Amphetamine-sensitized animals show a sensorimotor gating and neurochemical abnormality similar to that of schizophrenia

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Received 27 June 2002; received in revised form 6 December 2002; accepted 23 December 2002

Abstract

The aim of these studies was to examine whether amphetamine-induced sensitization in rats could be used as an animal model to study the basis of certain abnormalities seen in schizophrenia. Specifically, these experiments examined whether rats subjected to a sensitizing regimen of amphetamine would show the sensorimotor gating and greater amphetamine-induced displacement of radio-raclopride binding deficit that is observed in schizophrenia. In the first experiment, animals were divided into two groups with each rat receiving an intraperitoneal injection of amphetamine (AMPH) or saline (SAL) (1 ml/kg) three times per week for 3 weeks for a total of nine injections. AMPH dose was increased weekly from 1 mg/kg in the first week to 3 mg/kg in the third. Twenty-two days after the last injection, prepulse inhibition (PPI) of the acoustic startle response was tested. In addition, rats were tested for the effects of a challenge dose of 0.5 mg/kg AMPH on locomotor activity and \textsuperscript{3}H\textsuperscript{r}aclopride (RAC) binding potential (BP) in the striatum. The tests for PPI confirmed that sensorimotor gating was disrupted in the AMPH-induced sensitized-state rats at baseline. The AMPH-sensitized rats also exhibited higher locomotor response to AMPH and a lower binding of striatal \textsuperscript{3}H\textsuperscript{r}aclopride when challenged with the drug. The results were replicated and even more pronounced in rats that were treated with AMPH for 5 weeks, with doses ranging from 1mg/kg in the first week to 5 mg/kg in the fifth. These sensorimotor gating deficits and neurochemical (greater AMPH-induced displacement of radio-raclopride binding) abnormalities show similarities with the pathophysiology of schizophrenia and suggest that the AMPH-sensitized-state rats could be used to model certain aspects of schizophrenia.

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Keywords: Amphetamine; Schizophrenia; Sensitization; Dopamine; Prepulse inhibition; Raclopride

Schizophrenia is a complex neuropsychiatric disorder with psychotic, cognitive and affective features. Animal models are critical in pursuit of further knowledge about schizophrenia and in generating better therapeutic options. Since the etiology/ies of schizophrenia is not determined, it is not possible as yet to have an etiologically driven model. However, the development of models that demonstrate behavioural and neurochemical phenotypes analogous to that seen
in schizophrenia can lead to a much better insight into the disorder.

The most common approach to modelling dopaminergic pathophysiology in schizophrenia has been the acute challenge of a dopamine-releasing (e.g. amphetamine) or dopamine receptor stimulating (e.g. apomorphine) agent, and examining its effects on motor behaviour (see review by Robinson and Becker, 1986; Vanderschuren et al., 1999), sensorimotor gating (Braff and Geyer, 1990; Mansbach et al., 1988; Swerdlov et al., 2000) or learned associations (Moser et al., 2000; Broersen et al., 1999). This approach has several limitations. The hyperdopaminergic state of schizophrenia is a chronic and self-sustaining one, not an acute and exogenous one. In that regard, these acute challenges in normal rats may be much more akin to episodic drug abuse than they are to the condition of schizophrenia. In addition, the behavioural aberration is not reflected in the baseline state but in the challenged state of the animal. This is at odds with schizophrenia where the patients show abnormalities both at baseline and in response to challenges (Abi-Dargham et al., 1998, 2000; Laruelle, 2000; Seeman and Kapur, 2000). Finally, since these challenges create their behavioural endpoints via direct stimulation of the dopamine system, any drug that blocks the dopamine system, particularly the dopamine D2 system is effective in these models. As a result, the model tends to be self-referential and is thus unlikely to reveal truly novel methods of attenuating a hyperdopaminergic response. Thus, it is important to work with a model of hyperdopaminergia which is chronic, self-sustaining, does not require the contemporaneous drug administration and results in behavioural aberrations similar to schizophrenia in the baseline state of the animal.

