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Adolescent amphetamine exposure elicits dose-specific effects on monoaminergic neurotransmission and behaviour in adulthood

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Abstract

Despite the growing non-medical consumption of amphetamine (Amph) during adolescence, its long-term neurobiological and behavioural effects have remained largely unexplored. The present research sought to characterize the behavioural profile and electrophysiological properties of midbrain monoaminergic neurons in adult rodents after Amph exposure during adolescence. Adolescent rats were administered vehicle, 0.5, 1.5, or 5.0 mg/kg.d Amph from postnatal day (PND) 30–50. At adulthood (PND 70), rats were tested in an open-field test (OFT) and elevated plus maze (EPM), paralleled by *in-vivo* extracellular recordings of serotonin (5-HT), dopamine (DA) and norepinephrine (NE) neurons from the dorsal raphe nucleus, ventral tegmental area, and locus coeruleus, respectively. 5-HT firing in adulthood was increased in rats that had received Amph (1.5 mg/kg.d) during adolescence. At this regimen, DA firing activity was increased, but not NE firing. Conversely, the highest Amph dose regimen (5.0 mg/kg.d) enhanced NE firing, but not DA or 5-HT firing rates. In the OFT, Amph (1.5 mg/kg.d) significantly increased the total distance travelled, while the other doses were ineffective. In the EPM, all three Amph doses increased time spent in the open arms and central platform, as well as the number of stretch-attend postures made. Repeated adolescent exposure to Amph differentially augments monoaminergic neuronal firing in a dose-specific fashion in adulthood, with corresponding alterations in locomotion, risk assessment (stretch-attend postures and central platform occupancy) and risk-taking behaviours (open-arm exploration). Thus, adolescent Amph exposure induces long-lasting neurophysiological alterations that may have implications for drug-seeking behaviour in the future.

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Introduction

Amphetamine (Amph) and psychostimulants are among the most widely abused illicit drugs. The estimated percentage of adolescents who ever used Amph is 4.5% in Canada, 10.3% in the USA, and from 1% to 9% in Europe (UNODC, 2010). In the USA, full-time college students who were non-medical users of Amph, compared to non-Amph users, showed a higher intake of other illicit drugs such as marijuana and cocaine as well as higher consumption of alcohol, benzodiazepines and painkillers (SAaMHSAOoA, 2009). The behaviourally activating effects of Amph have long been thought to be due to its actions on the dopamine (DA) system (Creese & Iversen, 1974; Kalivas & Stewart, 1991; Sessions *et al.* 1980; Vanderschuren & Kalivas, 2000), although recent evidence suggests that norepinephrine (NE) and serotonin (5-HT) contribute also (Rothman & Baumann, 2006; Rothman *et al.* 2001). Interestingly, the locus coeruleus (LC), dorsal raphe nucleus (DRN), and ventral tegmental area (VTA), midbrain nuclei that are responsible for the output of NE, 5-HT, and DA, respectively, have been shown to interact with each other in an intricate manner (Guiard *et al.* 2008*b*).

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Therefore, it might be expected that alterations in this complex network might be responsible for the behavioural effects induced by repeated Amph exposure.

Adolescence is a critical developmental period characterized by neurobiological processes that profoundly influence behaviour later in adulthood. Adolescence also represents a period of increased impulsivity, risk taking, and novelty seeking, which further predisposes adolescents to seek out drugs of abuse (Spear, 2000). Indeed, the use of drugs usually begins during this developmental stage, with early use patterns predicting the development of substance use and mood disorders in adulthood (Chambers *et al.* 2003; Chen *et al.* 2009; Laviola *et al.* 1999). Repeated psychostimulant consumption during adolescence might interfere with this developmental neuroplasticity, leading to long-lasting behavioural alterations.

Despite the growing use and misuse of Amph during adolescence, its long-term effects on monoaminergic signalling and emotional responding remain poorly understood. Only a handful of studies have examined the effects of adolescent psychostimulant exposure in adulthood, and in most instances these were following a priming injection of the psychostimulant, thus preventing any evaluation of basal responding in the absence of drug (Caster *et al.* 2005; Estelles *et al.* 2007). Moreover, studies examining the effect of adolescent Amph exposure on monoamine function have been largely restricted to analyses of extracellular release in terminal subfields (Kuczenski & Segal, 1997, 1989; Kuczenski *et al.* 1995) or immediate early gene induction in monoamine cell bodies (McPherson & Lawrence, 2006); no research to date has formally characterized the effect of adolescent Amph exposure on monoaminergic neural firing under psychostimulant-free conditions. Given this, the first objective of the present study was to characterize the long-term effects of repeated adolescent Amph administration on basal 5-HT, DA, and NE firing properties from the DRN, VTA, and LC, respectively. The second objective was to determine whether repeated adolescent Amph exposure significantly alters measures of emotionality and risk assessment in the elevated plus maze (EPM) and open field test (OFT).

