European Journal of Neuroscience, Vol. 20, pp. 1647–1654, 2004

The induction of behavioural sensitization is associated with cocaine-induced structural plasticity in the core (but not shell) of the nucleus accumbens

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Keywords: cocaine, dendrites, dendritic spines, frontal cortex, rat, synaptic plasticity

Abstract

Repeated exposure to cocaine increases the density of dendritic spines on medium spiny neurons in the nucleus accumbens (Acb) and pyramidal cells in the medial prefrontal cortex (mPFC). To determine if this is associated with the development of psychomotor sensitization, rats were given daily i.p. injections of 15 mg/kg of cocaine (or saline) for 8 days, either in their home cage (which failed to induce significant psychomotor sensitization) or in a distinct and relatively novel test cage (which induced robust psychomotor sensitization). Their brains were obtained 2 weeks after the last injection and processed for Golgi–Cox staining. In the Acb core (AcbC) cocaine treatment increased spine density only in the group that developed psychomotor sensitization (i.e. in the Novel but not Home group), and there was a significant positive correlation between the degree of psychomotor sensitization and spine density. In the Acb shell (AcbS) cocaine increased spine density to the same extent in both groups; i.e. independent of psychomotor sensitization. In the mPFC cocaine increased spine density in both groups, but to a significantly greater extent in the Novel group. Furthermore, when rats were treated at Home with a higher dose of cocaine (30 mg/kg), cocaine now induced psychomotor sensitization in this context, and also increased spine density in the AcbC. Thus, the context in which cocaine is experienced influences its ability to reorganize patterns of synaptic connectivity in the Acb and mPFC, and the induction of psychomotor sensitization is associated with structural plasticity in the AcbC and mPFC, but not the AcbS.

Introduction

Repeated intermittent exposure to drugs of abuse can render animals hypersensitive (sensitized) to some drug effects, and to drug-related stimuli, even long after the discontinuation of drug treatment (Segal & Mandell, 1974; Robinson & Becker, 1986). Most studies have focused on the psychomotor activating effects of drugs (psychomotor sensitization), but repeated treatment with psychomotor stimulant drugs also facilitates Pavlovian conditioned motivational processes (Harmer & Phillips, 1998, 1999; Taylor & Horger, 1999; Wyvell & Berridge, 2001), the later acquisition of drug self-administration (Piazza *et al*., 1989; Horger *et al*., 1990; Pierre & Vezina, 1998), the acquisition of a preference for places associated with drug administration (Lett, 1989) and the 'breakpoint' achieved on a progressive ratio schedule (Mendrek *et al*., 1998; Lorrain *et al*., 2000). On the basis of these studies (for a review, see Vezina, 2004) it has been hypothesized that drug-induced adaptations in the brain associated with the induction of behavioural sensitization contribute to the development of addiction (Robinson & Berridge, 1993, 2003).

There has therefore been considerable interest in the neurobiology of sensitization, and many neurobiological correlates of sensitization have been reported, especially in the nucleus accumbens (Acb) and associated circuitry (Pierce & Kalivas, 1997; Vanderschuren & Kalivas, 2000). In most previous studies designed to identify neurobiological correlates of sensitization a drug is given repeatedly and then later the brain is examined to determine if drug treatment produced any persistent change in the neurobiological measure of interest. However, in such studies it is not possible to tell if any given drug-induced neuroadaptation is related specifically to the development of behavioural sensitization, or whether it would occur even if drug treatments failed to induce sensitization (i.e. as a function of mere drug history). We have developed a procedure to do this. There are doses of amphetamine or cocaine that are relatively ineffective in producing psychomotor sensitization if given in an animal's home cage, but induce robust psychomotor sensitization if given in a distinct and relatively novel test cage (Badiani *et al*., 1995; Crombag *et al*., 1996; Browman *et al*., 1998a). Thus, in this situation two randomly assigned groups (Home vs. Novel) have the same drug history, but only one group develops robust psychomotor sensitization. If any given drug-induced change in the brain is associated with the development of sensitization it should be present in the Novel, but not the Home group; unless dose is increased so that now drug treatments induce sensitization even at Home (Browman *et al*., 1998a,b).

The purpose of the present experiment was to manipulate the induction of psychomotor sensitization by varying environmental context and dose to determine whether one neurobiological consequence of exposure to cocaine, its ability to alter synaptic organization in the Acb and the medial frontal cortex (mPFC) (Robinson & Kolb, 1999; Robinson *et al*., 2001) is related to its ability to induce

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Received 27 October 2003, revised 18 June 2004, accepted 13 July 2004

doi:10.1111/j.1460-9568.2004.03612.x

psychomotor sensitization, or whether cocaine induces this form of structural plasticity independent of its ability to induce sensitization (i.e. merely as a function of drug history).

