Neural and Behavioral Plasticity Associated with the Transition from Controlled to Escalated Cocaine Use

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Background: *Rats given extended access to cocaine develop several symptoms of addiction, including a gradual escalation of drug intake, whereas rats given limited access do not. We asked here whether extended access to cocaine also produces drug-induced sensitization, a form of neurobehavioral plasticity implicated in addiction.*

Methods: *Rats were given limited (1 hour/session) or extended access (6 hours/session) to self-administered cocaine. Following a period of abstinence, rats were selected at random for assessment of their psychomotor response to cocaine or drug-seeking during extinction or for anatomic studies.*

Results: *When reexposed to cocaine, rats allowed extended drug access showed greater drug-seeking behavior and were hypersensitive (sensitized) to the psychomotor activating effects of cocaine compared with rats given limited access. Extended access to cocaine was also associated with a greater increase in the density of dendritic spines on neurons specifically in the core of the nucleus accumbens (and not in the shell or medial or orbital frontal cortex).*

Conclusions: *The transition from stable to escalated cocaine use, a hallmark of addiction, is associated with especially robust behavioral sensitization and synaptic reorganization in the core of the nucleus accumbens.*

Key Words: Addiction, dendritic spines, nucleus accumbens, prefrontal cortex, sensitization, structural plasticity

When given the opportunity, nonhuman animals will work to self-administer most drugs that people choose to self-administer (Yokel 1987). Therefore, self-administration procedures have been invaluable for elucidating neural systems that mediate the reinforcing and incentive motivational effects of potentially addictive drugs (Wise 2002). These studies have not, however, provided great insight into why drug use sometimes leads to addiction and at other times does not. This is because self-administration of a potentially addictive drug is not itself evidence of addiction. Addiction refers to a specific pattern of compulsive drug use that predominates over most other pursuits in life. To determine why some susceptible individuals undergo a transition from controlled drug use to addiction requires animal self-administration models that can differentiate mere drug-taking behavior from patterns of drug use characteristic of addiction (Ahmed and Koob 1998; Deroche-Gamonet et al 2004; Robinson 2004; Vanderschuren and Everitt 2004).

One way this has been achieved is by manipulating access to a drug, either by varying daily drug availability (Ahmed and Koob 1998) or by making drug available for weeks or months (Deroche-Gamonet et al 2004; Heyne and Wolffgramm 1998). Relative to rats given limited access, rats given extended access to self-administered cocaine develop a number of symptoms characteristic of addiction, including an escalation in drug consumption (Ahmed and Koob 1998; Bozarth and Wise 1985; Deneau et al 1969), increased motivation for cocaine (Paterson and Markou 2003), and continued pursuit of cocaine in the face of adverse consequences (Vanderschuren and Everitt 2004). These studies suggest that extended access to drugs is especially effective in producing some form of neurobehavioral plasticity that promotes the transition to addiction.

A specific form of drug-induced neurobehavioral plasticity hypothesized to be important for the transition to addiction is represented by the phenomenon of behavioral and neural sensitization (Robinson and Berridge 1993, 2003). Repeated intermittent treatment with psychostimulant drugs, as well as other drugs of abuse, produces an enduring hypersensitivity (sensitization) to their psychomotor activating (Post and Rose 1976; Robinson and Becker 1986; Segal 1975) and incentive motivational effects (Robinson and Berridge 2003; Vezina 2004). Indeed, the development of psychomotor sensitization has been directly related to increases in drug-motivated behavior (De Vries et al 1998, 2002). Furthermore, behavioral sensitization has been associated with neuroadaptations in brain regions that mediate incentive motivational processes, such as the nucleus accumbens (Acb), as well as brain regions involved in decision making and inhibitory control over behavior, such as the prefrontal cortex (PFC; Vanderschuren and Kalivas 2000). These neuroadaptations include structural alterations in dendritic morphology indicative of synaptic reorganization in these regions (Li et al 2004; Robinson and Kolb 2004).

If neuroadaptations that underlie sensitization are important for the transition to addiction, then the ability of drugs to induce this form of neurobehavioral plasticity should be sensitive to patterns of cocaine use that lead to escalation of intake and other symptoms of addiction. We hypothesized, therefore, that extended access to cocaine would lead to especially robust behavioral sensitization and that this would be associated with structural plasticity in the Acb, PFC, or both. We report, relative to rats given limited access to cocaine (1 hour/day), rats given extended access to cocaine (6 hours/day) showed 1) a marked escalation in drug consumption, 2) enhanced drug-seeking behavior, 3) especially robust behavioral sensitization, and 4) a greater increase in the density of dendritic spines on medium spiny neurons, specifically in the core of the Acb.

Methods and Materials

Subjects

Male Wistar rats (Harlan, Indianapolis, Indiana) weighing 250–275 g were individually housed (14:10–hour reversed light–dark cycle), and initially food and water were continually

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available. All testing was conducted during the dark phase of the light–dark cycle.

Surgical Procedures

After acclimation to the animal colony, rats were anesthetized using a mixture of ketamine and xylazine (100 + 10 mg/kg administered intraperitoneally [IP]) and were outfitted with a catheter in the right jugular vein using procedures described previously (Weeks 1972). Catheters were flushed with .1 mL of gentamicin (50 mg/kg, in .9% sterile bacteriostatic saline) before each self-administration session. Control animals underwent sham surgery in which the jugular vein was exposed and an incision was made on the back.

Cocaine Self-Administration

Five days after surgery, all rats were food restricted to maintain them at ~85% of free-feeding body weight, and the rats with catheters began cocaine self-administration training. Self-administration training was conducted in standard operant chambers (25 × 27 × 30 cm) located within sound-attenuating cabinets (Med Associates, Georgia, Vermont). Each chamber contained two nose-poke ports, a red house light, and a tone generator (2900 Hz). Infusion pumps were located outside of the operant chamber but within the sound-attenuating cabinets. At the start of each session, the red house light was illuminated and remained on for the entire session. Responding in one nose-poke port (inactive) had no consequences but was recorded. Responses in the other nose-poke port (active) resulted in the delivery of .4 mg/kg of cocaine HCl (weight of the salt) dissolved in 50 μl of saline administered over 1.6 sec. Drug delivery was accompanied by illumination of a light at the back of the active nose-poke port and presentation of the tone for 20 sec. Cocaine was available on a fixed-ratio 1 (FR1) schedule of reinforcement with a timeout of 20 sec. Rats were trained initially during daily 1-hour sessions for a total of five or six sessions.