Method

Animals

Twelve pregnant Sprague–Dawley female rats (Charles River, Canada) were housed individually in polycarbonate cages and maintained under standard laboratory conditions [12-h light/dark cycle (lights on 07:30 hours), temperature 20 ± 2 °C]. Water and food were available *ad libitum*. All procedures were approved by the McGill University Animal Care Center and the Canadian Office of Controlled Substances. On postnatal day 21 (PND 21), male pups were weaned, and randomly divided into four groups of animals receiving 0.5, 1.5, or 5.0 mg/kg i.p. of d-amphetamine sulfate (Amph; Sigma Aldrich, USA), or vehicle (0.9% saline). Littermates were counterbalanced across groups in an effort to minimize the contribution of litter effects on adult behaviour and electrophysiological correlates (Spear & File, 1996). Amph was prepared (dissolved in 0.9% saline) each day 1 h prior to administration. Injections coincided with the onset of early adolescence in rats (PND 30) and were given daily from PND 30 to PND 50. All groups were then subjected to a drug washout period of 20 d (PND 51–70), after which electrophysiological and behavioural experiments were conducted under drug-free conditions between PND 71 and PND 85 (see Fig. 1 for a scheme of the experimental design). Different cohorts of animals were used for electrophysiological and behavioural experiments to ensure that behavioural testing did not confound electrophysiological recordings.

In-vivo *electrophysiology experiments*

Single-unit *in-vivo* extracellular recordings were conducted in separate groups of animals. Rats were anaesthetized with chloral hydrate (400 mg/kg i.p.) and mounted in a stereotaxic frame with the skull positioned horizontally (incisor bar at -3.3 mm). A burr hole was drilled and the dura was removed. Extracellular recordings were performed using a glass single-barrel micropipette pulled from 2 mm (Narashige, Japan) and filled with 2% Pontamine Sky Blue dye in sodium acetate (0.5 M, pH 7.5). The micropipette tip was broken to a diameter of 1–3 mm. Electrode impedance ranged from 4 to 7 MΩ. A total of 4–6 descents were carried out per animal. Spontaneous activity of 5-HT, DA, and NE neurons were recorded for at least 240 s in order to establish basal firing rates. At the end of each experiment, recording sites were marked by iontophoretic ejection (5–10 μA, negative current for 15 min) of Pontamine Sky Blue for histological verification. The locations of recorded cells within each region were similar across groups. Neuronal signals were recorded and analysed offline using Spike2 software version 5.05 for Windows run on a system with a CED1401 interface (Cambridge Electronic Design, UK).
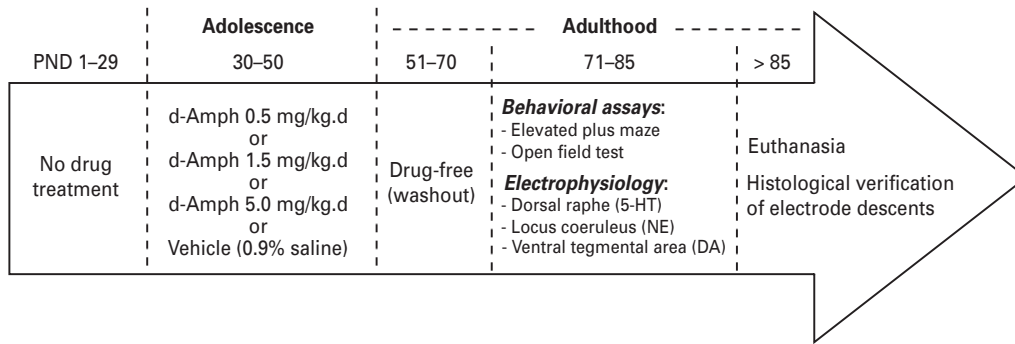
Drug treatment regimen

