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ORIGINAL INVESTIGATION

Expression of amphetamine sensitization is associated with recruitment of a reactive neuronal population in the nucleus accumbens core

R. E. Nordquist · L. J. M. J. Vanderschuren · A. J. Jonker · M. Bergsma · T. J. de Vries · C. M. A. Pennartz · P. Voorn

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Abstract
Rationale Repeated exposure to psychostimulant drugs causes a long-lasting increase in the psychomotor and reinforcing effects of these drugs and an array of neuroadaptations. One such alteration is a hypersensitivity of striatal activity such that a low dose of amphetamine in sensitized animals produces dorsal striatal activation patterns similar to acute treatment with a high dose of amphetamine.
Objectives To extend previous findings of striatal hypersensitivity with behavioral observations and with cellular activity in the nucleus accumbens and prefrontal cortex in sensitized animals.
Materials and methods Rats treated acutely with 0, 1, 2.5, or 5 mg/kg i.p. amphetamine and sensitized rats challenged with 1 mg/kg i.p. amphetamine were scored for stereotypy, rearing, and grooming, and locomotor activity recorded. c-*fos* positive nuclei were quantified in the nucleus accumbens and prefrontal cortex after expression of sensitization with 1 mg/kg i.p. amphetamine.
Results Intense stereotypy was seen in animals treated acutely with 5 mg/kg amphetamine, but not in the sensitized group treated with 1 mg/kg amphetamine. The c-*fos* response to amphetamine in the accumbens core was augmented in amphetamine-pretreated animals with a shift in the distribution of optical density, while no effect of sensitization was seen in the nucleus accumbens shell or prefrontal cortex.
Conclusions A lack of stereotypy in the sensitized group indicates a dissociation of behavioral responses to amphetamine and striatal immediate-early gene activation patterns. The increase in c-*fos* positive nuclei and shift in the distribution of optical density observed in the nucleus accumbens core suggests recruitment of a new population of neurons during expression of sensitization.

Keywords Behavioral sensitization · Immediate-early gene · Stereotypy · Locomotion · Striatum · Prefrontal cortex

R. E. Nordquist · A. J. Jonker · M. Bergsma · T. J. de Vries · P. Voorn (✉)
Department of Anatomy and Neurosciences,
Vrije Universiteit Medical Center,
Amsterdam, The Netherlands
e-mail: p.voorn@vumc.nl

L. J. M. J. Vanderschuren
Rudolf Magnus Institute of Neuroscience,
Department of Neuroscience and Pharmacology,
University Medical Center Utrecht,
Utrecht, The Netherlands

C. M. A. Pennartz
Animal Physiology and Cognitive Neuroscience,
Swammerdam Institute of Life Sciences,
University of Amsterdam,
Amsterdam, The Netherlands

R. E. Nordquist
Emotion and Cognition Program,
Department of Farm Animal Health,
Faculty of Veterinary Medicine, University Utrecht,
Marburglaan 2,
3584 CN Utrecht, The Netherlands
e-mail: r.e.nordquist@uu.nl

Introduction

Repeated exposure to psychostimulant drugs causes a long-lasting enhancement of certain behavioral responses to the drug, such as psychomotor activity and stereotypy, and behaviors related to incentive motivation, a process termed behavioral sensitization (Stewart and Badiani 1993). Be-

havioral sensitization is known to be associated with long-lasting functional changes within limbic corticostriatal systems (Pierce and Kalivas 1997; Robinson and Kolb 2004; Vanderschuren and Kalivas 2000). These systems comprise functionally and anatomically heterogeneous areas with a fine-grained specificity of anatomical projections connecting the divisions within the dorsal striatum, ventral striatum, and prefrontal cortex (Groenewegen et al. 1997; Voorn et al. 2004). This anatomical and functional heterogeneity is of potential importance to the roles of these areas in sensitization.

Within the dorsal striatum, subareas termed patches (or striosomes) show more reactivity to amphetamine than the surrounding matrix areas in sensitized animals (Canales and Graybiel 2000; Vanderschuren et al. 2002). Our previous studies demonstrated that this pattern of neuronal reactivity is also seen in acutely challenged animals with the important difference that sensitized animals show preferential activation in patches at much lower doses of amphetamine than those required to produce this type of differentiation in activation in drug-naive animals (Vanderschuren et al. 2002). An imbalance in patch–matrix activation has been suggested to underlie stereotyped behavior (Canales and Graybiel 2000). Because our drug treatment regimen caused robust locomotor sensitization, which is incompatible with profound stereotypy, we hypothesized that hyperreactivity of patch compartments is not sufficient to produce stereotypy (Vanderschuren et al. 2002). To extend our previous findings, we ran new experiments using the same doses and regimen of amphetamine administration previously used and measured locomotor activity, stereotypy, grooming, and rearing to establish whether our drug treatment regimen, which causes hyperreactivity of dorsal striatal patches, produces stereotypy.

The ventral striatum, specifically the nucleus accumbens (Acb), and the prefrontal cortex (PFC) are both involved in behavioral sensitization (Pierce and Kalivas 1997; Vanderschuren and Kalivas 2000), an involvement which has received particular attention because of the important role that both areas play in appetitive and consummatory properties of both natural and drug rewards (Everitt and Wolf 2002; Robbins and Everitt 2002; Salamone et al. 2003; Volkow and Li 2004). There are functional differences between subregions within both the Acb, i.e., core and shell, and within subregions of the PFC (Cardinal et al. 2002; Robbins and Everitt 2002). However, there is presently inconclusive evidence on the respective roles that the subregions play during the expression of behavioral sensitization to psychostimulants. Studies using neurochemistry, lesions, cellular activity markers, and study of morphological changes have suggested exclusive roles for the core (Cadoni et al. 2000; Li et al. 2004; Phillips et al. 2003) or the shell (Filip and Siwanowicz 2001; Hsieh et al. 2002; Pierce and Kalivas 1995; Todtenkopf et al. 2002a). Other studies, including previous work from our own laboratory, suggest a lack of sensitization of accumbens activity all together (Ostrander et al. 2003; Vanderschuren et al. 2002). The medial PFC, and particularly the prelimbic area, has been implicated in induction of psychostimulant sensitization (Tzschentke and Schmidt 1998, 2000), although conflicting results have been found for cocaine- vs. amphetamine-induced sensitization (Tzschentke and Schmidt 2000). The dorsomedial prefrontal cortex has been shown to be involved in the expression of sensitization (Pierce et al. 1998). However, the roles of the orbital and lateral areas in psychostimulant sensitization remain to be investigated. Thus, clarification of the specific roles of the Acb and PFC subregions is needed. To study the activation of the Acb and PFC in detail during the expression of behavioral sensitization, we examined levels of c-*fos*-like proteins (henceforth c-*fos*) in detail in the subregions of the Acb and PFC of the rat after an amphetamine challenge in behaviorally sensitized rats.

Materials and methods

Animals and drug treatments

All experiments were approved by the Animal Ethics Committee of the Vrije Universiteit and were conducted in agreement with Dutch laws (Wet op de Dierproeven 1996) and European regulations (Guideline 86/609/EEC).

A total of 48 male Wistar rats weighing 180–200 g upon arrival in the laboratory (as in, e.g., De Vries et al. 1996; Vanderschuren et al. 1999a, b, 2002) were housed in Macrolon cages in groups of two animals per cage under controlled laboratory conditions (lights on 0700 to 1900 hours). Food and water were available ad libitum. Drug treatment started after an acclimatization period of at least 1 week. Animals were briefly handled during the 2 days before all injections. In the acute amphetamine experiments, animals were injected with either saline or 1, 2.5, or 5 mg/kg D-amphetamine sulfate (O.P.G., Utrecht, The Netherlands; n=4 per dose). Sensitization regimens were according to a protocol previously established to produce locomotor sensitization in our laboratory (De Vries et al. 1996; Vanderschuren et al. 1999a, b), and doses were the same as used previously in a c-*fos* study in our laboratory (Vanderschuren et al. 2002). Animals received once daily injections for five consecutive days of 2.5 mg/kg D-amphetamine sulfate or saline in the home cage (pretreatment phase). Two weeks post treatment, half of the animals from each pretreatment group were given challenge injections of 1 mg/kg D-amphetamine sulfate while the other half was injected with saline. This gave a total of

four experimental groups, $n=8$ per group: amphetamine-pretreated, amphetamine-challenged (AA); amphetamine-pretreated, saline-challenged (AS); saline-pretreated, amphetamine-challenged (SA); and saline-pretreated, saline-challenged (SS).