Much progress has been made in understanding the sensorimotor gating deficits in patients with schizophrenia as measured by prepulse inhibition (PPI) of acoustic startle. PPI is the reduction of a startle response to a high-intensity stimulus that is preceded by a low-intensity prestimulus (Hoffman and Ison, 1980; Braff and Geyer, 1990). PPI is particularly valuable since it is possible to test animal models in methods very similar to that used in humans. Since disruption in PPI is seen in medicated patients as well as at baseline in schizophrenics (Braff et al., 2001), it is perhaps a trait abnormality, which should also be present at baseline in any proposed model of schizophrenia.

At a neurochemical level, neuroimaging data show increased striatal dopamine transmission following amphetamine challenge in patients with schizophrenia. Reduction in the binding potential (BP) of [123I]IBZM or [11C]raclopride indicated an increase in dopamine D2 receptor occupancy induced by amphetamine. As compared to healthy control subjects, schizophrenia patients showed a significant increase in amphetamine-induced reduction of the binding of the radiotracers (Abi-Dargham et al., 1998; Brier et al., 1997; Laruelle et al., 1996). Furthermore, an increase in the BP of [123I]IBZM was observed following depletion of endogenous dopamine with alpha-methyl-para-tyrosine in schizophrenics (Abi-Dargham et al., 2000). These results suggest that a greater portion of D2 receptors in schizophrenics are occupied by dopamine as compared to their controls—both at baseline and after a challenge of a dopamine-releasing agent (Abi-Dargham et al., 1998; Seeman and Kapur, 2000). Thus, an animal model of this illness should try and replicate this endophenotype.

It is well known that repeated exposure to stimulant drugs such as amphetamine induces behavioural and neurochemical sensitization in animals (Robinson and Becker, 1986). The phenomenon is of interest since amphetamine-induced psychosis resembles paranoid schizophrenia and it has been suggested that this model may have relevance for studying neurochemical and behavioural abnormalities associated with the psychotic aspects of schizophrenia (Robinson and Becker, 1986). Sensitization as defined by enhanced release of dopamine and behavioural activation in response to a drug or other challenge is, as is schizophrenia, a chronic and enduring state.

In examining the literature on psychostimulant-induced sensitization, there are a wide variety of regimens (number of injections, dose of drug, length of withdrawal period, etc.) that have been utilized. Although almost all the regimens show a behavioural sensitization, there is variance in the degree to which the dopaminergic system is hyperresponsive (Segal and Kuczenski, 1992; Kolta et al., 1985; Patrick et al., 1991; Kalivas and Stewart, 1991; Paulson and Robinson, 1995). Robinson et al. (1988) suggest that using gradually escalating intermittent doses of
amphetamine and separating the induction phase from challenge phase with a long period of withdrawal may lead to a more intense and robust sensitized state. Thus, we were interested in utilizing the escalating-dosing sensitization model since Robinson et al. (1988) had reported less individual variation as well as an increase in striatal dopamine release in animals sensitized by this regimen.

The aim of these studies was to examine whether rats that were sensitized by utilizing an escalating dose of AMPH regimen would show a sensorimotor gating (disruption of PPI) and neurochemical (greater amphetamine-induced displacement of radio-raclopride binding) abnormality similar to that of schizophrenia.

1. Methods and materials

1.1. Animals

Adult male Sprague–Dawley rats, weighing 200–225 g at the start of the experiment were used. They were housed two per cage with free access to food and water. The housing room was maintained at a constant temperature of 20 ± 2 °C on a 12:12 reverse light/dark cycle. Lights were off at 8:00 AM.

1.2. Locomotor activity testing

The activity boxes were similar to the home cages but were equipped with a row of six photocell beams placed 3 cm above the floor of the cage. A computer that detects the disruption of the photocell beams recorded the number of beam breaks. Prior to beginning any study the animals were allowed to habituate to the activity boxes 1 week before the drug treatment and during the last 2 weeks of the withdrawal phase. There were two habituation sessions before drug treatment and three sessions during withdrawal. The rats were placed in the activity boxes for a period of 2 h.

