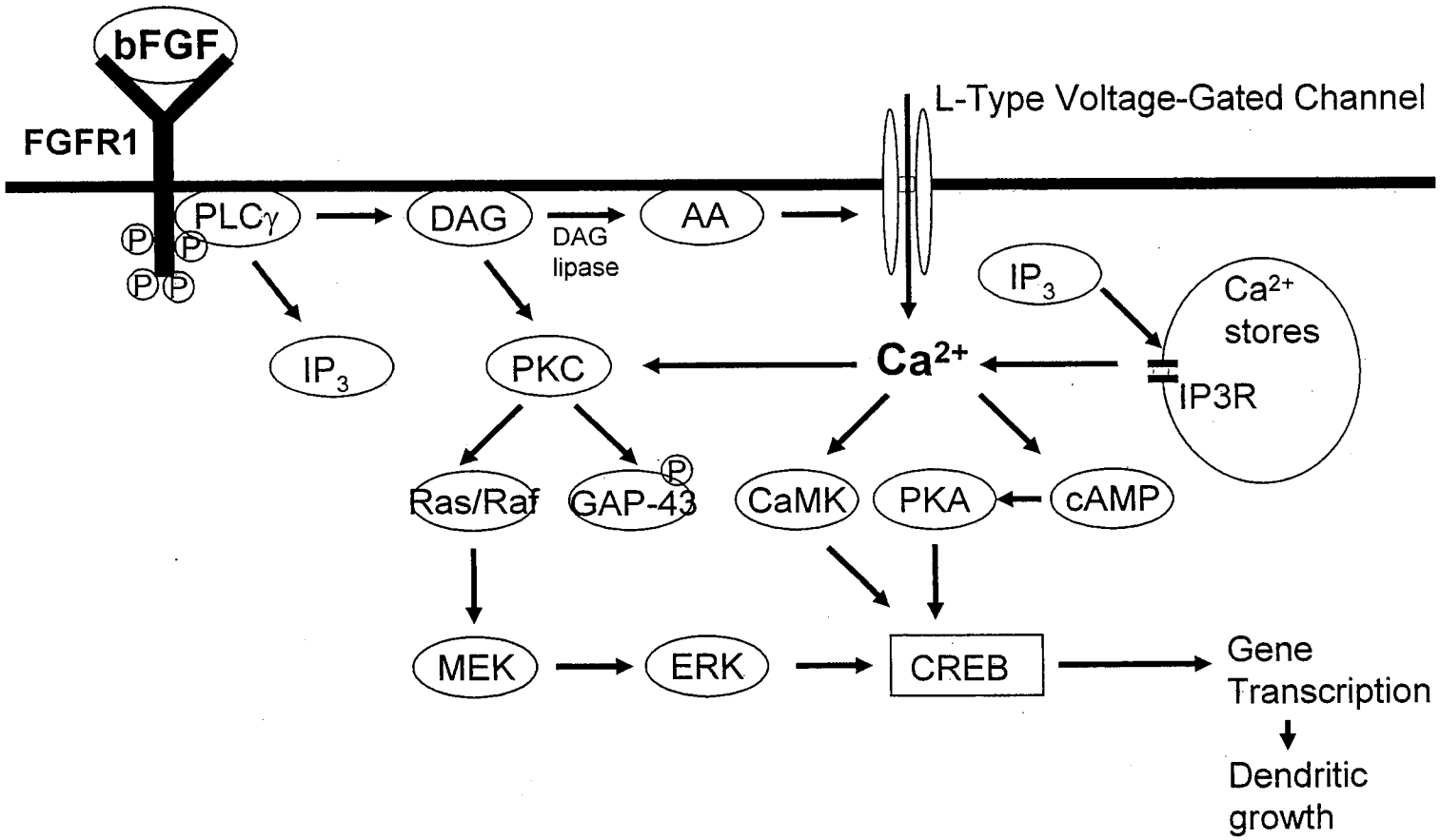
Figure 13. Synaptic model of the extracellular events in the VTA proposed to underlie the induction and expression of bFGF in response to amphetamine. The arrows highlight the flow of events starting with the amphetamine-induced *(A)* somatodendritic release of dopamine from VTA dopamine neurons, *(B)* the activation of D_1 dopamine receptors on afferent glutamatergic terminals, *(C)* the release of glutamate, *(D)* the induction and expression of bFGF in the astrocytes, and *(E)* the diffusion of bFGF and activation of its high affinity receptor, FGFR1, on the dopamine neurons in the VTA.


Astrocyte
D
C
B
D1
E
bFGF
A
FGFR1

sensitization (Bjijou et al., 1996; Stewart & Vezina, 1989; Vezina, 1996), found at the terminals of glutamate neurons (Kalivas, 1995). The augmented release of glutamate (Kalivas & Duffy, 1995; Kalivas & Duffy, 1998) could stimulate the induction and expression of astrocytic bFGF (Flores et al., 1998; Flores & Stewart, 2000b).

Astrocytes are known to mediate dendritic growth, and they do so, in part, by release of bFGF (Le Roux & Esquenazi, 2002). Thus, astrocytes could release bFGF into the extracellular space of the VTA where this protein could have actions directly on the dopamine neurons by binding to its high affinity, membrane-bound receptors (see Figure 13). Dopamine neurons have been shown to express the high affinity receptor for bFGF, FGFR1 (Gonzalez, Berry, Maher, Logan, & Baird, 1995; Walker, Terai, Matsuo, Beach, McGeer & McGeer, 1998; Claus, Werner, Timmer & Grothe, 2004). Activation of this receptor has been shown to trigger neurite growth in cultured neurons (Williams et al., 1994). The first evidence for a possible role of FGFR1 signaling in dendritic outgrowth *in vivo* comes from transgenic mice that over-express the FGFR1 receptor. In that study, aberrant growth of the apical dendrites of pyramidal neurons in the frontal and temporal areas was observed (Frantz, Bohner, Akers, & McConnell, 1994). The majority of evidence for a role of FGFR1 signaling in neurite growth, however, comes from studies of cultured neurons (Williams et al., 1994). Based on these studies, the scheme of signaling pathways activated upon FGFR1 phosphorylation includes two branches (Figure 14) that link the activated receptor with calcium entry (FGFR-Ca^{2+}) into the cytoplasm and with the mitogen-activated protein kinase (MAPK) pathway (FGFR-MAPK) via PKC.

Figure 14. Schematic model of intracellular signaling pathways in bFGF-mediated dendritic growth. See text for details.


bFGF
FGFR1
L-Type Voltage-Gated Channel
PLCγ
DAG
DAG lipase
AA
P
P
P
P
IP3
PKC
Ca2+
IP3
Ca2+ stores
IP3R
Ras/Raf
GAP-43
P
CaMK
PKA
cAMP
MEK
ERK
CREB
Gene Transcription
Dendritic growth

FGFR1 Signaling: Ca^{2+}

The FGFR-Ca^{2+} pathway activation is considered to be both necessary and sufficient for triggering neurite growth in culture. Ca^{2+} is a key second messenger that mediates neurite outgrowth and elongation (Lankford & Letourneau, 1989; Mattson & Kater, 1987). An increase in Ca^{2+} concentration has been detected in response to bFGF in PC12 cells (Archer, Doherty, Collins & Bolsover, 1999) and in cultured hippocampal neurons (Abe & Saito, 1992; Tanaka et al., 1996). The FGFR-Ca^{2+} signaling pathway includes FGFR1 and phospholipase C (PLCγ) activation, production of diacylglycerol (DAG) and arachidonic acid (AA), and, eventually, Ca^{2+} entering the cell via voltage-gated channels. PLCγ is activated upon binding to residue 766 of phosphorylated FGFR1. Activation of FGFR1 with a mutation at this site results in impaired neurite growth as compared to the growth induced by activation of wild-type FGFR1 (see Kiryushko et al., 2004). PLCγ recruitment results in the generation of the principal intracellular messengers inositoltriphosphate (IP_3) and DAG. IP_3 opens specific channels of intracellular Ca^{2+} stores, thereby releasing Ca^{2+} into the cytoplasm. The other messenger, DAG, is converted by DAG-lipase to AA. AA, in and of itself, has been shown to induce neurite growth (Williams et al., 1994; Kolkova, Novitskaya, Pedersen, Berezin & Bock, 2000), an effect that can be blocked by N- and L-type calcium channel antagonists (Williams et al., 1994). Based on these results, it is possible that AA generated from DAG activates or modulates the function of voltage-dependent Ca^{2+} channels and thereby triggers Ca^{2+} influx into the cytoplasm.

Activation of L-type calcium channels (Karler et al., 1991b), as well as the actions of endogenous bFGF (Flores et al., 2000b), have been shown to be necessary for the

development of behavioral sensitization. bFGF, upon binding to the FGFR1 receptor, triggers an intracellular cascade of events that can modify the function of L-type calcium channels to increase calcium influx (Williams et al., 1994; Kiryushko et al., 2004). Calcium is necessary for dendritic growth (Lankford & Letourneau, 1989; Mattson & Kater, 1987). Therefore, bFGF may mediate amphetamine-induced dendritic elongation via enhancement of calcium signaling.

Several important messengers act to couple the rise of cytosolic Ca^{2+} to subsequent morphological changes. First, Ca^{2+} influx has been shown to increase the level of cAMP in PC12 cells (Impey, Obrietan, Wong, Poser, Yano, Wayman, Deloulme, Chan & Storm, 1998). This, in turn, activates protein kinase A (PKA). Neurite growth can be blocked by antagonists of both cAMP and PKA, presumably by preventing PKA-induced cAMP response element binding protein (CREB) phosphorylation (Jessen, Novitskaya, Pedersen, Serup, Berezin & Bock, 2001). Interestingly, co-administration of a PKA inhibitor with repeated intra-VTA amphetamine injections fully blocks the development of behavioral sensitization (Tolliver, Ho, Fox & Berger, 1999).

In addition to the activation of transcription factors, such as CREB, increased cytoplasmic Ca^{2+} is known to affect two potential regulators of the cytoskeleton, calmodulin kinase II (CaMKII) and growth-associated protein-43 (GAP-43, also known as neuromodulin; see Figure 14). CaMKII has been implicated in many aspects of neuronal function, including synaptic plasticity, the maintenance of dendritic architecture, and the density of glutamatergic synapses (Kennedy, 2000). CaMKII has been demonstrated to mediate neurite growth initiated by AA through activation of CREB (Williams, Mittal, Walsh & Doherty, 1995). Interestingly, repeated amphetamine

exposure in juvenile rats induced a marked increase in CaMKII expression in the medial prefrontal cortex that coincided with an increase in neuronal dendritic length and spine density (Heijtz et al., 2003). In addition, intra-VTA administration of a CaMKII inhibitor, KN-93, prevented the augmentation of cocaine-induced behavioral hyperactivity following repeated injections (Licata, Schmidt & Pierce, 2004). Consistent with this finding, the development of behavioral sensitization to cocaine is attenuated in homozygous alpha-CaMKII knockout mice relative to wild-type mice (Licata et al., 2004). Thus, activation of CaMKII, stimulated by enhanced Ca^{2+} transmission, could contribute to the changes in dendritic cytoarchitecture observed following repeated administration of amphetamine.

GAP-43 is a membrane-associated protein that participates in neurite growth presumably by binding actin filaments and affecting the dynamics of actin polymerization (He, Dent, & Meiri, 1997; Meiri, Saffell, Walsh & Doherty, 1998). In agreement with a role during neurite formation, mice overexpressing GAP-43 develop aberrant interneuronal connections (Aigner, Arber, Kapfhammer, Laux, Schneider, Botteri, Brenner & Caroni, 1995). Activation of the FGFR1 receptor promotes GAP-43 translocation from the cytosol to the cytoskeleton, where it participates in neurite growth (Tejero-Diez, Rodriguez-Sanchez, Martin-Cofreces, & Diez-Guerra, 2000). GAP-43 is phosphorylated by stimulation of FGFR1 in neurons, an effect mediated by DAG-lipase (Meiri et al., 1998). The likely activator of GAP-43 is a membrane-associated isoform of PKC recruited by AA and/or the Ca^{2+} influx (Doherty, Williams & Williams, 2000). PKC has been demonstrated to phosphorylate GAP-43 both *in vivo* and *in vitro* (Coggins & Zwiers, 1991; Oestreicher, De Graan, Gispen, Verhaagen & Schrama, 1997).