Locomotor activity quantification and behavioral scoring

All injections for the acute experiments and challenge injections for the sensitization experiments took place in our locomotor activity setup. On the challenge injection day, animals were first placed in the Perspex cages (length×width×height=40×40×35 cm) in which locomotor activity was measured and allowed to acclimate for 2 h. After that period, challenge injections were administered and horizontal activity was measured in 10-min blocks for 90 min using a video tracking system (EthoVision, Noldus Information Technology B.V., Wageningen, The Netherlands), which determined the position of the animal five times per second. Behavior of the animals was also videotaped and scored afterwards for three mutually exclusive categories: grooming (rubbing two paws over head and/or body), rearing (both front paws off of the ground but not grooming), or stereotypical behavior (repeated movements without horizontal movement, e.g., head shaking). Behavior was scored for 5 min of every 15 min, giving a total of seven measurements per animal, by an observer unaware of the treatment of the animals using a time-sampling program written in PC Basic. Time spent performing each behavior was expressed as the percentage of total time for each 5-min block. Replication scoring several months after initial scoring produced results identical to initial observations, demonstrating the reliability of our scoring procedures.

c-*fos* Immunocytochemistry

At 90 min after the challenge injection, animals were decapitated, the brains were snap-frozen in isopentane and stored at −80°C until use. Sections of 20 μm were cut on a cryostat and mounted onto coated slides (SuperFrost Plus) which were dried and stored at −80°C until use. For visualization of c-*fos*, sections were defrosted and fixed in a 4% paraformaldehyde solution in phosphate-buffered saline (PBS; 0.1 M, pH 7.4). Sections were washed with Tris-buffered saline (TBS, 0.1 M, pH 7.4) then incubated with primary antibody against c-*fos* (1:1,800, Oncogene Research, Burlington, MA, USA) in TBS with 0.5% Triton-X and 0.5% bovine serum albumin (TBS-TX-BSA) overnight at 4°C. After rinsing with TBS, endogenous peroxidase activity was removed by incubation of sections in a 1% hydrogen peroxide solution for 15 min. Sections were rinsed with TBS, then incubated in biotinylated goat antirabbit antibody (1:100, Dako, Denmark) in TBS-TX-BSA for 1 h, washed in TBS and incubated in avidin–biotin complex with horseradish peroxidase (HRP) (1:100, Vector Laboratories, Burlingame, CA, USA) for 1 h. Sections were rinsed in Tris–HCl then incubated in 3′3-diaminobenzidine (DAB; Sigma Chemical, 0.05% DAB in Tris–HCl) and rinsed in Tris–HCl. Sections containing the prefrontal cortex were incubated with Hoechst 33258 (1:2,000; Molecular Probes, Eugene, OR, USA), a fluorescent nuclear stain used to visualize cytoarchitecture. Sections were dried and finally coverslipped with Merckoglas (Merck, Darmstadt, Germany).

Histological quantification

Quantification of c-*fos* immunopositive nuclei was performed using an MCID Elite imaging system (Imaging Research, Ontario, Canada). Images of the nucleus accumbens in the c-*fos* DAB immunostained sections were digitized using an objective magnification of ×10 on a Leica DM/RBE photomicroscope with a Xillix MicroImager digital camera (1,280×1,024 pixels). Digitized images were combined so that the core and the shell areas were included, using the MCID tiling tool. Three (in some cases, two) sections per rat were chosen for quantification at the rostral–caudal levels in which inputs from the prefrontal cortex, thalamus, and amygdala have been particularly well characterized (Wright and Groenewegen 1995). The prefrontal cortex was digitized in the same fashion with the exception that color digital images were acquired using a Sony HAD camera (Sony DXC 950v, 640×512 pixels) of both the DAB staining and the epifluorescence of the Hoechst 33258 staining. The core and shell areas of the nucleus accumbens were delineated on the basis of atlas drawings from sections stained for calbindin (Jongen-Relo et al. 1993). The prefrontal cortex was delineated into prelimbic, infralimbic, orbital, and lateral areas on the basis of cytoarchitectonic criteria visible in the Hoechst 33258 staining.

The c-*fos* immunopositive nuclei in the nucleus accumbens were segregated from background staining levels using several point operators and spatial filters combined in an algorithm designed to detect local changes in the relative optical density (ROD). Briefly, images underwent histogram equalization and smoothing (low-pass filter, kernel size 7×7). The unfiltered image was subtracted from the smoothed image, followed by a series of steps to optimize the processed image and make it a suitable measuring template for detecting objects the size and shape of c-*fos* immunopositive nuclei. This algorithm was preferred over ROD thresholding because it does not involve an observer-dependent operation. The number of nuclei counted was corrected with a factor indicating approximate size of a c-*fos* immunopositive nucleus, thus preventing two groups of

stained pixels touching one another in the image being mistakenly counted as one nucleus. The results of all counting were expressed as the number of nuclei per surface area (mm^2). Integrated ROD for each segmented immunopositive nucleus was determined. Segregation of c-*fos* positive nuclei in the color-digitized images was performed in a similar fashion.

Subsequently, we set out to compare c-*fos* positive density (i.e., the number of cells per surface area) in a manner that accounts for labeling intensity: dark, light, or midrange. First, histograms of c-*fos* nuclei ROD values (for black and white images) or intensity values (for color images) were constructed for each brain area for each treatment group and qualitatively compared. These histograms were used to determine the value of the 33rd and 66th percentile optical density within the SS group. Based on these values, all nuclei from all animals for each area were binned as "light" (ROD values under the 33rd percentile of the SS group), "midrange" (ROD values between the 33rd and 66th percentile of the SS group) or "dark" (ROD values above the 66th percentile of the SS group). The number of immunopositive nuclei per bin was counted per rat, and the group averages were determined from the rat averages. For the prefrontal cortex, the same technique was used, but as these areas were digitized in color, intensity was used to bin rather than optical density. An increase in the number of nuclei in the "dark" bin would signify a rightward shift in the histograms, indicating that the increased cellular activity measured was primarily the result of more c-*fos* expression in the same group of neurons. An increase in the number of nuclei in the "midrange" bin would signify an upward shift in the histograms, indicating that the increase in the total number of nuclei measured was the result of the addition of a new group of nuclei to the cellular response (Fig. 1).

The dorsal striatum of the AA group was qualitatively inspected by two observers, both blind to the experimental conditions. The distribution pattern of c-*fos* positive nuclei was described and compared with that in a series of closely adjacent sections from the same animals stained immunocytochemically for the μ-opioid receptor to visualize striatal patches (Vanderschuren et al. 2002).

Statistics

For the quantification of c-*fos* immunoreactivity, the experimental groups were compared for effects of pretreatment and challenge (saline vs. amphetamine) and for interactions between these effects using a two-way ANOVA test followed by a Tukey post hoc test. For locomotor activity and behavioral scores, a repeated-measures ANOVA was conducted using time as within-subjects factor and followed by a Tukey post hoc test.