1.3. Prepulse inhibition

Startle responses were assessed using SR-Lab Startle Response systems (San Diego Instruments, San Diego, CA, USA) as described by Sills (1999). The rats were placed in the startle apparatus and given 10 min to acclimatize to the equipment that had a 65-dB white noise in the background. After this period, subjects were presented with a series of five startle pulse-alone trials to control for habituation of the startle response. This series of stimuli was followed by 64 randomized trials consisting of no pulse (0 dB), a startle pulse (110 dB, 40 ms) or three prepulse intensities (70, 75 and 80 dB, 20 ms) presented alone or 100 ms preceding a startle pulse. Finally, another series of five startle pulse-alone trials were presented. The time between trials ranged from 10 to 20 s. The startle response was measured every 1 ms for a 100-ms period from the onset of the startle stimulus. The percent prepulse inhibition (PPI) was calculated by the following formula: PPI = 100 – (P+S/S)*100. Where P + S is the mean response amplitude for prepulse plus startle pulse trials and S is the mean response amplitude for the startle pulse-alone trials.

1.4. Amphetamine treatment—Experiment IA

This experiment investigated the effects of a sensitizing regimen of amphetamine given over 3 weeks on basal PPI of the acoustic startle reflex, and amphetamine stimulated locomotor activity.

Sixteen animals were randomly divided into two groups with each rat receiving an intraperitoneal injection of either D-amphetamine sulphate (AMPH; Sigma-RBI) or 0.9% saline (SAL; 1 ml/kg). Since the animals were housed in pairs, cage mates received the same drug treatment. Injections were given on Monday, Wednesday and Friday for 3 weeks. AMPH-pretreated rats received a total of nine AMPH injections over 3 weeks. The dose ranged from 1 to 3 mg/kg with an increase of 1 mg/kg each week. Immediately after the injection each rat was returned to its home cage. Following the last injection, the animals were left drug-free for 22 days. The rats were then tested for startle responses and PPI according to the protocol outlined above. The startle and PPI data of one rat (saline-pretreated) were not included since the startle response was very high at all prepulse stimulus intensities tested. The following day, the test for locomotor activity was carried out. Animals were allowed to habituate to the activity boxes for 30 min. Following this period, each rat was injected with saline intraperitoneally and the activity was recorded.
for 1 h. The animals were then injected with 0.5 mg/kg of AMPH intraperitoneally and the activity was measured for an additional 1 h.

1.5. \[^{3}H\]raclopride binding potential in striatum—Experiment IB

This experiment investigated the effects of an acute injection of AMPH on \[^{3}H\]raclopride displacement in AMPH-sensitized rats and their controls. Twenty-four rats were divided into four groups of six. Two groups of rats underwent the same AMPH dosing regimen as the animals in Experiment 1A. The remaining two groups were injected with saline. Twenty-eight days after the last injection, the animals that were AMPH-preexposed received either a SAL or an AMPH (3 mg/kg) challenge. The SAL-preexposed rats were also divided into a SAL- or AMPH-challenged group. Twenty minutes after this injection, the animals were given a tail vein injection of 7.5 \(\mu\)Ci \[^{3}H\]raclopride in 0.4 ml of saline. Thirty minutes after the RAC injection, the animals were sacrificed and the brains quickly dissected. Specific (striatum and nucleus accumbens) and nonspecific (cerebellum) binding was determined by scintillation counting. The percent occupancy of D\(_2\) receptors was calculated as described above in Experiment 1B.

1.6. Amphetamine treatment—Experiment II

In the second set of experiments, 24 rats were used. The AMPH-treated rats were given injections on Monday, Wednesday and Friday for 5 weeks. AMPH-pretreated rats received increasing doses of AMPH for a total of 15 injections over 5 weeks. The dose ranged from 1 to 5 mg/kg with an increase of 1 mg/kg each week. SAL-pretreated rats received injections of saline. Immediately after the injection each rat was returned to its home cage. The rats were tested for startle responses and PPI according to the protocol outlined above. The effect of pretreatment on basal PPI of startle reflex was examined on the 21st, 40th and 60th day after the sensitization regimen.

2. Results

2.1. Experiment IA

2.1.1. Locomotor activity

Animals used in this experiment were preexposed to amphetamine or saline for 3 weeks and tested 22 days after the last injection for their locomotor response to saline and a challenge of 0.5 mg/kg of amphetamine (Fig. 1A). There was no difference between the amphetamine- and saline-pretreated groups during the 30-min habituation period. However, there was a significant difference in total activity counts between the two groups during the 1 h following the saline injection \((t = 3.0, df = 14, p < 0.01, t\text{-test})\)
and during the 1 h following the amphetamine challenge \((t=10.2, df=14, p<0.001, t\text{-test})\) (Fig. 1B). Thus, amphetamine-sensitized rats showed significantly greater locomotor stimulation in response to injection with saline and amphetamine.