Importantly, repeated amphetamine administration induces an increase in the phosphorylation of GAP-43 in the rat striatum (Gnegy, Hong & Farrell, 1993; Iwata, Hewlett, Ferrell, Czernik, Meiri & Gnegy, 1996). In addition, a single dose of cocaine (20 mg/kg), sufficient to induce behavioral sensitization, was demonstrated to increase the expression of GAP-43 mRNA in the VTA and NAcc (Grignaschi, Burbassi, Zennaro, Bendotti & Cervo, 2004). Psychostimulant-induced increases in GAP-43 and CaMKII, through alterations in Ca^{2+} transmission, likely play a role in the dendritic growth of VTA dopamine neurons.

FGFR1 Signaling: MAPK

Besides launching the FGFR1-Ca^{2+} signaling cascade, activation of FGFR1 can also trigger the MAPK signaling pathway (see Figure 14). Stimulation of the classic MAPK cascade, including the activation of ERK, induces cell growth and proliferation (Seger & Krebs, 1995; Ujike, Takaki, Kodama & Kuroda, 2002). PKC has been proposed to participate in the activation of the MAPK cascade (Kolkova et al., 2000). PKC phosphorylates a MAP3 kinase (Raf kinase) which stimulates the MAPK signal transduction cascade (Seger & Krebs, 1995). Raf then phosphorylates and activates a MAP kinase kinase (MEK), which in turn phosphorylates the extracellular signal-regulated kinases (ERKs; Seger & Krebs, 1995). Upon activation, the ERKs become proline-directed, phosphorylating serine or threonine residues that neighbour prolines. Thus, ERKs have many substrates in both the cytoplasm and nucleus, including tyrosine hydroxylase (Haycock, Ahn, Cobb & Krebs, 1992), the rate-limiting enzyme in the synthesis of dopamine. Importantly, ERK can phosphorylate CREB, leading to gene

transcription and the putative expression of proteins involved in the regulation of the cytoskeleton (Kiryushko et al., 1994; Seger & Krebs, 1995). Indeed, protein synthesis in the VTA is necessary for the induction of locomotor sensitization to amphetamine and cocaine (Karler et al., 1993; Sorg & Ulibarri, 1995). Interestingly, repeated methamphetamine exposure has been shown to induce the synthesis of arc, an activity-regulated cytoskeleton-associated protein localized in cell bodies and dendritic processes that co-expresses with actin (Lyford et al., 1995), in several cortical and striatal regions (Ujike et al., 2002). Although the study did not include analysis of the VTA, this protein is considered to be a marker for dendritic elongation (Ujike et al, 2002).

The MAPK signaling pathway has been implicated as playing an important role in both neurite growth and in the development of behavioral sensitization to psychostimulant drugs. bFGF activates the MAP kinase signaling pathway to cause neurite outgrowth in PC12 cells via phosphorylation of MEK (Rockow, Tang, Xiong & Li, 1996). The blockade of neurite growth in PC12 cells, by inhibition of the FGFR1 signaling pathway, could be reversed by over-expression of MEK (Kolkova et al., 2000). Thus, increased phosphorylation of MEK is necessary for neurite growth. Interestingly, intra-VTA administration of a MEK inhibitor blocks the development of behavioral sensitization to cocaine (Pierce, Pierce-Bancroft & Prasad, 1999). This finding is consistent with evidence indicating that repeated cocaine exposure induces an increase in ERK activity in the VTA (Berhow, Hiroi & Nestler, 1996). The evidence indicates that both bFGF, through its actions at the FGFR1 receptor, and psychostimulant drugs activate MAPK signaling. Thus, bFGF may act to mediate the psychostimulant-induced changes in the VTA via the signaling pathways associated with activation of FGFR1.

Nuclear FGFR1

Alternatively, or in addition to extracellular signaling, bFGF could have intracellular actions within the astrocytes themselves. bFGF has been demonstrated to localize in the nucleus and cytoplasm of astrocytes and hippocampal neurons (Woodward, Nishi, Meshul, Williams, Coulombe & Eckenstein, 1992). The high affinity FGFR1 receptor, a typically membrane-associated protein, is released from endoplasmic reticulum membranes into the cytosol and translocates to the cell nucleus along with its ligand, bFGF (Stachowiak, Fang, Myers, Dunham, Berezney, Maher & Stachowiak, 2003; Stachowiak, Maher, Joy, Mordechai & Stachowiak, 1996). Thus, bFGF could have direct nuclear actions, inducing gene transcription (Nakanishi, Kihara, Mizuno, Yoshitake & Nishikawa, 1992) and glial proliferation (Engele & Bohn, 1991; Knusel et al., 1990). In fact, nuclear localization of bFGF is associated with astrocyte proliferation (Joy et al., 1997). Furthermore, stimulation of neuronal dendritic growth by bone morphogenetic protein-7, *in vitro*, induces the nuclear accumulation of FGFR1 and bFGF (Horbinski, Stachowiak, Chandrasekaran, Miuzukoshi, Higgins & Stachowiak, 2002). Thus, the actions of bFGF on dendritic growth following repeated drug exposure could be mediated by gene transcription directly regulated by the nuclear translocation of FGFR1, bound by bFGF.

Recruitment of Additional Neurotrophic Factors

In addition to promoting the expression of proteins involved in the regulation of the cytoskeleton (Kiryushko et al., 1994; Seger & Krebs, 1995; Stachowiak et al., 2003), bFGF could also alter the expression of other neurotrophic factors. bFGF has been

shown to induce the expression of nerve growth factor and transforming growth factor _ (TGF-_) in both neurons and astrocytes *in vitro* (Yoshida & Gage, 1991; Ferhat, Represa, Zouaoui-Aggoun, Ferhat, Ben-Ari & Khrestchatisky, 1997; Krieglstein, Reuss, Maysinger & Unsicker, 1998). In addition, bFGF induces the expression of glial cell line-derived neurotrophic factor (GDNF; Suter-Crazzolara & Unsicker, 1996; Lenhard, Schober, Suter-Crazzolara & Unsicker, 2002), which has been demonstrated to be neurotrophic for dopamine neurons (Lin, Doherty, Lile, Bektesh & Collins, 1993). bFGF has been proposed to act synergistically with GDNF and TGF-β to exert its neurotrophic effects (Lenhard et al., 2002). bFGF has also been shown to upregulate the receptor mRNA for brain-derived neurotrophic factor, trkB mRNA (Brumwell, Hossain, Morest & Bernd, 2000), suggesting that brain-derived neurotrophic factor and trkB may respond jointly to bFGF. Interestingly, bFGF has been shown to induce the expression of its own gene and protein (Flott-Rahmel et al., 1992; Moffett, Kratz, Florkiewicz & Stachowiak, 1996; Moffett, Kratz, Myers, Stachowiak, Florkiewicz & Stachowiak, 1998), which could exacerbate its actions on gene transcription and upregulation of other neurotrophic factors. Thus, the psychostimulant-induced augmentation in bFGF expression in the VTA could, in turn, induce the expression of additional neurotrophic factors. The actions of these additional factors could contribute to the changes in function and structure of dopamine neurons in the VTA.

Future Research

The present study demonstrates that the actions of endogenous bFGF are necessary for amphetamine-induced dendritic growth in dopamine neurons of the VTA *in*

vivo. The sequence of extracellular and intracellular events by which the induction and expression of bFGF occurs remains to be elucidated. In the adult rat, amphetamine-induced increased expression of astrocytic bFGF in the VTA has been demonstrated to be mediated by the actions of glutamate at the NMDA receptor (Flores et al., 1998). It remains to be addressed, however, whether the actions of glutamate are necessary for dendritic growth. As such, the dendritic length of VTA dopamine neurons should be assessed following concurrent treatment with repeated amphetamine and a NMDA receptor antagonist. If glutamate stimulates the induction and expression of bFGF, via NMDA ionotropic receptors, then it would be expected that NMDA receptor blockade would prevent amphetamine-induced dendritic growth. Interestingly, dendritic outgrowth of hippocampal neurons has been demonstrated to be glutamate dependent (Nuijtinck, Baker, Ter Gast, Struik & Mud, 1997).

Much work has been done to delineate the intracellular signaling pathways associated with the FGFR1 receptor in culture (Williams et al., 1994). Whether the activation of the membrane-bound isoform of this receptor is necessary for *in vivo* dendritic growth needs to be determined. The co-administration of amphetamine and a FGFR1 receptor antagonist that specifically blocks the membrane-bound isoform of this receptor, such as myo-inositol hexakis (IP6; Joy et al., 1997), would answer whether bFGF promotes dendritic growth in VTA dopamine neurons via activation of the membrane-bound FGFR1 receptor. If receptor blockade prevented dendritic growth in these neurons, then the intracellular signaling cascades involved would have to be determined. For example, a candidate molecule that may participate in altering the cytoarchitecture of dendrites is GAP-43. GAP-43 is expressed in neurons during the

initial establishment and remodeling of neural connections (Oestreicher et al., 1997). Over-expression of GAP-43 in adult transgenic mice induces neuronal sprouting (Aigner et al., 1995). Furthermore, GAP-43 has been demonstrated to enhance NGF-induced neurite outgrowth in PC12 cells (Yankner et al., 1990). Activation of the FGFR1 receptor, in culture, results in the phosphorylation of GAP-43 (Meiri et al., 1998) and promotes its translocation from the cytosol to the cytoskeleton (Tejero-Diez et al., 2000). Amphetamine-induced dendritic growth likely involves phosphorylated GAP-43. Thus, an increase in the expression of this cytoskeletal-associated factor is likely to be observed in the VTA following repeated exposure to amphetamine.

Although dendritic elongation was observed in VTA dopamine neurons of rats exposed to amphetamine during the early postnatal period, it remains unknown whether similar morphological changes occur following amphetamine exposure in the adult rat. The observation of similar changes in the adult would provide evidence that morphological alterations in VTA dopamine neurons contribute to the development of long-lasting behavioral and neurochemical sensitization to the effects of psychostimulant drugs.

References

Abe, K., & Saito, H. (1992). Selective enhancement by basic fibroblast growth factor of NMDA receptor-mediated increase of intracellular Ca^{2+} concentration in hippocampal neurons. Brain Research, 595, 128-132.

Ackerman, F.J., & White, F.J. (1990). A10 somatodendritic dopamine autoreceptor sensitivity following withdrawal from repeated cocaine treatment. Neuroscience Letters, 117, 181-187.

Aigner, L., Arber, S., Kapfhammer, J.P., Laux, T., Schneider, C., Botteri, F., Brenner, H.R., & Caroni, P. (1995). Overexpression of the neural growth-associated protein GAP-43 induces nerve sprouting in the adult nervous system of transgenic mice. Cell, 83, 269-278.

Aoyagi, A., Nishikawa, K, Saito, H., & Abe, K. (1994). Characterization of basic fibroblast growth factor-mediated acceleration of axonal branching in cultured rat hippocampal neurons. Brain Research, 661, 117-126.

Archer, F.R., Doherty, P., Collins, D., & Bolsover, S.R. (1999). CAMs and FGF cause a local submembrane calcium signal promoting axon outgrowth without a rise in bulk calcium concentration. European Journal of Neuroscience, 11, 3565-3573.

Baird, A., & Klagsbrun, M. (1991). The fibroblast growth factor family: An overview. Annals of the New York Academy of Science, 638, xi.

Bean, A.J., Elde, R., Cao, Y., Oellig, C., Tamminga, C., Goldstein, M., Petterson, R.F., & Hokfelt, T. (1991). Expression of acidic and basic fibroblast growth factors in

the substantia nigra of rat, monkey, and human. Proceedings of the National Academy of Science USA, 88, 10327-10241.

Beitner-Johnson, D., & Nestler, E.J. (1991). Morphine and cocaine exert common chronic actions on tyrosine hydroxylase in dopaminergic brain reward regions. Journal of Neurochemistry, 57, 344-347.

Beitner-Johnson, D., & Nestler, E.J. (1993). Chronic morphine impairs axoplasmic transport in the rat mesolimbic dopamine system. Neuroreport, 5, 57-60.

Beitner-Johnson, D., Guitart, X., & Nestler, E.J. (1992). Neurofilament proteins and the mesolimbic dopamine system: Common regulation by chronic morphine and chronic cocaine in the rat ventral tegmental area. The Journal of Neuroscience, 12, 2165-2176.