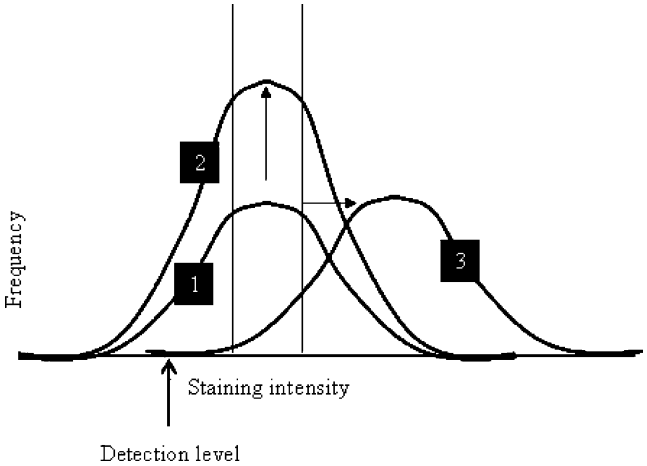

Fig. 1 Theoretical conceptualization of shifts in the ROD histograms. The total number of c-*fos* positive nuclei per mm^2 counted is represented as the area under the curves to the right of the detection level, indicated as an *arrow* on the *x*-axis. *Curve 1* indicates the control group; *vertical lines* indicate the ROD used to separate the neurons into light, midrange, and dark. An increase in the treatment group compared to the control group in the total number of c-*fos* positive nuclei per mm^2 could be the result of an increase in the frequency of c-*fos* positive nuclei, indicated by an increase in frequency in *curve 2*, causing an increase in the number of c-*fos* positive nuclei in the midrange. This would indicate that a new group of neurons is being recruited in the c-*fos* response, represented by the difference between *curves 1* and *2* in frequency. Alternatively, the same number of neurons could be active, but expressing more c-*fos* protein. This would cause a rightward shift in the curve (*curve 3*) and allowing more c-*fos* positive nuclei to come above the detection level and causing more c-*fos* positive nuclei to be measured in the dark range. A similar line of reasoning can be followed for other options, for instance a leftward shift in the case of reduced levels of c-*fos* protein (not illustrated)

Results

Behavioral results

For all behavioral measures, time was included as a within-subjects measure in a repeated-measures ANOVA followed by a post hoc test when significant time×pretreatment or time×challenge interactions were found. For brevity, only the most relevant of the results of these post hoc test results are described in this section and other results are presented in the figures.

Behavioral measures: responses to acute amphetamine

Locomotor activity was dose-dependently altered in animals treated acutely with amphetamine (Fig. 2a; main effect dose $F_{(3,10)}=43.914$; $p<0.001$). Saline-treated animals showed generally low activity levels, averaging a total of 1,335±514 cm traveled during the 90-min period. The groups treated with 1 mg/kg (14,045±1,836 cm) and 5 mg/kg amphetamine (14,906±2,321 cm) showed comparable levels

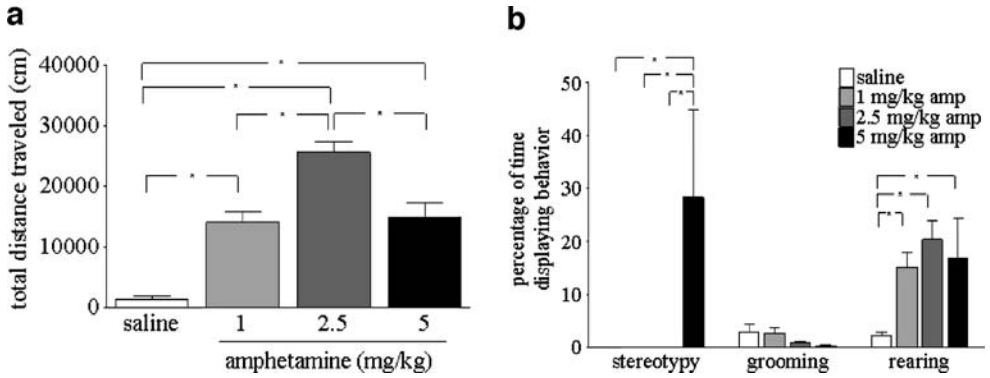

Fig. 2 Locomotor activity (**a**) and behavioral observations (**b**) for animals treated acutely with saline or 1, 2.5, or 5 mg/kg amphetamine. The highest levels of locomotor activity were seen in the 2.5-mg/kg treated group, which differed significantly from all other groups in the post hoc tests. Stereotypical behavior was observed in the animals treated with 5 mg/kg amphetamine, which differed significantly from all other groups, but no stereotypy was seen in any of the other treatment groups. No significant differences were seen in grooming behavior (**b**). All amphetamine-treated groups showed significantly more rearing than the saline-treated group (**b**), but the amphetamine treatment groups did not differ from one another. *Bars* indicate group averages, *error bars* represent SEM, $n=4$ per group. *Asterisks* indicate significant difference in post hoc testing ($p<0.05$)

of locomotor activity, whereas the highest locomotor response to amphetamine was observed in the 2.5 mg/kg group (25,543±1,704 cm). All amphetamine-treated groups differed from saline in the post hoc test. Acute amphetamine caused a significant increase in rearing ($F_{(3,12)}=7.971$; $p<0.005$; Fig. 2b), although not dose-dependently, as all doses differed significantly from the saline-treated group in post hoc testing, but there were no significant differences among the amphetamine-treated groups (average percent time spent rearing, saline=2.1±0.75; 1 mg/kg=15.2±2.7; 2.5 mg/kg=20.4±3.5; 5 mg/kg=16.8±7.7). No significant effect of amphetamine on grooming was seen (main effect of dose: $F_{(3,12)}=3.098$, n.s.; Fig. 2b). Stereotypy was exclusively observed in the group treated with 5 mg/kg of amphetamine (average percent time spent in stereotypical behavior, 5 mg/kg=28.4±16.5; all other groups average=0±0; Fig. 2b). A clear main effect of dose was seen ($F_{(3,12)}=11.724$; $p<0.005$). The 5-mg/kg group differed significantly from all other groups in the post hoc test.

Behavioral measures: responses to challenge after repeated amphetamine

Pretreatment with amphetamine caused a clear-cut augmentation of the locomotor response to amphetamine, as illustrated in Fig. 3a. Overall across the 90-min period, the AA group of amphetamine-pretreated rats that were challenged with amphetamine on the test day showed a 70% increase in activity compared to the SA group, which was pretreated with saline and challenged with amphetamine (total traveled distance during the 90-min test period=20,432±3,403 cm by AA compared to 11,924±2,149 cm by SA). Both groups that were challenged with saline on the test day (AS and SS, pretreated with amphetamine and saline, respectively)

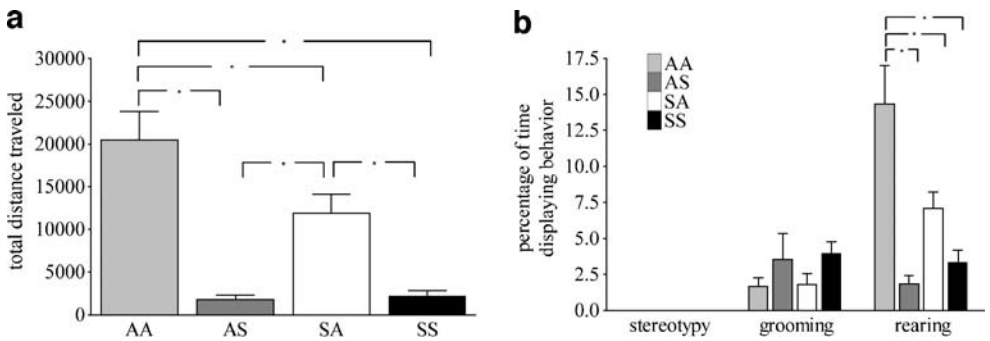

Fig. 3 Locomotor activity (**a**) and behavioral observations (**b**) in amphetamine-pretreated and amphetamine-challenged animals and control groups. *Bars* represent group average of locomotor activity (**a**) or percentage of time spent displaying each mutually exclusive category of behavior (grooming, rearing or stereotypical behavior; **b**). No bars are present representing stereotypical behavior because this behavior was not observed in any animals included in this experiment. *Error bars* indicate SEM, $n=6$ per group. *AA* amphetamine-pretreated, amphetamine-challenged; *SA* saline-pretreated, amphetamine-challenged; *AS* amphetamine-pretreated, saline-challenged; *SS* saline-pretreated, saline-challenged. *Asterisks* indicate significant difference in post hoc testing ($p<0.05$)

showed considerably less locomotor activity, showing approximately 10% of the activity displayed by the AA group (total traveled distance during 90 min, SS=1,770± 493 cm; AS=2,196±623 cm). These differences were reflected in significant main effects of pretreatment ($F_{(1,20)}$= 6.15; p<0.05) and challenge ($F_{(1,20)}$=62.07; p<0.001), and a significant pretreatment×challenge interaction ($F_{(1,20)}$= 5.029; p<0.05).