After initial training sessions, the rats with catheters were divided into two groups that were matched according to number of infusions taken per session during the training period: a long access group (LgA, $n = 27$) and a short access group (ShA, $n = 20$). Animals in the ShA group continued to receive daily 1-hour test sessions. In contrast, animals in the LgA group were shifted to daily 6-hour test sessions. For both groups, self-administration testing continued for 6 days per week for an additional 16 or 21 test sessions. After the last self-administration session, catheter patency was tested by injecting the short acting barbiturate Pentothal (thiopental sodium, 20 mg/mL in sterile water), and rats that did not become ataxic within 5 sec were excluded from the experiment.

Control rats without catheters were placed in holding chambers similar to the self-administration chambers for 1 hour per day for a total of 5 or 6 days (control, $n = 32$). After this, approximately half of the rats stayed in the chambers for 6 hours each day, and the other half stayed in the chambers for 1 hour each day for a total of 16 or 21 days. Thus, this group experienced the general effects of handling, transport, and time outside the animal colony similar to animals with self-administration experience.

After the last self-administration session, all rats were returned to food ad libitum and were left undisturbed for 1 month. Following this period of abstinence, subsets of rats were selected at random for 1) assessment of their psychomotor response to a cocaine challenge or drug-seeking under extinction conditions or 2) anatomic studies.

Cocaine Challenge Test

One month after their last exposure to cocaine (or control treatment), a subset of rats (ShA, $n = 10$; LgA, $n = 16$; control [Acute], $n = 16$) were given a cocaine challenge test. Rats were placed in an activity monitor (Digiscan, Omnitech Electronics, Columbus, Ohio) equipped with 32 photocell beams arrayed around the perimeter of the plexiglass chamber (42 × 42 × 30 cm). After a 1-hour habituation period, animals in the ShA, LgA, and Acute groups were given three consecutive injections of cocaine (7.5, 15, and 30 mg/kg, IP). This procedure generates within-subject dose–effect information (Li et al 2004; Uslaner et al 2003), and we have found it is more effective at revealing sensitization than using a single challenge dose. The 15 mg/kg dose was given 1 hour after the 7.5 mg/kg dose, and 30 mg/kg was given 1.5 hours after the 15 mg/kg dose. A fourth group of rats (control [Saline], $n = 11$) was given consecutive injections of an equivalent volume of saline. Both the Acute and Saline groups consisted of control rats that had never before been exposed to cocaine.

Locomotor activity was recorded as total number of beam breaks per 5-min interval. A video camera was mounted directly above the activity monitor and a video record of the entire test session was obtained using an Opticom Vista 480 Digital Video Recorder (Opticom Technologies, Vancouver, Canada). It was apparent from initial inspection of the video records that the automated measure of locomotor activity (beam breaks) did not capture obvious group differences in cocaine-induced stereotyped behavior. Therefore, a detailed analysis of the video record was conducted to examine repetitive stereotyped head movements produced by the cocaine challenge. This was done by first taking one 30-sec sample of behavior every 10 min for the first 40 min following administration of each dose of cocaine (this gave a total of four 30-sec samples/dose/rat). Behavior was examined during this specific time period because analysis of locomotor activity indicated that the onset and peak drug effect was captured within this time period. For each 30-sec sample of behavior, those periods that each rat spent "in place" were identified. Periods "in place" were defined as those in which both back paws remained in the same position on the floor for at least 2 sec (any time spent grooming was also excluded). The number of lateral head movements made during each period an animal was in place was counted, and the frequency of in place head movements was calculated by dividing the number of head movements by the time spent in place. This provided an index of the vigor of drug-induced stereotyped head movements (movements/sec) when animals were not engaged in other behaviors that might compete with stereotyped head movements, such as locomotion. The observer (CRF) was blind to treatment condition, and only video records in which the position of the animal and camera allowed discrete head movements to be detected and counted reliably were included in the analysis (ShA, $n = 10$; LgA, $n = 16$; Control [Acute], $n = 11$; Control [Saline], $n = 11$).

Drug-Seeking Test

After the cocaine challenge, a subset of rats was left undisturbed for 17 more days before drug-seeking was assessed (ShA, $n = 5$; LgA, $n = 8$). These rats were placed back into the self-administration chambers for 1 hour under conditions identical to previous self-administration sessions, with the exception that no cocaine was available. Nose-pokes into the previously active hole resulted in presentation of the light-tone conditioned stimulus, whereas nose-pokes into the inactive hole had no consequence.

Anatomic Procedures

One month after the last self-administration session, a separate subset of LgA, ShA, and Control rats were given an overdose of sodium pentobarbital and were then perfused intracardially with .9% saline. The brains were removed and processed for Golgi-Cox staining as previously described (Robinson and Kolb 1999). Based on our previous work (Robinson and Kolb 2004), cells from four brain regions were selected for analysis: medium spiny neurons in the shell (AcbS) and core (AcbC) of the Acb (cells were sampled throughout the rostral-caudal extent of the Acb), pyramidal cells in layer III of the orbital prefrontal cortex (oPFC, dorsal agranular insular-AID), and pyramidal cells in layer V of the medial prefrontal cortex (mPFC, Cg3), as defined by Zilles (1985). For cortical pyramidal cells, one third-order terminal tip from the apical dendritic tree and one fourth-order terminal tip from the basilar dendritic tree of each cell were identified and spines along these segments were counted. For medium spiny neurons, spines on one third-order or greater terminal tip per cell were counted. Slides were coded so that the person responsible for cell selection and analysis (G.G.) was blind to experimental condition.

Spine density was calculated using camera lucida procedures to trace at least a 10-μm length of dendrite, counting all visible spines along that length (final magnification of 2000×). The exact length of the analyzed branch was then determined, and spine density was expressed as the total number of spines per 10 μm. This measure was obtained for approximately five cells per hemisphere, which were then averaged, and hemisphere was used as the unit for statistical analysis (ShA, $n = 10$; LgA, $n = 10$; Control, $n = 10$), as described previously (Robinson and Kolb 1999).

Statistics

Self-administration data across sessions were analyzed with a mixed-model analysis of variance (ANOVA) using the MIXED procedure in the SPSS software program. This analysis has several advantages compared with other repeated-measures analyses; it is particularly well suited to examine changes over time and is unaffected by missing data (Gueorguieva and Krystal 2004). For this analysis, a Satterthwaite approximation for the denominator degrees of freedom was used, producing decimal places in these values. Group differences in the number of infusions received during the first self-administration test session versus the last self-administration test session were assessed using repeated-measures ANOVA. Group differences in cocaine seeking were assessed with a two-way ANOVA. Group differences in the locomotor activity time course were assessed using a mixed-model ANOVA. Group differences in dose-response function for locomotor activity, head movement frequency, and percent time in place were assessed using repeated-measures ANOVAs. Group differences in the density of dendritic spines were analyzed using one-way ANOVAs followed by pairwise Scheffé tests, when appropriate. The results of all statistical analyses are presented in the figure captions, and all statements in the results section regarding group differences are also documented in the captions.