Beitner-Johnson, D., Guitart, X., & Nestler, E.J. (1993). Glial fibrillary acidic protein and the mesolimbic dopamine system: Regulation by chronic morphine and Lewis-Fischer strain differences in the rat ventral tegmental area. Journal of Neurochemistry, 61, 1766-1773.

Bell, K., & Kalivas, P.W. (1996). Context-specific cross sensitization between systemic cocaine and intra-accumbens AMPA infusion in rats. Psychopharmacology, 127, 377-383.

Ben-Shahar, O., & Ettenberg, A. (1994). Repeated stimulation of the ventral tegmental area sensitizes the hyperlocomotor response to amphetamine. Pharmacology Biochemistry & Behavior, 48, 1005–1009.

Berhow, M.T., Hiroi, N., & Nestler, E.J. (1996). Regulation of ERK (extracellular signal regulated kinase), part of the neurotrophin signal transduction

cascade, in the rat mesolimbic dopamine system by chronic exposure to morphine or cocaine. The Journal of Neuroscience, 16, 4707-4715.

Berridge, K.C. (1999). Pleasure, pain, desire and dread: Hidden core processes of emotion. In D. Kahneman, E. Diener, & N. Schwarz (eds.) Well being: The foundations of hedonic psychology (pp. 527-559). New York: Sage Foundation.

Bjijou, Y., Stinus, L., Le Moal, M., Cador, M. (1996). Evidence for selective involvement of dopamine D1 receptors in the ventral tegmental area in the behavioral sensitization induced by intra-ventral tegmental area injections of d-amphetamine. Journal of Pharmacology and Experimental Therapeutics, 277, 1177-1187.

Bonci, A., & Malenka R.C. (1999). Properties and plasticity of excitatory synapses on dopaminergic and GABAergic cells in the ventral tegmental area. The Journal of Neuroscience, 19, 3723-3737.

Bonci, A., & Williams, J.T. (1996). A common mechanism mediates long-term changes in synaptic transmission after chronic cocaine and morphine. Neuron, 16, 631-639.

Bonhoeffer, T. (1996). Neurotrophins and activity-dependent development of the neocortex. Current Opinions in Neurobiology, 6, 119-126.

Bonnet, J-J, & Costentin, J. (1989). Correlation between [^{3}H]dopamine specific uptake and [^{3}H]GBR 12783 specific binding during the maturation of rat striatum. Life Sciences, 44, 1759-1765.

Borgland, S.L., Malenka, R.C., & Bonci, A. (2004). Acute and chronic cocaine-induced potentiation of synaptic strength in the ventral tegmental area:

Electrophysiological and behavioral correlates in individual rats. The Journal of Neuroscience, 24, 7482-7490.

Bouvier, M., & Mytilineou, C. (1995). Basic fibroblast growth factor increases division and delays differentiation of dopamine precursors *in vitro*. The Journal of Neuroscience, 15, 7141-7149.

Bowman, B.P., & Kuhn, C.M. (1996). Age-related differences in the chronic and acute response to cocaine in the rat. Developmental Psychobiology, 29, 597-611.

Brodie, M.S, & Bunney, E.B. (1996). Serotonin potentiates dopamine inhibition of ventral tegmental area neurons in vitro. Journal of Neurophysiology, 76, 2077-2082.

Bruinink, A., Lichtensteiger, W., & Schlumpf, M. (1983). Pre and postnatal ontogeny and characterization of dopaminergic D2, serotonergic S2, and spirodecanone binding sites in rat forebrain. Journal of Neurochemistry, 40, 1227-1236.

Brumwell, C.L., Hossain, W.A., Morest, D.K., & Bernd, P. (2000). Role for basic fibroblast growth factor (FGF-2) in tyrosine kinase (TrkB) expression in the early development and innervation of the auditory receptor: in vitro and in situ studies. Experimental Neurolology, 162, 121-145.

Bunnemann, B., Terron, A., Zantedeschi, V., Merlo Pich, E., & Chiamulera, C. (2000). Chronic nicotine treatment decreases neurofilament immunoreactivity in the rat ventral tegmental area. European Journal of Pharmacology, 393, 249–253.

Caday, C.G., Klagsbrun, M., Fanning, P.J., Mirzabegian, A., & Finklestein, S.P. (1990). Fibroblast growth factor (FGF) levels in the developing rat brain. Developmental Brain Research, 52, 241-246.

Cador, M., Bjijou, Y., & Stinus, L. (1995). Evidence of a complete independence of the neurobiological substrates for the induction and expression of behavioral sensitization to amphetamine. Neuroscience, 65, 385-395.

Cador, M., Bjijou, Y., Cailhol, S, & Stinus, L. (1999). D-amphetamine induced behavioural sensitization: Implications of a glutamatergic medial prefrontal cortex-ventral tegmental area innervation. Neuroscience, 94, 705-721.

Cameron, D.L., & Williams, J.T. (1993). Dopamine D_1 receptors facilitate transmitter release. Nature, 366, 344-347.

Carlezon, W.A., Jr., Nestler, E.J. (2002). Elevated levels of GluR1 in the midbrain: a trigger for sensitization to drugs of abuse? Trends in Neuroscience, 25, 610-615.

Carlezon, W.A., Jr., Boundy, V., Haile, C., Lane, S., Kalb, R., Neve, R., & Nestler, E.J. (1997). Sensitization to morphine induced by viral-mediated gene transfer. Science, 277, 812-814.

Carr, D.B., & Sesack, S.R. (2000). GABA-containing neurons in the rat ventral tegmental area project to the prefrontal cortex. Synapse, 38, 114-123.

Castner, S., & Goldman-Rakic, P. (1999). Long-lasting psychotomimetic consequences of repeated low-dose amphetamine exposure in rhesus monkeys. Neuropsychopharmacology, 20, 10-28.

Chadi, G., Cao, Y., Pettersson, R.F., & Fuxe, K. (1994). Temporal and spatial increase of astroglial basic fibroblast growth factor synthesis after 6-hydroxydopamine-induced degeneration of the nigrostriatal dopamine neurons. Neuroscience, 61, 891-910.

Chadi, G., Moller, A., Rosen, L., Janson, A., Agnati, L., Goldstein, M., Ogren, S-O, Pettersson, R., & Fuxe, K. (1993). Protective actions of human recombinant basic fibroblast growth factor on MPTP-lesioned nigrostriatal dopamine neurons after intraventricular infusion. Experimental Brain Research, 97, 145-158.

Chamak, B., Fellous, A., Glowinski, J., & Prochiantz, A. (1987). MAP2 expression and neuritic outgrowth and branching are coregulated through region-specific neuro-astroglial interactions. The Journal of Neuroscience, 7, 3163-3170.

Chapman, C.A., & Lacaille, J.-C. (1999). Intrinsic theta-frequency membrane potential oscillations in hippocampal CA1 interneurons of stratum lacunosum-moleculare. Journal of Neurophysiology, 81, 1296-1307.

Cheng, B., & Mattson, M.P. (1991). NGF and bFGF protect rat and human central neurons against hypoglycaemic damage by stabilizing calcium homeostasis. Neuron, 7, 1031-1041.

Cheng, B., Furukawa, K., O'Keefe, J., Goodman, Y., Kihiko, M., Fabian, T., & Mattson, M.P. (1995). Basic fibroblast growth factor selectively increases AMPA-receptor subunit GluR1 protein level and differentially modulates Ca^{2+} responses to AMPA and NMDA in hippocampal neurons. Journal of Neurochemistry, 65, 2525-2536.

Chiodo, L.A. (1988). Dopamine-containing neurons in the mammalian nervous system: electrophysiology and pharmacology. Neuroscience and Biobehavioral Reviews, 12, 49-91.

Cintra, A., Cao, Y., Oellig, C., Tinner, B., Bortolotti, F., Goldstein, M., Petterson, R.F., & Fuxe, K. (1991). Basic FGF is present in dopaminergic neurons of the ventral midbrain of the rat. Neuroreport, 2, 597-600.

Claus, P., Werner, S., Timmer, M., & Grothe, C. (2004). Expression of the fibroblast growth factor-2 isoforms and the FGF receptor 1-4 transcripts in the rat model system of Parkinson's disease. Neuroscience Letters, 360, 117-120.

Coggins, P.J., & Zwiers, H. (1991). B-50 (GAP-43): Biochemistry and functional neurochemistry of a neuron-specific phosphoprotein. Journal of Neurochemistry, 56, 1095-1106.

Cooper, D.C. (2002). The significance of action potential bursting in the brain reward circuit. Neurochemistry International, 41, 333-340.

Coyle, J.T., & Axelrod, J. (1972). Tyrosine hydroxylase in rat brain: Developmental characteristics. Journal of Neurochemistry, 19, 1117-1123.

Coyle, J.T., & Campochiaro, P. (1976). Ontogenesis of dopaminergic-cholinergic interactions in the rat striatum: A neurochemical study. Journal of Neurochemistry, 27, 673-678.

Cunningham, S.T., & Kelley, A.E. (1993). Hyperactivity and sensitization to psychostimulants following cholera toxin infusion into the nucleus accumbens. The Journal of Neuroscience, 13, 2342-2350.

Dai, Z., & Peng, H.B. (1995). Prsynaptic differentiation induced in cultured neurons by local application of basic fibroblast growth factor. The Journal of Neuroscience, 15, 5466-5475.

Dalia, A., Uretsky, N.J., & Wallace, L.J. (1998). Dopaminergic agonists administered into the nucleus accumbens: effects on extracellular glutamate and on locomotor activity. Brain Research, 788, 111–117.

Date, I., Yoshimoto, Y., Imaoka, T., Yasuyuki, M., Gohda, Y., Furuta, T., Asari, S., Ohmoto, T. (1993). Enhanced recovery of the nigrostriatal dopaminergic system in MPTP-treated mice following intrastriatal injection of basic fibroblast growth factor in relation to aging. Brain Research, 621, 150-154.

Deschepper, C.F. (1998). Peptide receptors on astrocytes. Frontiers in Neuroendocrinology, 19, 20-46.

Desire, L., Head, M.W., Fayein, zn.S., Courtois, Y., & Jeanny, J.C. (1998). Suppression of fibroblast growth factor 2 expression by antisense oligonucleotides inhibits embryonic chick neural retina cell differentiation and survival *in vivo*. Developmental Dynamics, 212, 63-74.

Di Chiara, G. (1999). Drug addiction as dopamine-dependent associative learning disorder. European Journal of Pharmacology, 375, 13-30.

Di Chiara, G., & Imperato, A. (1988). Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proceedings of the National Academy of Sciences USA, 85, 5274-5278.

Doherty, P., Williams, G., & Williams, E.J. (2000). CAMs and axonal growth: A critical evaluation of the role of calcium and the MAPK cascade. Molecular and Cellular Neuroscience, 16, 283-295.

Eckenstein, F.P., Andersson, C., Kuzis, K., & Woodward, W.R. (1994). Distribution of acidic and basic fibroblast growth factors in the mature, injured and developing rat nervous system. Progress in Brain Research, 103, 55-64.

Elliot, P.J., & Nemeroff, C.B. (1986). Repeated neurotensin administration in the ventral tegmental area : Effects on baseline and d-amphetamine-induced locomotor activity. Neuroscience Letters, 68, 239-244.

Engle, J., & Bohn, M. C. (1991). The neurotrophic effects of fibroblast growth factors on dopaminergic neurons *in vitro* are mediated by mesencephalic glia. The Journal of Neuroscience, 11, 3070-3078.

Ernfors, P., Lonnerberg, P., Ayer-Leivre, C., & Persson, H. (1990). Developmental and regional expression of basic fibroblast growth factor mRNA in the rat central nervous system. Journal of Neuroscience Research, 27, 10-15

Everitt, B.J., & Wolf, M.E. (2002). Psychomotor stimulant addiction: A neural systems perspective. The Journal of Neuroscience, 22, 3312-3320.

Fagan, A.M., Suhr, S.T., Lucidi-Phillipi, C.A., Peterson, D.A., Holtzman, D.M., & Gage, F.H. (1997). Endogenous FGF-2 is important for cholinergic sprouting in the denervated hippocampus. The Journal of Neuroscience, 17, 2499-2511.

