

Structural plasticity associated with exposure to drugs of abuse

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Abstract

Persistent changes in behavior and psychological function that occur as a function of experience, such those associated with learning and memory, are thought to be due to the reorganization of synaptic connections (structural plasticity) in relevant brain circuits. Some of the most compelling examples of experience-dependent changes in behavior and psychological function, changes that can last a lifetime, are those that accrue with the development of addictions. However, until recently, there has been almost no research on whether potentially addictive drugs produce forms of structural plasticity similar to those associated with other forms of experience-dependent plasticity. In this paper we summarize evidence that, indeed, exposure to amphetamine, cocaine, nicotine or morphine produces persistent changes in the structure of dendrites and dendritic spines on cells in brain regions involved in incentive motivation and reward (such as the nucleus accumbens), and judgment and the inhibitory control of behavior (such as the prefrontal cortex). It is suggested that structural plasticity associated with exposure to drugs of abuse reflects a reorganization of patterns of synaptic connectivity in these neural systems, a reorganization that alters their operation, thus contributing to some of the persistent sequela associated with drug use—including addiction.

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1. Introduction

Persistent changes in behavior and psychological function that occur as a consequence of experience are thought to be mediated by the reorganization or strengthening of synaptic connections in specific neural circuits. This idea has been a fundamental assumption underlying research on the neurobiology of learning and memory, as well as other forms of experience-dependent plasticity, since the time of Ramon y Cajal (1928), and Hebb (1949) formalized this postulate in his seminal book, “*The Organization of Behavior*”. Although experimental evidence directly relating structural plasticity in the brain to changes in specific behaviors is very limited, there are numerous examples

where experience has been shown to alter the physical structure of neurons and synapses (i.e., produce structural plasticity). For example, changes in behavior that result from learning (Chang and Greenough, 1982; Moser et al., 1994; Stewart and Rusakov, 1995; Leuner et al., 2003), living in an isolated versus complex environment (Greenough et al., 1990; van Praag et al., 2000; Kolb et al., 2003a) or recovery of function after brain damage (Kolb and Gibb, 1991; Jones et al., 1996; Biernaskie and Corbett, 2001) are all associated with structural alterations in relevant neural circuits. Thus, a major aim of modern research on the neurobiology of behavioral plasticity, including learning and memory, is elucidating the molecular mechanisms involved in the structural reorganization of neuronal circuits (Lamprecht and LeDoux, 2004).

A focus of much research on structural plasticity has been on the morphology of dendrites and dendritic spines. The vast majority of synaptic inputs onto

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neurons are on dendrites or dendritic spines, and the amount of synaptic input cells receive varies with the amount of dendritic surface available (Harris and Kater, 1994). Furthermore, it is estimated that over 90% of excitatory synapses are on dendritic spines, and synaptogenesis associated with experiences like learning or environmental complexity is reflected by changes in the number of dendritic spines (Greenough et al., 1990; Kolb et al., 1998; Woolley, 1999; Rampon et al., 2000). Even changes in the shape of existing spines may modify synaptic efficacy by altering the chemical micro-environment or the electrotonic properties of the synapse (Horner, 1993; Rusakov et al., 1996; Shepherd, 1996; Nimchinsky et al., 2002; Tsay and Yuste, 2004), or by altering fast synaptic neurotransmission (Kasai et al., 2003). Indeed, dendrites and dendritic spines are thought to be a locus of experience-dependent structural plasticity (Harris and Kater, 1994; Nimchinsky et al., 2002; Kasai et al., 2003; Lamprecht and LeDoux, 2004), and therefore, they provide an obvious focus of study in trying to understand how experiences can alter brain organization to produce life-long changes in behavior and psychological function.

Nearly all research on structural plasticity in the brain has involved how learning (Greenough and Bailey, 1988; Andersen and Trommald, 1995; Kolb and Whishaw, 1998; Lamprecht and LeDoux, 2004), long-term potentiation (Andersen and Soleng, 1998; Yuste and Bonhoeffer, 2001), stress (McEwen, 2000; Vyas et al., 2002), environmental manipulations (Greenough et al., 1990; van Praag et al., 2000), recovery of function (Kolb and Whishaw, 1998), changes in the hormonal milieu (Woolley, 1999; Leranath et al., 2003), pathological states (Fiala et al., 2002), etc., change synapses or dendritic structure. But one of the most compelling examples of experience-dependent plasticity, whereby experience at one point in life changes behavior and psychological function for a lifetime, is addiction. The propensity of addicts to relapse, even months to many years after the discontinuation of drug use, and long after withdrawal symptoms have subsided, provides stark evidence that drug use has long-lasting consequences for behavior and psychological function. Similarly, very long-lasting changes in behavior produced by repeated exposure to drugs of abuse have been described in controlled animal studies, as exemplified, for example, by phenomena like behavioral sensitization (Robinson and Becker, 1986). Repeated intermittent exposure to a variety of drugs of abuse can produce a hypersensitivity (sensitization) to their psychomotor activating and incentive motivational effects that can persist for months to years after the discontinuation of drug treatment (Paulson et al., 1991; Robinson and Berridge, 2003).

The behavioral evidence leaves no doubt that drugs of abuse can produce very persistent changes in brain

function. Furthermore, there is growing evidence that drugs of abuse usurp many of the same cellular and molecular mechanisms involved in other forms of synaptic plasticity (Berke and Hyman, 2000; Hyman and Malenka, 2001; Nestler, 2001). It is surprising, therefore, that until recently there has been almost no research on whether any of the long-lasting behavioral consequences of repeated exposure to drugs of abuse in adulthood are accompanied by the kinds of structural plasticity normally associated with other forms of experience-dependent plasticity. (Note that structural changes in dendrites have been described when drugs are given early in development, but this literature will not be reviewed here; see Stanwood and Levitt, 2004 for a recent review).

