Opposite Effects of Amphetamine Self-administration Experience on Dendritic Spines in the Medial and Orbital Prefrontal Cortex

We studied the long-term effects of amphetamine self-administration experience (or sucrose reward training) on dendritic morphology (spine density) in nucleus accumbens (Nacc), medial (MPC) and orbital prefrontal cortex (OFC), and hippocampus (CA1 and dentate). Independent groups of rats were trained under a continuous schedule of reinforcement to nose-poke for infusions of amphetamine (0.125 mg/kg/inf) or to receive sucrose pellets during 2 h daily test sessions for 14–20 days. One month after the last training session, the brains were collected and processed for Golgi-Cox staining. We found that: (i) amphetamine self-administration experience selectively increased spine density on medium spiny neurons in the Nacc and on pyramidal neurons in the MPC; (ii) in contrast, amphetamine self-administration decreased spine density in the OFC, whereas sucrose-reward training increased spine density; and (iii) both amphetamine self-administration and sucrose-reward experience increased spines in the CA1, but had no effect in the dentate gyrus. Thus, amphetamine self-administration experience produces long-lasting and regionally-selective morphological alterations in rat forebrain — alterations that may underlie some of the persistent psychomotor, cognitive and motivational consequences of chronic drug exposure.

Keywords: addiction, Golgi, hippocampus, nucleus accumbens, rat, sensitization

Introduction

Inquiry into the pathophysiology of drug addiction has focused on mesocorticolimbic dopamine projections originating in the ventral tegmental area and projecting to the nucleus accumbens (Nacc) and medial prefrontal cortex (MPC), because (i) this circuit is a critical substrate for the reinforcing and incentive motivational effects of drug- and non-drug rewards (Wise and Bozarth, 1984; Salamone, 1991; Berridge and Robinson, 1998; Ikemoto and Panksepp, 1999); and (ii) when given repeatedly, drugs such as amphetamine or cocaine produce persistent neuroplastic alterations in this system (Robinson and Becker, 1986; Kalivas et al., 1992; Robinson and Berridge, 1993; Vanderschuren and Kalivas, 2000; Vezina, 2004). Of particular relevance to the present study are reports of persistent psychostimulant drug-induced structural changes in dendritic branching and spine density on medium spiny neurons in the Nacc and pyramidal cells in the MPC (Robinson and Kolb, 1997, 1999b; Robinson et al., 2001). These structural changes are thought to reflect changes in pattern of synaptic connectivity in these brain regions, altering their operation, and thus contributing to some of the persistent behavioral sequela associated with repeated stimulant use.

However, recent studies demonstrate that stimulant drugs produce persistent alterations in other forebrain regions as well, in particular, in orbital frontal cortex (OFC). For example, imaging studies in human stimulant users have found persistent basal and drug-induced changes in metabolic activity (Volkow et al., 1992; Paulus et al., 2002; Adinoff et al., 2003; Bolla et al., 2003), DA receptor levels (Volkow et al., 1993; London et al., 2000) and gray matter volume in OFC (Fein et al., 2002; Franklin et al., 2002; Matochik et al., 2003). Furthermore, human cocaine or amphetamine users, and cocaine-exposed monkeys, show long-lasting performance deficits on cognitive tasks sensitive to MPC and/or OFC function (Jentsch and Taylor, 1999; Rogers et al., 1999; Ornstein et al., 2000). Finally, recent studies in rats have shown that sensitizing regimens of cocaine produce deficits in discrimination/reversal learning (Schoenbaum et al., 2004) and reinforcer devaluation (Setlow and Schoenbaum, 2004) — processes dependent on OFC function (Gallagher et al., 1999; McAlonan and Brown, 2003; Schoenbaum et al., 2003). Together, these studies indicate that, apart from changes in the ‘traditional’ reward circuit of the brain, psychostimulant drugs also produce alterations in other areas associated with higher-order associative learning and/or response selection/inhibition. Little is known, however, about the persistent neurobiological effects of psychostimulant drugs in prefrontal regions.