As shown in Fig. 3b, the evaluation of the percentage of time spent rearing revealed an increase in rearing in the AA group compared to all other groups (average over 90 min, AA=14.3±2.9%, AS=1.84±0.64%, SA=7.09±1.2%, SS= 3.34±0.92%). This was confirmed by a significant main effect of challenge ($F_{(1,20)}$=27.52; p<0.001) and pretreatment×challenge interaction ($F_{(1,20)}$=7.96; p<0.05). No main effect of pretreatment was observed for this parameter ($F_{(1,20)}$=3.41; n.s.).

Percentages of time spent grooming were generally low with average scores under 4% in all groups (average over 90 min, AA=1.66±0.64, AS=3.54±1.97, SA=1.78±0.86, SS=3.97±0.87; Fig. 3b). Despite the somewhat higher averages in the saline-challenged groups compared to the amphetamine-challenged groups, no significant effects of pretreatment, challenge, or interaction between the two were seen on grooming (pretreatment: $F_{(1,20)}$=0.64, n.s.; challenge: $F_{(1,20)}$=3.42, n.s.; pretreatment×challenge: $F_{(1,20)}$= 0.21, n.s.).

No stereotyped behavior was observed in any animal in any of the groups included in the repeated amphetamine experiments during any of the time periods scored (Fig. 3b).

Cellular reactivity results

In the nucleus accumbens and prefrontal cortex, the quantification of c-*fos* positive nuclei was tested for statistical significance by two-way ANOVAs using pretreatment and challenge as factors, followed by a Tukey HSD post hoc. For brevity, results of these post hoc tests are presented in the figures and in Table 1.

Sensitization alters distribution of reactivity in dorsal striatum

In the dorsal striatum, visual inspection of the sections from animals both pretreated and challenged with amphetamine (AA group) showed overall more c-*fos* positive nuclei compared to the AS group, and a differential distribution throughout the area (Fig. 4). c-*fos* positive nuclei were more abundant medially than laterally. Within the general distribution, heterogeneity of reactivity was seen conforming to patterns previously observed in patches in sensitized animals. Comparison of c-*fos* staining pattern with μ-opioid stained patches in sections from another series from the same animals confirmed the location of high concentrations of nuclei in μ-opioid stained patches.

Cellular reactivity sensitizes in nucleus accumbens core, but not accumbens shell or prefrontal cortex

Within the nucleus accumbens, the core and shell subdivisions showed different c-*fos* immunoreactivity response patterns to an amphetamine challenge after the sensitizing regimen (Fig. 5a–d and Fig. 6). The core showed a clear effect of sensitization with a number of c-*fos* positive nuclei in the AA group 48% higher than that of the SA group and more than 100% compared to both saline-challenged groups (Fig. 6). Significant main effects of pretreatment ($F_{(1,28)}$=10.59; p<0.005) and challenge ($F_{(1,28)}$=44.75; p< 0.001) and a significant pretreatment×challenge interaction ($F_{(1,28)}$=7.55; p<0.05) confirmed this observation. In the nucleus accumbens shell, more c-*fos* positive nuclei were counted in the amphetamine than saline-challenged groups, but no sensitization effect was seen (Fig. 6). A significant main effect of challenge ($F_{(1,28)}$=4.41; p<0.05), but not of pretreatment ($F_{(1,28)}$=0.57; n.s.), was observed, and no pretreatment×challenge interaction ($F_{(1,28)}$=0.04; n.s.) was present in the ANOVA of the nucleus accumbens shell data.

In the prefrontal cortex, an increase in c-*fos* positive nuclei in amphetamine-challenged groups compared to saline-challenged groups was observed in all four subregions measured. No differences were seen between amphetamine- and saline-pretreated animals in the number of c-*fos* positive nuclei after an amphetamine challenge (Fig. 6). Significant main effects of challenge were observed in all areas (PL: $F_{(1,28)}$=13.87, p<0.001; IL: $F_{(1,28)}$=6.94, p<0.05; orbital PFC: $F_{(1,28)}$=25.96, p<0.001; lateral PFC: $F_{(1,28)}$=20.55, p<0.001). No significant effects of pretreatment (PL: $F_{(1,28)}$=0.14, n.s.; IL: $F_{(1,28)}$=1.20, n. s.; orbital PFC: $F_{(1,28)}$=0.945, n.s.; lateral PFC: 0.79, n.s.) or pretreatment×challenge interactions (PL: $F_{(1,28)}$=0.08, n. s.; IL: $F_{(1,28)}$=0.037, n.s.; orbital PFC: $F_{(1,28)}$=0.039; lateral PFC: $F_{(1,28)}$=0.67) were observed.

Frequency distributions of relative optical densities across groups show sensitization of midrange in accumbens core

When comparing the ROD of the c-*fos* positive nuclei of each area across groups, significantly higher (=darker) averages were seen in amphetamine- compared to saline-challenged groups in the core (optical density 0.1216±0.0026 in saline-challenged, 0.1372±0.0040 in amphetamine-challenged; $F_{(1,28)}$=10.815, p<0.005), the shell (optical density 0.1208± 0.0023 in saline-challenged, 0.1307±0.0035 in amphetamine-challenged; $F_{(1,28)}$=5.513, p<0.05), the infralimbic area (intensity 0.7173±0.0057 in saline-challenged, 0.6985±

Table 1 Mean ± SEM density (number of cells per mm^2) of c-*fos* immunoreactive nuclei in light, dark, and midranges of cellular staining intensity

			Treatment group			
			AA	AS	SA	SS
Nucleus accumbens	Core	Light	21.4±6.9	17.6±4.2	17.9±3.2	21.2±4.2
		Midrange§	34.2±2.8$^{\&}$	19.0±3.2$^{\#}$	20.5±3.7	22.1±5.1
		Dark$^{\P/****}$	94.5±14.1$^{\&/+}$	35.6±4.6$^{\#}$	61.2±12.4	24.1±3.6$^{\#}$
	Shell	Light	28.8±8.7	26.9±7.3	28.8±5.9	38.3±6.4
		Midrange	42.3±3.2	41.7±9.2	34.5±6.6	40.3±6.6
		Dark*	96.1±17.1	63.6±9.2	86.5±20.7	44.5±7.6
Prefrontal cortex	Prelimbic	Light	48.2±5.0	34.9±6.7	39.3±5.4	32.2±3.8
		Midrange**	50.0±6.9	31.0±6.0	46.4±7.0	27.7±6.3
		Dark***	74.3±12.4	32.0±6.7$^{\%}$	82.28±18.8$^{+/\&}$	28.7±7.9$^{\%}$
	Infralimbic	Light	37.6±3.8	33.1±5.8	30.4±5.6	29.9±5.1
		Midrange	46.7±8.3	34.9±7.8	35.0±7.9	28.3±5.4
		Dark***	78.1±13.7^{+}	40.7±7.1	70.3±14.5	30.2±6.9$^{\#}$
	Orbital	Light	27.8±3.6	24.7±6.1	23.0±4.3	21.0±4.4
		Midrange**	61.6±8.5^{+}	32.6±7.7	45.0±8.4	30.7±5.9$^{\#}$
		Dark****	129.7±21.7$^{\&/+}$	32.6±8.6$^{\#}$	120.4±24.2^{+}	26.3±5.7$^{\#/\%}$
	Lateral	Light*	22.8±2.3	14.8±10.0	18.1±2.8	14.4±3.3
		Midrange*	39.9±5.2	19.3±5.1	27.4±7.6	18.4±3.7
		Dark****	69.5±11.6$^{\&/+}$	18.3±4.4$^{\#}$	65.1±15.6^{+}	16.7±4.6$^{\#/\%}$

Results of ANOVA testing are shown in the column indicating ROD range as follows: *paragraph mark* significant main effect of pretreatment, *asterisk* significant main effect of challenge, *section mark* significant pretreatment×challenge interaction; one symbol: $p<0.05$, two symbols: $p<0.01$, three symbols: $p<0.005$, four symbols: $p<0.001$. Results of post hoc testing are indicated in the cells containing the value of the average number of nuclei as follows: *plus sign* significantly different from SS, *percent sign* significantly different from SA, *ampersand* significantly different from AS, *number sign* significantly different from AA.