Fig. 1 . Scheme illustrating the amphetamine (Amph) treatment regimen during adolescence. On postnatal day 21 (PND 21), male pups were weaned and randomly divided into four groups of animals receiving either 0.5 mg/kg, 1.5 mg/kg, or 5.0 mg/kg of d-amphetamine sulfate or the equivalent volume of vehicle (0.9% saline). Injections coincided with the onset of early adolescence in rats (PND 30) and were given daily over a 20-d period (PND 30–50). All groups were then subjected to a withdrawal period of 20 d during which no injections were given (PND 51–70). Electrophysiological and behavioural experiments were conducted between PND 71 and PND 100, corresponding to the period of early adulthood in rats. (Adapted from Bambico *et al.* 2010.)

5-HT recordings. The micropipette was positioned using the following coordinates from lambda (Paxinos & Watson, 1986): AP +1.0–1.2 mm, L±1.0 mm, V 5.0–7.0 mm. The first DRN 5-HT neurons were encountered immediately below an electrically silent zone corresponding to the cerebral Sylvian aqueduct. 5-HT neurons were identified using the following criteria: a slow (0.3–3 Hz) regular firing rate and a broad positive action potential (0.8–3.5 ms; 1.4 ms first positive and negative deflections) (Allers & Sharp, 2003; Baraban & Aghajanian, 1980). Neuronal burst activity was also analysed as the percent of spikes occurring in a burst (ratio of the number of spikes contained in all bursts to the total number of spikes in the entire recording ×100), mean burst length, and mean number of spikes per burst. Based on criteria previously described (Gobbi *et al.* 2005), a burst was defined as a train of at least two spikes with an initial interspike interval (ISI) of <20 ms and a maximum termination ISI of 40 ms, within a regular low-frequency firing pattern.

DA recordings. For VTA recordings, the micropipette was positioned using the following coordinates from bregma (Paxinos & Watson, 1986): AP −6.0 to −5.4 mm, L ±1.0–0.6 mm, V 7.0–9.0 mm. DA neurons were identified according to the following properties: a low and irregular firing rate (0.5–5 Hz) with a characteristic low burst activity, a triphasic action potential with a marked negative deflection, a long duration (>2.5 ms), and a characteristic notch on the rising phase (Grace & Bunney, 1983). A burst was defined according to criteria previously described

(Grace & Bunney, 1983), as a train of at least two spikes with an initial ISI of <80 ms and a maximum termination ISI of 160 ms.

NE recordings. For analysis of LC NE firing activity, the micropipette was positioned according to the following coordinates from lambda (Paxinos & Watson, 1986): AP −1.0 to −1.2 mm, L ±1.0–1.5 mm, V 5.0–7.0 mm. NE neurons were selected on the basis of their regular firing rate (0.5–7.5 Hz) and broad positive action potential (0.8–1.2 ms), often associated with a notch on the rising phase. Moreover, a brief increase of the firing rate characterized by a series of bursts following a contralateral paw pinch was used as a selection criterion (Aghajanian & Vandermaelen, 1982). A burst was defined according to criteria previously described (Guiard *et al.* 2008*b*; Seager *et al.* 2004), as a train of at least two spikes with an initial ISI of <80 ms and a maximum termination ISI of 160 ms.

Behavioural experiments

Open-field test (OFT)

The OFT was performed in an open-field arena (80×80×30 cm) illuminated by a white-light lamp. Behavioural endpoints assessed included distance covered by locomotion and time spent in the peripheral (thigmotaxis) and central quadrants, and frequency of central-area entries. Area entries were defined as whether the rat's centre of gravity, defined by the automated tracking system (localized to the torso) crossed the predefined borders of the central

and peripheral quadrants. All sessions lasted a total of 10 min and data were analysed using an automated behavioural tracking system (Videotrack, ViewPoint Life Sciences Inc., Canada) equipped with infrared sensitive cameras as described previously (Bambico *et al.* 2010).

Elevated plus maze (EPM) test

The EPM apparatus consisted of a black maze with two open arms (50 × 10 cm) opposite each other crossed by two walled (closed) arms (50 × 10 × 40 cm). The maze was raised 80 cm above ground and had a 10 × 10 cm central area forming the intersection of the four arms. All sessions were conducted under white light and lasted for a total of 5 min. Behavioural endpoints assessed included time spent in the open arms, closed arms, central platform, and frequency of open-arm entries and stretch-attend postures (i.e. forward movement and retraction of the body without limb movement). Area entries were determined using the automated tracking system described above and all data were subsequently analysed offline as described previously (Bambico *et al.* 2010).

