

Amphetamine or cocaine limits the ability of later experience to promote structural plasticity in the neocortex and nucleus accumbens

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Drugs of abuse and many other kinds of experiences share the ability to alter the morphology of neuronal dendrites and spines, the primary site of excitatory synapses in the brain. We hypothesized, therefore, that exposure to psychostimulant drugs might influence later experience-dependent structural plasticity. We tested this hypothesis by treating rats repeatedly with amphetamine or cocaine and then housing them in either a complex environment or standard laboratory cages for 3–3.5 mo. The brains were processed for Golgi–Cox staining, and the number of dendritic branches and the density of dendritic spines on medium spiny neurons in the nucleus accumbens and pyramidal cells in the parietal cortex were quantified. On most measures, prior treatment with amphetamine or cocaine interfered with the ability of experience in a complex environment to increase dendritic arborization and spine density. We conclude that in some brain regions, repeated exposure to psychomotor-stimulant drugs limits the ability of later experience to produce this form of synaptic plasticity, which may contribute to the persistent behavioral and cognitive deficits associated with drug abuse.

complex environment | behavioral sensitization | dendrites | dendritic spines

The idea that experience-dependent changes in behavior are due to alterations in the physical structure of neurons can be traced to Ramon y Cajal (1), and Hebb (2) made this a central tenet of his influential neuropsychological theory. The first direct experimental evidence for experience-dependent changes in brain structure came from studies by Rosenzweig and colleagues in the 1960s (3), who compared the brains of animals housed in a relatively complex environment with those housed in standard laboratory cages. Since that time, there have been many demonstrations that experience in complex environments and learning experiences can increase the length of dendrites and the density of dendritic spines on cells throughout the neocortical mantle, in the hippocampus and striatum, and can produce associated changes in patterns of synaptic connectivity (4–8).

The ability of experience to alter dendritic structure is generally considered advantageous and is thought to be the primary mechanism by which past experience influences subsequent behavior (for reviews, see refs. 5–7 and 9). However, alterations in dendritic structure are also associated with pathological states (10, 11). In particular, we have shown that repeated treatment with psychostimulant drugs, such as amphetamine, cocaine, or nicotine, produces long-lasting increases in dendritic branching and spine density in some brain regions, changes that may be related to the development of behavioral sensitization and compulsive patterns of drug-seeking behavior (12–15).

That environmental manipulations and drugs of abuse can have apparently similar effects on neuronal morphology raises questions regarding the extent to which these two kinds of experiences may interact. Indeed, given that there likely are

limits on this form of neural plasticity and given the magnitude of the effects of drugs of abuse, we hypothesized that past experience with drugs of abuse might limit other forms of experience-dependent neural plasticity. To test this hypothesis, rats were first treated repeatedly with amphetamine or cocaine (or saline) and then placed into a complex environment or left in standard laboratory cages for 3–3.5 mo. We then processed their brains for Golgi–Cox staining and quantified dendritic branching and spine density on medium spiny neurons in the nucleus accumbens (NAcc) (shell) and pyramidal neurons in the parietal cortex (Par1). We report that prior experience with amphetamine or cocaine can limit the ability of experience in a complex environment to alter dendritic morphology.

Methods

Experiment (Exp.) 1: Amphetamine. Subjects and groups. The subjects were 28 female Sprague–Dawley rats weighing 200–225 g at the beginning of the experiment. The animals were initially housed singly in stainless-steel wire hanging cages in a room on a 14:10 h light/dark cycle, with food and water available ad lib. After 1 wk, each animal was removed from its cage once per day for 20 consecutive days and placed in an activity monitor. One hour later, the animals were given an i.p. injection of either *d*-amphetamine sulfate or 0.9% saline. Two hours after the injection, they were returned to their home cages. On the first test day, animals in the amphetamine group received 0.5 mg/kg (weight of the salt); for the next 18 days, they received 4.0 mg/kg; and on the last day, they again received 0.5 mg/kg. As expected (14), this protocol produced psychomotor sensitization [mean \pm SEM peak crossovers, first test session, 44.8 ± 6.8 , vs. last test session, 72.0 ± 9.1 ; $t(15) = 2.96$, $P < 0.01$].