0.0660 in amphetamine-challenged; $F_{(1,28)}=4.368$, $p<0.05$), the orbital prefrontal area (intensity 0.7181±0.0048 in saline-challenged, 0.6921±0.0073 in amphetamine-challenged; $F_{(1,28)}=8.258$, $p<0.001$), and the lateral prefrontal area (intensity 0.7208±0.0045 in saline-challenged, 0.6992±0.0067 in amphetamine-challenged; $F_{(1,28)}=6.753$, $p<0.05$). Significant differences were seen between the AA and SS groups and between the SA and SS group in the core in post hoc testing. No significant differences were seen in the shell in post hoc testing.

The distributional patterns of the cellular ROD suggested differences in the distributions of ROD across the treatment groups. To further analyze the optical densities and compare the frequencies of the various optical densities between groups, histograms of the optical densities of all cells per area, per group were constructed (Figs. 5e–f and 7a and b) and used to divide immunostained nuclei into light, midrange, and dark optical density (OD) ranges based on the 33rd and 66th percentile values in the SS group (Table 1), as described in the "Materials and methods"

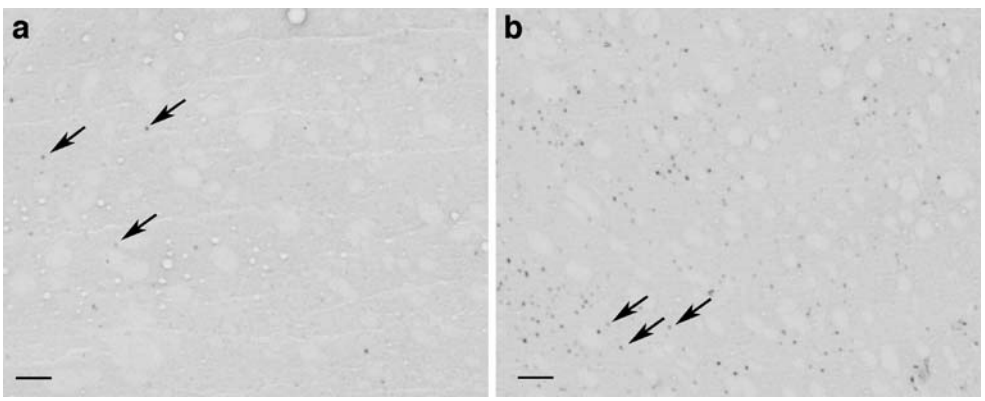

Fig. 4 Digital micrographs of c-*fos* immunopositive nuclei in the dorsal striatum of representative animals from groups pretreated with amphetamine and challenged with either saline (**a**, group AS) or amphetamine (**b**, group AA). Scale bars indicate 100 μm, *arrows* point to c-*fos* immunopositive nuclei. Note the higher number of c-*fos* positive nuclei in the AA animal compared to the SA animal, and the inhomogeneous distribution of nuclei in the AA animal

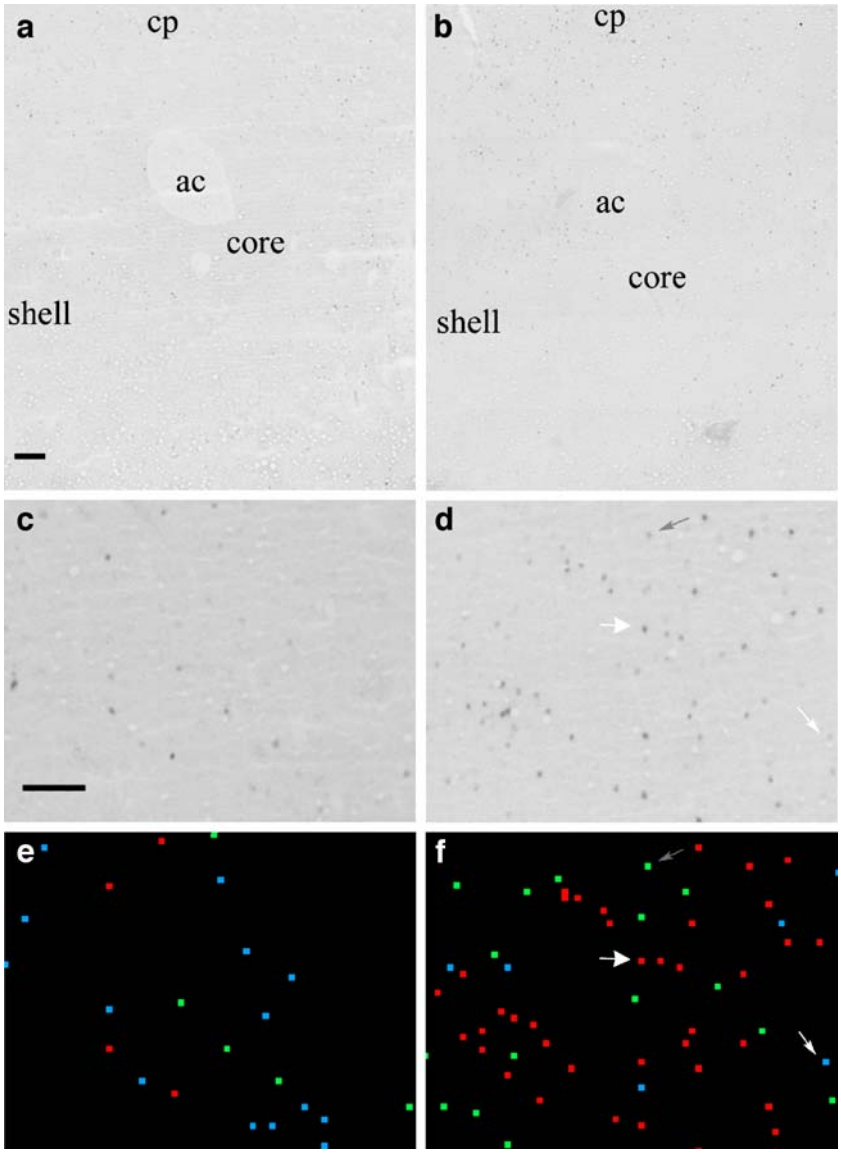

Fig. 5 Digital micrographs of c-*fos* immunopositive nuclei and representations of nuclei quantified in the nucleus accumbens from representative animals treated acutely with amphetamine (SA group; **a**, **c**, and **e**) and challenged with amphetamine after amphetamine pretreatment (AA group; **b**, **d**, and **f**). The low magnification overviews of nucleus accumbens in **a** (SA animal) and **b** (AA animal) show differences in the number of c-*fos* immunopositive nuclei between the two experimental groups. Corresponding detail images of the nucleus accumbens core, shown in **c** (detail from **b**) and **d** (detail from **b**), are medial to anterior commissure. Nuclei counted for quantification in **c** and **d** are illustrated in **e** and **f**, respectively. *Red squares* indicate immunoreactive nuclei classified as "dark," *green squares* as "midrange," and *blue squares* as "light" (see text for classification procedures). *Broad white arrows* in **d** and **f** indicate a "dark" immunopositive nucleus and its representation in the counted nuclei; *gray arrows* represent "midrange"; *narrow white arrows* indicate "light." Scale bar in **a** indicates 300 μm for **a** and **b**; scale bar in **c** represents 100 μm for **c** and **d**. *cp* caudate putamen, *ac* anterior commissure, *core* nucleus accumbens core, *shell* nucleus accumbens shell

section. Two-way ANOVAs for pretreatment×challenge were conducted, followed by Tukey HSD post hoc tests. For brevity, the results of the post hoc tests are indicated in Table 1.