We have begun to address this issue in a series of experiments in which we asked whether repeated exposure to cocaine, amphetamine, morphine or nicotine, in adult rats, whether administered by an experimenter (EA) or self-administered (SA), have long-lasting effects on the structure of dendrites and dendritic spines in brain regions thought to mediate drug-induced changes in incentive motivation and reward (such as the nucleus accumbens; Acb) and in cognitive function (such as the prefrontal cortex; PFC). The purpose of this paper is to summarize what we have learned thus far.

2. The method

A common approach to examine the impact of experience on synaptic organization is to use Golgi-stained material to quantify the structure of dendrites and the density of dendritic spines (Greenough, 1984; Greenough et al., 1990; Kolb et al., 1998) and that is the method used in most of the studies summarized here. In all cases, the structure of dendrites or the density of spines on neurons was quantified using one of three measures (Greenough and Chang, 1985; Kolb and Whishaw, 1998). (1) Total dendritic length was estimated by counting the number of ring intersections using an overlay of concentric rings (Sholl, 1981). (2) The total number of dendritic branches (indicated by bifurcations) was counted at each order away from the cell body (Coleman and Riesen, 1968). (3) Spine density was estimated along a specific segment of dendrite by tracing the dendritic segment, calculating its exact length, and counting the number of spines along that length (to yield spines/10 μm). In some experiments, the frequency of branched spines (i.e., spines with multiple heads) was also quantified (Comery et al., 1996).

There are, of course, limitations in interpreting changes in dendritic structure estimated from Golgi material that need to be kept in mind. This approach does not provide a direct measure of synapses, but only an

indirect measure based on alterations in the postsynaptic surface of cells. However, a strong relationship between measures of dendritic structure and synapses has been confirmed in many studies using electron microscopy to directly quantify synaptic density. Typically changes in the dendritic surface of cortical neurons assessed using Golgi-stained material are accompanied by changes in the number of synapses per neuron assessed with EM (Greenough and Bailey, 1988; Greenough et al., 1990; Kolb and Whishaw, 1998; Woolley, 1999). Of course, without ultrastructural studies one cannot be positive that changes in dendritic surface and spines are accompanied by an increase in synaptic contacts, but normally in adult rats nearly all spines in the cortex and striatum have a synaptic contact (Gray, 1959; Peters and Feldman, 1976; Wilson et al., 1983).

It is also important to emphasize that the kinds of structural alterations in neurons seen at this level of analysis provide no information about exactly how the operation of cells or circuits is altered. Indeed, very different changes in structure, such as an increase versus a decrease in spine density on a given dendritic segment of a cell, could have exactly the same outcome in terms of how cell signaling 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. Thus, it is impossible to tell how dendritic reorganization characterized at this level of structural analysis translates into alterations in properties of cells (although see Kasai et al., 2003). Some information may be gleaned from ultrastructural studies, which have the potential to tell how different inputs are rearranged, but even this will not tell exactly how the synaptic rearrangement changed cell signaling. This will require electrophysiological approaches. Nevertheless, as described below, the approach used here can provide a great deal of information about the structural plasticity associated with exposure to drugs of abuse—including, what brain regions are affected, which cells and which portions of the dendritic surface are altered, whether different drugs have similar or different effects, the conditions that lead to structural plasticity (the doses, treatment conditions, mode of drug administration, etc.), whether there is any relationship between persistent structural changes and persistent behavioral changes produced by exposure to drugs of abuse, and how drug-induced structural plasticity interacts with the structural plasticity associated other kinds of life experience. So, what have we learned?

3. Repeated exposure to drugs of abuse produces long-lasting changes in the structure of dendrites and dendritic spines

The most extensive data available are from studies with the psychomotor stimulant drugs, amphetamine and cocaine. For these drugs, the effects of both experimenter-administered (EA) and self-administered (SA) drug have been studied, primarily in the nucleus accumbens (Acb) and medial prefrontal cortex (mPFC) (Table 1). EA and SA cocaine and amphetamine have very similar effects on the density of spines in both the Acb and mPFC. Both amphetamine (Robinson and Kolb, 1997, 1999a; Ferrario et al., 2003; Heijtz et al., 2003; Kolb et al., 2003b; Li et al., 2003; Crombag et al., 2004) and cocaine (Robinson and Kolb, 1999a; Robinson et al., 2001; Li and Robinson, 2003; Norrholm et al., 2003), and both modes of drug administration, *increase* spine density on medium spiny neurons in both the Acb shell (AcbS) and the Acb core (AcbC; amphetamine SA data not available for AcbC, see Table 1). Where data are available (Table 2) these increases in spine density in the Acb are accompanied by increases in dendritic branching (Robinson and Kolb, 1997, 1999a; Robinson et al., 2001; Kolb et al., 2003b). Similarly, both amphetamine (Robinson and Kolb, 1997, 1999a; Ferrario et al., 2003; Heijtz et al., 2003; Crombag et al., 2004) and cocaine (Robinson and Kolb, 1999a; Robinson et al., 2001; Ferrario et al., 2003) and both modes of administration, *increase* spine density (and dendritic branching where studied) on the apical dendrites of pyramidal cells in the mPFC. Similar effects are seen on the basilar dendrites of mPFC pyramidal cells, but the effect of amphetamine here is much weaker than that of cocaine (Robinson and Kolb, 1999a). The effects of EA nicotine are very similar to the psychostimulants: nicotine *increases* spine density in both the AcbS and mPFC (Brown and Kolb, 2001; Gonzalez et al., 2004). Deprenyl, which is metabolized into amphetamine, also is reported to *increase* dendritic branching in prefrontal pyramidal cells in Bonnett monkeys (Shankaranarayana Rao et al., 1999). In sharp contrast, both EA and SA morphine markedly *decrease* spine density in the AcbS and the mPFC, and where studied (following EA; Table 3) this is accompanied by a decrease in dendritic branching (Robinson and Kolb, 1999b; Robinson et al., 2002). Chronic morphine treatment and withdrawal also alters the morphology of VTA dopamine neurons (Skclair-Tavron et al., 1996; Spiga et al., 2003). Thus, (1) exposure to four different drugs of abuse (cocaine, amphetamine, nicotine and morphine) produces structural plasticity; (2) the effects of the three stimulants are very similar (but not identical, see below)—they *increase* spines in the Acb and mPFC; (3) the effect of