Results

Self-Administration and Drug Seeking

During the initial 5- to 6-day training period, both groups (LgA and ShA) had 1 hour sessions, and they were selected to match drug intake so that they do not differ during this period (Figure 1).

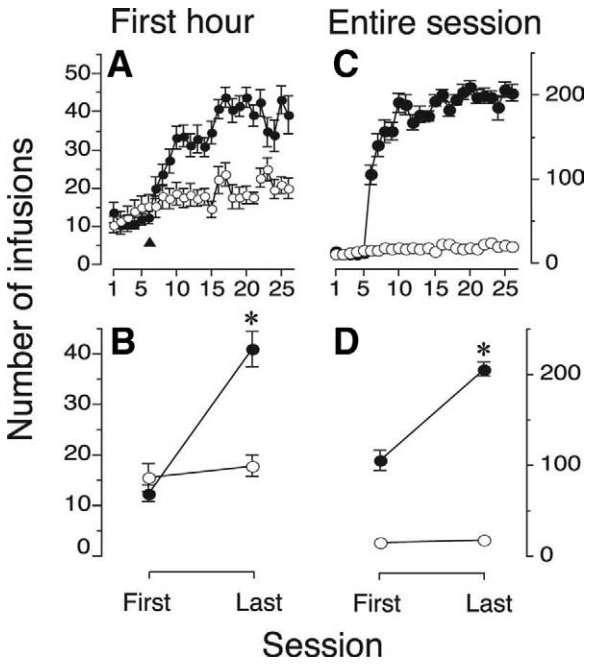

Figure 1. Mean (± SEM) number of cocaine infusions taken during the last 5 daily training sessions, which were 1 hour long for both groups, and then on each of the 16–21 test sessions (beginning where indicated by arrowhead), which were 1 hour long for the short access group (ShA, open circles) and 6 hours for the long access group (LgA, closed circles). The number of infusions taken during the first hour of each self-administration session are shown in **(A)** and **(B)**, and during the entire test session in **(C)** and **(D)**. There is a break in the graph before the last 5 test sessions because some rats were tested for 16 sessions and others for 21 sessions, so the *n* changes at that point. Because of this change in *n*, a mixed-model analysis of variance was conducted using the MIXED procedure in the SPSS software program (see Results for details). This revealed a significant group by session interaction for number of infusions taken in the first hour (group × session: $F_{(1,220)} = 34.6$, $p < .001$) as well as for total number of infusions (group × session: $F_{(1,220)} = 204$, $p < .001$). A summary of the change in the number of infusions between the first and last test sessions is shown in **(B)** (group × session: $F_{(1,45)} = 26.2$, $p < .001$) and **(D)** (group × session: $F_{(1,45)} = 38.5$, $p < .001$). There was no change between the number of infusions taken during the first and last test session for the ShA group (mean difference = 2.30, $p > .05$). In contrast, for the LgA group, there was a significant increase between the number of infusions taken during the first and last test session, both during the first hour of access (mean difference = 28.6, $p < .001$) and during the entire test session (mean difference = 100, $p < .001$), indicated by asterisks.

Beginning with the first test session (session 6 or 7) animals in the LgA group were given access to cocaine for 6 hours/session, whereas animals in the ShA group continued to have access to cocaine for 1 hour/session. Animals in the ShA group maintained a stable level of cocaine intake over the remaining 16-21 test sessions (Figure 1A, 1C). In contrast, animals in the LgA group showed a large and progressive increase in total cocaine intake (Figure 1C), which was evident even during the first hour of each test session (Figure 1A). The magnitude of the increase in cocaine intake between the first session of extended access to cocaine and the last self-administration test session is summarized in Figure 1B (first hour of each session) and Figure 1D (entire session). Throughout self-administration testing, responses in the inactive port were very low, and no group differences were observed (data not shown).

Forty-seven days following the last self-administration test session, a subset of animals was returned to the operant chambers, but with no cocaine available, and nose-pokes into the

previously active port resulted in presentation of the light-tone stimulus previously paired with cocaine delivery. Figure 2 shows that both the ShA and LgA groups made more responses in the active than in the inactive port, but the LgA group made significantly more responses into the active port than did the ShA group. Thus, even after 47 days of forced abstinence, the LgA group showed more vigorous drug-seeking behavior than the ShA group.

Psychomotor Activation Produced by a Cocaine Challenge: Locomotion

Figures 3 and 4 show the locomotor response (beam breaks) on the challenge test day 1 month after the last self-administration test session. Placement into the activity monitor for a 1-hour habituation period produced a large increase in locomotor activity. Locomotor activity decreased significantly over the next 40 min, and there was no effect of treatment condition on the rate of habituation of locomotor activity (Figure 3a). The animals were then given IP injections of increasing doses of cocaine, or an equivalent volume of saline, with each injection separated by 1-1.5 hours. It is important to note that rats in the Saline control group never received any cocaine but are illustrated along with animals receiving cocaine injections for ease of comparison. Figure 3B-D shows the time course of the locomotor response during the 40-min period following each injection. In all groups, all doses of cocaine produced a significant increase in locomotor activity relative to an injection of saline. There was, however, no effect of previous self-administration experience on the locomotor response produced by 7.5 or 15 mg/kg of cocaine compared with Acute control animals (Figure 3B, 3C). Following 30 mg/kg of cocaine, there were significant group differences in the pattern of locomotor activity over time. Both the ShA and LgA groups showed a marked decline in locomotor activity 15-20 min following the 30 mg/kg injection, followed by an increase in locomotor activity, whereas the Acute group showed relatively high levels of locomotor activity throughout the 40-min period (Figure 3D). The group given cocaine for the first time (Acute) differed statistically from the two groups that had prior cocaine self-administration experience (ShA, LgA), but these latter two groups did not differ from one another at the highest dose tested.

Although there were no significant effects of prior cocaine self-administration experience when the effects of the 7.5 or 15 mg/kg challenge doses were considered alone (Figure 3), examination of the full dose-effect functions revealed a clear effect of past cocaine experience. Figure 4 shows the dose-effect functions for cocaine-induced locomotor activity during the first 20 min (peak effect) following each injection of the three doses of cocaine. It is clear that the shape of the dose-effect function for animals given cocaine for the first time (a linear increase in locomotion with increasing dose in the Acute group) is different from that for the LgA and ShA groups (inverted-U shaped functions). In animals with prior cocaine self-administration experience, the dose-effect functions and the time course of locomotor activity following 30 mg/kg of cocaine (Figure 3D) suggest that these groups may have showed enhanced stereotyped behavior indicative of an increased drug effect (Lyon and Robbins 1975; Segal 1975). A detailed analysis of the video records indicates that this was indeed the case.