In both the core and the shell, amphetamine-challenged animals showed higher numbers of c-*fos* positive nuclei than saline-pretreated animals in the dark range (core challenge: $F_{(1,28)}=23.928$, $p<0.001$; shell challenge: $F_{(1,28)}=6.421$, $p<0.05$). A significant effect of pretreatment was seen in the core in this range ($F_{(1,28)}=5.202$, $p<0.05$), and no effect was seen in the shell ($F_{(1,28)}=0.949$, n.s.). No pretreatment×challenge interactions were observed in the core (pretreatment×challenge: $F_{(1,28)}=1.227$, n.s.; Fig. 7c and Table 1) or shell (pretreatment×challenge: $F_{(1,28)}=0.103$, n.s.; Fig. 7d and Table 1) in this range. In the midrange nuclei, the core area showed a distinct pattern of distribution compared to all other areas measured as a sensitization effect was confirmed by a significant pretreatment×challenge interaction ($F_{(1,28)}=4.901$, $p<0.05$; Fig. 7c), while no significant main effects were observed (pretreatment: $F_{(1,28)}=1.985$, n.s.; challenge: $F_{(1,28)}=3.195$, n.s.). No differences between groups were observed in the midrange nuclei in the shell (pretreatment: $F_{(1,28)}=0.474$, n.s.; challenge: $F_{(1,28)}=0.146$, n.s.; pretreatment×challenge: $F_{(1,28)}=0.229$, n.s.; Fig. 7d). No differences between groups were seen in the light range nuclei in the core (Fig. 7c; pretreatment: $F_{(1,28)}=0.000$, n.s.; challenge: $F_{(1,28)}=0.002$, n.s.; pretreatment×challenge: $F_{(1,28)}=0.532$, n.s.) or the shell (Fig. 7d; pretreatment: $F_{(1,28)}=0.624$, n.s.; challenge: $F_{(1,28)}=0.280$, n.s.; pretreatment×challenge: $F_{(1,28)}=0.632$, n.s.).

Within the prefrontal cortex, the areas measured showed a relatively homogenous pattern of distribution of c-*fos* immunoreactivity across the OD ranges (Table 1). In the

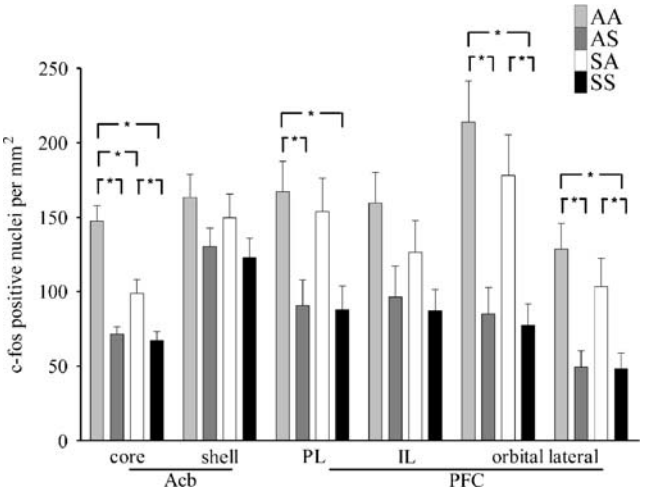

Fig. 6 Mean number of c-*fos* immunopositive nuclei in nucleus accumbens core and shell and prelimbic, infralimbic, orbital, and lateral prefrontal cortex. Two-way ANOVA analysis of accumbens data showed significant main effects of pretreatment and challenge and a pretreatment×challenge interaction in the nucleus accumbens core. A significant effect of challenge was observed in the nucleus accumbens shell and for all PFC areas measured. *Bars* represent the number of c-*fos* immunopositive nuclei per mm^2, *error bars* indicate SEM, n=8 per group. *Asterisk* indicates significant differences in post hoc testing. See Fig. 3 for abbreviations of treatment groups

dark range, all areas showed significantly more c-*fos* positive nuclei in the amphetamine-treated groups compared to the saline-treated groups (main effect challenge: PL: $F_{(1,28)}$=14.974, p<0.005; IL: $F_{(1,28)}$=11.563, p<0.005; orbital: $F_{(1,28)}$=31.370, p<0.001; lateral: $F_{(1,28)}$=23.751, p<0.001) but no effects of pretreatment (main effect pretreatment: PL: $F_{(1,28)}$=0.035, n.s.; IL: $F_{(1,28)}$=0.645, n.s.; orbital: $F_{(1,28)}$=0.209, n.s.; lateral: $F_{(1,28)}$=0.092, n.s.) or interaction between pretreatment and challenge (pretreatment×challenge: PL: $F_{(1,28)}$=0.207, n.s.; IL: $F_{(1,28)}$=0.014, n.s.; orbital: $F_{(1,28)}$=0.008, n.s.; lateral: $F_{(1,28)}$=0.015, n.s.). In the midrange, increased numbers of c-*fos* positive nuclei in amphetamine-challenged animals were seen in the prelimbic ($F_{(1,28)}$=8.081, p<0.01), orbital ($F_{(1,28)}$=7.941, p<0.01), and lateral (7.029, p<0.05) areas, but not the infralimbic area ($F_{(1,28)}$=1.545, n.s.). No effects were seen in the midrange segment of pretreatment (main effect pretreatment: PL: $F_{(1,28)}$=0.265, n.s.; IL: $F_{(1,28)}$=1.511, n.s.; orbital: $F_{(1,28)}$=1.453, n.s.; lateral: $F_{(1,28)}$=1.461, n.s.), and no pretreatment×challenge interactions were seen (pretreatment×challenge: PL: $F_{(1,28)}$=0.001, n.s.; IL: $F_{(1,28)}$=0.113, n.s.; orbital: $F_{(1,28)}$=0.928, n.s.; lateral: $F_{(1,28)}$=1.076, n.s.). In the light range, only the lateral