Fallon, J., Koziell, D., & Moore, R. (1978). Catecholamine innervation of the basal forebrain. Journal of Comparative Neurology, 180, 509–532.

Ferhat, L., Represa, A., Zouaoui-Aggoun, D., Ferhat, W., Ben-Ari, Y., & Khrestchatisky, M. (1997). FGF-2 induces nerve growth factor expression in cultured rat hippocampal neurons. European Journal of Neuroscience, 9, 1282-1289.

Ferrari, G., Minozzi, M-C, Toffano, G., Leon, A., & Skaper, S.D. (1989). Basic fibroblast growth factor promotes the survival and development of mesencephalic neurons in culture. Developmental Biology, 133, 140-147.

Fitzgerald, L., Ortiz, J., Hamedani, A., & Nestler, E.J. (1996). Drugs of abuse and stress increase the expression of GluR1 and NMDAR1 glutamate receptor subunits in the rat ventral tegmental area: Common adaptations among cross-sensitizing agents. The Journal of Neuroscience, 16, 274-282.

Flores, C., & Stewart, J. (2000a). Changes in astrocytic basic fibroblast growth factor expression during and after prolonged exposure to escalating doses of amphetamine. Neuroscience, 98, 287-293.

Flores, C., & Stewart, J. (2000b). Basic fibroblast growth factor as a mediator of the effects of glutamate in the development of long-lasting sensitization to stimulant drugs: Studies in the rat. Psychopharmacology, 151, 152-165.

Flores, C., Rodaros, D., & Stewart, J. (1998). Long-lasting induction of astrocytic basic fibroblast growth factor by repeated injections of amphetamine: Blockade by concurrent treatment with a glutamate antagonist. The Journal of Neuroscience, 18, 9547-9555.

Flores, C., Samaha, A.N., & Stewart, J. (2000). Requirement of endogenous basic fibroblast growth factor for sensitization to amphetamine. The Journal of Neuroscience, 20, RC55: 1-5.

Flores, C., Stewart, J., Salmaso, N., Zhang, Y., & Boksa, P. (2002). Astrocytic basic fibroblast growth factor expression in dopaminergic regions after perinatal anoxia. Biological Psychiatry, 52, 362-370.

Flott-Rahmel, B., Gerdes, W., Pechan, P.A., Brysch, W., Schlingensiepen, K.H., & Seifert, W. (1992). bFGF induces its own gene expression in astrocytic and hippocampal cell cultures. Neuroreport, 3, 1077-1080.

Frantz, G.D., Bohner, A.P., Akers, R.M., & McConnell, S.K. (1994). Regulation of the POU domain gene SCIP during cerebral cortical development. The Journal of Neuroscience, 14, 472-485.

Fujiwara, Y., Kazahaya, Y., Nakashima, M., Sato, M., & Otsuki, S. (1987). Behavioral sensitization to methamphetamine in the rat: An ontogenetic study. Psychopharmacology, 91, 316-319.

Garris, P.A., Ciolkowski, E.L., Pastor, P., & Wightman, R.M. (1994). Efflux of dopamine from the synaptic cleft in the nucleus accumbens of the rat brain. The Journal of Neuroscience, 14, 6084-6093.

Ghasemzadeh, M., Nelson, L., Lu, X-Y, & Kalivas, P. (1999). Neuroadaptations in ionotropic and metabotropic glutamate receptor mRNA produced by cocaine treatment. Journal of Neurochemistry, 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. The Journal of Neuroscience, 21, 6362–6369.

Giorgi, O., De Montis, G., Porceddu, M.L., Mele, S., Calderini, G., Toffano, G, & Biggio, G. (1987). Developmental and age-related changes in D1-dopamine receptors and dopamine content in the rat striatum. Developmental Brain Research, 35, 283-290.

Gnegy, M.E., Hong, P., & Ferrell, S.T. (1993). Phosphorylation of neuromodulin in rat striatum after acute and repeated, intermittent amphetamine. Molecular Brain Research, 20, 289-298.

Gomez-Pinilla, F., Lee, J.W.-K., & Cotman, C.W. (1994). Distribution of basic fibroblast growth factor in the developing rat brain. Neuroscience, 61, 911-923.

Gonzalez, A.M., Berry, M., Maher, P.A., Logan, A., & Baird, A. (1995). A comprehensive analysis of the distribution of FGF-2 and FGFR1 in the rat brain. Brain Research, 701, 201-226.

Gospodarowicz, D., Cheng, J., Lui, G-M, Baird, A., & Bohlen, P. (1984). Isolation of brain fibroblast growth factor by heparin-Sepharose affinity chromatography: Identity with pituitary fibroblast growth factor. Proceedings of the National Academy of Science USA, 81, 6963-6967.

Grace, A.A., & Bunney, B.S. (1983). Intracellular and extracellular electrophysiology of nigral dopaminergic neurons. Identification and characterization. Neuroscience, 10, 301-315.

Grace, A.A., & Onn, S-P (1989). Morphology and electrophysiological properties of immunocytochemically identified rat dopamine neurons recorded *in vitro*. The Journal of Neuroscience, 9, 3463-3481.

Greenough, W.T., & Bailey, C.H. (1988). The anatomy of a memory: Convergence of results across a diversity of tests. Trends in Neuroscience, 11, 142-147.

Greenough, W.T., Withers, G.S., & Wallace, C.S. (1990). Morphological changes in the nervous system arising from behavioral experience: What is the evidence that they are involved in learning and memory? In L.R. Squire, & E. Lindenlaub (Eds.), Synposia Medica Hoeschst (pp. 159-185). New York: Schattauder.

Grignaschi, G., Burbassi, S., Zennaro, E., Bendotti, C., & Cervo, L. (2004). A single high dose of cocaine induces behavioural sensitization and modifies mRNA encoding GluR1 and GAP-43 in rats. European Journal of Neuroscience, 20, 2833-2837.

Grothe, C., Schulze, A., Semkova, I., Muller-Ostermeyer, F., Rege, A., & Wewetzer, K. (2000). The high molecular weight fibroblast growth factor-2 isoforms (21,000 and 23,000 mol. wt) mediate neurotrophic activity on rat embryonic mesencephalic dopaminergic neurons *in vitro*. Neuroscience, 100, 73-86.

Guaza, C., Garcia-Andres, C., Sandi, C., Munoz-Willery, I., Cuevas, P., & Gimenez-Gallego, G. (1996). Fibroblast growth factor decreases locomotor activity in rats. Neuroscience, 75, 805–813.

Haile, C.N., Hiroi, N., Nestler, E.J., & Kosten, T.A. (2001). Differential behavioral responses to cocaine are associated with dynamics of mesolimbic dopamine proteins in Lewis and Fischer 344 rats. Synapse, 41, 179-190.

Hamam, B.N., & Kennedy, T.E. (2003). Visualization of the dendritic arbor of neurons in intact 500 μm thick brain slices. Journal of Neuroscience Methods, 123, 61-67.

Harris, K.M., & Kater, S.B. (1994). Dendritic spines: Cellular specializations imparting both stability and flexibility to synaptic function. Annual Review of Neuroscience, 17, 341-371.

Hartley, R.S., Margulis, M., Fishman, P.S., Lee, V.M.-Y., & Tang, C.-M. (1999). Functional synapses are formed between NTera2 (NT2N, hNT) neurons grown on astrocytes. Journal of Comparative Neurology, 407, 1-10.

Hatten, M.E., Lynch, M., Ryder, R.E., Sanchez, J., Joseph-Silverstein, J., Moscattelli, D., & Rifkin, D.B. (1988). *In vitro* neurite extension by granule neurons is dependent upon astroglial-derived fibroblast growth factor. Developmental Biology, 125, 280-289.

Haycock, J.W., Ahn, N.G., Cobb, M.H., & Krebs, E.G. (1992). ERK1 and ERK2, two microtubule-associated protein 2 kinases, mediate the phosphorylation of tyrosine hydroxylase at serine-31 *in situ*. Proceedings of the National Academy of Sciences USA, 89, 2365-2369.

He, Q., Dent, E.W., & Meiri, K.F. (1997). Modulation of actin filament behavior by GAP-43 (Neuromodulin) is dependent on the phosphorylation status of serine 41, the protein kinase C site. The Journal of Neuroscience, 17, 3515-3524.

Heijtz, R.D., Kolb, B., & Forssberg, H. (2003). Can a therapeutic dose of amphetamine during pre-adolescence modify the pattern of synaptic organization in the brain? European Journal of Neuroscience, 18, 3394-3399.

Hooks, M.S., Jones, G.H., Liem, B.J., & Justice, J.B., Jr. (1992). Sensitization and individual differences to IP amphetamine, cocaine or caffeine following repeated intracranial amphetamine infusions. Pharmacology Biochemistry & Behavior, 43, 815-823.

Horbinski, C., Stachowiak, E.K., Chandrasekaran, V., Miuzukoshi, E., Higgins, D., & Stachowiak, M.K. (2002). Bone morphogenetic protein-7 stimulates initial dendritic growth in sympathetic neurons through an intracellular fibroblast growth factor signaling pathway. Journal of Neurochemistry, 80, 54-63.

Horcholle-Bossavit, G., Gogan, P., Ivanov, Y., Korogod, S., & Tyc-Dumont, S. (2000). The problem of the morphological noise in reconstructed dendritic arborizations. Journal of Neuroscience Methods, 95, 83-93.

Horikawa, K., & Armstrong, W.E. (1988). A versatile means of intracellular labeling: injection of biocytin and its detection with avidin conjugates. Journal of Neuroscience Methods, 25, 1-11.

Hotta, M., Kuriyama, H., Arai, K., Takano, K., & Shibasaki, T. (2001). Fibroblast growth factor inhibits locomotor activity as well as feeding behavior of rats. European Journal of Pharmacology, 416, 101-106.

Hou, J-G.G., Cohen, G., & Mytilineou, C. (1997). Basic fibroblast growth factor stimulation of glial cells protects dopamine neurons from 6-hydroxydopamine toxicity: Involvement of the glutathione system. Journal of Neurochemistry, 69, 76-83.

Hu, X.T., Koeltzow, T.E., Cooper, D.C., Robertson, G.S., White, F.J., & Vezina, P. (2002). Repeated ventral tegmental area amphetamine administration alters dopamine D1 receptor signaling in the nucleus accumbens. Synapse, 45, 159-170.

Hyman, S.E., & Malenka, R.C. (2001). Addiction and the brain: The neurobiology of compulsion and its persistence. Nature Reviews Neuroscience, 2, 695-703.

Impey, S., Obrietan, K., Wong, S.T., Poser, S., Yano, S., Wayman, G., Deloulme, J.C., Chan, G., & Storm, D.R. (1998). Cross talk between ERK and PKA is required for Ca2+ stimulation of CREB-dependent transcription and ERK nuclear translocation. Neuron, 21, 869-883.

Ishiyama, J., Saito, H, & Abe, K. (1991). Epidermal growth factor and basic fibroblast growth factor promote the generation of long-term potentiation in the dentate gyrus of anaesthetized rats. Neuroscience Research, 12, 403-411.

Iwata, S., Hewlett, G.H., Ferrell, S.T., Czernik, A.J., Meiri, K.F., & Gnegy, M.E. (1996). Increased in vivo phosphorylation state of neuromodulin and synapsin I in striatum from rats treated with repeated amphetamine. Journal of Pharmacology and Experimental Therapeutics, 278, 1428-1434.

Jake-Matthews, C., Jolly, D., Queen, A.L., Brose, J., & Vezina, P. (1997). The competitive NMDA receptor antagonist CGS19755 blocks the development of sensitization of the locomotor and dopamine activating effects of amphetamine. Society for Neuroscience Abstracts, 23, 1092.

Jessen, U., Novitskaya, V., Pedersen, N, Serup, P., Berezin, V., & Bock, E. (2001). The transcription factors CREB and c-Fos play key roles in NCAM-mediated neuritogenesis in PC12-E2 cells. Journal of Neurochemistry, 79, 1149-1160.

Johnston, L.D., O'Malley, P.M., & Bachman, J.G. (2001). Monitoring the future: National survey results on drug use, 1975–2000, vol. II. College students and adults ages 19–40. Bethesda, MD: National Institute on Drug Abuse. (NIH No. 01-4925)

Jones, S.W. (1998). Overview of voltage-dependent calcium channels. Journal of Bioenergetics and Biomembranes, 30, 299-312.