Thus, the primary reason for the experiment reported here was to examine the effects of amphetamine on dendritic morphology in both OFC and MPC, as well as in Nacc and hippocampus (CA1 and dentate gyrus). Additionally, we sought to determine if amphetamine self-administration experience (versus instrumental training with sucrose-reward) has effects on dendritic morphology similar to those seen previously with experimenter-administered amphetamine. That is, in previous studies on structural plasticity associated with amphetamine treatment, the drug was always administered by an experimenter (Robinson and Kolb, 1997, 1999b). This is an important consideration because the behavioral and neurobiological effects of drugs, including morphine’s effects on dendritic morphology in some regions, vary depending on whether drug is experimenter-administered (i.e. non-contingently) or is self-administered (contingent on the animal performing an operant response) (Dworkin et al., 1995; Robinson and Kolb, 1999a).

Materials and Methods

Subjects

Subjects were male Sprague-Dawley rats (Harlan, Indianapolis, IN), weighing 250–300g at the start of the experiment. Throughout the experiment the rats were housed individually in a climate-controlled animal colony maintained on a 14/10 h light/dark cycle (lights on at 0800 h), and food and water were always freely available (except during daily test sessions). All procedures used followed the Guide for the Care
Behavioral Procedures
Rats were transported from their housing cage to the operant chamber each day for 14-20 days, where they were allowed to NP for either intravenous infusions of amphetamine (AMPH condition) or for delivery of sucrose pellets (SUC condition) on a continuous reinforcement schedule (FR1). A training session commenced with the illumination of the house-light and the activation of the NP stimulus light for 20s. In the AMPH condition, responses into the active NP activated an infusion of amphetamine (0.125 mg/kg D-amphetamine over 2.8 s. In the SUC condition responses into the active NP delivered a single bolus infusion of 0.125 mg/kg d-amphetamine and sucrose pellets (Research Diets, Inc.) could be delivered was located centrally between the two NP holes.

Apparatus
Behavioral testing was conducted in standard operant chambers (Med Associates, Inc., Georgia, VT) containing an acrylic front hinged loading door, acrylic side panels and a stainless steel back panel (22 × 18 × 13 cm). The chambers were located in sound- and light-attenuating cabinets equipped with fans providing constant ventilation and low level background noise. Two nose-poke (NP) holes, containing a white cue-light, were located on one side wall of the chamber at ~3 cm above a grid floor. Responding into one (active) NP hole resulted in simultaneous delivery of the amphetamine- or sucrose-reward (see below) and activation of the cue-light. Responding into the second (inactive) NP hole was recorded but had no programmed consequences. Finally, for the sucrose condition, a recessed receptacle into which 45 mg Noyes pellets (Research Diets, Inc.) could be delivered was located centrally between the two NP holes.

Anatomical Procedures
One month after the last training session, rats were deeply anesthetized with sodium pentobarbital and perfused transectially with 0.9% saline. The brains were removed, placed in vials containing Golgi–Cox solution for 14 days followed by 30% sucrose solution for 3 days. The brains were cut into 200 μm thick coronal sections using a vibrating microtome and stained using procedures described previously (Gibb and Kolb, 1998). Neurons in the following regions were examined (Fig. 1): (i) medium spiny neurons in the Nacc; (ii) pyramidal neurons in layer V of area Cg3 (MPC) and layer III of agranular insular cortex (OFc); and (iii) pyramidal neurons in hippocampus region CA1 and granule cells in dentate gyrus. Neurons were identified at low power (100–125×), and in order to be included in the analysis their dendritic tree had to be well stained and visible, and not obscured by blood vessels or glia or other cells. For prefrontal pyramidal neurons one third-order terminal tip from both the basilar and apical dendritic trees was identified, and the total number of visible spines along the length of the dendritic segment (at least 20 μm long) was counted. For medium spiny neurons spines were counted on one terminal tip (third order or up) per neuron. For pyramidal neurons in hippocampus region CA1, spines were counted on one third-order tip from only basilar dendrites. For granule cells in the dentate gyrus, the dendritic layer was divided into three layers of roughly equal width (inner, middle and outer molecular layer) and spine density was determined for each layer separately. Spine density was determined with a total magnification on the scope of 1600×, and with additional magnification from the camera lucida, the total magnification was ~2000×. Spine counts were obtained from at least five cells in each hemisphere of each rat, and statistical analyses were conducted after averaging across cells within hemispheres.