Statistical analysis

SPSS version 15 (SPSS Inc., USA) was used to analyse data. All data were expressed as mean ± S.E.M. and were analysed using one-way ANOVA followed by Fisher's least significant differences (LSD) test for *post-hoc* comparisons where appropriate. Statistical values reaching $p < 0.05$ were considered significant.

Results

DRN 5-HT firing

A total of 113 5-HT neurons were recorded from the DRN in a group of 18 animals (4–6 rats per group). One-way ANOVA revealed a significant effect of adolescent Amph treatment on the firing rate of DRN 5-HT neurons ($F_{3,109} = 3.132$, $p = 0.029$). *Post-hoc* comparisons revealed that the medium (1.5 mg/kg) dose of Amph significantly increased the firing rate of DRN 5-HT neurons in adulthood compared to vehicle-treated rats ($p = 0.004$) (Fig. 2*a*). There was also a trend towards an enhanced firing rate in the low (0.5 mg/kg) Amph treatment group relative to control ($p = 0.073$) and a non-significant increase in the 5-HT firing rate in the high-dose group ($p = 0.123$) (Fig. 2*a*). There was also trend towards a significant effect of adolescent Amph exposure on the firing rate of non-bursting 5-HT neurons ($F_{3,78} = 2.562$, $p = 0.061$), but no

main effect among the burst-firing population ($F_{3,33} = 0.796$, $p = 0.506$). There was also no effect of adolescent Amph treatment on the mean percent of spikes occurring in a burst ($F_{3,33} = 1.229$, $p = 0.316$), the mean number of spikes per burst ($F_{3,33} = 0.552$, $p = 0.651$), or the mean burst length ($F_{3,33} = 0.236$, $p = 0.870$) (see Table 1).

VTA DA firing

A total of 55 DA neurons were recorded in the VTA in a distinct group of 19 animals (4–5 rats per group). A one-way ANOVA revealed a significant main effect of adolescent Amph treatment on the firing rate of VTA DA neurons in adulthood ($F_{3,52} = 3.050$, $p = 0.037$). *Post-hoc* comparisons further showed that the mean firing rate of VTA DA neurons was significantly enhanced in the group receiving 1.5 mg/kg Amph compared to firing rates in vehicle ($p = 0.018$), 0.5 mg/kg ($p = 0.058$) and 5.0 mg/kg ($p = 0.01$) treatment groups (Fig. 2*b*). Among burst-firing neurons, there was no significant effect of adolescent Amph treatment on the mean percent of spikes occurring in a burst ($F_{3,52} = 0.418$, $p = 0.741$), mean number of spikes per burst ($F_{3,52} = 0.431$, $p = 0.732$), mean burst ISI ($F_{3,52} = 1.081$, $p = 0.366$), or mean burst length ($F_{3,52} = 1.020$, $p = 0.392$) (see Table 1).