Methods

Male Sprague–Dawley rats (200–225 g) obtained initially from Harlan (Indianapolis, IN, USA) were housed individually in an animal colony room (14-h light : 10-h dark cycle) in clear plastic hanging cages (24 × 21.6 × 20.3 cm) with shredded paper bedding for 7 days prior to any experimental manipulation. Food and water were available at all times. All experimental procedures were approved by the University of Michigan Committee on the Use and Care of Animals.

Experiment 1

Procedures

After 7 days, rats were assigned randomly to one of two groups. Rats in one group (Home) were transferred from the animal colony to a room where they were housed in transparent plastic rectangular cages (41 × 25.4 × 20.3 cm) that contained a clear plastic insert in the centre of the cage (23 × 6.3 × 20.3 cm). This insert formed a corridor around the perimeter of the cage where the animals could walk. Ground corncob bedding was located on the floor. These cages were located inside a frame that contained photocell emitters and detectors that could be used to record the movement of an animal from one end of the cage to the other (crossovers). The animals remained in these cages for the duration of the experiment. Animals in the other group (Novel) were left in the animal colony room.

After seven additional days of habituation to these conditions, during which time all animals were periodically handled, rats in the Novel group were transferred from their home cages to photocell-equipped test cages that were identical to the ones in which the Home group lived. After 60 min in these test cages, the animals were given an intraperitoneal (i.p.) injection of either 1 mL/kg of 0.9% saline (Novel/Saline, $n = 8$) or 15 mg/kg of cocaine HCl (Novel/Cocaine, $n = 12$; cocaine from NIDA) and behaviour recorded for an additional 90 min before the animals were returned to their home cages in the animal colony room. This procedure was repeated each day for a total of eight consecutive days. At the same time as the animals in the Novel group received an injection, animals in the Home group were also given an i.p. injection of either saline (Home/Saline, $n = 8$) or 15 mg/kg of cocaine (Home/Cocaine, $n = 12$), and were then placed immediately back into their home cage and behaviour monitored. After the last injection of saline or cocaine, the animals were left undisturbed for 2 weeks before their brains were obtained.

Experiment 2

Procedures

The procedures for this experiment were exactly the same as for experiment 1, except for the following modifications: (i) there was only a Home group, and half of these animals received cocaine ($n = 12$) and half saline ($n = 11$); (ii) on the first test day, cocaine-treated animals received injections of escalating doses of cocaine (7.5, 15 and 30 mg/kg), with 1 h between each injection (and saline-treated animals received three injections of saline). On each of the next seven daily test sessions, the animals received a single injection of 30 mg/kg of cocaine (or saline). On the last test day, cocaine-treated animals again received escalating doses of cocaine (7.5, 15 and 30 mg/kg), with 1 h between each injection (and saline-treated animals received three injections of saline). This procedure allows the construction of a dose–response function on the first and last day of treatment. Sensitization is indicated by a shift to the left in the dose–response function. As in experiment 1, the animals were left undisturbed for 2 weeks after the last test session before their brains were obtained.

Note that in these experiments we did not test independent groups of animals after 2 weeks of withdrawal to ensure that sensitization persisted in the Novel group and did not appear in a delayed fashion in the Home group (e.g. there were no saline-treated groups that received a drug challenge). This was because we have reported the effects of a withdrawal period in seven different published papers using these procedures, many involving multiple behavioural experiments. In all cases the Home/Novel difference described here was also evident after a period of withdrawal (Badiani *et al*., 1995; Crombag *et al*., 1996; Browman *et al*., 1998a). Therefore, we were confident this would be the case here as well, and did not run independent groups to limit the number of animals used.

Anatomical procedures

After the 2-week drug-free period, the animals were deeply anaesthetized with an overdose of sodium pentobarbital and perfused through the heart with 0.9% saline. The brains were removed and placed in vials containing Golgi–Cox solution (Gibb & Kolb, 1998) and after 14 days transferred to vials containing a 30% sucrose solution. After at least 3 days in the sucrose solution, the brains were cut into 200-μm thick coronal sections using a vibrating microtome, and stained using procedures described by Gibb & Kolb (1998).

We focused our analyses on medium spiny neurons in the shell of the nucleus accumbens (AcbS) and the core of the nucleus accumbens (AcbC), because (i) these regions are embedded in very different circuitry (Groenewegen *et al*., 1991; Zahm, 2000) (ii) they play different roles in the behaviours and psychological processes that undergo sensitization (Cardinal *et al*., 2002) and (iii) repeated drug treatments have different effects on the AcbC and AcbS (Cadoni & Di Chiara, 2000; Cadoni *et al*., 2000). In experiment 1, we analysed pyramidal cells in the mPFC as well, because we previously found sensitization-related structural adaptations in these cells (Robinson & Kolb, 1997, 1999).