Results
Two rats in the AMPH condition had leaking or occluded catheters after day 14 of training, and these rats, together with two randomly selected rats from both the sucrose and the untreated control conditions, were excluded from further training. However, their brains were processed for Golgi–Cox and analyzed for alterations in spine density. Thus, the results were obtained from 2 rats/group that received 14 days of training and from 5–6 rats/group that received 20 days of training.

Behavior
Figure 2a shows the mean (± SEM) number of responses into the active and inactive NP hole and the number of drug infusions received by rats in the AMPH condition. Active NP
responding for AMPH infusions progressively increased during the first 2 weeks of training, after which NP responding and drug-intake remained relatively stable. By the end of the 14–20 day self-administration phase, cumulative intake of amphetamine was on average 29.5 ± 2.01 mg/kg.

Figure 2b shows the mean (± SEM) number of responses into the active and inactive NP hole and the number of pellets received by rats in the SUC condition. Not surprisingly, acquisition was fast, responding stabilized rapidly and the overall response rate in the SUC condition was much higher than for the AMPH condition.

For both conditions responding into the inactive (non-reinforced) NP hole was low and remained relatively stable over the entire training period (Fig. 2).

**Anatomy**

**Nucleus Accumbens**

Figure 3 shows the effects of AMPH self-administration or SUC-reward training on dendritic spine density on medium spiny neurons in the Nacc shell. A one-way ANOVA indicated that there was a significant effect of Group \( F(2,33) = 35.02, P < 0.001 \) and Fisher’s post-hoc comparisons revealed that, relative to the UNTR and SUC groups, AMPH self-administration experience significantly increased spine density (Fisher’s PLSD test, \( P_s < 0.05 \)). This effect was unique to AMPH self-administration experience because SUC-reward training did not significantly alter Nacc spine density relative to UNTR control rats.

**Prefrontal Cortex**

Figure 4 shows the results for basilar (Fig. 4a,c) and apical (Fig. 4b,d) dendrites of pyramidal neurons in the MPC (Fig. 4a,b) and OFC (Fig. 4c,d). For the MPC, a one-way analysis of variance (ANOVA) resulted in a significant effect of Group for both basilar \( F(2,33) = 14.98, P < 0.001 \) and apical dendrites \( F(2,33) = 31.25, P < 0.001 \). Post-hoc tests indicated that AMPH self-administration significantly increased spine density on apical and basilar dendrites relative to both UNTR and SUC groups (Ps < 0.05), and the latter groups were not significantly different.

For the OFC, a one-way ANOVA also resulted in a significant effect of Group for both basilar \( F(2,51) = 44.4, P < 0.001 \) and apical dendrites \( F(2,51) = 99.95, P < 0.001 \). Post-hoc tests revealed this effect of group was due to a decrease (rather than increase) in dendritic spines on apical and basilar dendrites in the AMPH self-administration condition relative to both UNTR control and SUC conditions (Ps < 0.05). Finally, SUC reward training produced a significant increase in dendritic spines on apical dendrites \( P < 0.05 \) in the OFC, but this effect did not reach significance on basilar dendrites.

**Figure 2.** The mean (± SEM) number of responses into the active (black circles) and inactive (open circles) NP holes, and the number of infusions or pellets received (gray circles). Rats were allowed to NP on an FR1 schedule of reinforcement to obtain intravenous injections of 0.2 mg/kg/inf of amphetamine (top) or to receive sucrose pellets (bottom).

**Figure 3.** Mean (± SEM) spine density (the number of spines/10 µm) on medium spiny neurons in the Nacc as a function of amphetamine self-administration experience (gray bars) or sucrose-reward training (hatched bars). The untreated control group is shown in the open bars. Asterisk and swords indicate a significant difference from UNTR and SUC conditions, respectively \( P < 0.05 \).