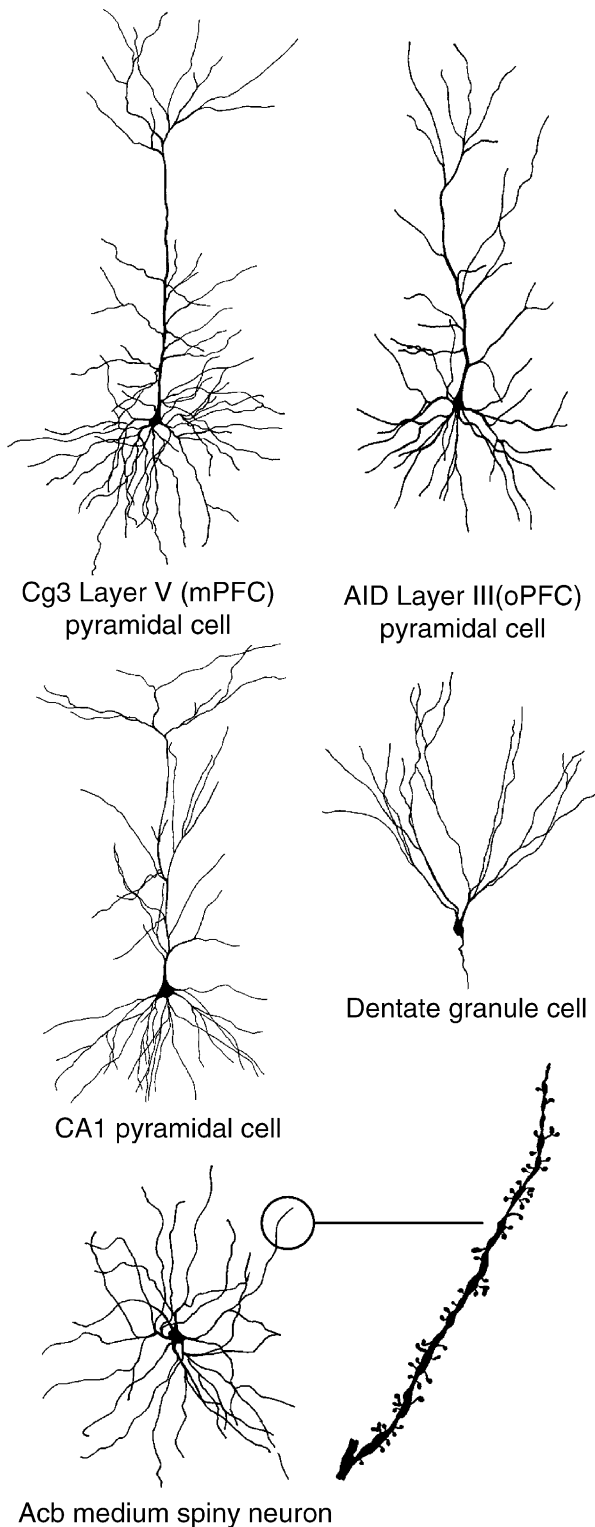


Fig. 1. Camera lucida drawings (courtesy of Grazyna Gorny) of the major cell types discussed in this paper (not drawn to scale). A high power illustration of a distal dendrite on a medium spiny neuron illustrating dendritic spines is also shown at the bottom right. Zilles' (1985) terminology: Cg3 (cingulate cortex, area 3; medial frontal cortex [mPFC]); AID (agranular insular cortex; which we refer to as orbital frontal cortex [oPFC]); CA1 (field CA1 of Ammon's horn [hippocampus]); Dentate (dentate gyrus); Acb (nucleus accumbens).

the narcotic, morphine, is very different—it *decreases* spine density in the Acb and mPFC.

3.1. Persistence

For all drugs studied thus far structural changes are evident long after the discontinuation of drug treatment. Amphetamine- and nicotine-induced increases in spine density are seen up to 3.5 months after the last drug treatment (Kolb et al., 2003b; Gonzalez et al., 2004), although most studies have been conducted approximately one month after the last treatment (Robinson and Kolb, 1997, 1999a). For cocaine, spine changes have been found as little as 24–48 h after the last of 28 daily injections (Norrholm et al., 2003), and as long as 2 weeks (Li et al., 2004) to a month later (Robinson and Kolb, 1999a; Robinson et al., 2001). Similarly, the structural plasticity associated with morphine treatment has been described a month after the last treatment (Robinson and Kolb, 1999b; Robinson et al., 2002). Interestingly, in one experiment, rats were given a cocaine treatment regimen that produced only relatively transient behavioral sensitization; i.e., psychomotor sensitization dissipated by 3 months after the last treatment. In this case spine changes were also no longer evident after 3 months of withdrawal (Kolb et al., 2003b). This suggests that, depending on the treatment regimen, spine changes may be reversible, and that they wax and wane as drug-induced behavioral plasticity waxes and wanes.

3.2. Treatment regimens

There have been no systematic studies on the dose or number of drug treatments required to produce this form of structural plasticity, but we can glean some information from the variety of treatment regimens that have been used. In most cases fairly aggressive treatment regimens have been used [e.g., for amphetamine, 5 weeks of treatment with doses escalating from 1 to 8 mg/kg (Robinson and Kolb, 1997); 20 injections of 3 mg/kg (Robinson and Kolb, 1999a)—for cocaine, 20 injections of 15 mg/kg (Robinson and Kolb, 1999a)]. But such aggressive dosing regimens do not seem to be necessary to produce structural plasticity. For example, a single injection of 2 mg/kg of amphetamine produces a very small, but significant, increase in spine density in the AcbS, although the effects of repeated treatments are much greater (Kolb et al., 2003b). Also, eight daily injections of 15 mg/kg of cocaine are sufficient to see spine changes when animals are studied 2 weeks after the last treatment (Li et al., 2004).