Note that experiments 1 and 2 were conducted ≈ 1 year apart and the tissue was analysed using different scopes and procedures (e.g. NEUROLUCIDA® software was used in experiment 1 with a final magnification of 4000× and in experiment 2 manual camera lucida procedures with a final magnification of 2000× were used), although the same experimenter (Li) conducted the anatomical analysis in both experiments.

In experiment 1, a Leica model DMRE microscope equipped with an Ludl XYZ motorized stage and NEUROLUCIDA® software (http://www.microbrightfield.com) was used to identify cells, trace dendritic segments and count dendritic spines. Cells from each brain region were first identified at low power (100–125×). To be included in the analyses, the dendritic tree of a cell had to be well-stained and visible, and not obscured by blood vessels, glia or other cells. Given the relatively small number of cells that were well-stained and met these criteria, nearly all the available cells in a given section were included in the analyses. In addition, cells were sampled over the entire extent of the AcbC and AcbS, so the sample does reflect any specific subregion within these structures. Most importantly, the person who selected and analysed cells (Li)

was blind to the experimental condition, so the same criteria were applied to all groups. Thus, any variation in spine density due to variation in the location of cells contributed to the experimental error effect.

For medium spiny neurons, one third-order (or greater) terminal tip was identified, and the total number of visible spines along the length of the dendritic segment (at least 20-μm long) were counted. This was performed with a total magnification on the scope of 1600×, and with additional magnification from the video signal the final magnification on the video screen was ≈4000×. We focused on third order or greater terminal dendritic segments because we found previously that amphetamine treatment increases spine density on distal, but not proximal, dendrites of medium spiny neurons (Li *et al*., 2003). For pyramidal cells, spines were counted on one terminal tip per neuron (third order or greater) from both the apical and basilar dendritic tree. The NEUROLUCIDA® program calculated the total length of the dendritic segment and the number of spines/10 μm of dendrite. These measures were obtained from at least five cells in each hemisphere of each animal, and statistical analyses were conducted after averaging across cells within a hemisphere (i.e. hemisphere was the unit of analysis). In experiment 1, the data represent a total sample of 385 cells in the AcbC, 391 cells in the AcbS, 314 (for apical dendrites) and 346 (for basilar dendrites) cells in the mPFC. Data were not available for every hemisphere in every brain region because of tissue damage or poor staining, and therefore, the *n* for the saline-treated groups varies from 14–16, and for the cocaine-treated groups from 20–24. In experiment 2, cell selection and quantification was the same as in experiment 1, except conventional camera lucida procedures were used, with a total magnification of 2000×.

Results

Experiment 1

Behaviour

Figure 1 shows the number of cage crossovers, an index of locomotor activity, during the first and last (8th) test session in animals treated with cocaine or saline. A two-way ANOVA comparing the two groups that received cocaine resulted in a significant main effect of group ($F = 11.99$, $P < 0.002$), of test session ($F = 27.17$, $P < 0.0001$), and a significant group × test session interaction ($F = 14.92$, $P < 0.002$). Planned between-subjects comparisons indicated that there was no difference between the Home/Cocaine and Novel/Cocaine groups on the first day of testing ($t = 0.53$, $P = 0.6$), but there was on the last day of testing ($t = 3.86$, $P = 0.0009$). Planned within-subjects paired *t*-tests showed that there was a significant increase in crossovers between day 1 and day 8 of testing in the Novel/Cocaine group ($t = 5.28$, $P = 0.0003$), but not in the Home/Cocaine group ($t = 1.08$, $P = 0.3$). Thus, only the Novel/Cocaine group showed behavioural sensitization, and the Novel/Cocaine group changed over time to a greater extent than the Home/Cocaine group (as indicated by the interaction effect).

Anatomy

Nucleus accumbens. Figure 2 shows the effect of cocaine treatment on the density of dendritic spines on medium spiny neurons in the AcbC and AcbS. For the AcbC, a two-way ANOVA resulted in a significant group by drug interaction ($F = 6.1$, $P = 0.016$). Post-hoc Fisher's tests indicated that cocaine significantly increased spine density in the Novel group ($P < 0.0001$), but not in the Home group ($P = 0.78$). For the AcbS a two-way ANOVA resulted in no significant effect of group (Home vs. Novel, $F = 0.02$, $P = 0.89$), a significant effect of drug (Saline vs. Cocaine, $F = 31.7$, $P < 0.0001$) but no group × drug interaction ($F = 0.02$, $P = 0.9$). Thus, in the AcbS cocaine significantly increased spine density to the same extent in the Home and Novel groups.