**Figure 4.** Mean (± SEM) spine density (the number of spines/10 µm) on apical (a, c) and basilar (b, d) dendrites of pyramidal neurons in the MPC (Cg3) and OFC (AID) in the amphetamine self-administration (gray bars), sucrose-reward training (hatched bars) or in untreated control groups (open bars). The asterisk and daggers indicate a significant difference from UNTR and SUC conditions, respectively \( P < 0.05 \).
Hippocampus

Figure 5 shows the effects of AMPH self-administration or SUC-reward training on spine density on pyramidal neurons in CA1 (top) and granule cells in the dentate gyrus (bottom). Both AMPH self-administration and SUC-reward training significantly increased the number of dendritic spines in hippocampus region CA1 relative to the UNTR group, and these two former groups did not differ [effects of Group, \( F(2,33) = 35.02, P < 0.001; P_s < 0.05 \) for post-hoc tests]. No significant group differences in spine density were found on dentate gyrus granule cells, and this was true when the inner, middle and outer segments of the molecular layer were analyzed separately (see inset).

Discussion

Robinson and co-workers (Robinson and Kolb 1997, 1999b; Robinson et al., 2001) reported that experimenter-administered amphetamine or cocaine, or cocaine self-administration, produce persistent alterations in dendritic morphology in Nacc and MPC. The present results extend these earlier findings in several ways. (i) We report that amphetamine self-administration experience, but not instrumental training experience with sucrose-reward, increases the density of spines on medium spiny neurons in the Nacc and pyramidal neurons in MPC. (ii) In contrast to the MPC, amphetamine self-administration experience produces a marked decrease in dendritic spine density in the OFC, whereas instrumental experience with sucrose reward produces an increase in spines in this region. (iii) Both amphetamine and sucrose training produce comparable increases in spine density on hippocampal CA1 pyramidal neurons, but have no effect on granule cells in the dentate gyrus.

Alterations in Spine Density and Behavioral Contingencies

This is the first report showing that amphetamine self-administration, rather than experimenter-administered amphetamine, increases dendritic spine density in different forebrain regions including the Nacc and MPC. This is obviously important when considering the neurobiological consequences of drug taking in humans. Indeed, there is some experimental evidence suggesting that the neurobiological effects of psychostimulant (or opiate) drugs in these brain regions (e.g. dopamine neurotransmitter turnover) can vary depending on whether drug administrations are contingent on a rat performing some action (Smith et al., 1980; Dworkin and Smith, 1986; Stefanski et al., 1999). Also, morphine’s effects on spine density in parietal cortex (Par1) and CA1 differ depending on whether the drug is self-administered or experimenter-administered (Robinson and Kolb, 1999a).

However, the effects of amphetamine self-administration on spine density in Nacc and MPC reported here appear similar to those previously found following experimenter-administered amphetamine (Robinson and Kolb, 1997, 1999b). A similar cross-experiment comparison suggests the same is true for the effects of cocaine on spine morphology (Robinson and Kolb, 1999b; Robinson et al., 2001). Thus, barring differences between studies in dosing regimen and route of administration, it seems fair to conclude that at the level of spine changes and for the brain regions assayed here, the ability of psychostimulant drugs to produce persistent alteration in synaptic connectivity are relatively unaffected by whether drug administration is contingent on an action or not.

In this regard, it is important to note that most changes in spine density produced by amphetamine self-administration were not produced by instrumental training per se. That is, allowing rats to nose-poke for sucrose pellets had no effect on spine density in Nacc and MPC, and produced an increase, rather than decrease, in spines in OFC (see below). Thus, the effects of amphetamine are not readily accounted for by experiential factors related to the development of a motor skill, the repetitive motor actions associated with the nose poke response, or by simple appetitive conditioning. This was not the case, however, in the CA1 region of the hippocampus. Here, both the amphetamine and sucrose training conditions increased spine density. Although the hippocampus proper (i.e. the CA fields) has been associated with a variety of behavioral processes, traditionally its role in spatial and/or working memory has been emphasized (Jarrard, 1995). With respect to morphological alterations, for instance, experience in the Morris water maze (a spatial memory task) or trace eyeblink conditioning (a working memory task) increases dendritic spine density in CA1 region (Moser et al., 1994; Leuner et al., 2003). However, evidence that the dorsal hippocampus is directly involved in reward-learning and/or motivation has been lacking (Baldwin et al., 2000; Corbit et al., 2002, but see Schmeleizis and Mittleman, 1996). As such, our results provide novel, albeit correlative, evidence suggesting a role of CA1 region in appetitive instrumental conditioning. What that role is remains to be determined.