In general, animals allowed to self-administer drug take much greater amounts than used in studies where drug is EA, and therefore, this approach may lead to

Table 1
Effects of stimulant drugs on spine density

		Acb		mPFC		oPFC		Par1		Ocl	
		C	S	A	B	A	B	A	B	A	B
Cocaine	EA	↑	↑	↑	↑	–	–	–	–	–	–
	SA	↑	↑	↑	↑	NC	NC	↑	↑	NC	↓
Amphetamine	EA	↑	↑	↑	NC	–	–	↓	↓	↓	↓
	SA	–	↑	↑	↑	↓	↓	–	–	–	–
Nicotine	EA	–	↑	↑	↑	–	–	NC	NC	–	–

Abbreviations: EA, experimenter-administered; SA, self-administered; C, core; S, shell; A, apical; B, basilar; NC, no change; –, no data; ↑, increase; ↓, decrease (see text for references).

Table 2
Effects of stimulant drugs on dendritic branching

		Acb		mPFC		Par1		Ocl	
		C	S	A	B	A	B	A	B
Cocaine	EA	–	↑	↑	↑	–	–	–	–
	SA	–	↑	↑	↑	NC	↑	NC	NC
Amphetamine	EA	↑	↑	↑	NC	NC	NC	NC	NC
Nicotine	EA	–	↑	NC	↑	NC	NC	–	–

Abbreviations: see Table 1.

Table 3
Effects of morphine in spine density and dendritic branching

		Acb		mPFC		oPFC		Par1		Ocl		HPC	
		C	S	A	B	A	B	A	B	A	B	CA1	DG
Spines	EA	–	↓	↓	↓	↑	↑	↓	↓	NC	↓	NC	NC
	SA	–	↓	↓	↓	↑	↑	NC	NC	↓	↓	↓	↓
Branches	EA	–	↓	↓	↓	–	–	↓	↓	–	–	–	–

Abbreviations: see Table 1.

especially robust structural plasticity. But even here the amount of drug intake has an effect. For example, rats given 6 h of daily access to intravenous cocaine over approximately 20 days, by which time they have dramatically escalated their intake (Ahmed and Koob, 1998), show a significantly greater increase in spine density in the AcbC than animals given 1 h of daily access (who show a stable pattern of intake; unpublished studies). In conclusion, although there are no systematic studies, it seems likely that both the magnitude of the structural changes and their persistence depend on dose, the number of drug treatments and even the pattern of intake.

3.3. Locus

The locus of structural alterations on neurons provides some information about what synaptic inputs are

possibly changed as a consequence of drug experience. This issue has been addressed for medium spiny neurons in the Acb and the caudate-putamen (CPu). These neurons receive different inputs onto the proximal versus distal portions of their dendritic tree (Sesack and Pickel, 1990; Smith and Bolam, 1990; Groenewegen et al., 1991). The distal dendrites are the primary locus of DA inputs arising from the midbrain, as well as excitatory (presumably glutamate) inputs from the neocortex, hippocampus and amygdala. Typically, excitatory inputs form asymmetric synapses on the heads of spines, whereas the DA inputs make symmetric contacts with the neck of spines or the dendritic shaft, forming the so-called “triads” that are thought to provide the means by which DA modulates the excitatory drive on these cells. It is interesting, therefore, that changes in both dendritic branching (Robinson and Kolb, 1999a) and spine density (Li et al., 2003)

produced by amphetamine or cocaine are confined to distal dendrites of medium spiny neurons in both the Acb and CPu. Furthermore, in the Acb there is not only an increase in the number of spines, but an especially large increase in the number of spines with multiple heads (Robinson and Kolb, 1997, 1999a; see below). Thus, the morphological evidence suggests that prior exposure to psychostimulant drugs may change synaptic efficacy primarily on that portion of medium spiny neurons that integrates DA and glutamate signaling. This is especially important because both DA and glutamate have been prominently implicated in persistent forms of drug experience-dependent behavioral plasticity, such as behavioral sensitization (Kalivas, 1995; Wolf, 1998), as well as in drug experience-dependent changes in synaptic signaling measured electrophysiologically (Thomas et al., 2001; Li and Kauer, 2004). Of course, these same transmitters have been implicated in other forms of synaptic plasticity and in learning (Berke and Hyman, 2000; Hyman and Malenka, 2001; Lamprecht and LeDoux, 2004).

3.4. Branched spines

In a number of experiments there was an especially intriguing change in the shape of dendritic spines on medium spiny neurons in the Acb following treatment with amphetamine or cocaine (Robinson and Kolb, 1997, 1999a). Amphetamine or cocaine approximately doubled the proportion of spines with multiple heads (branched spines). In most brain regions branched spines are quite rare (Sorra et al., 1998), but there is increasing evidence that they are associated with synaptic plasticity. Trommald et al. (1990, 1996) have reported that after long-term potentiation (LTP) evoked by stimulation of the perforant path there is an increase in the number of branched spines on dendrites of dentate granule cells, and Geinisman and Morrell (1989) found a similar effect after kindling induced by perforant path stimulation. Comery et al. (1996) reported that there is an increase in branched spines on medium spiny neurons in the CPu of rats raised in a complex environment.

Although little is known about branched spines (Harris and Kater, 1994), recent evidence suggests they may represent a more radical alteration in synaptic organization than might seem at first glance. A common hypothesis is that branched spines are formed by “splitting” an existing presynaptic bouton to form two new synapses (Sorra et al., 1998). But in an evaluation of serial EM sections in CA1 of the hippocampus Sorra et al. (1998) found that of 91 branched spines, “Different branches of the same spine never synapsed with the same presynaptic bouton”, and that the boutons on each branch head, “were not even neighboring boutons splitting along the same axon” (p. 236). Similarly, in their LTP material Trommald et al. (1996)

reported that, “Each of the branches . . . made contact with a standard perforant path bouton and showed all the normal attributes of an asymmetric excitatory spine synapse” (pp. 224–5), and, “after reconstruction of 27 bifurcating spines no case was found in which the same two spine heads were served by the same axon” (see Andersen and Soleng, 1998 for a review). We do not know if the same holds true for branched spines in the Acb, but if it does, branched spines may reflect a fundamental reorganization of synaptic inputs onto the distal dendrites of medium spiny neurons in the Acb as a consequence of past drug experience.