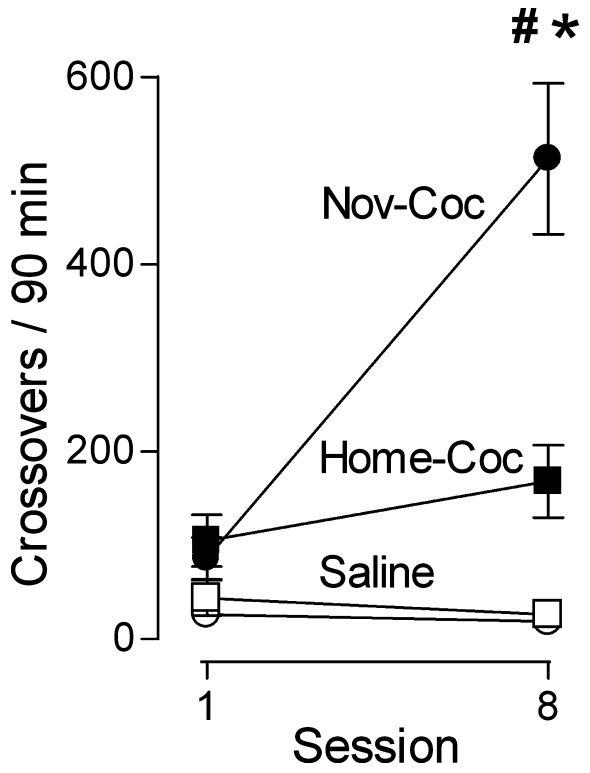

FIG. 1. The mean (± SEM) number of crossovers (locomotor activity) during the 1st and 8th 90 min test sessions in animals given either 15 mg/kg of cocaine (■) or saline (□) in their home cage, or 15 mg/kg of cocaine (●) or saline (○) in a novel test cage. Activity during the 60 min habituation period is not shown. There was a significant increase in locomotor activity between the 1st and 8th test session in animals given repeated injections of cocaine in the Novel condition (i.e. this group sensitized), but no change in animals given cocaine at Home (i.e. this group failed to sensitize). *Differs from Day 1; #Differs from the Home-Cocaine group.

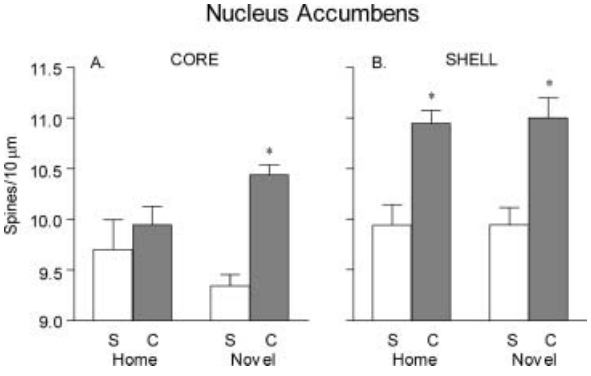

FIG. 2. The mean (± SEM) number of dendritic spines per 10 μm on medium spiny neurons in the core (A) of the nucleus accumbens (AcbC) and the shell (B) of the nucleus accumbens (AcbS), as a function of treatment condition. Repeated treatment with cocaine (C) in the Novel condition significantly increased spine density in the AcbC, relative to saline-treated animals (S). There was no effect of cocaine treatment in the AcbC in animals given cocaine at Home. Cocaine increased spine density in the AcbS to the same extent in animals-treated with cocaine at Home or in the Novel test cage.

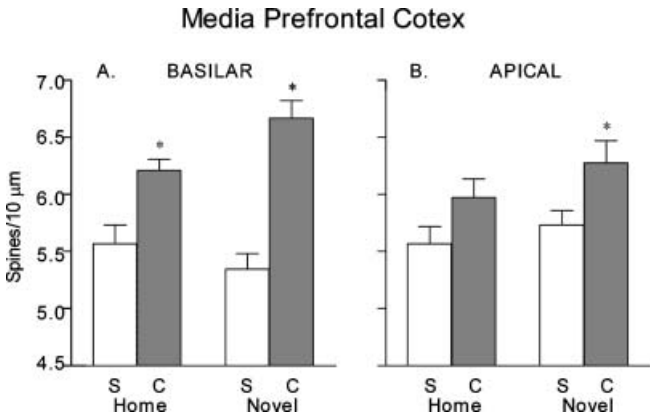

FIG. 3. The mean (± SEM) number of dendritic spines per 10 µm on the basilar (A) and apical (B) dendrites of layer V pyramidal cells in the medial prefrontal cortex, as a function of treatment condition. For basilar dendrites cocaine treatment increased spine density when given either at Home or in the Novel condition, but the effect in the Novel group was significantly greater than in the Home group. For apical dendrites the effect of cocaine treatment was significant only in the Novel group.