Figure 5. Mean (±SEM) spine density (the number of spines/10 µm) in hippocampus regions CA1 (top panel) and dentate gyrus (bottom panel) of rats in the amphetamine self-administration (gray bars), sucrose-reward training (hatched bars) or in untreated control groups (open bars). The inset shows the mean (± SEM) spine density when analyzed separately for the inner, middle and outer segments of the molecular layer. The asterisk and daggers indicate a significant difference from UNTR and SUC conditions, respectively (\( P < 0.05 \)).
Alterations in Spine Density in Nacc and MPC

The present findings extend previous reports showing that amphetamine or cocaine produce persistent increases in dendritic spine number (and branching) in Nacc and MPC — two regions implicated in the psychomotor and rewarding effects of drug- and non-drug reward (Robinson and Kolb, 1997, 1999b; Kolb et al., 2003; Li et al., 2003; Norrholm et al., 2003). These types of drug-induced changes in spine density are particularly interesting, given the well-established biochemical changes in dopamine and glutamate neurotransmission produced by repeated exposure to psychostimulant drugs of abuse (Robinson and Becker, 1986; Kalivas and Stewart, 1991; Vanderschuren and Kalivas, 2000). As discussed in more detail elsewhere (Robinson and Kolb, 1997), dendritic spines on Nacc medium spiny neurons and MPC pyramidal neurons receive dopaminergic inputs (from tegmentum and substantia nigra), as well as glutamatergic inputs (from prefrontal cortex, basal amygdala and hippocampus) (Sesack and Pickel, 1990; Smith and Bolam, 1990; Meredith and Totterdell, 1999). As such, alterations in dendritic spines may provide the structural mechanism by which drug-induced alterations in DA and glutamate neurotransmission occur (Robinson and Kolb, 1997).

Alterations in DA and/or glutamate neurotransmission in Nacc and MPC are also strongly implicated in the long-term behavioral changes associated with repeated drug exposure, including sensitization to the psychomotor activating effects (psychomotor sensitization), Pavlovian conditioned motivational effects (incentive sensitization) and the ability of drugs to support self-administration (sensitization of drug reward). For instance, pretreatment with DA or glutamate (NMDA or AMPA) receptor specific antagonists blocks the development of amphetamine psychomotor sensitization (Karler et al., 1989; Vezina and Stewart, 1989; Wolf and Jezierski, 1993; Vanderschuren and Kalivas, 2000), sensitization to the reinforcing effects of amphetamine (Schenk et al., 1993a,b; Pierre and Vezina, 1998), amphetamine-potentiated responding for a conditioned reward (Kelley and Delfs, 1991; Kelley and Throne, 1992; Wolterink et al., 1993; Burns et al., 1994) and reinstatement of extinguished drug seeking by drug- or drug-associated stimuli (Bespalov et al., 2000; Cornish and Kalivas, 2000; Crombag et al., 2002). It has been proposed, therefore, that chronic psychostimulant exposure reorganizes dopamine and glutamate inputs onto Nacc and MPC neurons in a way that renders animals persistently hypersensitive to stimuli that engage this circuitry, including drugs themselves and drug-associated stimuli (Robinson and Berridge, 1993; Hyman, 1996; Robinson and Kolb, 1997; Kalivas et al., 1998; Berke and Hyman, 2000; Vezina, 2004).