2.1.2. Prepulse inhibition of acoustic startle

Pretreatment had no effect on startle response to pulse-alone trials (means ± S.E.M. startle amplitudes were 370.9 ± 42.9 and 319.2 ± 31.1 for SAL- and AMPH-preexposed groups, respectively, \([F(1,13)=0.984, p=0.34])\). Although there was no effect of pretreatment on startle response to prepulse + pulse trials, \([F(1,13)=1.36, p<0.26]\), when the data was expressed as %PPI scores, it revealed significant effects of pretreatment (AMPH vs. SAL) \([F(1,13)=5.10, p=0.042]\) and prepulse intensities (70, 75 and 80 dB) \([F(2,26)=33.7, p<0.001]\), with AMPH-sensitized animals showing lower %PPI. Post hoc comparisons using Tukey’s test revealed that amphetamine pretreatment reduced PPI levels at the prepulse intensity of 70 dB \((p<0.01)\) but the difference at 75 and 80 dB prepulse intensities was not significant (Fig. 2A).
2.2. Experiment IB

2.2.1. $[^3]$Hraclopride binding potential in striatum

The $[^3]$Hraclopride binding potential (BP) was determined in the striata of saline- and amphetamine-treated animals 28 days after the last injection. Those animals that were saline-pretreated and challenged with saline were used as the control group (Fig. 3A). A two-way ANOVA was used to analyze the binding potential. There was a main effect of pretreatment $[F(1,20)=36.24, p<0.001]$ as well as a main effect of the challenge $[F(1,20)=12.40, p<0.05]$. Post hoc comparisons using Tukey’s test showed that when challenged with 3 mg/kg of amphetamine, saline-pretreated rats showed a decrease (12%) in BP but this was not significantly different from controls. On the other hand, AMPH-sensitized rats showed a significant decrease in striatal BP compared to the control group following an acute SAL (24%; $p<0.01$) or AMPH (36%; $p<0.001$) injection.

2.3. Experiment II

2.3.1. Prepulse inhibition of acoustic startle

Pretreatment (AMPH vs. SAL) had no effect on startle response to pulse-alone trials (means ± S.E.M. startle amplitudes were 257.9 ± 30.3 and 233.1 ± 25.3 for SAL- and AMPH-preexposed groups, respectively $[F(1,22)=0.397, p=0.53]$). There was no main effect of pretreatment on startle response to
prepulse + pulse trials \( F(1,22) = 0.60, p = 0.45 \), but there was a significant pretreatment \( \times \) prepulse stimulus intensity interaction \( F(2,44) = 4.37, p = 0.02 \). A post hoc analysis showed the interaction was being driven by a highly significant difference between AMPH vs. SAL at the 70-dB stimulus intensity.

When the data was expressed as %PPI scores, there were significant effects of pretreatment \( F(1,22) = 9.42, p < 0.01 \) as well as pretreatment \( \times \) prepulse stimulus interaction \( F(2,44) = 14.77, p < 0.001 \). Post hoc comparisons using Tukey’s test revealed that AMPH pretreatment reduced PPI levels at the prepulse intensity of 70 dB \( (p < 0.001) \) but the difference at 75 dB \( (p = 0.62) \) and 80 dB \( (p = 1.0) \) prepulse intensities was not significant (Fig. 2B).

Fig. 3. Tritiated raclopride binding potential (BP) in striatum of rats treated for (A) 3 weeks and for (B) 5 weeks. Animals were challenged with either SAL or AMPH (3 mg/kg) 28 days after the last drug injection. Those animals that were SAL-pretreated and challenged with saline (Sal/Sal) served as controls. SAL-pretreated rats showed a slight decrease in BP when challenged with amphetamine (Sal/Amp). AMPH-pretreated rats showed a significant decrease in BP following a saline (Sen/Sal) or amphetamine challenge (Sen/Amp). Values are means \( \pm \) S.E.M. for five to six rats per group \( (* p < 0.01, ** p < 0.001 \text{ vs. control}).) \)