The day after the last injection, animals were subdivided into two additional treatment conditions. Animals in one group (standard housed) were transferred from single cages and placed in double-sized standard stainless-steel wire hanging cages $40.6 \times 24.1 \times 17.9$ cm high in groups of three. Rat chow was available on the floor of the cage. Animals in the second group (complex housed) were transferred from single cages to stainless-steel monkey cages $61 \times 61 \times 72.4$ cm high, where they were housed in groups of six to eight per cage. These latter cages were divided into multiple levels by use of wire-mesh bridges and ramps, so animals could move vertically within the enclosure. There was also a chain hanging from the roof of each cage that animals could climb, multiple “tunnels” made from poly(vinyl chloride) piping, and miscellaneous “toys” placed in the cage, all of which resulted in a relatively complex environment. The animals could also climb the walls of this cage and frequently did. Food was located within the enclosure, and periodically these animals were given spiral-shaped pasta, which was intended to

Abbreviations: Par1, parietal cortex; Exp. *n*, experiment *n*; NAcc, nucleus accumbens.

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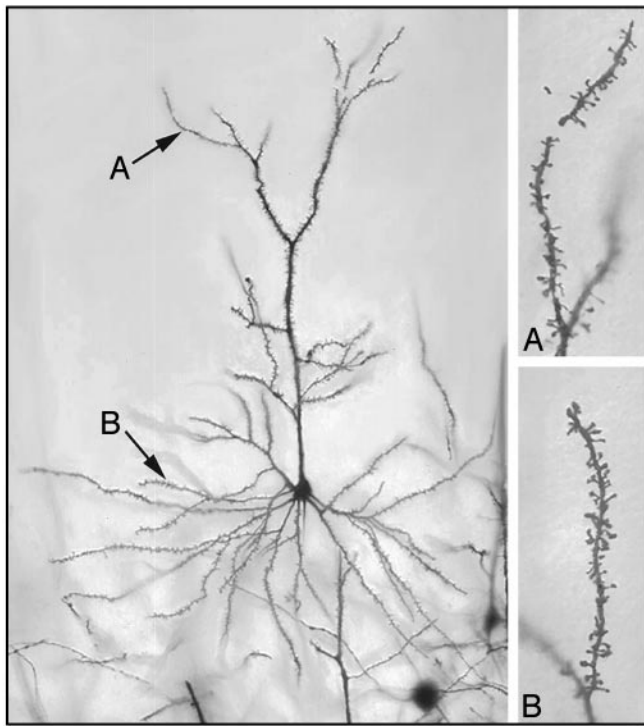


Fig. 1. Photomicrograph of a Golgi-stained layer III pyramidal cell in Par1. To get as much of the dendritic field in focus as possible, multiple photographs were taken at different focal planes, and these were then merged to create the composite image shown here. (Insets) Apical (A) and basilar (B) dendritic segments at higher power.

encourage tactile manipulation of objects. In addition, the objects in the cage were rearranged once per week to encourage continued exploration of the environment. The animals remained in this environment for 3.5 mo.

At the end of the experiment, there was a total of four groups: (i) a group treated with saline and group housed in standard laboratory cages ($n = 6$); (ii) a group treated with saline and group housed in the complex environment ($n = 6$); (iii) a group treated with amphetamine and group housed in standard laboratory cages ($n = 8$); and (iv) a group treated with amphetamine and group housed in the complex environment ($n = 8$).

Anatomical analysis. After 3.5 mo in their respective environments, animals were deeply anesthetized with sodium pentobarbital and perfused through the heart with 0.9% saline. The brains were removed and placed in light-tight vials containing Golgi-Cox solution. After 14 days, the brains were transferred to vials containing 30% sucrose and, after at least 3 days in the sucrose solution, they were cut into 200- μ m sections with a vibrating microtome, mounted on glass slides, and stained by using procedures described previously (16).

Specific cell types in two brain regions were selected for analysis because they had previously been shown to be sensitive to either psychostimulant drugs or housing conditions: pyramidal cells in layer III of Par1 and medium spiny neurons in the shell of the NAcc. Fig. 1 shows a photomicrograph of a layer III pyramidal cell in Par1 to illustrate the quality of the staining. The relevant brain regions were first identified at low power ($\times 100$), and five cells from each hemisphere were drawn by using camera lucida ($\times 250$). To be included in the analysis, the dendritic tree of a cell had to be intact (i.e., largely in the 200- μ m section and not obscured by blood vessels or astrocytes). The dendritic arbor of each cell was quantified by counting the number of dendritic branches (indicated by bifurcations), as described by Coleman