4. Different drugs produce different effects on dendritic structure in different regions

It is already obvious from the above that different drugs have different effects on dendritic structure. The most striking differences are between the stimulants, all of which increase spine density in the Acb and mPFC, versus morphine, which decreases spine density in these regions. Nevertheless, it is worth emphasizing that even closely related drugs like amphetamine and cocaine, although similar in many respects, do not produce identical patterns of structural plasticity. For example, we have consistently found that in adult rats EA cocaine increases spine density on the basilar dendrites of pyramidal cells in the mPFC (Robinson and Kolb, 1999a; Robinson et al., 2001; Ferrario et al., 2003; Li et al., 2004), but EA amphetamine has either no effect or a weak effect on these dendrites [in one study the effect of amphetamine was non-significant (Robinson and Kolb, 1997) and in another only marginally significant and much less than for cocaine (Robinson and Kolb, 1999a)]. In contrast, EA amphetamine has been reported to increase spine density on the basilar dendrites of mPFC pyramidal cells when given to juvenile (P22–P34) rats (Heijtz et al., 2003).

One of the most striking regional differences comes from comparing the effects of drugs on pyramidal cells in the mPFC versus the orbital prefrontal cortex (oPFC). The mPFC and oPFC are two closely related frontal regions; e.g., they both receive projections from the mediodorsal nucleus of the thalamus (defining them as prefrontal cortex), and they are the primary cortical targets of mesocortical DA inputs in rats (Kolb, 1990). Experience with SA amphetamine or EA nicotine increases spine density in the mPFC, but *decreases* spine density in the oPFC (Crombag et al., 2004; Gonzalez et al., 2004). Experience with SA cocaine increases spine density in the mPFC, but has no effect on spine density in the oPFC (Ferrario et al., 2003). Both EA and SA morphine decrease spine density in the mPFC, but increase spine density in the oPFC (Table 1; Robinson et al., 2002). It appears, therefore,

that different drugs reorganize these closely related prefrontal regions in very specific and very different ways.

Another curious drug-dependent difference is seen in the motor cortex. A priori we would not anticipate that psychomotor stimulant drugs would affect the motor cortex, especially if the mode of action is via effects on dopamine neurotransmission. As expected, amphetamine does not affect dendritic branching in motor cortex (unpublished observations). But [Brown and Kolb \(2001\)](#) found that nicotine does increase branching in motor cortex, although more recent studies have found it has no effect on the adjacent somatosensory cortex ([Gonzalez et al., 2004](#)). Of course, nicotine has actions on cholinergic afferents to the cortex so this may be the route of action but what is puzzling is why the effects are specific to motor cortex.

Finally, a lesson learned from examining other brain regions is that drugs can have different effects on spines versus branches. For example, EA amphetamine has no effect on dendritic branching on pyramidal cells in either the parietal cortex (Par1) or occipital cortex (Oc1), but decreases spine density on these cells (while increasing branching and spine density on pyramidal cells in the mPFC) ([Robinson and Kolb, 1997](#); [Kolb et al., 2003b](#)).

It is not clear what accounts for differences in the effects of even closely related drugs like amphetamine and cocaine. Some differences may be related to pharmacokinetics or even potency (e.g., the doses of amphetamine typically used would increase extracellular DA to a much greater extent than the doses of cocaine used). A more interesting possibility is that the differences in structural plasticity between amphetamine and cocaine (and other drugs) reflect differences in their spectrum of action. For example, cocaine (unlike amphetamine) has approximately equal affinity for DA, 5-HT and NE transporters. Therefore, these two drugs produce quite different patterns of change in monoamine transmission, and this may be reflected by different patterns of structural plasticity.

5. Whether drugs are EA or SA influences structural plasticity in some brain regions but not others

In most studies on drug-induced structural plasticity (and behavioral plasticity for that matter) drugs are administered by an experimenter (EA) rather than SA. This is potentially an important issue because the neurobiological effects of drugs may vary depending on whether it is SA or EA ([Smith and Dworkin, 1990](#); [Wilson et al., 1994](#); [Dworkin et al., 1995](#); [Mark et al., 1999](#); [Stefanski et al., 1999](#)). Furthermore, the rate at which intravenously administered cocaine reaches the brain has a dramatic effect on its ability to induce immediate early genes in mesocorticolimbic structures ([Samaha et al., 2004](#)), and therefore, mode of drug

administration is also an important factor. Thus, to the extent that studies of drug experience-dependent plasticity in animals are intended to model some of the changes that may occur in addiction it is important to determine whether EA (usually i.p.) and SA (usually i.v.) drugs have similar or different effects.

In some brain regions both EA and SA drug produce the same changes in spine density. Thus, both EA and SA amphetamine and cocaine increase spine density in the Acb and mPFC ([Robinson and Kolb, 1999a](#); [Robinson et al., 2001](#); [Ferrario et al., 2003](#); [Crombag et al., 2004](#)), and both EA and SA morphine decrease spine density in the Acb and mPFC ([Robinson and Kolb, 1999b](#); [Robinson et al., 2002](#)). There are, however, brain regions in which EA and SA morphine have different effects. For example, in the Par 1 of the rat EA morphine decreases spine density on pyramidal cells, but SA morphine has no effect. In the hippocampus SA morphine decreases spine density but EA morphine has no effect ([Robinson et al., 2002](#)).

It is not clear what accounts for differences between EA and SA drug administration on structural plasticity. Some of the difference may simply be pharmacokinetic, or related to the total amount of drug exposure. This usually varies considerably between studies using EA versus SA drug, which also typically involve different routes of administration. Furthermore, there is evidence the neurobiological impact of drugs varies as a function of rate of drug delivery ([Porrino, 1993](#)), even when the same route of administration is used ([Samaha et al., 2004](#)). On the other hand, some of the differences between EA and SA drug administration could be related to other “psychological” differences between these two modes of drug administration.