Jones, S. & Gutlerrner, J.L. (2002). Addictive drugs modify excitatory synaptic control of midbrain dopamine cells. Neuroreport, 13, 29-33.

Jones, S., Kornblum, J.L, & Kauer, J.A. (2000). Amphetamine blocks long-term synaptic depression in the ventral tegmental area. The Journal of Neuroscience, 20, 5575-5580.

Joy, A., Moffett, J., Neary, K., Mordechai, E., Stachowiak, E.K., Coons, S., Rankin-Shapiro, J., Florkiewicz, R.Z., & Stachowiak, M.K. (1997). Nuclear accumulation of FGF-2 is associated with proliferation of human astrocytes and glioma cells. Oncogene, 14, 171-183.

Joyce, E.M., & Iversen, S.D. (1979). The effect of morphine applied locally to mesencephalic dopamine cell bodies on spontaneous motor activity in the rat. Neuroscience Letters, 14, 207-212.

Kalivas, P.W. (1985). Interactions between neuropeptides and dopamine neurons in the ventromedial mesencephalon. Neuroscience and Biobehavioral Reviews, 9, 573-587.

Kalivas, P.W. (1993). Neurotransmitter regulation of dopamine neurons in the ventral tegmental area. Brain Research Reviews, 18, 75-113.

Kalivas, P.W. (1995). Interactions between dopamine and excitatory amino acids in behavioral sensitization to psychostimulants. Drug and Alcohol Dependency, 37, 95-100.

Kalivas, P.W., & Alesdatter, J.E. (1993). Involvement of NMDA receptor stimulation in the VTA and amygdale in behavioral sensitization to cocaine. Journal of Pharmacology and Experimental Therapeutics, 267, 486-495.

Kalivas, P.W., & Duffy, P. (1990). Effect of acute and daily neurotensin and enkephalin treatments on extracellular dopamine in the nucleus accumbens. The Journal of Neuroscience, 10, 2940-2949.

Kalivas, P.W., & Duffy, P. (1991). A comparison of axonal and somatodendritic dopamine release using in vivo microdialysis. Journal of Neurochemistry, 56, 961-967.

Kalivas, P.W., & Duffy, P. (1993). Time course of extracellular dopamine and behavioral sensitization to cocaine. II. Dopamine perikarya. The Journal of Neuroscience, 13, 276-284.

Kalivas, P.W., & Duffy, P. (1995). D1 receptors modulate glutamate transmission in the ventral tegmental area. The Journal of Neuroscience, 15, 5379-5388.

Kalivas, P.W., & Duffy, P. (1998). Repeated cocaine administration alters extracellular glutamate in the ventral tegmental area. Journal of Neurochemistry, 70, 1497-1502.

Kalivas, P.W., & Stewart, J. (1991). Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. Brain Research Reviews, 16, 223-244.

Kalivas, P.W., & Taylor, S. (1985). Effect of daily neurotensin injection into the ventral tegmental area. Brain Research, 358, 70-76.

Kalivas, P.W., & Weber, B. (1988). Amphetamine injected into the ventral mesencephalon sensitizes rats to peripheral amphetamine and cocaine. Journal of Pharmacology and Experimental Therapeutics, 245, 1095-1102.

Kalivas, P.W., Bourdelais, A., Abhold, R., & Abbott, L. (1989). Somatodendritic release of endogenous dopamine: In vivo dialysis in the A10 dopamine region. Neuroscience Letters, 22, 215-220.

Kalsbeek, A., Voorn, P., & Buijs, R.M. (1992). Development of dopamine-containing systems in the CNS. In A. Bjorklund, T. Hokfelt, & M. Tohyama (Eds.), Ontogeny of transmitters and peptides (pp. 63-112). Amsterdam: Elsevier.

Karler, R., Calder, L.D., & Turkanis, S.A. (1991a). DNQX blockade of amphetamine behavioral sensitization. Brain Research, 552, 295-300.

Karler, R., Calder, L.D., Thai, L.H., & Bedingfield, J.B. (1994). A dopaminergic-glutamatergic basis for the action of amphetamine and cocaine. Brain Research, 658, 8-14.

Karler, R., Finnegan, K.T., & Calder, L.D. (1993). Blockade of behavioral sensitization to cocaine and amphetamine by inhibitors of protein synthesis. Brain Research, 12, 19-24.

Karler, R., Turkanis, S.A., Partlow, L.M., & Calder, L.D. (1991b). Calcium channel blockers and behavioral sensitization. Life Sciences, 49, 165-170.

Katsuki, H, Itsukaichi, Y., & Matsuki, N. (2000). Distinct signalling pathways involved in multiple effects of basic fibroblast growth factor on cultured rat hippocampal neurons. Brain Research, 885, 240-250.

Kawamata, T., Dietrich, W., Schallert, T., Gotts, J., Cocke, R., Benowitz, L., & Finklestein, S. (1997). Intracisternal basic fibroblast growth factor enhances functional recovery and up-regulates the expression of a molecular marker of neuronal sprouting

following focal cerebral infarction. Proceedings of the National Academy of Science USA, 94, 8179-8184.

Keller, H.H., Bartholini, G., & Pletscher, A. (1973). Spontaneous and drug-induced changes of cerebral dopamine turnover during postnatal development of rats. Brain Research, 64, 371-378.

Kennedy, M.B. (2000). Signal-processing machines at the postsynaptic density. Science, 290, 750-754.

Kirksey, D.F., & Slotkin, T.A. (1979). Concomitant development of [^{3}H]-dopamine and [^{3}H]-5-hydroxytryptamine uptake systems in rat brain regions. British Journal of Pharmacology, 67, 387-391.

Kiryushko, D., Berezin, V., & Bock, E. (2004). Regulators of neurite outgrowth: Role of cell adhesion molecules. Annals of the New York Academy of Sciences, 1014, 140-154.

Kline, S., & Felton, D.L. (1985). Ventral tegmental area of the rabbit brain: a developmental Golgi study. Brain Research Bulletin, 14, 485-92.

Knusel, B., Michel, P.P., Schwaber, J.S., & Hefti, F. (1990). Selective and nonselective stimulation of central cholinergic and dopaminergic development *in vitro* by nerve growth factor, basic fibroblast growth factor, epidermal growth factor, insulin and the insulin-like growth factors I and II. The Journal of Neuroscience, 10, 558-570.

Kolkova, K., Novitskaya, V., Pedersen, N, Berezin, V., & Bock, E. (2000). Neural cell adhesion molecule-stimulated neurite outgrowth depends on activation of protein kinase C and the Ras-mitogen-activated protein kinase pathway. The Journal of Neuroscience, 20, 2238-2246.

Kolta, M.G., Shreve, P., De Souza, V., & Uretsky, N.J. (1985). Time course of the development of the enhanced behavioral and biochemical responses to amphetamine after pre-treatment with amphetamine. Neuropharmacology, 24, 823-829.

Kolta, M.G., Scalzo, F.M., Ali, S.F., & Holson, R.R. (1990). Ontogeny of the enhanced response to amphetamine in amphetamine-pretreated rats. Psychopharmacology, 100, 377-382.

Krieglstein, K., Reuss, B., Maysinger, D., & Unsicker, K. (1998). Transforming growth factor-beta mediates the neurotrophic effect of fibroblast growth factor-2 on midbrain dopaminergic neurons. European Journal of Neuroscience, 10, 2746-2750.

Kuzis, K., Reed, S., Cherry, N.J., Woodward, W.R., & Eckenstein, F.P. (1995). Developmental time course of acidic and basic fibroblast growth factors' expression in distinct cellular populations of the rat central nervous system. The Journal of Comparative Neurology, 358, 142-153.

Lacey, M.G., Mercuri, N.B., & North, R.A. (1989). Two cell types in rat substantia nigra zona compacta distinguished by membrane properties and the actions of dopamine and opioids. The Journal of Neuroscience, 9, 1233-1241.

Lankford, K.L., & Letourneau, P.C. (1989). Evidence that calcium may control neurite outgrowth by regulating the stability of actin filaments. Journal of Cell Biology, 109, 1229–1243.

Lenhard, T., Schober, A., Suter-Crazzolara, C., & Unsicker, K. (2002). Fibroblast growth factor-2 requires glial-cell-line-derived neurotrophic factor for exerting its neuroprotective actions on glutamate-lesioned hippocampal neurons. Molecular and Cellular Neuroscience, 20, 181-197.

Le Roux, P.D., & Esquenazi, S. (2002). Astrocytes mediate cerebral cortical neuronal axon and dendrite growth, in part, by release of fibroblast growth factor. Neurological Research, 24, 81-92.

Le Roux, P., & Reh, T. (1994). Regional differences in glial-derived factors that promote dendritic outgrowth from mouse cortical neurons in vitro. The Journal of Neuroscience, 14, 4639-4655

Le Roux, P., & Reh, T. (1995). Maturing glia support primary dendritic but not axonal outgrowth from mouse cortical neurons in vitro. Neuroscience Letters, 198, 5-8.

Le Roux, P., & Reh, T. (1996). Reactive glia promote primary dendritic but not axonal outgrowth from mouse cortical neurons in vitro. Experimental Neurology, 137, 49-65.

Li, Y., Vartanian, A., White, F.J., Xue, C-J, & Wolf, M.E. (1997). Effects of the AMPA receptor antagonist NBQX on the development and expression of behavioral sensitization to cocaine and amphetamine. Psychopharmacology, 134, 266-276.

Licata, S.C., & Pierce, R.C. (2003). The roles of calcium/calmodulin-dependent and Ras/mitogen-activated protein kinases in the development of psychostimulant-induced behavioral sensitization. Journal of Neurochemistry, 85, 14-22.

Licata, S.C., Freeman, A.Y., Pierce-Bancroft, A.F., & Pierce, R.C. (2000). Repeated stimulation of L-type calcium channels in the rat ventral tegmental area mimics the initiation of behavioral sensitization to cocaine. Psychopharmacology, 152, 110-118.

Licata, S.C., Schmidt, H.D., & Pierce, R.C. (2004). Suppressing calcium/calmodulin-dependent protein kinase II activity in the ventral tegmental area enhances the acute behavioural response to cocaine but attenuates the initiation of

cocaine-induced behavioural sensitization in rats. European Journal of Neuroscience, 19, 405-414.

Lin, L.F., Doherty, D.H., Lile, J.D., Bektesh, S., and Collins, F. (1993). GDNF: A glial cell line-derived neurotrophic factor for dopaminergic midbrain neurons. Science, 260, 1130-1132.

Lu, W., & Wolf, M.E. (1999). Repeated amphetamine administration alters AMPA receptor subunit expression in rat nucleus accumbens and medial prefrontal cortex. Synapse, 32, 119-131.

Lu, W., Monteggia, L.M., & Wolf, M.E. (1999). Withdrawal from repeated amphetamine administration reduces NMDAR1 expression in the rat substantia nigra, nucleus accumbens and medial prefrontal cortex. European Journal of Neuroscience, 11, 3167-3177.

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.

Lyford, G.L., Yamagata, K., Kaufmann, W.E., Barnes, C.A., Sanders, L.K., Copeland, N.G., Gilbert, D.J., Jenkins, N.A., Lanahan, A.A., & Worley, P.F. (1995). Arc, a growth factor and activity-regulated gene, encodes a novel cytoskeleton-associated protein that is enriched in neuronal dendrites. Neuron, 14, 433-445.

Malenka, R.C., & Nicoll, R. (1999). Long-term potentiation – a decade of progress? Science, 285, 1870-1874.

Manabe, T., Renner, P., & Nicoll, R.A. (1992). Postsynaptic contribution to long-term potentiation revealed by the analysis of miniature synaptic currents. Nature, 355, 50-55.

Mansour, A., Meador-Woodruff, J.H., Zhou, Q., Civelli, O., Akil, H., & Watson, S.J. (1992). A comparison of D_1 receptor binding and mRNA in rat brain using receptor autoradiographic and in siytu hybridization techniques. Neuroscience, 46, 959-971.

Margolis, E.B., Hjelmstad, G.O., Bonci, A., & Fields, H.L. (2003). K-Opioid agonists directly inhibit midbrain dopaminergic neurons. The Journal of Neuroscience, 23, 9981-9986.

Masserano, J., Baker, I., Natsukari, N., & Wyatt, R. (1996). Chronic cocaine administration increases tyrosine hydroxylase activity in the ventral tegmental area through glutamatergic- and dopaminergic D2-receptor mechanisms. Neuroscience Letters, 217, 73-76.