### 2.3.2. [\textsuperscript{3}H]raclopride binding potential in striatum

The [\textsuperscript{3}H]raclopride binding potential in the striata of animals treated with amphetamine for 5 weeks showed similar results as in Experiment IB. A two-way ANOVA on the binding potential revealed a main effect of pretreatment \( F(1,20) = 34.56, p < 0.001 \) as well as a main effect of the challenge \( F(1,20) = 10.64, p < 0.05 \). Post hoc Tukey comparison indicated that saline-pretreated rats, when challenged with 3 mg/kg of amphetamine, showed a decrease \( (~ 14\%) \) in BP but this was not significantly different from the control group. However, the AMPH-sensitized rats showed a significant decrease in BP as compared to controls after SAL \( (25\%; p < 0.01) \) or AMPH injection \( (41\%; p < 0.001) \) (see Fig. 3B).
2.4. Experiment III

2.4.1. Confirmation and duration of PPI abnormality

On completion of Experiments I and II, it was noticed that whereas PPI was markedly disrupted by AMPH in the 5 mg/kg group (either in absolute terms or in terms of %PPI), the effect of the drug was not as robust in the 3 mg/kg group (where %PPI was significantly different, but, absolute startle values did not reach significance). We were concerned that this reflected a lack of power in the 3 mg/kg group as opposed to a qualitative difference. Therefore, we repeated the same 3-week escalating dose of AMPH regimen with a larger sample size (N=16 vs. 8 rats per group) and examined the effect in more detail (i.e. not only 21, but also, after 40 and 60 days of withdrawal).

As before, pretreatment (AMPH vs. SAL) had no effect on startle response to pulse-alone trials on any of the days examined: day 21 (means ± S.E.M. startle amplitudes were 244.8 ± 10.6 and 240.4 ± 8.1 for SAL- and AMPH-preexposed groups, respectively \[ F(1,30) = 0.11, p = 0.75 \]), day 40 (means ± S.E.M. startle amplitudes were 250.9 ± 11.9 and 255.1 ± 13.0 for SAL- and AMPH-preexposed animals, respectively, \[ F(1,30) = 0.055, p = 0.82 \]) or day 60.

Fig. 4. Effect of escalating dose of amphetamine on startle magnitude and prepulse inhibition of acoustic startle after various withdrawal periods.

(A) Mean ± S.E.M. startle magnitude for 21, 40 and 60 days after last injection. Saline-preexposed rats received either startle pulse (Sal/P-alone) or a prepulse stimulus followed by the startle pulse (Sal/P + 70) trials. Amphetamine-preexposed rats received either startle pulse (Amp/P-alone) or a prepulse stimulus followed by the startle pulse (Amp/P + 70) trials. (B) Prepulse inhibition of acoustic startle in rats sensitized to amphetamine. Animals received either saline (SAL) or amphetamine (AMPH) for 3 weeks and tested 21, 40 and 60 days after their last injection. N=16 rats per group (*p < 0.05, **p < 0.001).
(means ± S.E.M. startle amplitudes were 254.5 ± 12.2 and 251.7 ± 11.4 for SAL- and AMPH-preexposed rats, respectively \(F(1,30) = 0.028, p = 0.87\). However, pretreatment (AMPH vs. SAL) had a significant effect on startle response to prepulse + pulse trials, animals sensitized with AMPH showed a higher startle magnitude in prepulse + pulse trials on all the days measured: day 21 \(F(1,30) = 7.45, p < 0.01\); day 40 \(F(1,30) = 4.38, p = 0.045\) and day 60 \(F(1,30) = 4.30, p = 0.047\) (Fig. 4).