LC NE firing

A total of 131 NE neurons were recorded from the LC in a distinct group of 19 animals (4–5 rats per group). A one-way ANOVA revealed a significant main effect of adolescent Amph exposure on the firing rate of LC NE neurons in adulthood ($F_{3,127} = 3.514$, $p = 0.017$). *Post-hoc* comparisons revealed that the mean firing rate in the high-dose Amph group (5.0 mg/kg) was significantly elevated compared to the firing rates of vehicle ($p = 0.006$), 0.5 mg/kg ($p = 0.023$) and 1.5 mg/kg ($p = 0.013$) treatment groups (Fig. 2*c*). A one-way ANOVA revealed that adolescent Amph exposure did not significantly modify the firing rate of single firing LC NE neurons in adulthood ($F_{3,38} = 1.530$, $p = 0.222$). Among LC NE neurons displaying burst activity, there was a main effect of adolescent Amph treatment on the firing rate of this population ($F_{3,85} = 4.605$, $p = 0.005$). *Post-hoc* analyses revealed that the 5.0 mg/kg treatment group exhibited a significantly elevated firing rate relative to control ($p = 0.001$), 0.5 mg/kg ($p = 0.006$), and 1.5 mg/kg ($p = 0.017$) treatment groups. Moreover, there was a main effect of adolescent Amph treatment on the mean percentage of spikes occurring in a burst in this population ($F_{3,85} = 7.844$, $p < 0.001$). *Post-hoc* comparisons again
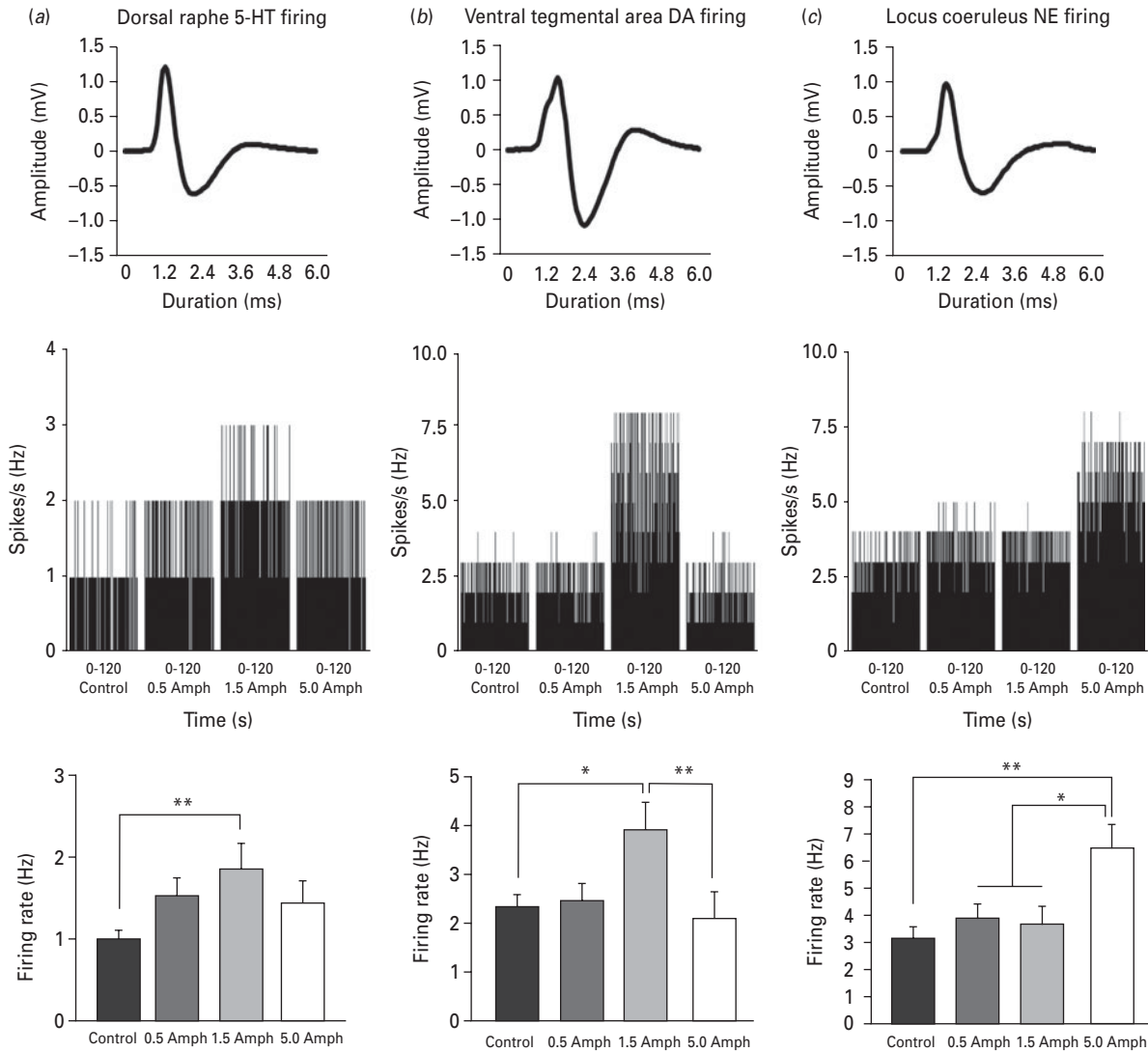
Fig. 2. The effect of repeated adolescent amphetamine (Amph) exposure on the firing activity of midbrain monoaminergic neurons in adulthood. The top row represents a typical waveform for each monoamine cell subtype. The middle row shows a representative interstimulus interval histogram for each monoamine cell subtype according to different treatment groups over a total of 120 s. The bottom row shows the effect of adolescent Amph exposure on the firing rate of each monoamine cell subtype. (*a*) Serotonin (5-HT) neurons exhibit a broad biphasic/triphasic waveform with a slow (0.3–3 Hz) and prominently regular firing rate. The 1.5 mg/kg Amph dose regimen significantly increased the firing rate of 5-HT neurons in the dorsal raphe nucleus (DRN) relative to vehicle-treated rats. (*b*) Dopamine (DA) neurons exhibit a low irregular firing rate (0.5–5 Hz) with a long (>2.5 ms) triphasic waveform characterized by a marked negative deflection and a notch on the rising phase. Repeated adolescent Amph exposure at a dose of 1.5 mg/kg significantly increased the firing rate of DA neurons in the ventral tegmental area (VTA) relative to vehicle-treated and 5.0 mg/kg Amph-treated groups. (*c*) Norepinephrine (NE) neurons also exhibited a regular firing rate (0.5–7.5 Hz) and a broad (0.8–1.2 ms) biphasic/triphasic waveform with a notch on the rising phase and a characteristic burst discharge in response to a nociceptive pinch of the contralateral hind paw. In contrast to 5-HT and DA neuronal populations, only the highest dose of Amph (5.0 mg/kg) during adolescence significantly increased the mean firing rate of NE neurons in the locus coeruleus (LC), and this increase was significant relative to all other Amph- and vehicle-treated groups. All data are presented as the mean ± S.E.M. * $p < 0.05$, ** $p < 0.01$; two-way ANOVA, followed by Fisher's LSD *post-hoc* test.