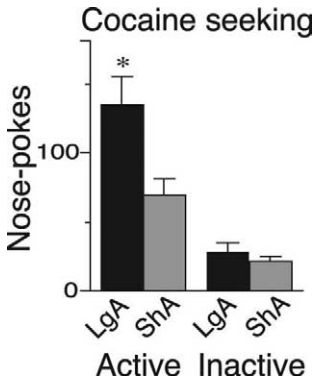

Figure 2. Mean (± SEM) number of active and inactive nose-pokes made by the long-access (LgA) and short-access (ShA) groups during the test for cocaine seeking behavior (under extinction conditions). A two-way analysis of variance resulted in a significant effect of port ($F_{(1,11)} = 45.9$, $p < .001$), indicating that both groups made more active than inactive nose-pokes. However, there was also a significant group × port interaction ($F_{(1,11)} = 6.50$, $p < .03$), indicating the LgA group made significantly more nose-pokes in the active port than the ShA group.

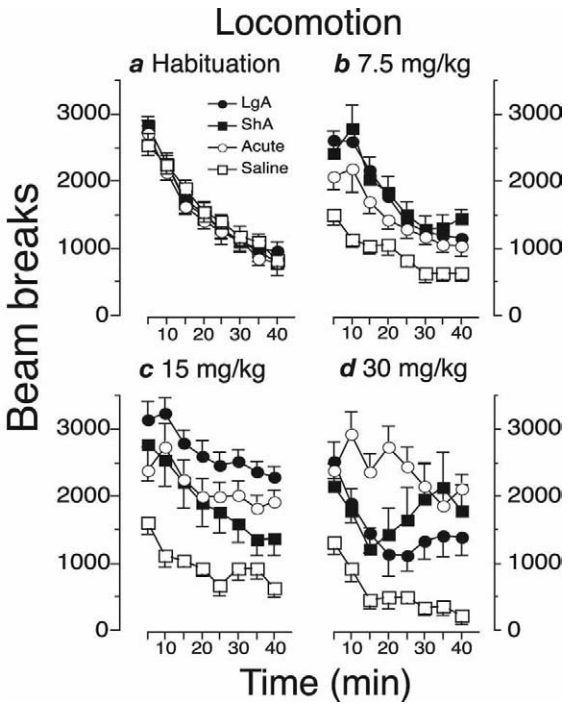

Figure 3. Mean (± SEM) locomotor activity (number of beam breaks) during the initial 40-min period following placement into the activity monitor (habituation, panel A) and following each of three challenge injections with saline or cocaine (7.5, 15, and 30 mg/kg or saline, intraperitoneal injection; panels B–D). It is important to note that rats in the Saline group never received any cocaine but are graphed in Figures 3–5 for ease of comparison. The Acute group received cocaine for the first time, where as the short-access (ShA) and long-access (LgA) groups previously self-administered cocaine for 1 or 6 hours/session, respectively. There were no group differences during the habituation period. In all groups, all doses of cocaine produced a significant increase in locomotor activity, relative to animals given saline (main effect of group, $Fs_{1,21.5–37.9)} = 13.4 – 85.2$, $ps \leq .001$). The saline group was not included in any subsequent statistical analyses of locomotor activity. There was no effect of prior self-administration experience on the locomotor response to a challenge with either 7.5 or 15 mg/kg cocaine (i.e., the LgA and ShA groups did not differ from the Acute group), although following 15 mg/kg the LgA and ShA groups did differ from one another (main effect of group; $F_{(1,26.2)} = 5.99$, $p < .05$). Following the 30 mg/kg challenge, the time course of the locomotor response of the LgA and ShA groups was significantly different from the Acute group, as indicated by significant group × time interactions (Acute vs. ShA; $F_{(7,165)} = 2.39$, $p < .05$, Acute vs. LgA; $F_{(7,200)} = 2.91$, $p < .02$), although the LgA and ShA groups did not differ from one another.

Stereotyped Head Movements

Figure 5A shows the percent time in place (both back paws on the floor) as a function of dose and treatment condition. Animals given saline were in place for approximately 75% of the sample period. Animals given cocaine for the first time showed a dose-related decrease in time in place, consistent with their dose-related increase in locomotor activity (Figure 4). In contrast, for animals with prior cocaine self-administration experience, the low dose of cocaine decreased time in place, followed by a dose-related increase in time in place.

Figure 5B shows the frequency of head movements during periods in place, as an index of the intensity of drug-induced stereotyped behavior. It is clear from Figure 5B that there was a large effect of treatment condition on the psychomotor response to the cocaine challenge, using this index of cocaine-induced stereotyped behavior. All groups given cocaine showed a dose-related increase in the frequency of head movements (Figure 5B); however, the dose-effect function was shifted significantly to the left in both groups that had prior cocaine self-administration experience. Most importantly, the magnitude of the shift in the dose-effect function was much greater in animals that previously had extended access to cocaine (LgA group) than in animals that previously had limited access to cocaine (ShA group). That is, animals that previously had extended access to cocaine (6 hours/session) were markedly hypersensitive (sensitized) to cocaine, as indicated by its ability to induce stereotyped head movements, relative to animals given limited access to the drug (1 hour/session).

Anatomy

Figure 6 shows the effects of prior cocaine self-administration experience on the density of dendritic spines on pyramidal cells in the mPFC (Figure 6A) and oPFC (Figure 6B). There was no effect of cocaine self-administration experience on spine density in the oPFC (Figure 6B). Prior cocaine self-administration experience significantly increased spine density on both the apical and basilar dendrites of cells in the mPFC, but there was no effect of duration of access to cocaine (Figure 6A).

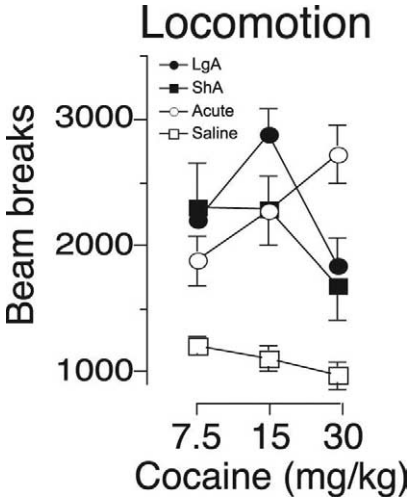

Figure 4. Mean (± SEM) number of beam breaks during the first 20 min after each cocaine injection (peak effect), as a function of dose. There were significant group differences in the shape of the dose–effect functions (group × dose interaction; $F_{(4,80.7)} = 4.03$, $p < .01$). Animals given cocaine for the first time (Acute) showed a linear increase in locomotion as dose increased, where as the long-access (LgA) and short access (ShA) groups had inverted U-shaped dose–effect functions (group × dose interactions, Acute vs. ShA, $F_{(2,51.8)} = 3.62$, $p < .05$; Acute vs. LgA, $F_{(2,61)} = 6.77$, $p < .01$).