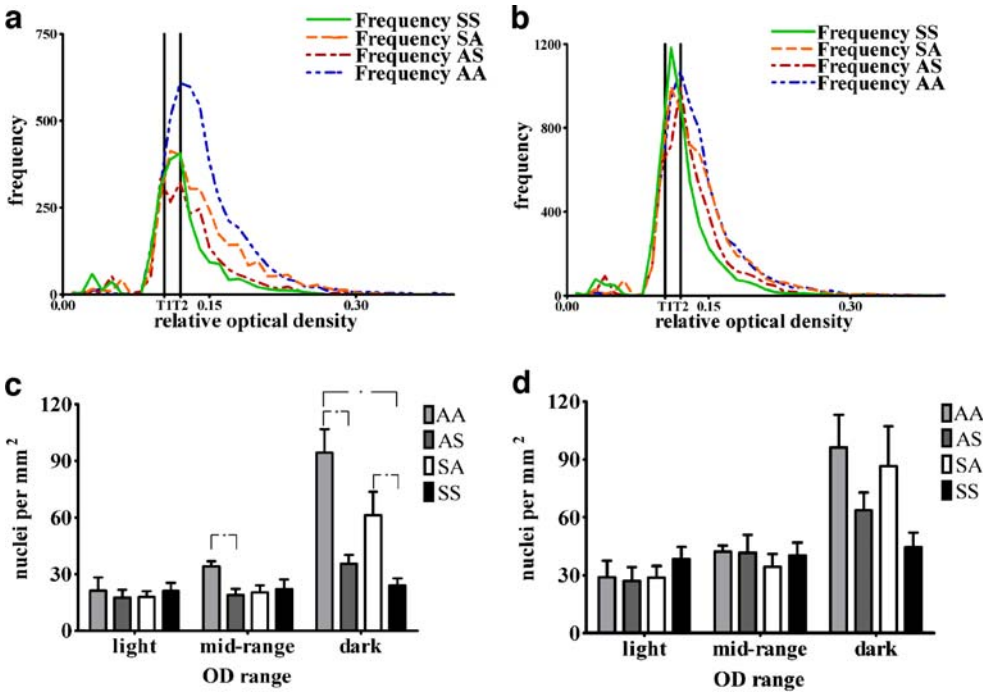

Fig. 7 Representations of distributions of optical densities in the nucleus accumbens. In **a** and **b**, histograms of relative optical densities of individual c-*fos* immunopositive nuclei in nucleus accumbens core (**a**) and shell (**b**) are depicted. *T1* and *T2 lines* represent the 33rd and 66th percentile values, respectively, for the SS group. In the nucleus accumbens core, the AA group shows more neurons in the midrange segment (between T1 and T2), as confirmed by the analysis of number of cells per segment (**c**). A significant pretreatment×challenge interaction is present in the midrange of the nucleus accumbens core. The amphetamine-challenged groups both contain more neurons in the dark segment of both core (**c**) and shell (**d**) compared to saline-challenged groups, as evidenced by a significant effect of challenge in the ANOVA test. No significant differences were observed in post hoc testing in the shell; *asterisks* indicate significant differences in post hoc testing for core in **c** (p<0.05). *Line colors* and *patterns* or *bar fillings* indicating groups are as shown in **a** and **c**. For **c** and **d**, *bars* represent the number of c-*fos* positive nuclei in each OD segment per mm^2, *error bars* indicate SEM, n=8 per group. See Fig. 2 for abbreviations of treatment groups

prefrontal cortex showed a significant increase in c-*fos* positive nuclei in the amphetamine-challenged groups (main effect challenge: $F_{(1,28)}=4.228$, $p<0.05$). No other challenge effects were observed (main effect challenge: PL: $F_{(1,28)}=3.751$, n.s.; IL: $F_{(1,28)}=0.243$, n.s.; orbital: $F_{(1,28)}=0.302$, n.s.). No effects of pretreatment (main effect pretreatment: PL: $F_{(1,28)}=1.225$, n.s.; IL: $F_{(1,28)}=1.060$, n.s.; orbital: $F_{(1,28)}=0.863$, n.s.; lateral: $F_{(1,28)}=0.793$, n.s.) or of sensitization (pretreatment×challenge interaction: PL: $F_{(1,28)}=0.356$, n.s.; IL: $F_{(1,28)}=0.156$, n.s.; orbital: $F_{(1,28)}=0.015$, n.s.; lateral: $F_{(1,28)}=0.573$, n.s.) were seen in the light range of the PFC c-*fos* immunopositive nuclei.

Discussion

In the present study, we set out to characterize the behavioral response to amphetamine in drug-naive and amphetamine-pretreated animals, as we had previously observed that treatment with a high dose (5 mg/kg) of amphetamine in drug-naive animals resulted in a similar pattern of cellular reactivity in the dorsal striatum as a challenge with an intermediate dose (1 mg/kg) in amphetamine-pretreated, behaviorally sensitized rats (Vanderschuren et al. 2002). Furthermore, we aimed to characterize the reactivity of subregions within the nucleus accumbens and prefrontal cortex during the expression of psychostimulant sensitization. Our results showed that previously demonstrated similarity of cellular activation patterns in the dorsal striatum after amphetamine in sensitized or drug-naive rats was not accompanied by similar behavioral responses. Thus, animals given acute amphetamine at a dose that produces heightened patch activation (5 mg/kg) displayed intense stereotypy, which was not seen during the expression of sensitization at a challenge dose (1 mg/kg) that also produces higher activation of patches than surrounding matrix. Cellular activation was sensitized in response to a drug challenge in the nucleus accumbens core, but not the shell or the prefrontal cortex. Examination of the distribution patterns of the optical densities of the individual immunopositive nuclei revealed an upward shift in the histograms of the core in the AA group, resulting in a significant increase in the midrange stained nuclei in the accumbens core in sensitized animals.

Sensitization of locomotor activity and rearing without stereotypy

After amphetamine pretreatment, an augmented locomotor and rearing response to amphetamine was seen compared to saline-pretreated, amphetamine-challenged animals, thus supporting the notion that stereotypy and patch hyperreactivity could take place independently. The behavioral pattern of the amphetamine-pretreated, amphetamine-challenged group most resembled the animals treated acutely with 1.0 or 2.5 mg/kg of amphetamine. No stereotyped behavior was observed in the sensitized animals after an amphetamine challenge, while intense stereotypy was seen in animals treated acutely with 5 mg/kg of amphetamine. In our previous paper (Vanderschuren et al. 2002), we noted that the decrease in locomotion normally observed in animals treated acutely with a high dose of amphetamine was likely a result of stereotypy, an idea which is strengthened by the present observations.

In previous studies by our laboratory and others, animals treated acutely with the relatively high dose of 5 mg/kg of amphetamine (Graybiel et al. 1990; Vanderschuren et al. 2002) or with the same amphetamine regimen for sensitization followed by a challenge with 1 mg/kg amphetamine used in the present study (Vanderschuren et al. 2002) show enhanced ratios of response in patches compared to the matrix in the dorsal striatum, findings visually confirmed in the present set of experiments. We concluded on the basis of our previous results that the changes seen in striatal reactivity represent a shift in sensitivity to amphetamine rather than long-term adaptations in circuitry. If the increased sensitivity to amphetamine underlying behavioral sensitization paralleled the hyperreactivity of patches, the same pattern of behavior would be expected in animals showing similar immediate-early gene expression patterns, namely, the 5-mg/kg acutely challenged group and the amphetamine-sensitized, amphetamine-challenged group. However, the expression of stereotypy in the 5-mg/kg group, not seen in the sensitized animals, and the erratic locomotor activity seen in the 5-mg/kg group, clearly indicate behavioral differences after the two treatments. It is interesting to note that the dissociation of hyperreactivity of patches and sensitized behavioral responses manifests in a lack of stereotypical behavior in sensitized animals, as increased reactivity of patches compared to the matrix has been suggested to underlie stereotypical behavior (Canales and Graybiel 2000). Given the fact that the sensitization regimen and challenge dose used in the present study causes heightened patch to matrix ratios of c-*fos* expression, but does not cause stereotyped behavior, changes in striatal patterns of activity are apparently not causally related to stereotypy.

Sensitization of immediate-early gene activation in a specific neural population of the nucleus accumbens core

The sensitized immediate-early gene expression we observed in the nucleus accumbens core during the expression of psychostimulant sensitization after a withdrawal period of 14 days contradicts a number of previous immediate-

early gene studies where no sensitization effect was observed in the core (Ostrander et al. 2003; Todtenkopf et al. 2002a), including one study from our own laboratory (Vanderschuren et al. 2002). There are two main differences between the present study and the previous study from our group. First, in our previous study, a 3-week abstinence period was observed, while in the present study, animals were tested after 14 days of abstinence. It is interesting to note that studies examining immediate-early gene expression after shorter abstinence periods (2 days) do show sensitization of reactivity within the core (Hedou et al. 2002; Todtenkopf et al. 2002a), indicating that abstinence time is of potential importance for sensitization of c-*fos* in the nucleus accumbens core. However, this explanation seems unsatisfactory as the study by Totenkopf et al. (2002a) also tested c-*fos* expression after 2 weeks of abstinence and found no sensitization of c-*fos* expression in the nucleus accumbens core. This discrepancy might be explained by a second difference between the studies where no effect was observed and the present study: in studies finding no effect of sensitization on the nucleus accumbens core, animals were at least partially pretreated and challenged in the same environment. Exposure to amphetamine in a relatively novel environment has been shown to potentiate the c-*fos* response to the drug in the nucleus accumbens core (Ostrander et al. 2003).The importance of the testing environment was recently underscored by a study showing that sensitized c-*fos* responses to cocaine in the nucleus accumbens only occurred when cocaine was always administered in a discrete environment outside of the home cage (Hope et al. 2006).