When the data was expressed as %PPI scores, they confirmed the previous finding that AMPH sensitization disrupts PPI and that this was most prominent at the 70-dB prepulse intensity. On each of the three withdrawal durations the findings were similar. On day 21 of the withdrawal period, there were significant effects of pretreatment \(F(1,30) = 12.9, p = 0.001\) as well as pretreatment × prepulse stimulus interaction \(F(2,60) = 7.2, p = 0.002\). On withdrawal day 40, there was a main effect of pretreatment \(F(1,30) = 15.6, p < 0.001\) as well as a significant pretreatment × prepulse stimulus interaction \(F(2,60) = 23.3, p < 0.001\) with the difference being most prominent at 70 dB \(p < 0.001, \text{Tukey’s test}\). On withdrawal day 60, there was a main effect of pretreatment \(F(1,30) = 31.5, p < 0.001\) as well as a pretreatment × prepulse stimulus interaction \(F(2,60) = 25.7, p < 0.001\). Post hoc comparisons revealed that AMPH pretreatment significantly reduced PPI scores at the 70-dB level \(p < 0.001\). Again, there were no differences between pretreatment at the other two prepulse intensities.

3. Discussion

The present study shows that amphetamine sensitization disrupts PPI at baseline and this was accompanied by a lower level of raclopride binding to dopamine D2 receptors in the sensitized animals under saline and drug challenge. In addition, the sensitized state model induced sensitization not just to a challenge dose of amphetamine, but also, to an injection of saline—an effect that may reflect a sensitized stress response.

Two previous studies (Druhan et al., 1998; Zhang et al., 1998) have examined PPI disruption after sensitizing regimens of amphetamine. In their study, Druhan et al. (1998) found that amphetamine induced sensitization of locomotor activity but did not disrupt PPI following an amphetamine challenge. Zhang et al. (1998), using similar drug administration procedures but with repeated startle testing, showed sensitization to the disruptive effect of amphetamine on PPI although they did not report PPI in nonchallenged rats. It should be noted that, in our study, we found a decrease in PPI at baseline in the absence of any challenge. In this regard, the sensitized state model, at baseline, is more like patients with schizophrenia (Braff et al., 1978, 2001), as both show PPI impairments without an additional challenge.

A recent report (Russig, 2001) showed a disruption of latent inhibition during withdrawal from a repeated escalating dose of amphetamine regimen. Latent inhibition and PPI are often used as measures of ‘gating deficits’ although they do not represent the same construct: latent inhibition is a model of ‘gating’ of attention through the learning of the nonsignificance of stimuli, whereas PPI is a model of ‘gating’ of a sensorimotor reflex. However, both are reported to be disrupted in schizophrenic patients (Braff et al., 1992; Swerdlow et al., 1994; Gray et al., 1995) and it is intriguing in this regard that both are also abnormal in the amphetamine-sensitized rats.

Recently, Murphy et al. (2001) reported a disruption in latent inhibition in rats treated with an escalating dose of amphetamine. However, they did not observe PPI disruption during any of the withdrawal days tested. Although their sensitization procedures are similar to ours (e.g. dosages and withdrawal times), there were some notable differences. In our study, AMPH injections were administered once daily over several weeks with intermittent drug-free days (refer to Methods and materials). In their study, Murphy et al. (2001) had administered amphetamine three times daily over 6 days. There were no drug-free days during their schedule of escalating dosages. Robinson and Becker (1986) had previously reported that a more intense and robust sensitized state is produced when AMPH is given intermittently. Furthermore, Robinson and Becker (1986) pointed out that when AMPH injections are administered too close together, this was similar to continuous administration of the drug, which could lead to neurotoxicity. In addition, we had used a different strain of rats—Sprague–Dawley vs. Wistars. In a recent review by Geyer et al. (2001), it was shown that there...
are considerably more studies reporting the use of Sprague–Dawley than Wistar rats for testing the effects of amphetamine on PPI. Furthermore, a large percentage of these studies using Sprague–Dawley rats showed amphetamine disrupted PPI. These differences between Murphy et al. (2001) and our study may account for the dissimilar observations.

Previously, Sills (1999) examined the effect of an acute injection of amphetamine at various doses on PPI. The disruptive effects of amphetamine were evident only when the prepulse was just a few decibels above the background noise. Although the precise mechanism for the disruption of PPI is not evident, it is reasonable to propose that the heightened dopaminergic transmission associated with amphetamine-sensitized state may be contributory.