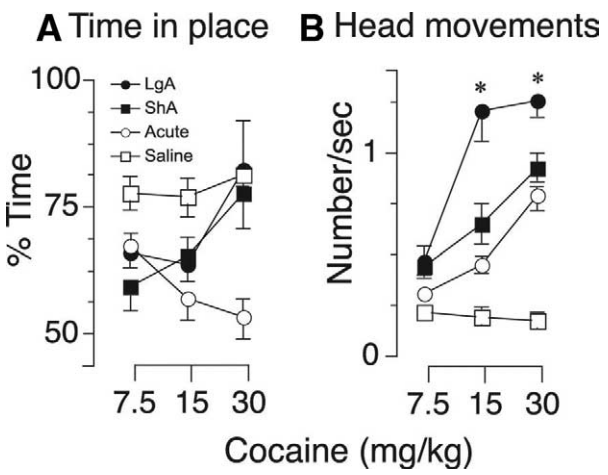

Figure 5. Mean (± SEM) percent time "in place" (both rear feet on the floor > 2 sec) and frequency of in place head movements, as a function of cocaine challenge dose. **(A)** Time in place: in the group given cocaine for the first time (Acute), cocaine produced a dose-related decrease in time spent in place (Saline vs. Acute, group × dose interaction, $F_{(2,40)} = 4.88$, $p < .02$). In contrast, in animals with prior cocaine self-administration experience, the lowest dose of cocaine decreased the amount of time spent in place, followed by a dose-related increase in time in place. The dose–effect functions for the two groups with previous self-administration experience were significantly different than for the Acute group (group × dose interactions, Acute vs. short-access group (ShA), $F_{(2,38)} = 7.52$, $p < .01$; Acute vs. long-access (LgA), $F_{(2,50)} = 11.55$, $p < .001$), but the LgA and ShA groups did not differ from one another. **(B)** Frequency of in place head movements: cocaine produced a dose-related increase in the frequency of head movements in all groups, relative to animals given repeated injections of saline (group × dose interactions, $Fs_{(2,38-50)} = 10.8 - 20.9$, $ps < .001$). Relative to animals given cocaine for the first time (Acute), the dose–effect functions for both groups with prior cocaine self-administration experience were shifted significantly to the left (Acute vs. ShA, main effect of group, $F_{(1,19)} = 9.22$, $p < .01$, group × dose interaction, $F_{(2,38)} = .14$, $p > .05$; Acute vs. LgA, main effect of group, $F_{(1,25)} = 19.9$, $p < .01$, group × dose interaction, $F_{(2,50)} = 7.09$, $p < .01$). Furthermore, the dose–effect function for the LgA group was shifted significantly to the left of that for the ShA group, indicated by asterisks (main effect of group, $F_{(1,21)} = 7.73$, $p < .02$, group × dose interaction, $F_{(2,48)} = 4.62$, $p < .02$).

Prior cocaine self-administration experience increased spine density on medium spiny neurons in both the AcbS and AcbC (Figures 7 and 8). In the AcbS, there was no effect of duration of access to cocaine (i.e., there was no difference between the LgA and ShA groups). In the AcbC, however, extended access to cocaine (LgA) produced a much greater increase in spine density than did limited access to cocaine (ShA). Indeed, the effect of extended access was approximately double the effect of limited access.

Discussion

Animals given 1 hour of access to cocaine showed a stable level of cocaine intake, whereas animals given 6 hours of access progressively escalated their intake of cocaine, as expected (Ahmed and Koob 1998). We were able to ask, therefore, whether the transition from stable, controlled cocaine use to an escalated pattern of use typical of addiction, was accompanied by the development of psychomotor sensitization and structural plasticity. We found that, indeed, when given a cocaine challenge 1 month after the last self-administration test session, the group that had extended access to cocaine showed especially

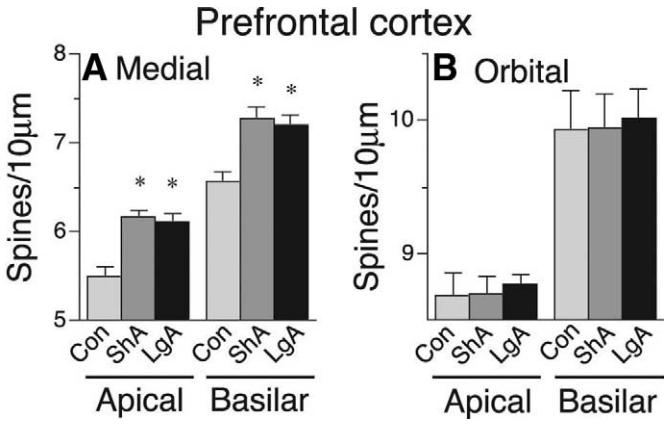

Figure 6. Mean (± SEM) number of dendritic spines per 10 μm of dendrite on the apical and basilar dendrites of pyramidal cells in **(A)** the medial prefrontal cortex (mPFC) and **(B)** the orbital prefrontal cortex (oPFC). Cocaine self-administration experience significantly increased the density of dendritic spines on both apical and basilar dendrites of cells in the mPFC (indicated by asterisks), but duration of access to cocaine had no significant effect (apical, $F_{(2,27)} = 12.1$, $p < .001$; basilar, $F_{(2,27)} = 15.2$, $p < .001$; pairwise Scheffé tests, $p < .05$). There were no significant effects of cocaine self-administration experience on spine density in the oPFC. Con, control group; LgA, long-access group; ShA, short-access group.

robust psychomotor sensitization. These animals also showed greater drug-seeking behavior when returned to the self-administration context under extinction conditions. Prior experience with cocaine increased the density of dendritic spines on pyramidal cells in the mPFC (but not oPFC) and on medium spiny neurons in both the core (AcbC) and shell (AcbS) of the Acb, consistent with previous reports (Li et al 2004; Robinson et al 2001; Robinson and Kolb 1999, 2004). Animals given extended

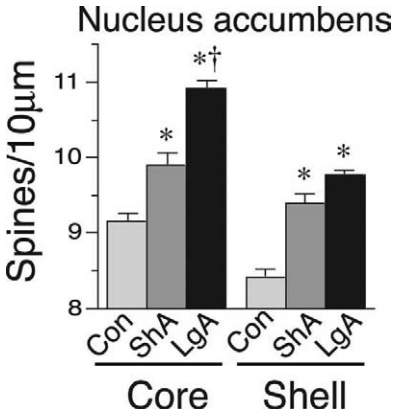

Figure 7. Mean (± SEM) number of dendritic spines per 10 μm of dendrite on medium spiny neurons in the core and shell of the nucleus accumbens. In the shell (AcbS), cocaine self-administration experience significantly increased the density of dendritic spines in both the short-access (ShA) and long-access (LgA) groups (indicated by asterisks), but the ShA and LgA groups did not differ significantly from one another ($F_{(2,27)} = 43.4$, $p < .001$, pairwise Scheffé tests, $p < .05$). In the core (AcbC), cocaine self-administration experience also significantly increased the density of dendritic spines in both the ShA and LgA groups (indicated by asterisks), but the magnitude of this increase was significantly greater in the LgA group than in the ShA group (indicated by a dagger, $F_{(2,27)} = 48.2$, $p < .001$, pairwise Scheffé tests, $p < .05$). Con, control group.