Matsuzaki, K., Yoshitake, Y., Matuo, Y., Sasaki, H., & Nishikawa, K. (1989). Monoclonal antibodies against heparin-binding growth factor II/basic fibroblast growth factor that block its biological activity: Invalidity of the antibodies for tumor angiogenesis. Proceedings of the National Academy of Sciences USA, 86, 9911-9915.

Mattson, M.P., & Kater, S.B. (1987). Calcium regulation of neurite elongation and growth cone motility. The Journal of Neuroscience, 7, 4034–4043.

Mattson, M.P., Murrain, M., Guthrie, P.B., & Kater, S.B. (1989). Fibroblast growth facto rand glutamate : Opposing roles in the generation and degeneration of hippocampal neuroarchitecture. The Journal of Neuroscience, 9, 3728-3740.

Mattson, M.P., Wang, H., & Michaelis, E.K. (1991). Developmental expression, compartmentalization, and possible role in excitotoxicity of a putative NMDA receptor protein in cultured hippocampal neurons. Brain Research, 565, 94-108.

Mattson, M.P., Kumar, K., Cheng, B., Wang, H., & Michaelis, E.K. (1993). Basic FGF regulates the expression of a functional 71 kDa NMDA receptor protein that mediates calcium influx and neurotoxicity in cultured hippocampal neurons. The Journal of Neuroscience, 13, 4575-4588.

McAllister, A., Katz, L., & Lo, D. (1999). Neurotrophins and synaptic plasticity. In W. Cowan, E. Shooter, C. Stevens, & R. Thompson (Eds.), Annual Review of Neuroscience (pp. 295-318). Palo Alto, CA: Annual Reviews.

McDougall, S.A., Duke, M.A., Bolanos, C.A., & Crawford, C.A. (1994). Ontogeny of behavioural sensitization in the rat: Effects of direct and indirect dopamine agonists. Psychopharmacology, 116, 483-490.

McDougall, S.A., Collins, R.L., Karper, P.E., Watson. J.B., & Crawford, C.A. (1999). Effects of repeated methylphenidate treatment in the young rat: Sensitization of both locomotor activity and stereotyped sniffing. Experimental and Clinical Psychopharmacology, 7, 208-218.

Meiri, K.F., Saffell, J.L., Walsh, F.S., & Doherty, P. (1998). Neurite outgrowth stimulation by neural cell adhesion molecules requires growth-associated protein-43 (GAP-43) function and is associated with GAP-43 phosphorylation in growth cones. The Journal of Neuroscience, 18, 10429-10437.

Meyer, J.S., & Yacht, A.C. (1993). Lack of behavioural sensitization to repeated cocaine administration from postnatal days 1 to 10. International Journal of Neuroscience, 72, 107-113.

Miserendino, M.J.D., & Nestler, E.J. (1995). Behavioral sensitization to cocaine: Modulation by the cyclic AMP system in the nucleus accumbens. Brain Research, 674, 299-306.

Mitsumoto, Y., Watanabe, A., Miyauchi, T., Jimma, F., & Moriizumi, T. (2001). Stimulation of the regrowth of MPP+-damaged dopaminergic fibers by the treatment of mesencephalic cultures with basigin. Journal of Neural Transmission, 108, 1127-1134.

Moffett, J., Kratz, E., Florkiewicz, R., & Stachowiak, M.K. (1996). Promoter regions involved in density-dependent regulation of basic fibroblast growth factor gene expression in human astrocytic cells. Proceedings of the National Academy of Sciences USA, 93, 2470-2475.

Moffett, J., Kratz, E., Myers, J., Stachowiak, E.K., Florkiewicz, R.Z., & Stachowiak, M.K. (1998). Transcriptional regulation of fibroblast growth factor-2 expression in human astrocytes: Implications for cell plasticity. Molecular Biology of the Cell, 9, 2269-2285.

Mugnaini, E., & Oertel, W. (1985). An atlas of the distribution of GABAergic neurons and terminals in the rat CNS as revealed by GAD immunohistochemistry. In: Handbook of chemical neuroanatomy, pp 436-608. Amsterdam: Elsevier.

Murrin, L.C. (1982). In vivo studies of dopamine receptor ontogeny. Life Sciences, 31, 971-980.

Nagai, T., McGeer, P.L., & McGeer, E.G. (1983). Distribution of GABA-T intensive neurons in the rat forebrain and midbrain. Journal of Comparative Neurology, 218, 220-238.

Nakanishi, Y., Kihara, K., Mizuno, K., Masamune, Y., Yoshitake, Y., & Nishikawa, K. (1992). Direct effects of basic fibroblast growth factor on gene transcription in a cell-free system. Proceedings of the National Academy of Sciences USA, 89, 5216-5220.

Nestler, E.J. (1992). Molecular mechanisms of drug addiction. The Journal of Neuroscience, 12, 2439-2450.

Nestler, E.J., Terwilliger, R.Z., Walker, J.R., Sevarino, K.A., & Duman, R.S. (1990). Chronic cocaine treatment decreases levels of the G protein subunits $G_{i\alpha}$ and $G_{o\alpha}$ in dicrete regions of rat brain. Journal of Neurochemistry, 55, 1079-1082.

Nestler, E.J., Guitart, X., Ortiz, J., Trevisan, L. (1994). Second messenger and protein phosphorylation mechanisms underlying possible genetic vulnerability to alcoholism. Annals of the New York Academy of Sciences, 708, 108–118.

Nicoll, R., & Malenka, R.C. (1999). Expression mechanisms underlying NMDA receptor-dependent long-term potentiation. Annals of the New York Academy of Sciences, 868, 515-525.

Noble, M., Fok-Seang, J., & Cohen, J. (1984). Glia are a unique substrate for the in vitro growth of central nervous system neurons. The Journal of Neuroscience, 4, 1892-1903.

Noisin, E.L., & Thomas, W.E. (1988). Ontogeny of dopaminergic function in the rat midbrain tegmentum, corpus striatum and frontal cortex. Brain Research, 469, 241-252.

Nomura, Y., Naitoh, F., & Segawa, T. (1976). Regional changes in monoamine content and uptake of the rat brain during postnatal development. Brain Research, 101, 305-315.

Norris, C.R., & Kalil, K. (1991). Guidance of callosal axons by radial glia in the developing cerebral cortex. The Journal of Neuroscience, 11, 3481-3492.

Nuijtinck, R.R., Baker, R.E., Ter Gast, E., Struik, M.L., & Mud, M.T. (1997). Glutamate dependent dendritic outgrowth in developing neuronal networks of rat hippocampal cells *in vitro*. International Journal of Developmental Neuroscience, 15, 55-60.

Oestreicher, A.B., De Graan, P.N., Gispen, W.H., Verhaagen, J., & Schrama, L.H. (1997). B-50, the growth associated protein-43: Modulation of cell morphology and communication in the nervous system. Progress in Neurobiology, 53, 627-686.

Ohgoh, M., Kimura, M., Ogura, H., Katayama, K., & Nishizawa, Y. (1998). Apoptotic cell death of cultured cerebral cortical neurons induced by withdrawal of astroglial trophic support. Experimental Neurology, 149, 51-63.

Ortiz, J., Fitzgerald, L.W., Charlton, M., Lane, S., Trevisan, L., Guitart, X., Shoemaker, W., Duman, R.S., Nestler, E.J. (1995). Biochemical actions of chronic ethanol exposure in the mesolimbic dopamine system. Synapse, 21, 289-298.

Otterson, O.P., & Storm-Mathisen, J. (1984). Neurons containing or accumulating transmitter amino acids. In: Handbook of chemical neuroanatomy, pp 141-245. Amsterdam: Elsevier.

Otto, D., & Unsicker, K. (1993). FGF-2 mediated protection of cultured mesencephalic dopaminergic neurons against MPTP and MPP^+: Specificity and impact of culture conditions, non-dopaminergic neurons and astroglial cells. Journal of Neuroscience Research, 34, 382-393.

Otto, D., Frotscher, M., & Unsicker, K. (1990). Basic FGF reverses chemical and morphological deficits in the nigrostriatal system of MPTP-treated mice. The Journal of Neuroscience, 10, 1912-1921.

Overton, P.G., & Clark, D. (1992). Iontophoretically administered drugs acting at the N-methyl-D-aspartate receptor modulate burst firing in A9 dopamine neurons in the rat. Synapse, 10, 131-140.

Overton, P.G., Richards, C.D., Berry, M.S., & Clark, D. (1999). Long-term potentiation at excitatory amino acid synapses on midbrain dopamine neurons. Neuroreport, 10, 221-226.

Paladini, C.A., Fiorillo, C.D., Morikawa, H., & Williams, J.T. (2001). Amphetamine selectively blocks inhibitory glutamate transmission in dopamine neurons. Nature Neuroscience, 4, 275-28.

Pardo, J.V., Creese, I., Burt, D.R., & Snyder, S.H. (1977). Ontogenesis of dopamine receptor binding in the corpus striatum of the rat. Brain Research, 125, 376-382.

Park, Y.H., Kantor, L., Wang, K.K.W., & Gnegy, M.E. (2002). Repeated, intermittent treatment with amphetamine induces neurite outgrowth in rat pheochromocytoma cells (PC12 cells). Brain Research, 951, 43-52.

Park Y.H., Kantor L., Guptaroy B., Zhang M., Wang K.K., & Gnegy, M.E. (2003). Repeated amphetamine treatment induces neurite outgrowth and enhanced amphetamine-stimulated dopamine release in rat pheochromocytoma cells (PC12 cells) via a protein kinase C- and mitogen activated protein kinase-dependent mechanism. Journal of Neurochemistry, 87, 1546-57.

Paulson, P.E., & Robinson, T.E. (1995). Amphetamine-induced time-dependent sensitization of dopamine transmission in the dorsal and ventral striatum: A microdialysis study in behaving rats. Synapse, 19, 56-65.

Paulson, P.E., Camp, D.M., & Robinson, T.E. (1991). Time course of transient behavioural depression and persistent behavioural sensitization in relation to regional brain monoamine concentrations during amphetamine withdrawal in rats. Psychopharmacology, 103, 480-492.

Patterson, P.H., & Nawa, H. (1993). Neuronal differentiation factors/cytokines and synaptic plasticity. Cell, 72, 123-137.

Paulson, P.E., & Robinson, T.E. (1995). Amphetamine-induced time-dependent sensitization of dopamine transmission in the dorsal and ventral striatum: A microdialysis study in behaving rats. Synapse, 19, 56-65.

Pechan, P.A., Chowdhury, K., Gerdes, W., & Seifert, W. (1993). Glutamate induces the growth factors NGF, bFGF, the receptor FGF-R1 and c-fos mRNA expression in rat astrocyte culture. Neuroscience Letters, 153, 111-114.

Perron, J.C., & Bixby, J.L. (1999). Distinct neurite outgrowth signalling pathways converge on ERK activation. Molecular and Cellular Neuroscience, 13, 362-378.

Perugini, M., & Vezina, P. (1994). Amphetamine administered to the ventral tegmental area sensitizes rats to the locomotor effects of nucleus accumbens amphetamine. Journal of Paharmacology and Experimental Therapeutics, 270, 690-696.

Pettit, H.O., Pan, H-T, Parsons, L.H., & Justice, J.B., Jr. (1990). Extracellular concentrations of cocaine and dopamine are enhanced during chronic cocaine administration. Journal of Neurochemistry, 55, 798-804.

Phillipson, O.T. (1979). A Golgi study of the ventral tegmental area of Tsai and interfascicular nucleus in the rat. Journal of Comparative Neurology, 187, 99-115.

Pierce, R.C., & Kalivas, P.W. (1997). A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants. Brain Research Reviews, 25, 192-216.

Pierce, R.C., Born, B., Adams, M., & Kalivas, P.W. (1996). Repeated intra-ventral tegmental area administration of SKF-38393 induces behavioural and neurochemical sensitization to a subsequent cocaine challenge. Journal of Pharmacology and Experimental Therapeutics, 278, 384-392.

Pierce, R.C., Bell, K., Duffy, P., & Kalivas, P.W. (1996). Repeated cocaine augments excitatory amino acid transmission in the nucleus accumbens only in rats having developed behavioral sensitization. The Journal of Neuroscience, 16, 1550-1560.