and Riesen (17). Spine density was calculated by tracing a length of dendrite (at least 20 μ m long) at $\times 1,000$. The exact length of the dendritic segment was calculated, and the number of spines along the entire length was counted. For cortical pyramidal neurons, spines were counted on one-third-order terminal tip from both the basilar and apical dendrites; for medium spiny neurons, spines were counted on one terminal tip per neuron. No attempt was made to correct for the fact that some spines were obscured from view, so the measure of spine density necessarily underestimates total spine number. The values for cells in each hemisphere of each rat were averaged, and hemisphere was used as the unit of analysis. Anatomical analyses were performed by someone blind to experimental conditions.

Exp. 2: Amphetamine Dose Effect. Female Sprague-Dawley rats were initially treated with 0.75 mg/kg *d*-amphetamine sulfate, followed by nine consecutive daily injections of either 1 or 5 mg/kg amphetamine (or saline). Animals were then left undisturbed for 20–22 days, when they received a challenge injection of 2 mg/kg amphetamine (or saline). Brains were obtained 2–3 days later and processed for Golgi-Cox staining. There were, therefore, four groups: (i) animals treated with saline ($n = 8$); (ii) animals given a single injection of 2 mg/kg amphetamine ($n = 8$); (iii) animals given repeated injections of amphetamine (total cumulative dose of 12 mg/kg; $n = 12$); and (iv) animals given repeated injections of amphetamine (total cumulative dose of 44 mg/kg; $n = 12$).

This experiment and Exp. 3 were conducted primarily to determine whether the drug-environment interaction in the NAcc seen in Exp. 1 was due to a ceiling effect (see below) and therefore, only cells in the NAcc were examined.

Exp. 3: Cocaine. The design of this experiment was the same as for Exp. 1, except rats were treated with cocaine. Rats received one injection (i.p.) of 15 mg/kg cocaine HCl (or saline) each weekday (not on weekends) for a total of 4 weeks. The animals were then housed in the complex environment or standard laboratory cages ($n = 10$ –16 per group), as described above, for a total of 3 mo. Brains were then obtained and processed for Golgi-Cox staining, and cells in the NAcc (shell) were selected for analysis.

This procedure produced behavioral sensitization, in that the locomotor response (photocell beam breaks in activity monitors) after the 20th injection was significantly greater than the response after the first injection ($P > 0.05$). However, we also treated independent groups of animals the same way but tested for behavioral sensitization by giving a cocaine challenge after 3 mo of withdrawal. In these animals, locomotor sensitization was no longer evident, as indicated by photocell-beam breaks in activity monitors (mean \pm SEM beam breaks; saline pretreated, $n = 10$, 988 ± 177 ; cocaine pretreated, $n = 12$, 1034 ± 162 ; $t = 0.04$; $P = 0.85$). Therefore, with this dosing regimen, locomotor sensitization to cocaine had dissipated by 3 mo after the discontinuation of drug treatment, consistent with previous reports (18).

Results

Exp. 1: Amphetamine. Overall, the main effects of amphetamine treatment and of environmental complexity on neuronal morphology were as expected from previous studies. However, the most interesting findings were indicated by significant drug-environment interactions. On most measures, the effect of environment varied as a function of past drug experience.

NAcc. In the NAcc, experience in the complex environment increased both dendritic arborization and spine density on medium spiny neurons, as did amphetamine followed by housing in a standard laboratory cage. In animals previously treated with