The present study examined the binding of [3H]raclopride to striatal dopamine D2 receptors in the presence and absence of an amphetamine challenge. These experiments were carried out in an effort to try and replicate the abnormalities noted in patients with schizophrenia after an amphetamine challenge, as measured by [11C]raclopride and [123I]IBZM (Laruelle et al., 1996; Brier et al., 1997; Abi-Dargham et al., 1998). Similar to schizophrenic subjects, the sensitized rats showed a lower binding of striatal [3H]raclopride compared to their controls when challenged with amphetamine. In a manner that is dissimilar from schizophrenia, the amphetamine-sensitized rats showed a lower level of [3H]raclopride even when challenged with saline. These findings were replicated in two different cohorts and are likely to be reliable. However, the precise mechanism for this finding is not clear. The binding of [3H]raclopride in vivo represents a dynamic competition between the tracer amounts of the radioligand and endogenous dopamine for the D2 receptors (Laruelle, 2000). An increased release of dopamine in response to a challenge, a decreased number of dopamine receptors or a change in their affinity could, in principle, contribute to the findings observed. Microdialysis studies have shown that amphetamine-sensitized animals show a higher dopamine release to amphetamine (Paulson and Robinson, 1995) as well as a nonpharmacological stressor such as foot shock (Hamamura and Fibiger, 1993). It is possible that the saline injection in sensitized rats was a sufficient stressor to provoke dopamine release. Indeed, the behavioural finding that amphetamine-sensitized rats showed increased locomotor activity in response to a saline injection provides some evidence that amphetamine-sensitized rats are more responsive than nonsensitized rats to this manipulation. Thus, in keeping with the microdialysis findings, the data is likely to represent an alteration in dopamine release, however, the alternative explanations cannot be ruled out and we are currently undertaking in vivo/in vitro experiments to resolve this issue.

We are not aware of any previous efforts in rodents to examine [11C]raclopride or other ligand binding after amphetamine sensitization, however, Castner et al. (2000) found that whereas low doses of amphetamine in rhesus monkeys induced behavioural changes (e.g. fine motor and oral stereotypies, static posturing, etc.), a decrease in the percentage of amphetamine-induced displacement of [123I]IBZM in the striatum was observed. There are several differences between the present study and theirs: differences in techniques (between-group ex vivo radioligand binding vs. within-subject SPECT neuroimaging); species (rodents vs. monkeys); sample size, the doses of amphetamine used and the sensitization regimen and challenge. Rather than speculate on the precise cause for this discrepancy, it may be more prudent to wait until this discrepancy is replicated.

There are of course, several differences between schizophrenia and this sensitized state animal model. Schizophrenia is not induced by repeated psychostimulant injections, though it should be noted that chronic amphetamine abuse does lead to a picture quite indistinguishable from schizophrenia, exhibiting both positive and negative symptoms (Harris and Batki, 2000). Thus, the amphetamine-induced sensitized state model shown here is a model replicating the pathophysiology of schizophrenia, not its etiology. Secondly, there is an increasing consensus that the dopaminergic abnormalities in schizophrenia are not primary, but secondary to a prefrontal or a glutamate dysfunction (Weinberger, 1987; Deutch, 1992; Grace, 1991). It is of interest in this regard that alterations in excitatory amino acids, and hypodopaminergia in the prefrontal cortex have been implicated causally in the development of the amphetamine-induced sensitized state (Wolf, 1998; Karler et al., 1998). As such, the sensitized state model may provide a link between the classical dopamine hypothesis and the more encom-
passing glutamate hypothesis and the prefrontal neuro-developmental hypothesis of schizophrenia.

In summary, after discontinuing treatment with escalating doses of amphetamine, rats in their sensitized state showed a hyperlocomotor response to novelty, a heightened displacement of $[^3]$H]raclopride in response to saline and amphetamine as well as a disruption of PPI. This behavioural and neurochemical findings bear similarity to the pathophysiology of schizophrenia. Given that these findings are likely long-lasting and do not require acute pharmacological challenges to maintain them, the present results indicate that the amphetamine-sensitized state may serve as a valuable pathophysiological model of schizophrenia.

Acknowledgements

The authors would like to thank Karin Korth, Barbara Brownlee, Alex Kecojevic and Suzi Vander-Spek for their technical assistance, and Drs. T.L. Sills and T.E. Robinson for their expert advice. This work was supported in part by the Canadian Institutes of Health Research Chair in Schizophrenia and Therapeutic Neuroscience, as well as operating grants from the CIHR (Canada).

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