Medial prefrontal cortex. Figure 3 shows the effect of cocaine treatment on the density of dendritic spines on the basilar and apical dendrites of layer V pyramidal cells in the mPFC. For the basilar dendrites, a two-way ANOVA resulted in a significant group × drug interaction ($F = 5.94$, $P = 0.017$). *Post hoc* Fisher's tests indicated that cocaine significantly increased spine density in the Novel group ($P < 0.0001$) and in the Home group ($P = 0.002$), but the significant interaction indicates that cocaine increased spine density to a significantly greater extent in the Novel group than in the Home group (and these two groups differed, $P = 0.012$, Fisher's test). For the apical dendrites, a two-way ANOVA resulted in no significant effect of group (Home vs. Novel, $F = 1.9$, $P = 0.17$), a significant effect of drug (Saline vs. Cocaine, $F = 7.6$, $P = 0.008$) but no significant group × drug interaction ($F = 0.16$, $P = 0.69$). However, *post hoc* Fisher's tests indicated that cocaine significantly increased spine density in the Novel group ($P = 0.029$) but the effect in the Home group was not statistically significant ($P = 0.10$).

Finally, we used the difference in the number of crossovers between day 8 of testing and day 1 of testing (day 8 minus day 1) as an index of the degree of sensitization in individual cocaine-treated animals, and averaged spine density values from the two hemispheres to obtain one value per rat, for the purposes of a correlational analysis (complete data were available from 11 animals/group). There was a significant positive correlation between the degree of psychomotor sensitization and spine density in the AcbC ($n = 22$, $r = 0.52$, $P = 0.011$), but no other correlations (AcbS, mPFC) were significant.

Experiment 2

Figure 4 shows the dose–response function for locomotor activity (crossovers) in the Home condition on the first and last (9th) test session. Animals received a single injection of 30 mg/kg (or saline) on the intervening days. There was a marked shift to the left in the dose–response function between the first and last test session, indicating that this higher dose of cocaine produced robust psychomotor sensitization, even though it was given at home ($F = 5.81$, $P = 0.005$). Figure 5 shows that under these conditions, cocaine also produced a significant increase in the density of dendritic spines on

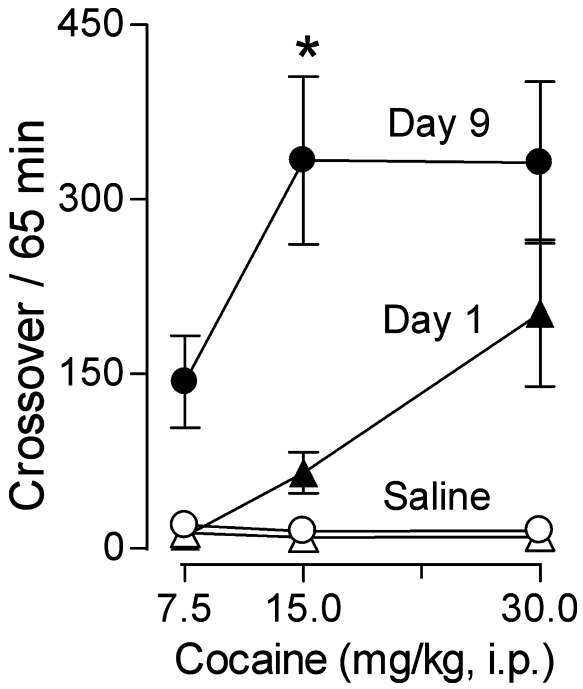

FIG. 4. The mean (± SEM) number of crossovers during the 1st and last (9th) test session for animals treated in their home cage (experiment 2) with either saline (open symbols) or escalating doses of cocaine (7.5, 15 and 30 mg/kg; filled symbols). During the remaining daily test sessions (2–8) the cocaine-treated group was given a single injection of 30 mg/kg of cocaine each day. Thus, sensitization is indicated here by a shift in the dose–response function between the 1st and last test session. Note that under these conditions, cocaine produced robust behavioural sensitization, even though all drug treatments were given in the home cage.