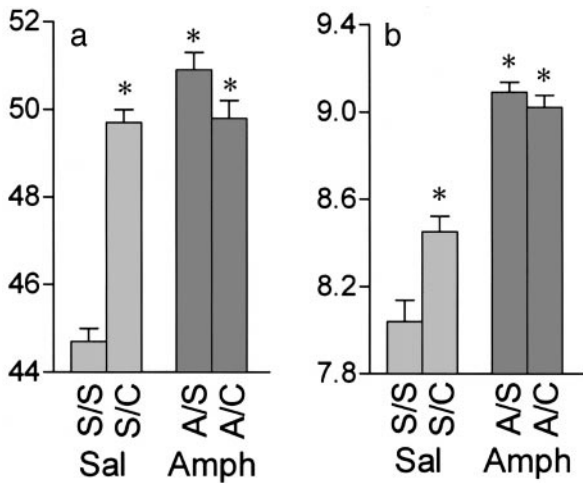


Fig. 2. Effects of amphetamine and housing in a complex environment on dendritic branches (a) and spines (b) on medium spiny neurons in the shell of the NAcc. Both experience in the complex environment [saline treated/housed in standard cage (S/S) vs. saline treated/housed in a complex environment (S/C)] and amphetamine [S/S vs. amphetamine treated/housed in a standard environment (A/S) or amphetamine treated/housed in a complex environment (A/C)] significantly increased both dendritic branching and spine density, but in amphetamine-treated animals, there was no incremental effect of housing in a complex environment. Two-way ANOVA for branches: main effect of amphetamine ($F = 73.0$, $P < 0.0001$), main effect of environment ($F = 27.2$, $P < 0.0001$), and amphetamine by environment interaction ($F = 66.3$, $P < 0.0001$). Spines: main effect of amphetamine ($F = 142.9$, $P < 0.0001$), main effect of environment ($F = 6.3$, $P = 0.015$), and amphetamine by environment interaction ($F = 12.7$, $P < 0.001$). *, Differs from S/S (Fisher's test).

amphetamine, however, there was no incremental effect of experience in the complex environment on either measure of dendritic morphology (see Fig. 2).

Par1. Experience in the complex environment increased the number of basilar and apical dendritic branches on Par1 pyramidal cells, whereas amphetamine treatment followed by housing in a standard laboratory cage had no effect on branching of either basilar or apical dendrites, consistent with earlier findings (13). Interestingly, past exposure to amphetamine completely eliminated the effect of experience in the complex environment on dendritic branching in this brain region (Fig. 3).

Experience in the complex environment increased spine density on both apical and basilar dendrites of Par1 cells, but amphetamine treatment decreased spine density, again, consistent with our earlier work (13). In animals previously exposed to amphetamine, experience in the complex environment also increased spine density, but only to levels well below those found in saline-treated animals housed in the complex environment (Fig. 3).

The interaction between amphetamine treatment and experience in a complex environment on dendritic branching in Par1 provides compelling evidence that prior exposure to amphetamine can limit or even block the subsequent effects of experience. In the NAcc, however, the data are more difficult to interpret. It appeared that prior treatment with amphetamine interfered with the ability of experience in a complex environment to increase dendritic branching and spine density in the NAcc, in that experience produced no incremental effect over and above that seen with amphetamine alone. Alternatively, it is possible that the effect of amphetamine was already maximal, and no further morphological plasticity was possible (i.e., there was a ceiling effect). Exps. 2 and 3 were conducted to further explore the nature of the drug–environment interaction, specif-