Alterations in Spine Density in Orbital Frontal Cortex

In contrast to Nacc and MPC, amphetamine self-administration produced a persistent decrease in dendritic spine number in OFC of rats. As first sight, the decrease in spine density in OFC appears in line with recent findings showing OFC abnormalities and/or OFC-dependent cognitive deficits in human stimulant users. For instance, amphetamine and/or cocaine users were shown to have alterations in metabolic activity (Volkow et al., 1992; Paulus et al., 2002; Adinoff et al., 2003; Bolla et al., 2003), DA receptor levels (Volkow et al., 1993; London et al., 2000) and gray matter volume (Fein et al., 2002; Franklin et al., 2002; Matohich et al., 2003) in OFC. Furthermore, amphetamine and/or cocaine users show pervasive performance deficits on a range of neuropsychological tasks involving attentional, working memory and/or decision-making processes similar to those seen in patients with focal OFC lesions (Rogers et al., 1999; Ornstein et al., 2000; Bolla et al., 2003). There are now a few controlled studies looking at the effects of cocaine on OFC function in laboratory animals (to our knowledge, there are presently no studies looking at the effects of amphetamine). Monkeys given repeated cocaine administrations, for instance, show enduring impairments of object discrimination learning when inhibition of a previously conditioned response is required (Jentsch et al., 2002). Furthermore, in a recent series of studies using rats, Schoenbaum and colleagues found that cocaine exposed rats fail to show normal reversal learning (Schoenbaum et al., 2004) and reinforcer devaluation (Setlow and Schoenbaum, 2004) — performance on these tasks depends (in part) on OFC function (Gallagher et al., 1999; McAlonan and Brown, 2003; Schoenbaum et al., 2003). Based on their results, the authors suggest that drug-induced deficits in the ability to alter behavior depending on changes in outcome, could partly explain why addict’s behavior often appears ‘habit-like’ and why their drug seeking and taking persists despite its many aversive consequences (Tiffany, 1990; White, 1996; Jentsch and Taylor, 1999; Robbins and Everitt, 1999a,b; Schoenbaum et al., 2004).

The picture that is emerging from these and other recent electrophysiological, lesion and human imaging studies is that the OFC, together with the basolateral amygdala, form part of a circuit that plays a unique role in processing affective or motivational information attached to cues (Baxter et al., 2000; Schoenbaum and Setlow, 2001; Gottfried et al., 2003; Holland and Gallagher, 2004). That is, under conditions where environmental stimuli provide relevant information regarding the incentive value of outcomes (i.e. rewards), this circuit is important for forming and storing stimulus-reward associations and using them to guide goal-directed behavior. If so, the persistent increase in spine density in OFC seen as a function of sucrose-reward training could reflect storage of such stimulus-outcome associations (e.g. between the cue-light/lever/context and the affective and motivation value of sucrose-reward). Furthermore, the fact that experience with amphetamine decreases spine density in OFC may explain why Schoenbaum and colleagues found that repeated treatment with cocaine produces deficits in reversal learning and devaluation. Support for this notion requires knowing (i) whether amphetamine (self-administration) experience produces similar functional deficits as those reported following experimenter-administered cocaine; and (ii) whether cocaine experience produces similar structural alterations in OFC. With respect to the latter, however, it appears that this may not be the case and that cocaine self-administration experience, which increases spines in Nacc and MPC, does not alter spine density in OFC (Ferrario et al., 2003).

Different Drugs Have Different Effects in Different Regions

Whatever the case, together with the functional evidence from human and non-human animal studies, the present study reminds us that drugs of abuse produce persistent changes in many brain regions and these effects are not uniform throughout the brain. Indeed, one of the more striking findings is that, even in closely related areas such as MPC and OFC,
amphetamine self-administration produced completely opposite effects. In considering the importance of these region-specific changes for understanding addiction, it is important to note that the pattern of results reported here with amphetamine self-administration is the exact opposite of what was previously found following morphine self-administration. That is, morphine decreases spine density in MPC (and Nacc) and increases spine density in OFC (Robinson et al., 2002). Additionally, as mentioned, we have recently found that cocaine self-administration, which increases spine density in Nacc and MPC, does not alter spine density in OFC even when rats were allowed to self-administer cocaine for 6 h/day for 30 days (Ferrario et al., 2003). This triple dissociation raises the possibility that chronic drug users will show different patterns of cognitive deficits depending on their ‘drug-of-choice’. In fact, there is now some evidence that this may be the case. Using a computerized decision-making task, Rogers et al. (1999) reported that, on some dependent measures, long-time amphetamine users showed deficits more similar to patients with focal OFC damage than opiate users (see also Ornstein et al., 2000). The challenge for the future, therefore, will be to understand what these drug-specific functional consequences are and how they could contribute to the process of addiction.

Notes
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