5.1. Role of learning

An interesting issue addressed by comparing EA and SA drug is the extent to which drug-associated structural plasticity is induced “unconditionally” as a consequence of drug actions on the brain, or whether they are related to learning about the relationship between an action and drug delivery (i.e., operant or instrumental learning). Many of the structures in which we have seen drug-induced changes in dendritic structure are brain regions that have been implicated in various aspects of learning ([Cardinal et al., 2002](#); [Killcross and Coutureau, 2003](#); [Cardinal and Everitt, 2004](#); [Holland and Gallagher, 2004](#); [Kelley, 2004](#)). For example, [Kelley et al. \(1997\)](#) have suggested that the Acb is critical in instrumental (response-reinforcement) learning. If EA and SA drug have the same effect on dendrites, presumably learning about the contingency between an action (a lever press or nose-poke) and delivery of a drug reward is not responsible for the

structural plasticity. Thus, the fact that EA and SA amphetamine and cocaine have similar effects on spines in the Acb and mPFC suggests this structural plasticity is not due to instrumental learning. (An important caveat is required here. Although EA and SA psychostimulant administration has similar effects on spine density in the Acb and mPFC, we do not know whether the synaptic reorganization reflected by these changes is qualitatively the same; see Section 2 above).

Another way to address the role of instrumental learning in structural plasticity is to study whether learning an instrumental action for a non-drug reward (food) has a similar effect as learning an action for a drug reward. We have found that learning to work for a food reward has no effect on spine density in the Acb and mPFC (Robinson et al., 2001; Ferrario et al., 2003; Crombag et al., 2004). In contrast, we have found that in the hippocampus responding for either a food reward or for amphetamine increases spine density (Crombag et al., 2004). Thus, the available evidence suggests that drug-induced spine changes in the Acb and the mPFC are not a consequence of instrumental learning, whereas in the hippocampus it is possible the spine changes are related to instrumental learning.

It is much more difficult to determine the extent to which the kinds of changes in dendritic structure we have described here with EA and SA drug are related to Pavlovian learning about the relationship between stimuli in the environment and drug administration (Cardinal et al., 2002). This kind of Pavlovian learning occurs whether drugs are EA or SA. That is, in both of these situations stimuli in the environment (especially the context) are paired with drug administration (the US) and these stimuli can acquire conditioned stimulus (CS) properties, whereby subsequent exposure to the CS can either elicit a conditioned response or modulate the ability (set the occasion) of other stimuli to elicit responses (Anagnostaras and Robinson, 1996; Anagnostaras et al., 2002; Cardinal et al., 2002). It is possible, therefore, that some of the changes in dendrites described here are due to Pavlovian learning (or even higher level cognitive learning), rather than to an unconditional drug effect.

Although learning may contribute to drug-induced structural plasticity, there are reasons to believe that at least some of the changes in dendritic structure are due to “unconditional” drug effects, rather than a consequence of associative learning. Probably the most compelling reason is that different rewards produce very different effects. The psychostimulants increase spine density in the Acb and mPFC, whether EA or SA, whereas morphine decreases spine density, and food has no effect. If the changes in spine density in these regions were related to Pavlovian learning one might expect to see the same changes whether the reward was cocaine, morphine or food. Nevertheless, this is not a

completely convincing argument, because learning about different rewards could have different effects. Nevertheless, at least we know that the structural changes are relatively stimulus (drug) specific.

5.2. Stress

Of course, psychostimulant drugs are also stressors, activating the HPA axis, and some of their effects on dendritic structure could be related to these actions. Repeated intermittent stress alters dendritic morphology, for example, decreasing dendritic branching on pyramidal cells in the CA3 region of the hippocampus (McEwen, 1999) and increasing dendritic branching in the amygdala (Vyas et al., 2002). We have not studied the CA3 region or amygdala, but amphetamine SA experience increases spine density on CA1 pyramidal cells and has no effect on dentate granule cells (Crombag et al., 2004). Interestingly, like psychostimulant drugs, sodium depletion (a stressful experience) increases dendritic branching on medium spiny neurons in the AcbS, and enhances (sensitizes) the later psychomotor response to an amphetamine challenge (Roitman et al., 2002).

In summary, there are a number of different actions of drugs that may contribute to structural plasticity in different brain regions. It will be a major challenge to untangle the extent to which drug-induced structural changes in any given brain region, or any given cell population, are related to unconditional drug effects, learning, the actions of drugs as stressors, etc., or to interactions amongst these factors.

6. Structural changes in some brain regions are associated with the development of behavioral plasticity (sensitization) whereas changes in other brain regions are not

One form of behavioral plasticity produced by the repeated intermittent administration of psychostimulant drugs, whether they are EA or SA, is behavioral sensitization (Robinson and Berridge, 2000; Vezina, 2004). Sensitization refers to an increase in a drug effect that occurs as a consequence of past drug administration, and a number of different drug effects have been reported to sensitize. Sensitization to the psychomotor activating effects of drugs have been best characterized, but sensitization of drug reward and incentive-sensitization have also been reported (Robinson and Berridge, 2003, for review), and all these forms of sensitization persist for long periods of time after the discontinuation of drug treatment. It has been suggested that drug sensitization is due to non-associative changes in the neural substrates that mediate unconditional drug effects, including psychomotor and incentive motivational effects, although the expression of behavioral sensitization can come under

strong CS control (i.e., its expression is modulated by learning; Stewart, 1992; Stewart and Badiani, 1993; Anagnostaras and Robinson, 1996; Anagnostaras et al., 2002). It has also been suggested that the neuroadaptations responsible for sensitization are critical in the transition from recreational or circumstantial drug use to the compulsive patterns of drug-seeking and drug-taking behavior that characterize addiction (Robinson and Berridge, 1993, 2000, 2003). We have been interested, therefore, in the extent to which drug-induced structural plasticity in specific brain regions is related to the development of behavioral sensitization.