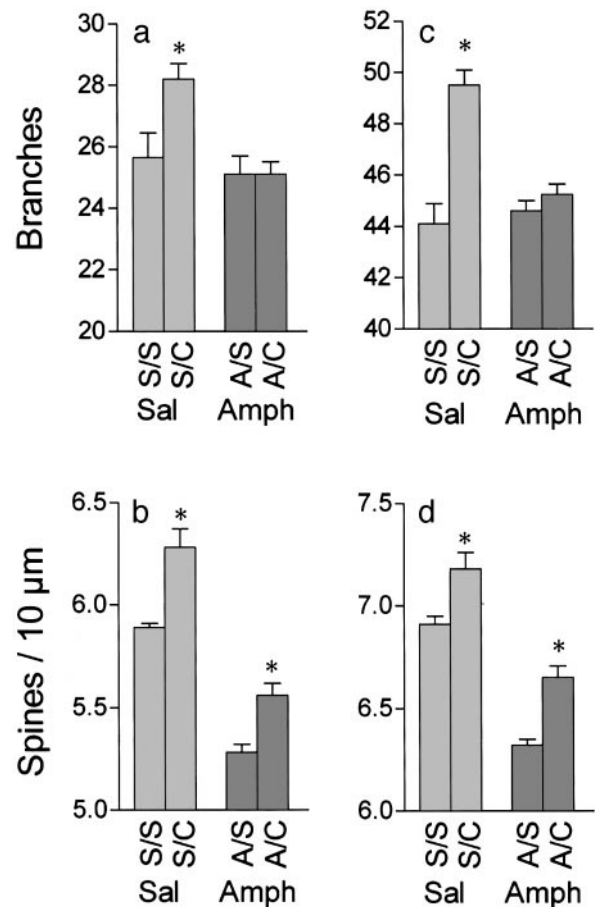


Fig. 3. Effects of amphetamine and housing in a complex environment on dendritic branches (a and c) and spines (b and d) on both apical (a and b) and basilar (c and d) dendrites of layer III pyramidal cells in Par1. Branches: housing in a complex environment increased the number of both apical (a) and basilar (c) branches, but amphetamine had no effect. Prior amphetamine completely blocked the effect of housing in a complex environment. Two-way ANOVA; apical branches: main effect of amphetamine ($F = 9.79$, $P = 0.003$), environment ($F = 4.41$, $P = 0.041$), and interaction ($F = 5.06$, $P = 0.029$); basilar branches: main effect of amphetamine ($F = 12.54$, $P = 0.0009$), environment ($F = 32.7$, $P < 0.0001$), and interaction ($F = 20.61$, $P < 0.0001$). Spines: housing in a complex environment increased the number of spines on both apical and basilar dendrites, and amphetamine decreased spine density. Spine density in the A/C group remained significantly lower than in the control group (S/S). Two-way ANOVA; apical spines: main effect of amphetamine ($F = 127$, $P < 0.0001$), environment ($F = 32.1$, $P < 0.0001$), and interaction ($F = 0.76$, $P = 0.38$); basilar spines: main effect of amphetamine ($F = 102$, $P < 0.0001$), environment ($F = 28.96$, $P < 0.0001$), and interaction ($F = 0.44$, $P = 0.51$). Abbreviations are as in Fig. 2 legend. *, Differs from S/S (Fisher's tests).

ically in the NAcc. Therefore, in the next two experiments, only NAcc medium spiny neurons were examined. The data suggest the former interpretation is correct; in the NAcc, prior drug treatment does indeed limit morphological plasticity.

Exp. 2: Amphetamine Dose Effect. Fig. 4 compares the effects of different doses of amphetamine on spine density in the NAcc with the effects obtained in Exp. 1. Amphetamine treatment significantly increased spine density relative to control in all three experimental groups (Fig. 4a). Note that spine density in the saline-treated control group is the same in both experiments. Also note that spine density in animals given a cumulative dose of 44 mg/kg was considerably higher than in amphetamine-treated animals in Exp. 1. Perhaps more important than the different doses is the fact that, in Exp. 2, animals were examined

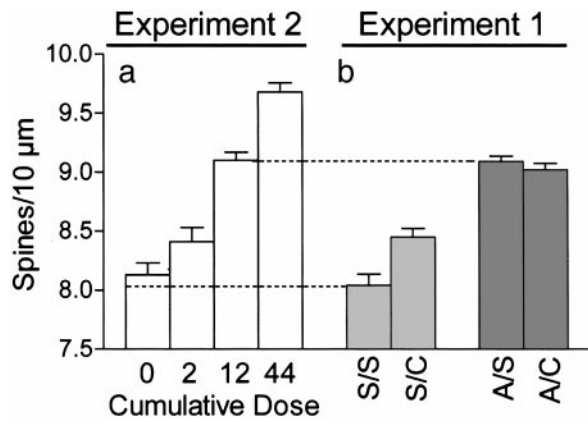


Fig. 4. A comparison of the effects of repeated amphetamine treatment on the density of dendritic spines in the NAcc seen in Exps. 1 and 2. (a) Amphetamine treatment significantly increased spine density ($F = 59.6, P < 0.0001$) in a cumulative dose-dependent manner (all groups differ from all other groups, Fisher's tests). (b) Data from Exp. 1 are replotted for comparison with Exp. 2. Note that spine density was the same in the control groups (S/S and dose of 0) and that spine density after a cumulative dose of 44 mg/kg amphetamine was considerably higher than in amphetamine-treated animals in Exp. 1. Abbreviations are as in Fig. 2 legend.

after only 1 mo of withdrawal and 2–3 days after an additional challenge injection, whereas in Exp. 1, animals were examined after 3.5 mo of withdrawal. Whatever the critical variable, comparison of the two experiments suggests that the density of spines seen in Exp. 1 was not maximal (i.e., there was no ceiling effect).