Pierce, R.C., Pierce-Bancroft, A.F., & Prasad, B.M. (1999). Neurotrophin-3 contributes to the initiation of behavioral sensitization to cocaine by activating the

Ras/mitogen-activated protein kinase signal transduction cascade. The Journal of Neuroscience, 19, 8685-8695.

Piontek, J., Regnier-Vigouroux, A., & Brandt, R. (2002). Contact with astroglial membranes induces axonal and dendritic growth of human CNS model neurons and affects the distribution of the growth-associated proteins MAP1B and GAP43. Journal of Neuroscience Research, 67, 471-483.

Plotnikov, A.N., Schlessinger, J., Hubbard, S.R., & Mohammadi, M. (1999). Structural basis for FGF receptor dimerization and activation. Cell, 98, 641-650.

Porcher, W., & Heller, A. (1975). Regional development of catecholamine biosynthesis in rat brain. Journal of Neurochemistry, 19, 1917-1930.

Pothos, E.N., Creese, I., & Hoebel, B.G. (1995). Restricted eating with weight loss selectively decreases extracellular dopamine in the nucleus accumbens and alters dopamine response to amphetamine, morphine, and food intake. The Journal of Neuroscience, 15, 6640–6650.

Ramirez, J., Finklestein, S., Keller, J., Abrams, W., George, M., & Parakh, T. (1999). Basic fibroblast growth factor enhances axonal sprouting after cortical injury in rats. Neuroreport, 10, 1201-1204.

Rao, P.A., Molinoff, P.B., & Joyce, J.N. (1991). Ontogeny of dopamine D1 and D2 receptor subtypes in rat basal ganglia: A quantitative autoradiographic study. Developmental Brain Research, 60, 161-177.

Ray, J., Peterson, D.A., Schinstine, M., & Gage, F.H. (1993). Proliferation, differentiation, and long-term culture of primary hippocampal neurons. Proceedings of the National Academy of Sciences USA, 90, 3602-3606.

Reid, M.S., & Berger, S.P. (1996). Evidence for sensitization of cocaine-induced nucleus accumbens glutamate release. Neuroreport, 7, 1325-1329.

Reisert, I., Schuster, R., Zienecker, R., & Pilgrim, C. (1990). Prenatal development of mesencephalic and diencephalic dopaminergic systems in the male and female rat. Developmental Brain Research, 53, 222-229.

Reith, M., Meisler, B., Sershen, H., & Lajtha, A. (1986). Structural requirements for cocaine cogeners to interact with dopamine and serotonin uptake sites in mouse brain and to induce stereotyped behavior. Biochemical Pharmacology, 35, 123–9.

Reuss, B., & Unsicker, K. (2000). Survival and differentiation of dopaminergic mesencephalic neurons are promoted by dopamine-mediated induction of FGF-2 in striatal astroglial cells. Molecular and Cellular Neuroscience, 16, 781-792.

Riva, M.A., & Mocchetti, I. (1991). Developmental expression of the basic fibroblast growth factor gene in the rat brain. Developmental Brain Research, 62, 45-50.

Robinson, T. E., & Becker, J. B. (1986). Enduring changes in brain and behavior produced by chronic amphetamine administration: A review and evaluation of animal models of amphetamine psychosis. Brain Research Reviews, 396, 157-198.

Robinson, T.E., & Berridge, K.C. (1993). The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Research Reviews, 18, 247-291.

Robinson T.E., & Berridge, K.C. (2000). The psychology and neurobiology of addiction: An incentive-sensitization view. Addiction, 95, S91–S117.

Robinson, T.E., & Berridge, K.C. (2003). Addiction. Annual Review of Psychology, 54, 25-53.

Robinson, T.E., & Kolb, B. (1997). Persistent structural modifications in nucleus accumbens and prefrontal cortex neurons produced by previous experience with amphetamine. The Journal of Neuroscience, 17, 8491-8497.

Robinson, T.E., & Kolb, B. (1999). Alterations in the morphology of dendrites and dendritic spines in the nucleus accumbens and prefrontal cortex following repeated treatment with amphetamine or cocaine. European Journal of Neuroscience, 11, 1598–1604.

Robinson, T.E., Gorny, G., Mitton, E., & Kolb, B. (2001). Cocaine self-administration alters the morphology of dendrites and dendritic spines in the nucleus accumbens and neocortex. Synapse, 39, 257-266.

Robinson, T.E., Jurson, P.A., Bennett, J.A., & Bentgen, K.M. (1988). Persistent sensitization of dopamine neurotransmission in ventral striatum nucleus accumbens produced by prior experience with +-amphetamine: A microdialysis study in freely moving rats. Brain Research, 462, 211-222.

Rockow, S., Tang, J., Xiong, W., & Li, W. (1996). Nck inhibits NGF and basic FGF induced PC12 cell differentiation via mitogen-activated protein kinase-independent pathway. Oncogene, 12, 2351-2359.

Rousselet, A., Fetler, L., Chamak, B., & Prochiantz, A. (1988). Rat mesencephalic neurons in culture exhibit different morphological traits in the presence of media conditioned on mesencephalic or striatal astroglia. Developmental Biology, 129, 495-504.

Rousselet, A., Autillo-Touati, A., Araus, D., & Prochiantz, A. (1990). *In vitro* regulation of neuronal morphogenesis and polarity by astrocyte-derived factors. Developmental Biology, 137, 33-45.

Rowntree, S., & Kolb, B. (1997). Blockade of basic fibroblast growth factor retards recovery from motor cortex injury in rats. European Journal of Neuroscience, 9, 2432-2441.

Rydel, R.E. & Greene, L.A. (1987). Acidic and basic fibroblast growth factors promote stable neurite outgrowth and neuronal differentiation in cultures of PC12 cells. The Journal of Neuroscience, 7, 3639-3653.

Saal, D., Dong, Y., Bonci, A., & Malenka, R.C. (2003). Drugs of abuse and stress trigger a common synaptic adaptation in dopamine neurons. Neuron, 37, 577-582.

Sales, N., Martres, M.P., Bouthenet, M.L., & Schwartz, J.C. (1989). Ontogeny of dopaminergic D2 receptors in the rat nervous system: Characterization and detailed autoradiographic mapping with [^{125}H]iodosulpiride. Neuroscience, 28, 637-700.

Scalzo, F.M., & Holson, R.R. (1992). The ontogeny of behavioral sensitization to phencyclidine. Neurotoxicology and Teratology, 14, 7-14.

Seiden, L.S., Sabol, K.E., & Ricaurte, G.A. (1993). Amphetamine: Effects on catecholamine systems and behavior. Annual Review of Pharmacology & Toxicology, 33, 639–677.

Seger, R., & Krebs, E.G. (1995). The MAPK signaling cascade. FASEB Journal, 9, 726–735.

Self, D.W., & Nestler, E.J. (1995). Molecular mechanisms of drug reinforcement and addiction. Annual Review of Neuroscience, 18, 463-495.

Shin, D.M., Korada, S., Raballo, R., Shashikant, C.S., Simeone, A., Taylor, J.R., & Vaccarino, F. (2004). Loss of glutamatergic pyramidal neurons in frontal and temporal cortex resulting from attenuation of FGFR1 signaling is associated with spontaneous hyperactivity in mice. The Journal of Neuroscience, 24, 2247-2258.

Shitaka, Y., Matsuki, N., Saito, H., & Katsuki, H. (1996). Basic fibroblast growth factor increases functional L-type Ca^{2+} channels in fetal rat hippocampal neurons: Implications for neurite morphogenesis *in vitro*. The Journal of Neuroscience, 16, 6476-6489.

Sholl, D.A. (1956). The organization of the cerebral cortex. London: Methuen.

Shults, C.W., Ray, J., Tsuboi, K., & Gage, F.H. (2000). Fibroblast growth factor-2-producing fibroblasts protect the nigrostriatal dopaminergic system from 6-hydroxydopamine. Brain Research, 883, 192-204.

Sklair-Tavron, L., Shi, W-X, Lane, S.B., Garris, H.W., Bunney, B.S., & Nestler, E.J. (1996). Chronic morphine induces visible changes in the morphology of mesolimbic dopamine neurons. Proceedings of the National Academy of Sciences USA, 93, 11202-11207.

Smith, G.M., Ruitishauser, Y., Silver, J., & Miller, R.H. (1990). Maturation of astrocytes *in vitro* alters the extent and molecular basis of neurite outgrowth. Developmental Biology, 138, 377-390.

Smith, S. (1999). Dissecting dendrite dynamics. Science, 283, 1860-1861.

Snyder, K.J., Katovic, N.M., & Spear, L.P. (1998). Longevity of the expression of behavioral sensitization to cocaine in preweanling pups. Pharmacology Biochemistry and Behavior, 60, 909-914.

Soderling, T.R., Tan, S.E., McGlade-McCulloh, E., Yamamoto, H., & Kukunaga, K. (1994). Excitatory interactions between glutamate receptors and protein kinases. Journal of Neurobiology, 25, 304-311.

Sorg, B.A., & Ulibarri, C. (1995). Application of a protein synthesis inhibitor into the ventral tegmental area, but not into the nucleus accumbens, prevents behavioral sensitization to cocaine. Synapse, 20, 217-224.

Srivastava, L.K., Morency, M.A., & Mishra, R.K. (1992). Ontogeny of dopamine D2 receptor mRNA in rat brain. European Journal of Pharmacology, 225, 143-150.

Stachowiak, M.K., Fang, X., Myers, J.M., Dunham, S.M., Berezney, R., Maher, P.A., & Stachowiak, E.K. (2003). Integrative nuclear FGFR1 signaling (INFS) as a part of a universal "feed-forward-and-gate" signaling module that controls cell growth and differentiation. Journal of Cellular Biochemistry, 90, 662-691.

Stachowiak, M.K., Maher, P.A., Joy, A., Mordechai, E., & Stachowiak, E.K. (1996). Nuclear accumulation of fibroblast growth factor receptors is regulated by multiple signals in adrenal medullary cells. Molecular Biology of the Cell, 7, 1299-1317.

Steketee, J.D., & Kalivas, P.W. (1990). Modulation of dopamine neurons by apamin. Journal of Pharmacology and Experimental Therapeutics, 254, 711-719.

Steketee, J.D., & Kalivas, P.W. (1991). Sensitization to psychostimulants and stress after injection of pertussis toxin into the A10 dopamine region. Journal of Pharmacology and Experimental Therapeutics, 259, 916–924.

Stewart, J., & Druhan, J.P. (1993). Development of both conditioning and sensitization of the behavioral activating effects of amphetamine is blocked by the non-competitive NMDA receptor antagonist, MK-801. Psychopharmacology, 110, 125-132.

Stewart, J., & Vezina, P. (1989). Microinjections of SCH-23390 into the ventral tegmental area and substantia nigra pars reticulate attenuate the development of sensitization to the locomotor effects of systemic amphetamine. Brain Research, 495, 401-406.

Stoop, R., & Poo, M. (1996). Synaptic modulation by neurotrophic factors. Progress in Brain Research, 109, 359-364.

Stroemer, R.P., Kent, T.A., & Hulsebosch, C.E. (1998). Enhanced neocortical sprouting, synaptogenesis, and behavioral recovery with D-amphetamine therapy after neocortical infarction in rats. Stroke, 29, 2381-2395.

Sulzer, D., Chen, T.K., Lau, Y.Y., Kristensen, H., Rayport, S., & Ewing, A. (1995). Amphetamine redistributes dopamine from synaptic vesicles to the cytosol and promotes reverse transport. The Journal of Neuroscience, 15, 4102-4108.

Suter-Crazzolara, C., & Unsicker, K. (1996). GDNF mRNA levels are induced by FGF-2 in rat C6 glioblastoma cells. Molecular Brain Research, 41, 175-182.

Takayama, H., Ray, J., Raymon, H., Baird, A, Hogg, J., Fisher, L., & Gage, F. (1995). Basic fibroblast growth factor increases dopaminergic graft survival and function in a rat model of Parkinson's disease. Nature Medicine, 1, 53-58.

Tanabe, L.M., Suto, N., Creekmore, E., Steinmiller, C.L., & Vezina, P. (2004). Blockade of D2 dopamine receptors in the VTA induces a long-lasting enhancement of the locomotor activating effects of amphetamine. Behavioral Pharmacology, 15, 387-395.