Expression of c-*fos* in the striatum can be elicited by stimulation of dopamine receptors (Berretta et al. 1992), which have been demonstrated to be crucially involved in the expression of amphetamine sensitization (Vanderschuren and Kalivas 2000). As mentioned in the "Introduction", a number of studies point to the sensitization of dopamine transmission in the accumbens core during the expression of sensitization (Cadoni et al. 2000; Phillips et al. 2003), and increased dopamine is also seen in the core compared to the shell in yoked controls in cocaine and heroin self-administration studies (Lecca et al. 2007a; Lecca et al. 2007b). It is thus possible that dopamine may play a role in eliciting the increased c-*fos* response seen in the accumbens core in the present study.

In the accumbens core, more cells in the midrange of optical densities were present in the amphetamine-pretreated, amphetamine-challenged group than in all other groups. This "upward" shift in sensitized animals, visible in the histogram of optical density frequencies, indicates that the increase in c-*fos* positive nuclei is not due to nuclei that were under our detection threshold for c-*fos* increasing their reactivity enough to be measured. In the case of increased c-*fos* expression within the same population of nuclei that normally respond to amphetamine, a rightward shift in the histogram of optical densities would be expected, which would manifest in a sensitization effect in the dark range and increase of the average optical density. No increase was seen in the overall average optical density, no significant interaction observed between pretreatment and challenge in the dark group, and there is no significant difference between the AA and SA groups in the dark group. However, an obvious trend to sensitization in the darkly stained group is visible in Fig. 6. Although the lack of significance precludes firm conclusions, it might be that some neurons which normally respond to amphetamine by producing c-*fos* react by producing more c-*fos* after sensitization.

The increase of frequency of neurons stained in the midrange without a significant shift in average optical density suggests the recruitment of a new population of nuclei becoming responsive to amphetamine in the sensitized animals. The population most likely to be involved in that sensitized response is the dopamine D_2 receptor-containing, enkephalin-positive population that project via the subthalamic nucleus to the substantia nigra pars reticulata and entopenduncular nucleus. Indeed, sensitization of cocaine-induced c-*fos* expression in the nucleus accumbens in animals treated with the drug in a discrete environment was only found in enkephalin-positive cells, but not in dynorphin-positive, dopamine D_1 receptor-expressing neurons that directly project to the substantia nigra (Hope et al. 2006). Differences in responsivity to amphetamine in these two cellular populations have also been demonstrated in a study where dopamine D_2 receptor-containing neurons became activated when amphetamine is administered in a novel environment (Badiani et al. 1999). Remarkably, c-*fos* expression in dopamine D_1 receptor-containing accumbens neurons was shown to be important for the induction of cocaine sensitization (Zhang et al. 2006), suggesting that these two different cell populations play distinct roles in the induction and expression of behavioral sensitization.

Immediate-early gene responses to acute amphetamine in the nucleus accumbens shell and prefrontal cortex

The cellular response of the nucleus accumbens shell to acute amphetamine, which did not sensitize after repeated amphetamine, corresponds well with previous results from our laboratory demonstrating the same effect (Vanderschuren et al. 2002). The role of the shell in the expression of psychostimulant sensitization is not clear-cut. On the one hand, psychostimulant sensitization-induced long-term changes in cellular reactivity or cellular morphology are not generally found in the nucleus accumbens shell after

psychostimulant administration (Cadoni et al. 2000; Li et al. 2004; Todtenkopf et al. 2002a). Moreover, post induction lesions of the shell leave the expression of cocaine sensitization intact (Todtenkopf et al. 2002b), although preinduction lesions of a subarea of the shell result in reduced sensitized, but not acute responses to cocaine (Brenhouse and Stellar 2006). On the other hand, microinjection of amphetamine or cocaine into the shell (but not core) produces sensitized psychomotor responses and augmented dopamine levels in animals pretreated with cocaine (Filip and Siwanowicz 2001; Pierce and Kalivas 1995), and sensitized cellular reactivity has been observed in specific subareas within the accumbens shell after repeated cocaine administration (Brenhouse et al. 2006; Todtenkopf et al. 2002a). Discrepancies between studies finding the effects of sensitization in the shell and the present study could be due to the use of cocaine pretreatment and/or challenges in other studies and amphetamine pretreatment and challenge in the present study. Cocaine and amphetamine have been observed to produce different activation patterns in the dorsal striatum with acute amphetamine producing activation of patches and acute cocaine producing a more homogenous staining pattern (Graybiel et al. 1990); it is possible that cocaine and amphetamine pretreatment produce different patterns of reactivity in the ventral striatum as well.

Within the prefrontal cortex, all areas showed c-*fos* expression in response to amphetamine, but there was no effect of amphetamine preexposure. To our knowledge, this is the first examination of c-*fos* expression in orbital and lateral prefrontal areas. However, the lack of sensitization of c-*fos* expression in the medial prefrontal cortex corresponds well with results from other groups showing similar results after 2 weeks of abstinence (Todtenkopf et al. 2002a). Sensitization of c-*fos* immunoreactivity does occur when animals are challenged after 2 days of abstinence (Hedou et al. 2002; Todtenkopf et al. 2002a, b), suggesting a role in the induction or early phases of sensitization. In support of a role for the prefrontal cortex in the induction and early phases of expression, lesion studies have demonstrated region-specific effects in the involvement of prefrontal areas in the induction of cocaine sensitization (Tzschentke and Schmidt 2000), and lesions of the entire medial prefrontal cortex have been shown to prevent the induction of amphetamine sensitization (Wolf et al. 1995; Cador et al. 1999, but see Tzschentke and Schmidt 2000). Studies examining expression of psychostimulant sensitization suggest that the dorsal areas of the medial prefrontal cortex that project to the nucleus accumbens core are involved in the expression of psychostimulant sensitization (Steketee 2003). Although no sensitization of immediate-early gene expression was observed in the present study, a dorsal–ventral gradient was seen in acute response to amphetamine with the prelimbic area showing a 74% increase in the SA group over the SS group and the infralimbic area showing a smaller increase at 45%. The high responsivity of lateral prefrontal neurons to amphetamine (114% increase in SA over SS) is also interesting in this respect, as the projections from this area to the nucleus accumbens are nearly exclusive to the nucleus accumbens core (Berendse et al. 1992). The highly active prefrontal cortical inputs projecting to the core may play a role in enhancing the activation of the core during the expression of sensitization.

Remarkably, the highest responsivity to acute amphetamine was found in the orbital frontal area, a prefrontal cortex area that projects primarily to the dorsal striatum and only very sparsely to the nucleus accumbens (Berendse et al. 1992). Metabolic and structural changes within the orbital prefrontal cortex have been demonstrated in human drug addicts and nonhuman primates and rodents with a history of drug self-administration (Crombag et al. 2005; Porrino and Lyons 2000; Volkow and Li 2004). A recent study showed that rats sensitized to cocaine are impaired on a task that is sensitive to orbital prefrontal lesions (Schoenbaum et al. 2004). The high responsivity of this area to acute amphetamine indicates that corticostriatal circuits not involving the nucleus accumbens also play an important role in the acute response to amphetamine.

In conclusion, challenging animals sensitized to amphetamine with a dose of the drug that produces preferential activation of dorsal striatal patches did not produce stereotyped behavior, unlike the behavioral pattern observed in animals treated acutely with a dose of amphetamine that causes the same pattern of neural activation. Sensitized immediate-early gene activity was found in the nucleus accumbens core, but not the accumbens shell or the prefrontal cortex. The gradients observed in the present study in cellular responses to amphetamine in subareas of the prefrontal cortex with specific afferents to the nucleus accumbens suggest a specific role for various corticostriatal loops in the behavioral responses to psychostimulant drugs, further supporting the importance of accumbens–prefrontal cortex interactions in drug addiction (Volkow and Li 2004; Robbins and Everitt 2002).

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