Exp. 3: Cocaine. In this experiment, experience in a complex environment increased both dendritic branching and spine density on medium spiny neurons in the NAcc, replicating the effect in Exp. 1 (Fig. 5). However, when examined 3 mo after the last

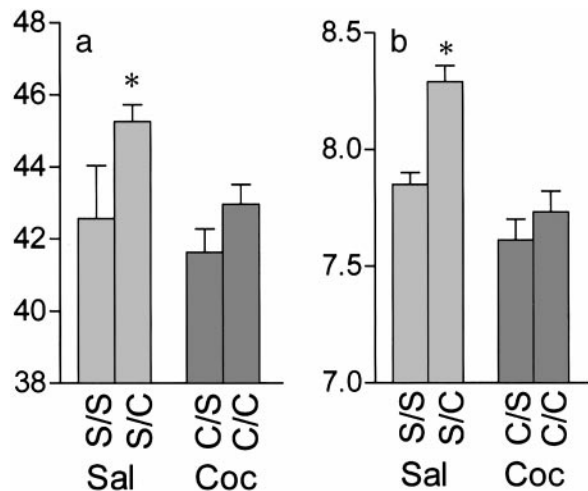


Fig. 5. Effects of cocaine and housing in a complex environment on dendritic branches (a) and spines (b) on medium spiny neurons in the shell of NAcc. Experience in a complex environment increased both dendritic branches and spine density (S/S vs. S/C). Cocaine alone had no effect (S/S vs. C/S) but blocked the effect of housing in a complex environment. Branches: main effect of cocaine ($F = 4.34, P = 0.043$), main effect of environment ($F = 6.74, P = 0.013$), and cocaine by environment interaction ($F = 78, P = 0.38$). Spines: main effect of cocaine ($F = 24.2, P < 0.0001$), main effect of environment ($F = 11.82, P = 0.001$), and cocaine by environment interaction ($F = 3.9, P = 0.05$). *, Differs from S/S (Fisher's tests). Abbreviations are as in Fig. 2 legend.

injection of cocaine, there was no measurable effect of cocaine treatment on dendritic morphology in animals housed in standard laboratory cages. Nevertheless, prior exposure to cocaine completely blocked the effects of experience in a complex environment on both dendritic branching and spine density (Fig. 5).

In earlier studies (14, 15), we found that cocaine did produce an increase in spine density in the NAcc, but in these studies, brains were obtained after only 1 mo of withdrawal. We hypothesize, therefore, that in the present study, cocaine initially induced morphological changes, but that these had reversed by 3 mo of withdrawal (see *Discussion*).

Discussion

The cellular and molecular mechanisms responsible for neuroadaptations that occur as a consequence of experience bear many similarities to those that occur as a consequence of exposure to drugs of abuse (19, 20). Indeed, it has been argued that drugs of abuse usurp the normal mechanisms responsible for experience-dependent plasticity, which include changes in patterns of synaptic connectivity (13, 19). Viewed in this light, changes in synaptic organization produced by drugs of abuse should interact with those produced by experience. To our knowledge, however, this hypothesis has never been tested directly. We did so here with an approach that provides an indirect index of synaptic organization: the analysis of dendritic structure. This approach has proven to be sensitive for studying the synaptic plasticity associated with experience in a complex environment (5, 7, 21), learning (4, 22, 23), long-term potentiation (24–26), gonadal hormone manipulations (27, 28), and early cortical injury (29), as well as treatment with drugs of abuse (12–15).

We report that exposure to psychomotor stimulant drugs influences the ability of later experience in a complex environment to shape the structure of dendrites in a neocortical region that mediates sensory-motor functions (Par1), as well as a subcortical region important for incentive motivation and reward (NAcc). On most measures, prior exposure to amphetamine or cocaine interfered with the ability of subsequent experience to increase dendritic arborization and the number of dendritic spines. This result suggests that repeated exposure to drugs of abuse can limit the ability of later experiences to promote synapse formation and/or synaptic reorganization, at least in some brain regions. Of course, the analysis of Golgi material provides only an indirect index of synaptic organization, but there is typically a good correspondence between the kinds of alterations in Golgi material described here and alterations in synaptic organization studied at the ultrastructural level (5, 6, 21, 28). This correspondence may be because spines represent the primary site of excitatory signaling in the brain and are thought to be the major locus whereby plastic changes alter synaptic signaling (30, 31).