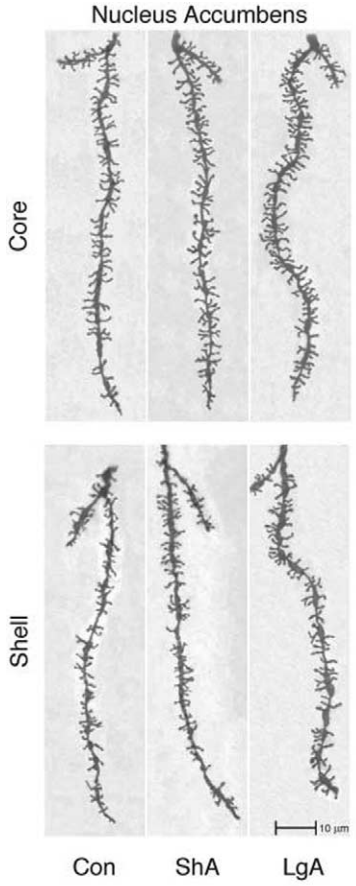

Figure 8. Digital images of Golgi-stained medium spiny neuron terminal tips in the nucleus accumbens core and shell. Images were captured at 1600× magnification by a Polaroid DMC video camera attached to a Zeiss Axioskop. Once a dendritic segment was identified, multiple images were taken at different focal planes and then merged to create the composite images shown here. In addition, the full dendritic segment was drawn by hand for use as a reference. No digital enhancement was used to capture these images, but brightness and contrast levels were equalized across individual video captures to produce the final images. Con, control group; LgA, long-access group; ShA, short-access group.

access to cocaine showed a significantly greater increase in spine density in the AcbC (but not in the AcbS or mPFC), however, than animals given limited access to cocaine. Thus, the transition from stable (controlled) cocaine use to an escalated pattern of use characteristic of addiction is associated with both the development of especially robust psychomotor sensitization and morphologic changes in the AcbC.

Behavioral Sensitization

These findings are consistent with reports that limited access to self-administered cocaine (1- to 3-hour test sessions) induces both neurochemical and psychomotor sensitization (Hooks et al 1994; Phillips and Di Ciano 1996; Zapata et al 2003). They are inconsistent, however, with one report that extended access to cocaine (6 hours/session) results in a loss of sensitization (Ben-Shahar et al 2004). The reason for the apparent discrepancy may be that the behavioral measure used by Ben-Shahar et al (2004) was not sensitive to the qualitative changes in behavior associated with robust behavioral sensitization.

A major theme of much of the early literature on behavioral sensitization is that sensitization does not involve simply quantitative changes in a given behavior (e.g., more and more locomotion), but complex qualitative changes in patterns of behavior that vary as a function of many factors, including dose, number of treatments, time since the last treatment, context, and so on (Post and Rose 1976; Robinson and Becker 1986; Segal 1975). As a result, over-reliance on any one behavioral measure, especially when faced with negative findings, can lead to erroneous conclusions. As Segal (1975) warned many years ago, "characterization of the various components of the behavioral response is required because drug effects on behavior may be competitively related" (p. 248). This may be why Ben-Shahar et al (2004), who on their challenge test for sensitization relied on one behavioral measure and one dose of cocaine, failed to find evidence of sensitization. Indeed, had we relied only on the locomotor response to either the 7.5 or 15 mg/kg challenge dose (Figure 3), we, too, would have reported no effect of past cocaine self-administration experience. Examination of the shape of dose-effect functions, however, and of the time course of the locomotor response, revealed a pattern of behavior typical of animals that had undergone a transition from psychostimulant-induced behavior dominated by locomotor hyperactivity to behavior dominated by progressively more stereotyped activity.

The presence of behavior dominated by stereotyped actions has long been thought to reflect increased drug effects characterized by "increasing response rates within a decreasing number of response categories" (Lyon and Robbins 1975, p. 84). This is evident here in the dose-related increase in the frequency of head movements and "in place" activity of animals that had prior self-administration experience and typifies behavioral sensitization (Post and Rose 1976; Robinson and Becker 1986; Segal 1975). We conclude, therefore, that one long-term consequence of cocaine self-administration experience is to render hypersensitive the brain systems that mediate the psychomotor activating effects of cocaine (Hooks et al 1994; Phillips and Di Ciano 1996; Zapata et al 2003), and that extended access to cocaine, with escalation of intake, produces greater sensitization than that produced by more limited access to the drug.

Mesocorticolimbic circuits that mediate the psychomotor activating effects of psychostimulant drugs also mediate their incentive motivational properties (Wise and Bozarth 1987). Therefore, treatments capable of producing psychomotor sensitization might also be expected to produce incentive sensitization. Indeed, the development of psychomotor sensitization has been directly related to increases in drug-motivated behavior (De Vries et al 1998, 2002); treatments that produce psychomotor sensitization also facilitate the later acquisition of drug self-administration behavior (Horger et al 1990; Piazza et al 1989) and conditioned place preferences (Lett 1989), the motivation for drug assessed by "breakpoint" on a progressive ratio schedule (Mendrek et al 1998; Vezina 2004), and Pavlovian conditioned motivational processes (Harmer and Phillips 1998; Wyvell and Berridge 2001). In most of these studies, drug was administered by an experimenter, so it is especially important to note that prior cocaine self-administration experience also produces incentive sensitization (Deroche et al 1999; Morgan and Roberts 2004). Furthermore, consistent with the results reported here, Paterson and Markou (2003) found that extended access to cocaine (6 hours/session) produces an especially large "increase in the incentive motivational value of self-administered cocaine" (p. 2229), relative to limited access conditions. We conclude, therefore, that extended access to cocaine produces both especially robust psychomotor sensitization and incentive sensitization.