One approach to this question is to determine whether manipulations that influence behavioral sensitization have a similar effect on structural plasticity (i.e., the extent to which the two phenomena are dissociable). A simple example is that as dose increases the degree of behavioral sensitization increases (Kalivas and Stewart, 1991), and so does the magnitude of the increase in spine density in the Acb (Kolb et al., 2003b). More interesting is a procedure that allows one to hold drug history constant while manipulating whether drug treatment produces behavioral sensitization or not. There are doses of cocaine that induce robust behavioral sensitization when cocaine is given in a distinct test environment (“novel” condition) but not when cocaine is given in the home cage (“home” condition; Badiani et al., 1995; Browman et al., 1998). If drug-induced changes in dendritic structure are related to the development of behavioral sensitization they should be seen in the “novel” condition but not the “home” condition, even though both groups receive the same drug treatments (just in different environments). Indeed, Li et al. (2004) found that in the AcbC repeated cocaine treatment increased spine density only in the “novel” group (the group that developed behavioral sensitization), but not in the “home” group (that failed to sensitize). In contrast, cocaine increased spine density in the AcbS in both groups, that is, independent of sensitization. Furthermore, if the dose of cocaine (and number of treatments) was increased, such that cocaine induced behavioral sensitization even when given at “home”, an increase in spine density was now seen in the AcbC (Li et al., 2004). Thus, the induction of this particular form of behavioral plasticity (sensitization) is associated with structural plasticity in the AcbC, but not the AcbS, although mere exposure to cocaine is sufficient to produce structural plasticity in the AcbS (but not the AcbC). It appears, therefore, that the conditions necessary for cocaine to produce structural changes in these two subregions of the Acb are quite different, and only changes in the AcbC are associated with a form of drug experience-dependent behavioral plasticity that has been linked to the compulsive pursuit of drugs.

7. Drug experience-dependent structural plasticity may influence the structural plasticity associated with other experiences

As discussed above, drugs of abuse usurp many of the cellular and molecular mechanisms responsible for experience-dependent plasticity, and both experience and drugs alter dendritic structure. This raises the possibility that changes in synaptic organization produced by experience may interact with those produced by exposure to drugs of abuse. We recently tested this hypothesis by studying the influence of treatment with amphetamine or cocaine on an especially well-studied form of experience-dependent structural plasticity; that associated with variation in environmental complexity (Kolb et al., 2003b). There is a wealth of evidence that housing adult rats in a relatively complex environment (compared to standard cages) increases dendritic branching, spine density and the number of synapses in a variety of brain regions (Greenough et al., 1990; van Praag et al., 2000; Kolb et al., 2003a). Therefore, we used this procedure to study the effect of past exposure to psychostimulant drugs on the ability of housing in a complex environment to alter dendritic structure.

Rats were given repeated injections of amphetamine or cocaine, using an injection regimen that produced behavioral sensitization, and after the last injection they were housed for 3–3.5 months either in a complex environment or socially in standard lab cages. After this, their brains were obtained and dendritic branching and spine density on cells in the AcbS and somatosensory cortex (Par1) were quantified. We found that past treatment with amphetamine or cocaine interfered with the ability of experience in a complex environment to produce structural plasticity in these brain regions (Kolb et al., 2003b). Although the topic requires much further investigation, this study suggests that in some situations, and in some brain regions, exposure to drugs of abuse may limit or occlude the ability of subsequent experience to promote synapse formation and/or synaptic reorganization.

This notion has important implications for thinking about the long-term consequences of drug use on behavior and psychological function. For example, many other kinds of experiences besides housing in a complex environment are associated with structural plasticity, including learning and recovery of function following brain damage, and the synaptic reorganization associated with these experiences is thought to have desirable functional consequences. Thus, if prior exposure to drugs of abuse interferes with the ability of experience to reorganize neural circuits this could interfere with the behavioral/cognitive advantages that accrue with experience. Consistent with this notion, Gonzalez et al. (2004) recently found that treatment with nicotine can affect motor learning. Rats were

given nicotine while they learned one skilled motor task and then went drug free for 2 months. They then were trained on a new skilled motor task, but did not receive further drug treatment. Past treatment with nicotine interfered with the learning of the new motor task and even with extended training nicotine treated rats failed to learn the task. Saline-treated animals learned the new task in only a few days. Thus, prior exposure to nicotine may have impaired the process of synaptic reorganization required for new learning.

These studies also raise an interesting way to think about some of the behavioral and psychological deficits seen in addicts. There is accumulating evidence that addicts present with a variety of neuropsychological deficits indicative of frontal cortical dysfunction (Bolla et al., 1998; Rogers and Robbins, 2001), and these cognitive deficits are often attributed to either a kind of “lesion” effect, that is, frank neurotoxicity, or at least a kind of “functional” lesion. But the interaction between drugs and other experiences in producing structural plasticity suggests an alternative hypothesis. It is possible that some of the neuropsychological deficits seen in addicts are due to limits on structural and synaptic plasticity imposed by drug use, rather than to a kind of “lesion effect”.

8. Mechanisms

Of course, there is considerable interest in the molecular mechanisms responsible for structural plasticity associated with both experience and exposure to drugs of abuse (Hyman and Malenka, 2001; Nestler, 2001; Ujike et al., 2002; Bolanos and Nestler, 2004; Chao and Nestler, 2004; Lamprecht and LeDoux, 2004). For example, these mechanisms are thought to involve a host of changes initiated in part by calcium entry via glutamate NMDA receptors, activation of numerous intracellular signaling cascades that alter gene expression, and eventually to changes in growth factors, cytoskeletal and adhesion molecules, and many other proteins needed to form new synapses. A discussion of these putative mechanisms is beyond the scope of this article, but suffice it to say that the molecular mechanisms involved in development and in mediating other forms of experience-dependent plasticity are likely to be shared with those involved in mediating the structural plasticity associated with drugs of abuse.