Tanaka, T., Saito, H., & Matsuki, N. (1996). Basic fibroblast growth factor modulates synaptic transmission in cultured rat hippocampal neurons. Brain Research, 723, 190-195.

Tanaka, H., Grooms, S.Y., Bennett, M.V., & Zukin, R.S. (2000). The AMPAR subunit GluR2: Still front and center-stage. Brain Research, 886, 190-207.

Tejero-Diez, P., Rodriguez-Sanchez, P., Martin-Cofreces, N.B., & Diez-Guerra, F.J. (2000). bFGF stimulates GAP-43 phosphorylation at Ser41 and modifies its intracellular localization in cultured hippocampal neurons. Molecular and Cellular Neuroscience, 16, 766-780.

Terlau, H., & Seifert, W. (1990). Fibroblast growth factor enhances long-term potentiation in the hippocampal slice. European Journal of Neuroscience,2, 973-977.

Thomas, M.J., Malenka, R.C., & Bonci, A. (2000). Modulation of long-term depression by dopamine in the mesolimbic system. The Journal of Neuroscience, 20, 5581-5586.

Thomas, M.J., Beurrier, C., Bonci, A., Malenka, R.C. (2001). Long-term depression in the nucleus accumbens: A neural correlate of behavioral sensitization to cocaine. Nature Neuroscience, 4, 1217-1223.

Tirelli, E. (2001). Short-term contextual sensitization and conditioned hyperkinesia produced by cocaine in suckling rats aged 4-10 days and 14-20 days. Psychopharmacology, 156, 42-52.

Tirelli, E., Laviola, G., & Adriani, W. (2003). Ontogenesis of behavioral sensitization and conditioned place preference induced by psychostimulants in laboratory rodents. Neuroscience and Biobehavioral Reviews, 27, 163-178.

Tolliver, B.K., Ho, L.B., Fox, L.M., & Berger, S.P. (1999). Necessary role for ventral tegmental area adenylate cyclase and protein kinase A in induction of behavioral sensitization to intraventral tegmental area amphetamine. Journal of Pharmacology and Experimental Therapeutics, 289, 38-47.

Tong, Z.-Y., Overton, P.G., & Clark, D. (1995). Chronic administration of (+)-amphetamine alters the reactivity of midbrain dopaminergic neurons to prefrontal cortex stimulation in the rat. Brain Research, 674, 63-74.

Trent, F., Nakamura, S., & Tepper, J.M. (1991). Amphetamine exerts anomolous effects on dopaminergic neurons in neonatal rats in vivo. European Journal of Pharmacology, 204, 265-272.

Tsuchida, K., Ujike, H., Kanzaki, A., Fujiwa, Y., & Akiyama, K. (1994). Ontogeny of enhanced striatal dopamine release in rats with methamphetamine-induced behavioral sensitization. Pharmacology Biochemistry and Behavior, 47, 161-168.

Ujike, H., Tsuchida, K., Akiyama, K., Fujiwara, Y., & Kuroda, S. (1995). Ontogeny of behavioral sensitization to cocaine. Pharmacology Biochemistry and Behavior, 50, 613-617.

Ujike, H., Takaki, M., Kodama, M., & Kuroda, S. (2002). Gene expression related to synaptogenesis, neuritogenesis, and MAP kinase in behavioral sensitization to psychostimulants. Annals of the New York Academy of Sciences, 965, 55-67.

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.

Unsicker, K., Grothe, C., Ludecke, G., Dorte, O., & Westermann, R. (1993). Fibroblast growth factors: Their role in the central and peripheral nervous system. In S. Loughlin & J. Fallon (Eds.), Neurotrophic Fcators (pp. 313-338). San Diego, CA: Academic Press.

Vanderschuren, L.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.

Vezina, P. (1993). Amphetamine injected into the ventral tegmental area sensitizes the nucleus accumbens dopaminergic responses to systemic amphetamine: an in vivo microdialysis study in the rat. Brain Research, 605, 332–337.

Vezina, P. (1996). D_1 dopamine receptor activation is necessary for the induction of sensitization by amphetamine in the ventral tegmental area. The Journal of Neuroscience, 16, 2411-2420.

Vezina, P. (2004). Sensitization of midbrain dopamine neuron reactivity and the self-administration of psychomotor stimulant drugs. Neuroscience and Biobehavioral Reviews, 27, 827-839.

Vezina, P., & Queen, A.L. (2000). Induction of locomotor sensitization by amphetamine requires the activation of NMDA receptors in the rat ventral tegmental area. Psychopharmacology, 151, 184-191.

Vezina, P., & Stewart, J. (1984). Conditioning and place-specific sensitization of increases in activity induced by morphine in the VTA. Pharmacology Biochemistry & Behavior, 20, 925-934.

Vezina, P., & Stewart, J. (1990). Amphetamine administered to the ventral tegmental area but not to the nucleus accumbens sensitizes rats to systemic morphine: Lack of conditioned effects. Brain Research, 516, 99-106.

Vezina, P., Kalivas, P.W., & Stewart, J. (1987). Sensitization occurs to the locomotor effects of morphine and the specific mu opioid receptor agonist, DAGO, administered repeatedly to the VTA but not to the nucleus accumbens. Brain Research, 417, 51-58.

Wagner, F.A., & Anthony, J.C. (2002). From first drug use to drug dependence: Developmental periods of risk for dependence upon marijuana, cocaine, and alcohol. Neuropsychopharmacology, 26, 479-488.

Wagner, J.P., Black, I.B., & DiCocco-Bloom, E. (1999). Stimulation of neonatal and adult brain neurogenesis by subcutaneous injection of basic fibroblast growth factor. The Journal of Neuroscience, 19, 6006-6016.

Waite, W.L., & Kolb, B.E. (2003). Pre- or postnatal FGF-2 administration stimulates functional recovery and brain plasticity after perinatal cortical lesions in rats. The Society for Neuroscience Abstracts, 29, 459.9.

Walicke, P.A. (1988). Basic and acidic fibroblast growth factors have trophic effects on neurons from multiple CNS regions. The Journal of Neuroscience, 8, 2618-2627.

Walicke, P., Cowan, W.M., Ueno, N., Baird, A., & Guillemin, R. (1986). Fibroblast growth factor promotes survival of disassociated hippocampal neurons and enhances neurite extension. Proceedings of the National Academy of Sciences USA, 83, 3012-3016.

Walker, D.G., Terai, K., Matsuo, A., Beach, T.G., McGeer, E.G., & McGeer, P.L. (1998). Immunohistochemical analyses of fibroblast growth factor receptor-1 in the human substantia nigra. Comparison between normal and Parkinson's disease cases. Brain Research, 794, 181-187.

Wanaka, A., Johnson, E.M., Jr., & Milbrandt, J. (1991). Localization of FGF receptor mRNA in the adult rat central nervous system by in situ hybridization. Neuron, 5, 267-281.

Wang, R.Y. (1981a). Dopaminergic neurons in the rat ventral tegmental area. I. Identification and characterization. Brain Research Reviews, 3, 123-140.

Wang, R.Y. (1981b). Dopaminergic neurons in the rat ventral tegmental area. II. Evidence for autoregulation. Brain Research Reviews, 3, 141-151.

White, F.J. (1996). Synaptic regulation of mesocorticolimbic dopamine neurons. Annual Review of Neuroscience, 19, 405–436.

White, F.J., & Kalivas, P.W. (1998). Neuroadaptations involved in amphetamine and cocaine addiction. Drug and Alcohol Dependence, 51, 141–53.

White, F.J., & Wang, R.Y. (1984). A10 dopamine neurons: Role of autoreceptors in determining firing rate and sensitivity to dopamine agonists. Life Sciences, 34, 1161-1170.

Williams, E.J., Furness, J., Walsh, F.S., & Doherty, P. (1994). Characterization of the second messenger pathway underlying neurite outgrowth stimulated by FGF. Development, 120, 1685–1693.

Williams, E.J., Mittal, B., Walsh, F.S., & Doherty, P. (1995). A Ca2+/calmodulin kinase inhibitor, KN-62, inhibits neurite outgrowth stimulated by CAMs and FGF. Molecular and Cellular Neuroscience, 6, 69-79.

Wise, R.A. (1989). The brain and reward. In J.M. Liebman & S.J. Cooper (Eds.), The neuropharmacological basis of reward (pp. 377-424). New York: Oxford University Press.

Wise, R.A. (2004). Dopamine, learning and motivation. Nature Reviews Neuroscience, 5, 483-494.

Wood, R.D., Tirelli, E., Snyder, K.J., Heyser, C.J., LaRocca, T.M., & Spear, L.P. (1998). Evidence for behavioral sensitization to cocaine in preweanling rat pups. Psychopharmacology, 138, 114-123.

Wolf, M.E. (1998). The role of excitatory amino acids in behavioral sensitization to psychomotor stimulants. Progress in Neurobiology, 54, 679-720.

Wolf, M.E. (2003). LTP may trigger addiction. Molecular Interventions, 3, 205-219.

Wolf, M.E., & Xue, C.-J. (1998). Amphetamine and D1 receptor agonists produce biphasic effects on glutamate efflux in rat ventral tegmental area: Modification by repeated amphetamine administration. Journal of Neurochemistry, 70, 198-209.

Wolf, M.E., White, F.J., & Hu, X-T (1994). MK-801 prevents alterations in the mesoaccumbens dopamine system associated with behavioral sensitization to amphetamine. The Journal of Neuroscience, 14, 1735-1745.

Wolf, M.E., Frank, F.J., Nassar, R.N., Brooderson, R.J., & Khansa, M.R. (1993). Differential development of autoreceptor supersensitivity and enhanced dopamine release

during amphetamine sensitization. Journal of Pharmacology and Experimental Therapeutics, 264, 249-255.

Woodward, W.R., Nishi, R., Meshul, C.K., Williams, T.E., Coulombe, M., & Eckenstein, F.P. (1992). Nuclear and cytoplasmic localization of basic fibroblast growth factor in astrocytes and CA2 hippocampal neurons. The Journal of Neuroscience, 12, 142-152.

Xue, C-J, Ng, J.P., Li, Y., & Wolf, M.E. (1996). Acute and repeated systemic amphetamine administration: Effects on extracellular glutamate, aspartate, and serine levels in rat ventral tegmental area and nucleus accumbens. Journal of Neurochemistry, 67, 352-363.

Yankner, B.A., Benowitz, L.I., Villa-Komaroff, L., & Neve, R.L. (1990). Transfection of PC12 cells with the human GAP-43 gene: effects on neurite outgrowth and regeneration. Molecular Brain Research, 7, 39-44.

Yazaki, N., Hosoi, Y., Kawabata, K., Miyake, A., Minami, M., Satoh, M., Ohta, M., Kawasaki, T., & Itoh, N. (1994). Differential expression patterns of mRNAs for members of the fibroblast growth factor receptor family, FGFR-1-FGFR-4, in rat brain. Journal of Neuroscience Research, 37, 445-452.

Yoshida, K., & Gage, F.H. (1991). Fibroblast growth factors stimulate nerve growth factor synthesis and secretion by astrocytes. Brain Research, 538, 118-126.

Zaheer, A., Zhong, W., & Lim, R. (1995). Expression of mRNAs of multiple growth factors and receptors by neuronal cell lines: Detection with RT-PCR. Neurochemical Research, 20, 1457-1463.

Zamburlin, P., Gilardino, A., Ariano, P., Lovisolo, D., & Distasi, C. (2003). GDNF and bFGF are differentially involved in glial cell differentiation and neurite bundle formation in cultures from chick embryonic ciliary ganglia. Neuroreport, 14, 2343-2347.

Zavala, A.R., Nazarian, A., Crawford, C.A., & McDougall, S.A. (2000). Cocaine-induced behavioral sensitization in the young rat. Psychopharmacology, 151, 291-298.

Zeng, W., Hyttel, J., & Murrin, L.C. (1988). Ontogeny of dopamine D1 receptors in rat striatum. Journal of Neurochemistry, 50, 862-867.

Zhang, X., 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. Journal of Pharmacology and Experimental Therapeutics, 281, 699-706.

Zhou, D. & Difiglia, M. (1993). Basic fibroblast growth factor enhances the growth of postnatal neostriatal GABAergic neurons *in vitro*. Experimental Neurology, 122, 171-188.