Structural Plasticity

Repeated intermittent administration of psychostimulant drugs is accompanied by many neuroadaptations in mesocorticolimbic circuits, including changes in multiple neurotransmitter systems and intracellular signaling cascades (Vanderschuren and Kalivas 2000). Of particular relevance here are reports that psychostimulant drugs also induce long-lasting structural modifications in these same circuits. Repeated treatment with amphetamine or cocaine produces a long-lasting increase in the density of dendritic spines on pyramidal cells in the mPFC and medium spiny neurons in the Acb (Li et al 2004; Robinson et al 2001; Robinson and Kolb 1999, 2004) and caudate-putamen (Li et al 2003). Consistent with these studies, we found that prior cocaine self-administration experience, whether for 1 or 6 hours/session, increased spine density in the AcbC, AcbS, and mPFC. Furthermore, these effects are not seen in animals trained to nose poke for food rewards (Crombag et al 2005; Robinson et al 2001). The interesting new finding is that the robust behavioral sensitization associated with an escalation of cocaine intake was accompanied by a further increase in spine density in the AcbC, but not in the AcbS, oPFC, or mPFC. This suggests that structural plasticity in the AcbC may be especially important in the development of cocaine-induced sensitization (as reported by Li et al 2004) and in the escalation of drug intake.

Cocaine self-administration experience had no effect on spine density in the oPFC. This brain region has been implicated in some of the cognitive deficits seen in addicts (Bolla et al 1998; Rogers et al 1999), and we previously reported that amphetamine (Crombag et al 2005) and morphine (Robinson et al 2002) self-administration experience has opposite effects on spine density in the mPFC and oPFC. The reason for the differences between the effects of cocaine and amphetamine self-administration experience on dendritic organization in the oPFC is unknown.

Implications for Addiction

The reason rats given extended access to cocaine escalate their drug intake is not known. It could be due to alterations in prefrontal cortical function that lead to a loss of inhibitory control over behavior or increased "impulsivity" (Jentsch and Taylor 1999; Rogers et al 1999). We found no effects of extended access to cocaine on spine density in the mPFC or oPFC, however. This is consistent with a recent report that extended access conditions that produce symptoms of addiction do not produce symptoms of "impulsivity and disinhibition" (Deroche-Gamonet et al 2004; also see Schoenbaum and Setlow 2004). Instead, escalation of intake was associated with increased spine density in the AcbC (but not AcbS). The AcbC is uniquely important in mediating the motivational impact of Pavlovian conditioned stimuli on behavior (Cardinal et al 2002), and Pavlovian conditioned motivational processes undergo sensitization as a consequence of repeated exposure to psychostimulant drugs (Harmer and Phillips 1998; Robinson and Berridge 2003; Wyvell and Berridge 2001). Furthermore, sensitization has been associated with neuroadaptations specifically in the AcbC, and not the AcbS (Cadoni et al 2000; Di Chiara 2002; Li et al 2004; Pierce et al 1996), although there are exceptions (e.g., Pierce and Kalivas 1995). Thus, rats may exhibit some symptoms of addiction, such as escalation of drug intake, due to a sensitization-related synaptic reorganization of the AcbC that leads to a pathologic increase in the

incentive value of drugs and drug-associated stimuli—that is, an increase in drug "wanting" (Robinson and Berridge 2003). This could also explain why extended access to cocaine leads to increased motivation for drug, as assessed by increased "breakpoint" (Paterson and Markou 2003) and continued drug-seeking in the face of adverse consequences (Vanderschuren and Everitt 2004).

In conclusion, extended access to cocaine leads to at least three well-established symptoms of addiction: escalation of drug intake, continued drug seeking in the face of adverse consequences, and increased incentive motivation for drug. We show here that it also leads to especially robust behavioral sensitization and synaptic reorganization in the AcbC. We speculate, therefore, that the transition from mere drug use to addiction may involve a pathologic increase (sensitization) in incentive motivational functions mediated by the AcbC due to a drug-induced reorganization of synaptic connections in this brain region.

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Ahmed SH, Koob GF (1998): Transition from moderate to excessive drug intake: Change in hedonic set point. *Science* 282:298–300.

Ben-Shahar O, Ahmed SH, Koob GF, Ettenberg A (2004): The transition from controlled to compulsive drug use is associated with a loss of sensitization. *Brain Res* 995:46–54.

Bolla KI, Cadet JL, London ED (1998): The neuropsychiatry of chronic cocaine abuse. *J Neuropsychiatry Clin Neurosci* 10:280–289.

Bozarth MA, Wise RA (1985): Toxicity associated with long-term intravenous heroin and cocaine self-administration in the rat. *JAMA* 254:81–83.

Cadoni C, Solinas M, Di Chiara G (2000): Psychostimulant sensitization: Differential changes in accumbal shell and core dopamine. *Eur J Pharmacol* 388:69–76.

Cardinal RN, Parkinson JA, Hall J, Everitt BJ (2002): Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex. *Neurosci Biobehav Rev* 26:321–352.

Crombag HS, Gorny G, Li Y, Kolb B, Robinson TE (2005): Opposite effects of amphetamine self-administration experience on dendritic spines in the medial and orbital prefrontal cortex. *Cereb Cortex* 15:341–348.

De Vries TJ, Schoffelmeer AN, Binnekade R, Mulder AH, Vanderschuren LJ (1998): Drug-induced reinstatement of heroin- and cocaine-seeking behaviour following long-term extinction is associated with expression of behavioural sensitization. *Eur J Neurosci* 10:3565–3571.

De Vries TJ, Schoffelmeer AN, Binnekade R, Raaso H, Vanderschuren LJ (2002): Relapse to cocaine- and heroin-seeking behavior mediated by dopamine D2 receptors is time-dependent and associated with behavioral sensitization. *Neuropsychopharmacology* 26:18–26.

Deneau G, Yanagita T, Seevers MH (1969): Self-administration of psychoactive substances by the monkey. *Psychopharmacologia* 16:30–48.

Deroche V, Le Moal M, Piazza PV (1999): Cocaine self-administration increases the incentive motivational properties of the drug in rats. *Eur J Neurosci* 11:2731–2736.

Deroche-Gamonet V, Belin D, Piazza PV (2004): Evidence for addiction-like behavior in the rat. *Science* 305:1014–1017.

Di Chiara G (2002): Nucleus accumbens shell and core dopamine: Differential role in behavior and addiction. *Behav Brain Res* 137:75–114.

Gueorguieva R, Krystal JH (2004): Move over ANOVA. *Arch Gen Psychiatry* 61:310–317.

Harmer CJ, Phillips GD (1998): Enhanced appetitive conditioning following repeated pretreatment with d-amphetamine. *Behav Pharmacol* 9:299–308.

Heyne A, Wolffgramm J (1998): The development of addiction to d-amphetamine in an animal model: Same principles as for alcohol and opiate. *Psychopharmacology* 140:510–518.

Hooks MS, Duffy P, Striplin C, Kalivas PW (1994): Behavioral and neurochemical sensitization following cocaine self-administration. *Psychopharmacology (Berl)* 115:265–272.

Horger BA, Shelton K, Schenk S (1990): Preexposure sensitizes rats to the rewarding effects of cocaine. *Pharm Biochem Behav* 37:707–711.