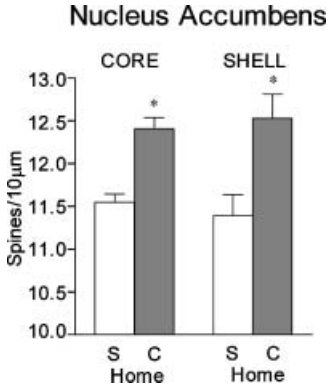

FIG. 5. The mean (± SEM) number of dendritic spines per 10 µm on medium spiny neurons in the core (left) of the nucleus accumbens (AcbC) and the shell (right) of the nucleus accumbens (AcbS), in animals-treated with either saline or cocaine (30 mg/kg) at Home (experiment 2). Note that under these conditions, cocaine treatment significantly increased spine density in both the AcbC and AcbS.

medium spiny neurons in both the AcbC (left, $t = 2.88$, $P = 0.009$) and the AcbS (right, $t = 4.96$, $P < 0.0001$).

Discussion

When rats were treated repeatedly with 15 mg/kg of cocaine in the Home condition there was no significant increase in psychomotor

effect over time (i.e. this dose failed to induce sensitization), but when the same dose was given in the Novel condition it induced robust psychomotor sensitization. These data are consistent with our previous studies using this environmental manipulation (Badiani *et al*., 1995; Browman *et al*., 1998a), and indicate that in Experiment 1 we achieved our goal of holding drug history constant while varying the ability of cocaine to induce psychomotor sensitization. We were able to ask therefore whether structural plasticity was produced by mere exposure to cocaine, or whether structural plasticity was associated with the ability of cocaine to induce psychomotor sensitization. The answer to this question differed depending on the brain region studied. Cocaine altered synaptic organization in some brain regions (AcbS and mPFC) whether it was given in a Novel environment or at Home, and therefore, independent of whether drug treatment induced psychomotor sensitization. However, only when cocaine was given under the condition that promoted psychomotor sensitization did it change synaptic organization AcbC, and its effect in the mPFC was of greater magnitude.

The environmental manipulation used here does not gate sensitization in an all-or-none fashion, but as dose is increased robust sensitization is seen even when drugs are given at Home. Environmental context merely shifts the dose–response function for inducing psychomotor sensitization (Browman *et al*., 1998a,b). We predicted therefore that if the dose of cocaine were increased such that it now induced behavioural sensitization, even when given at Home, and if structural plasticity in the AcbC is related to the ability of cocaine to induce sensitization, then under these conditions cocaine should come to increase spine density in the AcbC. We confirmed this prediction in experiment 2. Repeated injections of 30 mg/kg of cocaine at Home (vs. 15 mg/kg in experiment 1) produced robust behavioural sensitization, and increased spine density in both the AcbC and AcbS.

There are previous studies suggesting that psychomotor sensitization preferentially involves alterations in AcbC-related circuitry. For example, Cadoni, Di Chiara and colleagues (Cadoni & Di Chiara, 1999, 2000; Cadoni *et al*., 2000) have reported that sensitization to morphine, nicotine, cocaine and amphetamine is associated with increased dopamine (DA) release in the AcbC (but not AcbS, where in fact DA is sometimes decreased). Recently Hédou *et al*., 2002) reported that amphetamine sensitization is accompanied by increased Fos expression in the AcbC (and not AcbS). Lastly, the AcbC (and not AcbS) projects both directly and indirectly to the subthalamic nucleus (STN) (Zahm, 2000), and locomotor sensitization is associated with increased c-*fos* mRNA expression in the STN (Uslaner *et al*., 2003a). However, this literature is not consistent and for every example of a neural correlate of sensitization in the AcbC one can find examples of a change in the AcbS (e.g. Pierce & Kalivas, 1995; Todtenkopf *et al*., 2002). Indeed, even a cursory review of the literature on the neurobiology of sensitization reveals that many so-called neural correlates of sensitization have been reported in many brain regions. Repeated treatment with psychomotor stimulant drugs, using protocols that induce behavioural sensitization, has been reported to alter nearly every neurotransmitter system in the Acb, including DA, norepinephrine, 5-HT, acetylcholine, glutamate, GABA, endogenous opioid transmitters, etc., and a host of intracellular signalling molecules, growth factors, transcription factors, etc., and many of these changes have been found in the AcbS as well as the AcbC (Stewart & Badiani, 1993; Pierce & Kalivas, 1997; White & Kalivas, 1998; Wolf, 1998; Robinson & Berridge, 2000; Vanderschuren & Kalivas, 2000; Kalivas, 2004). However, it is possible that many of these drug-induced changes in the brain are not actually related to whether the drug treatments produced sensitization, but occur as a consequence of mere drug history, and this may account for some of the apparent inconsistencies in the literature.