We are aware of very few studies that directly examine the molecular basis of drug-induced structural plasticity. Norrholm et al. (2003) recently reported that repeated exposure to cocaine increases spine density in the Acb, and that this is prevented by inhibition of cyclin-dependent kinase 5 (Cdk5). This kinase is known to be involved in regulating cytoskeletal proteins and neurite outgrowth (e.g., Sasaki et al., 2002; Hallows

et al., 2003; Chao and Nestler, 2004 for review) as well as some behavioral effects of cocaine, and is a downstream target of delta-FosB, which is involved in actions of cocaine and is altered by repeated cocaine administration (Kelz et al., 1999; Chao and Nestler, 2004). The potential role of neurotrophic factors in mediating long-term behavioral and neurobiological adaptations produced by drugs of abuse is also an important area of investigation (Bolanos and Nestler, 2004; Flores and Stewart, 2000). For example, Flores and their colleagues have shown that astrocytic basic fibroblast growth factor (bFGF) expression is enhanced by repeated treatment with amphetamine, and that this is necessary for the induction of psychomotor sensitization (Flores et al., 1998; Flores and Stewart, 2000; Flores et al., 2000).

Another approach to exploring potential mechanisms comes from recent studies from Margaret Gnegy's lab at Michigan using cultured PC12 cells. These cells contain endogenous DA (and NE) that is released by amphetamine, and repeated intermittent treatment with amphetamine enhances later amphetamine-stimulated DA release from PC12 cells (Kantor et al., 2002), a phenomenon that has been associated with behavioral sensitization in vitro (Robinson and Becker, 1982) and in vivo (Robinson et al., 1988; Vezina, 2004). Recently Park et al. (2002) have shown that repeated intermittent treatment with amphetamine also causes neurite outgrowth in PC12 cells, and the conditions that lead to neurite outgrowth (and enhanced DA release) are very similar to the conditions required to see robust behavioral sensitization and enhanced DA release in the intact animal. For example: (1) Amphetamine produces much greater neurite outgrowth when given intermittently than when given continuously or a single time. (2) The degree of neurite outgrowth is greater after a period of withdrawal than immediately after the last treatment. (3) The effect of amphetamine is attenuated by blocking the DA transporter. Park et al. (2003) have also explored the intracellular signaling pathways responsible for this amphetamine-evoked enhancement in neurite outgrowth and amphetamine-stimulated DA release. They found that inhibition of MAP kinase or PKC (but not PKA) prevented the neurite outgrowth and enhanced DA release produced by repeated amphetamine treatment, whereas inhibition of PKA prevented the enhancement in DA release but not neurite outgrowth. Importantly, these intracellular signaling pathways have been implicated in mediating the actions of psychostimulant drugs, behavioral and neurochemical sensitization, various forms of experience-dependent plasticity, as well as structural plasticity (Nestler, 2001; Chao and Nestler, 2004).

Although the studies on drug-induced changes in dendritic structure reviewed here do not address potential mechanisms they do have important implications in

thinking about mechanisms, and how to go about studying them. For example, the regional specificity of the effects, and the variation in the effects of different drugs, suggests that repeated drug treatment does not alter dendritic structure due actions on some ubiquitous, brain-wide growth factor. To the extent that drug-induced structural plasticity is mediated by molecules that regulate neuronal and synaptic structure (Hyman and Malenka, 2001; Nestler, 2001; Chao and Nestler, 2004; Lamprecht and LeDoux, 2004) the actions of such agents must also vary regionally, and as a function of mode of drug administration. Indeed, the specificity of the effects described here may provide an excellent avenue to delineate causal mechanisms. If one hypothesizes, for example, that a given molecule is critical for morphine to alter synaptic organization (at least as indicated by changes in spine density), then in Par1 only EA morphine should have an effect on the molecule under study, in the hippocampal formation only SA morphine should have an effect, and in mPFC and oPFC one might predict opposite effects (Table 3). Of course, it is also possible that parsimony does not rule, and that the effects of different drugs of abuse on synaptic organization in different types of cells in different brain regions are mediated by entirely different mechanisms. Whatever the case, the available data strongly suggest that to delineate the molecular mechanisms for structural plasticity, and associated changes in patterns of synaptic connectivity, will require studying very specific brain regions and cell types, and perhaps even specific portions of a dendritic tree.

9. Conclusions

The available literature establishes that repeated exposure to a number of different drugs of abuse (amphetamine, cocaine, nicotine and morphine) alters the morphology of dendrites and dendritic spines on cells in brain regions associated with incentive motivation, reward and learning, such as the Acb, CPU and prefrontal cortex. Drug-induced structural plasticity is evident long (many months) after the discontinuation of drug treatment, suggesting that drugs of abuse produce a persistent reorganization of patterns of synaptic connectivity in these brain regions. The form of drug-induced changes in dendritic structure (e.g., whether branching or spine density increases or decreases) varies depending on the drug, the brain region and even the portion of the dendritic field on a cell. This suggests that drugs do not produce these effects by promoting a ubiquitous “growth factor”, but drug-induced structural plasticity represents very specific patterns of synaptic reorganization in very specific circuits. It is not known which drug-induced structural changes in which brain regions are related to which actions and

effects of drugs. Some may be produced unconditionally as a consequence of exposure to drugs, and some may be related to learning about the relationship between actions or stimuli associated with drug delivery. Some may be related to the induction of forms of behavioral plasticity, and others not. For example, cocaine-induced changes in spine density in the AcbS are seen whether cocaine induces behavioral sensitization or not (i.e., they occur as a function of mere drug history), but changes are seen in the AcbC only if cocaine induces behavioral sensitization.

It is not known how these structural changes alter the operation of cells and circuits, but presumably the reorganization of these brain regions contributes to some of the persistent sequelae associated with repeated drug use, including the hypersensitivity to the incentive motivational effects of drugs and drug-related stimuli, and cognitive alterations, that are the hallmarks of addiction (Robinson and Berridge, 2003). Finally, it is important that repeated exposure to drugs of abuse influences the ability of other life experiences to produce structural plasticity. Our initial studies suggest that repeated exposure to drugs of abuse limits the ability of other experiences to reorganize synapses in some brain regions. Thus, some of the long-term behavioral and cognitive deficits seen in addicts could be to drug-induced limits on plasticity (Kolb et al., 2003b). However, to end on a positive note, given that drugs and other experiences interact in remodeling synapses, it is also possible that some life experiences could mitigate the ability of drugs to reorganize synapses, and thus mitigate some of the negative neurobehavioral consequences of drug abuse. That will be a topic of future study.

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