Jentsch JD, Taylor JR (1999): Impulsivity resulting from frontostriatal dysfunction in drug abuse: Implications for the control of behavior by reward-related stimuli. *Psychopharmacology* 146:373–390.

Lett BT (1989): Repeated exposures intensify rather than diminish the rewarding effects of amphetamine, morphine, and cocaine. *Psychopharmacology (Berlin)* 98:357–362.

Li Y, Kolb B, Robinson TE (2003): The location of persistent amphetamine-induced changes in the density of dendritic spines on medium spiny neurons in the nucleus accumbens and caudate-putamen. *Neuropsychopharmacology* 238:1082–1085.

Li Y, Acerbo MJ, Robinson TE (2004): Cocaine-induced dendritic plasticity in the core (but not shell) of the nucleus accumbens is associated with the induction of psychomotor sensitization. *Eur J Neurosci* 20:1647–1654.

Lyon M, Robbins TW (1975): The action of central nervous system stimulant drugs: A general theory concerning amphetamine effects. In: Essman W, Valzelli L, editors. *Current Developments in Psychopharmacology,* Vol. 2. New York: Spectrum 80–163.

Mendrek A, Blaha CD, Phillips AG (1998): Pre-exposure of rats to amphetamine sensitizes self-administration of this drug under a progressive ratio schedule. *Psychopharmacology* 135:416–22.

Morgan D, Roberts DC (2004): Sensitization to the reinforcing effects of cocaine following binge-abstinent self-administration. *Neurosci Biobehav Rev* 27:803–812.

Paterson NE, Markou A (2003): Increased motivation for self-administered cocaine after escalated cocaine intake. *Neuroreport* 14:2229–2232.

Phillips AG, Di Ciano P (1996): Behavioral sensitization is induced by intravenous self-administration of cocaine by rats. *Psychopharmacology (Berl)* 124:279–281.

Piazza PV, Deminière JM, Le Moal M, Simon H (1989): Factors that predict individual vulnerability to amphetamine self-administration. *Science* 245:1511–1513.

Pierce RC, Bell K, Duffy P, Kalivas PW (1996): Repeated cocaine augments excitatory amino acid transmission in the nucleus accumbens only in rats having developed behavioral sensitization. *J Neurosci* 16:1550–1560.

Pierce RC, Kalivas PW (1995): Amphetamine produces sensitized increases in locomotion and extracellular dopamine preferentially in the nucleus accumbens shell of rats administered repeated cocaine. *J Pharmacol Exp Ther* 275:1019–1029.

Post RM, Rose H (1976): Increasing effects of repetitive cocaine administration in the rat. *Nature* 260:731–732.

Robinson TE (2004): Addicted rats. *Science* 305:951–953.

Robinson TE, Becker JB (1986): Enduring changes in brain and behavior produced by chronic amphetamine administration: A review and evaluation of animal models of amphetamine psychosis. *Brain Res Rev* 11:157–198.

Robinson TE, Berridge KC (1993): The neural basis of drug craving: An incentive-sensitization theory of addiction. *Brain Res Rev* 18:247–291.

Robinson TE, Berridge KC (2003): Addiction. *Annu Rev Psychol* 54:25–53.

Robinson TE, Gorny G, Mitton E, Kolb B (2001): Cocaine self-administration alters the morphology of dendrites and dendritic spines in the nucleus accumbens and neocortex. *Synapse* 39:257–66.

Robinson TE, Gorny G, Savage VR, Kolb B (2002): Widespread but regionally specific effects of experimenter- versus self-administered morphine on dendritic spines in the nucleus accumbens, hippocampus, and neocortex of adult rats. *Synapse* 46:271–279.

Robinson TE, Kolb B (1999): Alterations in the morphology of dendrites and dendritic spines in the nucleus accumbens and prefrontal cortex following repeated treatment with amphetamine or cocaine. *Eur J Neurosci* 11:1598–1604.

Robinson TE, Kolb B (2005): Structural plasticity associated with exposure to drugs of abuse. *Neuropharmacology* 47(suppl 1): 33–46.

Rogers RD, Everitt BJ, Baldacchino A, Blackshaw AJ, Swainson R, Wynne K, et al (1999): Dissociable deficits in the decision-making cognition of chronic amphetamine abusers, opiate abusers, patients with focal damage to prefrontal cortex, and tryptophan-depleted normal volunteers: Evidence for monoaminergic mechanisms. *Neuropsychopharmacology* 20:322–339.

Schoenbaum G, Setlow B (2004): Cocaine makes actions insensitive to outcomes but not extinction: Implications for altered orbitofrontal-amyg-

dalar function. *Cereb Cortex.* Advance Access published on May 2, 2005; doi: 10.1093/cercor/bhh216.
Segal DS (1975): Behavioral and neurochemical correlates of repeated d-amphetamine administration. *Adv Biochem Psychopharmacol* 13:247–262.
Uslaner JM, Crombag HS, Ferguson SM, Robinson TE (2003): Cocaine-induced psychomotor activity is associated with its ability to induce c-fos mRNA expression in the subthalamic nucleus: Effects of dose and repeated treatment. *Eur J Neurosci* 17:2180–2186.
Vanderschuren LJ, Everitt BJ (2004): Drug seeking becomes compulsive after prolonged cocaine self-administration. *Science* 305:1017–1019.
Vanderschuren LJ, Kalivas PW (2000): Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: A critical review of preclinical studies. *Psychopharmacology* 151:99–120.
Vezina P (2004): Sensitization of midbrain dopamine neuron reactivity and the self-administration of psychomotor stimulant drugs. *Neurosci Biobehav Rev* 27:827–839.
Weeks JR (1972): Long-term intravenous infusions. In: Meyers RD, editor. *Methods in Psychobiology,* Vol. 2. London: Academic Press, 155–168.
Wise R (2002): Brain reward circuitry: insights from unsensed incentives. *Neuron* 36:229–240.
Wise RA, Bozarth MA (1987): A psychomotor stimulant theory of addiction. *Psychol Rev* 94:469–492.
Wyvell CL, Berridge KC (2001): Incentive sensitization by previous amphetamine exposure: increased cue-triggered "wanting" for sucrose reward. *J Neurosci* 21:7831–7840.
Yokel RA (1987): Intravenous self-administration: Response rates, the effects of pharmacological challenges, and drug preferences. In: Bozarth MA, editor. *Methods of Assessing the Reinforcing Properties of Abused Drugs.* New York: Springer-Verlag, 1–34.
Zapata A, Chefer VI, Ator R, Shippenberg TS, Rocha BA (2003): Behavioural sensitization and enhanced dopamine response in the nucleus accumbens after intravenous cocaine self-administration in mice. *Eur J Neurosci* 17:590–596.
Zilles K (1985): *The Cortex of the Rat*. Berlin: Springer-Verlag.