To our knowledge, in the entire literature on neurobiological correlates of sensitization, there has never been an attempt to dissociate the effects of mere drug history from the ability of drugs to induce sensitization (with one exception – see below). That is, in studies that have reported neurobiological correlates of sensitization (including our own, e.g. Robinson *et al*., 1988) typically a drug is given repeatedly, and this induces both sensitization and a change in some brain measure. However, this procedure does not allow one to determine whether it was necessary for the drug treatment to induce sensitization to produce a given change in brain, or whether drug exposure itself was sufficient, because the effects of mere drug history are confounded with the effects of drug-induced sensitization (although there have been attempts to see if treatments that prevent sensitization also block a given neural correlate of sensitization; e.g. Wolf *et al*., 1994). The results reported here clearly establish that mere exposure to cocaine, in the absence of measurable psychomotor sensitization, can produce structural plasticity in the AcbS. In contrast, structural plasticity was seen in the AcbC only if cocaine treatment also produced sensitization. Given that neuroadaptations responsible for sensitization may contribute to the transition from casual or experimental drug use to the compulsive patterns of drug pursuit that characterize addiction (Robinson & Berridge, 1993; Vanderschuren & Kalivas, 2000; Robinson & Berridge, 2003; Vezina, 2004), it will be important to know which drug-induced changes in brain are related to sensitization, and which are not. Procedures such as those described here may be valuable in this regard.

We are aware of only one previous study that attempted to dissociate drug history from sensitization. Pierce *et al*. (1996) explicitly divided cocaine-treated animals into those that sensitized vs. those that did not and then examined effects on the AcbC vs. AcbS. Interestingly, Pierce *et al*. (1996) found that a cocaine challenge increased extracellular glutamate in the AcbC (not AcbS) in animals sensitized to cocaine, but not in cocaine-treated rats that failed to develop sensitization. However, one short-coming of this approach as a method to dissociate drug history from drug-induced sensitization is that it is impossible to tell whether group differences in the brain are due to differential drug treatment effects (neural plasticity), or whether the two groups differed *prior* to any drug treatment. Indeed, in the Pierce *et al*. study (1996), the behavioural response to the first injection of cocaine was different in the subset of animals that sensitized than in the subset that did not sensitize (see their table 1), suggesting pre-existing differences. If differences in the brain in these selected subgroups reflect two different phenotypes from different portions of a normally distributed population, this would be akin to comparing so-called high responder and low responder subgroups, which also sensitize differentially, but are known to have very different brains even before they are ever exposed to drug (Piazza *et al*., 1998). This problem is avoided in the present study because animals in the population are assigned randomly to two groups; they are not selected based on some phenotype.

Nevertheless, it is interesting that Pierce *et al*. (1996) found that sensitization increased glutamate release in the AcbC and not AcbS, because glutamate synapses are found on the heads of dendritic spines, and in the Acb and mPFC spines are thought to be the locus of DA/glutamate interactions (Sesack & Pickel, 1990; Smith & Bolam, 1990; Groenewegen *et al*., 1991; Goldman-Rakic *et al*., 1992). Furthermore, we have found that amphetamine preferentially increases spine density on the distal portion of the dendritic tree of medium spiny neurons (Li *et al*., 2003), which is the location of DA and glutamate synapses on these cells. Therefore, to the extent that the

changes in spine density reported here reflect changes in the number of synapses, which is the case for many other forms of experience-dependent plasticity (Greenough *et al*., 1990; Kolb *et al*., 1998; Woolley, 1998), our data suggest that sensitization-related changes in patterns of synaptic connectivity specifically in the AcbC (and mPFC) may represent the structural basis of changes in DA/glutamate signalling that has been associated with sensitization.

Of course, we are assuming that the structural changes in dendritic spines described here reflect changes in synapses, changes that alter the operation of the relevant circuits. It is important to acknowledge therefore that it is impossible to tell how (or if) dendritic reorganization characterized at this level of structural analysis alters the operation of cells or circuits. Indeed, very different changes in structure, such as an increase vs. a decrease in spine density on a given dendritic segment of a cell, could have exactly the same outcome in terms of how cell signalling and the operation of the circuit is altered – depending on how different synaptic inputs are rearranged around the altered postsynaptic surface. By the same token, apparently similar alterations in dendrites produced by different treatments could have very different outcomes for the operation of cells and circuits, if synaptic inputs are rearranged differently. To determine how the kind of synaptic reorganization described here alters the operation of cells and circuits will require both ultra-structural and electrophysiological approaches. We also do not know the neurobiological mechanisms by which environmental context modulates drug-induced plasticity. However, environmental context does modulate the ability of psychostimulant drugs to induce immediate early genes in many brain regions (Badiani *et al*., 1998, 1999; Day *et al*., 2001; Uslaner *et al*., 2003a,b), and this could be related to the effect of context on synaptic plasticity reported here.