The effect of amphetamine on dendritic morphology seen here is consistent with previous reports that amphetamine increases dendritic branching and spine density in the NAcc (13, 14). However, we have also reported that cocaine has similar effects (14), but no such effects were evident in the present study. This apparent discrepancy is probably due to the length of time after the discontinuation of drug treatment brains were obtained and to differences in the persistence of amphetamine- vs. cocaine-induced neuroplastic adaptations. There are many reports that amphetamine treatment regimens similar to that used here can produce neurobehavioral adaptations that persist for many months, if not longer (32, 33). Even a single injection of amphetamine produces neurobehavioral sensitization lasting for at least 1 mo (34). However, the effects of some cocaine treatment regimens may not be so long-lasting. For example, Henry and White (18) reported that after twice-daily injections

of 10 mg/kg cocaine (for a total of 28 injections), both behavioral and neurophysiological sensitization was evident after 1 week and 1 mo of withdrawal but not after 2 mo. In the present experiment, in which animals were given 20 injections of 15 mg/kg, we found that locomotor sensitization had largely dissipated after 3 mo of withdrawal (see *Methods*). Thus, we have found cocaine-induced changes in dendritic morphology after 1 mo of withdrawal (when animals are behaviorally sensitized) but not after 3 mo of withdrawal (when behavioral sensitization is no longer evident). This result raises the interesting possibility that drug-induced changes in dendritic morphology may wane over time, and the time course of these effects may vary depending on the ability of different drugs and treatment regimens to produce persistent behavioral sensitization. Additional experiments will be required to test this hypothesis. Whatever the case, the most important result here is that prior treatment with cocaine completely blocked the effect of experience in a complex environment on dendritic morphology in the NAcc.

Given that ultrastructural studies confirm that psychostimulant drugs' influence, the ability of experience to promote synapse formation, and/or synaptic reorganization, and assuming for a moment that the interaction between drugs and experience is because both alter synaptic organization through a similar mechanism, what might the mechanism be? One possibility is that both effects involve common actions on neurotrophic factors, which have been implicated in experience-dependent neuroplasticity (35) and psychostimulant drug-induced behavioral sensitization (36–38). For example, Flores and Stewart (39) showed that repeated treatment with amphetamine increases the expression of basic fibroblast growth factor (bFGF) in the NAcc. Furthermore, housing animals in a complex environment increases bFGF expression throughout the cerebral hemispheres (9). Thus, both amphetamine and environmental complexity could potentially stimulate synaptogenesis through the action of bFGF. Prior treatment with amphetamine may limit the effects of subsequent environmental complexity in these brain regions, because there is a limit to either the increased expression of bFGF or the effect of bFGF on synapse formation. Of course, the effects of drugs and experience on neurotrophic factors also require explanation; the obvious one is that they have common actions on gene expression (19). In this regard, it is interesting that both amphetamine (40, 41) and environmental complexity (42) induce expression of

the immediate early gene, *arc*, which has been implicated in other forms of neuronal plasticity (43, 44).

Perhaps the most important issue raised by the current study concerns the long-term consequences of drug use for behavior and psychological function. If the effects described here generalize to other forms of experience-dependent plasticity, such as those involved in learning and memory or recovery of function after brain damage, and given that experience-dependent synaptic reorganization has desirable functional consequences, the present study makes a simple prediction. At least some of the cognitive/behavioral advantages that accrue with experience may be diminished as a function of prior exposure to psychostimulant drugs. We have no direct evidence this is the case, but there is accumulating evidence that amphetamine and cocaine addicts have numerous neuropsychological deficits (45, 46). Behavioral and cognitive deficits in addicts are usually attributed to either frank neurotoxic effects of drugs or their ability to directly render specific brain systems dysfunctional. Our data suggest, however, an alternative way by which repeated drug use might produce persistent alterations in behavioral or cognitive function: it may impair the ability of specific circuits to change as a consequence of experience. That is, some of the behavioral and cognitive deficits seen in addicts may be due to limits on synaptic plasticity imposed by drug use, rather than to some kind of "lesion effect."

On a more positive note, if exposure to psychostimulant drugs can alter the effects of subsequent experience, experience may be able to influence the later effects of drugs. There is considerable evidence that early environmental manipulations alter the effects of psychostimulant drugs in adulthood (47), including susceptibility to sensitization (48). Whether these or other experiences, such as exposure to a complex environment in adulthood, alter the ability of psychostimulant drugs to induce structural changes in dendrites is unknown, but it is possible some experiences may counteract the effects of psychostimulant drugs.

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