Given that this is the first report of a drug-induced change in the brain that has been specifically linked to whether drug administration produced sensitization or not, and given that sensitization was associated specifically with structural plasticity in the AcbC (but not AcbS), it behoves us to briefly consider what behavioural/psychological functions are mediated by the AcbC vs. the AcbS (Cardinal *et al*., 2002). There is increasing evidence that the AcbC is especially important in mediating the motivational impact of Pavlovian conditioned stimuli (CSs) on behaviour, as assessed by: (i) Pavlovian conditioned approach – that is, the tendency to approach CSs; (ii) conditioned reinforcement (CRf) – the process by which a Pavlovian CS reinforces instrumental responses and (iii) Pavlovian-instrumental transfer (PIT) – the process by which noncontingent presentation of a CS facilitates ongoing instrumental actions. Everitt, Robbins and their colleagues (for reviews, see Everitt *et al*., 2000; Cardinal *et al*., 2002) have found that the AcbC (but not AcbS) is critical for both Pavlovian conditioned approach (Parkinson *et al*., 1999; Di Ciano *et al*., 2001) and PIT (Hall *et al*., 2001; de Borchgrave *et al*., 2002; compare Corbit *et al*., 2001), and the noncontingent presentation of a Pavlovian CS increases extracellular DA in the AcbC, but not AcbS (Ito *et al*., 2000). This may be related to reports that AcbC-related circuitry is critical for the reinstatement of drug-seeking behaviour following extinction of self-administration (e.g. McFarland & Kalivas, 2001; for review, see Kalivas & McFarland, 2003). Lesions of both the AcbC and AcbS impair aspects of CRf, but in different ways – the AcbS appears to be especially important in mediating the potentiating effects of psychostimulant drugs on CRf (Parkinson *et al*., 1999), but the AcbC appears to be especially important for the ability of conditioned reinforcers to maintain instrumental actions (Ito *et al*., 2004). The AcbS also appears to be especially important in mediating the impact of primary reinforcers, including food and drugs, as well as aversive stimuli (Cardinal *et al*., 2002).

It is especially relevant that all of these aspects of Pavlovian conditioned motivation have been reported to be enhanced by past drug treatment (i.e. they appear to sensitize). Prior exposure to psychostimulant drugs facilitates Pavlovian conditioned approach (Harmer & Phillips, 1998, 1999; Taylor & Jentsch, 2001), PIT (Wyvell & Berridge, 2001) and the potentiative effects of psychostimulants on CRf (Taylor & Horger, 1999; Mead *et al*., 2004). Indeed, the facilitatory effects of past drug treatment on the acquisition of drug self-administration behaviour (Piazza *et al*., 1989; Horger *et al*., 1990), conditioned place preference (Lett, 1989) and progressive ratio responding (Mendrek *et al*., 1998; Lorrain *et al*., 2000) could all be secondary to sensitization of Pavlovian motivational processes, that is, to sensitization of incentive salience (Robinson & Berridge, 2000, 2003). Thus, drug-induced synaptic reorganization in the AcbC may be especially important for both psychomotor sensitization and for incentive sensitization (assuming that both forms of sensitization are sensitive to the environmental manipulation used here).

In closing, the extent to which the effects of past drug history on subsequent behaviour and psychological function are due to neuroadaptations that accompany the development of any form of sensitization, vs. those that occur independent of sensitization, is almost completely unexplored. This is an interesting question given that the development of psychomotor sensitization has been related directly to increases in drug motivated behaviour (De Vries *et al*., 1998, 2002). Thus, if forms of synaptic plasticity responsible directly for sensitization play an especially important role in the process by which repeated drug use leads to addiction, the data reported here suggest that synaptic reorganization in specifically the AcbC (and mPFC) may be critically involved in the transition to addiction.

Acknowledgements

This research was supported by a grant to T.E.R. from the National Institute on Drug Abuse (R01 DA013398). T.E.R. was also supported by a NIDA Senior Scientist Award (K05 DA00473). We thank Kent Berridge, Barry Everitt, Carrie Ferrario, Bryan Kolb and Jane Stewart for helpful comments on an earlier draft.

Abbreviations

Acb, nucleus accumbens; AcbC, Acb core; AcbS, Acb shell; DA, dopamine; mPFC, medial prefrontal cortex; STN, subthalamic nucleus.

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