revealed that 5.0 mg/kg Amph treatment during adolescence significantly increased the percentage of spikes occurring in a burst compared to control ($p = 0.001$), 0.5 mg/kg ($p < 0.001$) and 1.5 mg/kg ($p < 0.001$) treatment groups. There was no significant change in the mean number of spikes per burst

Table 1. Effect of repeated adolescent amphetamine (Amph) administration on burst-firing properties of midbrain monoaminergic nuclei in adulthood

		Control	0.5 mg/kg Amph	1.5 mg/kg Amph	5.0 mg/kg Amph
5-HT (DRN)	% spikes in burst	14.7 ± 3.0	8.8 ± 2.4	5.8 ± 1.9	12.7 ± 3.7
	No. spikes/burst	2.0 ± 0.0	2.2 ± 0.1	2.2 ± 0.2	2.1 ± 0.1
DA (VTA)	% spikes in burst	38.4 ± 8.6	34.8 ± 11.6	28.0 ± 7.4	36.6 ± 8.1
	No. spikes/burst	3.3 ± 0.4	4.0 ± 1.0	3.3 ± 0.7	4.1 ± 0.6
NE (LC)	% spikes in burst	27.5 ± 6.5	24.0 ± 6.6	23.5 ± 8.1	$62.3 \pm 7.1^*$
	No. spikes/burst	4.9 ± 1.3	10.2 ± 5.2	21.4 ± 18.7	226.3 ± 178.2

5-HT, Serotonin; DRN, dorsal raphe nucleus; DA, dopamine, VTA, ventral tegmental area, NE, norepinephrine; LC, locus coeruleus.

* Denotes significance from all other treatment groups at the 0.05 level.

($F_{3,85} = 1.099$, $p = 0.354$) or mean burst length ($F_{3,85} = 1.201$, $p = 0.315$) (see Table 1).

OFT

A total of 29 rats (7–8 rats per group) that received chronic Amph or vehicle injections during adolescence were tested in the OFT under drug-free conditions during adulthood. One-way ANOVA revealed a main effect of drug treatment on the total distance travelled in the open field ($F_{3,25} = 3.109$, $p = 0.044$) (Fig. 3a). *Post-hoc* comparisons further revealed that rats treated with 1.5 mg/kg Amph travelled a significantly greater distance than vehicle ($p = 0.042$) and 5.0 mg/kg Amph ($p = 0.008$) treatment groups, but not compared to the 0.5 mg/kg group ($p = 0.233$). There was no effect of treatment on the distance travelled in the central quadrant of the open field ($F_{3,25} = 1.253$, $p = 0.312$) (Fig. 3b), or the mean number of entries into the central quadrant of the open field ($F_{3,25} = 0.687$, $p = 0.568$) (Fig. 3c). There was also no effect of treatment on the percentage of time spent in the inner quadrant of the open field, a measure of thigmotaxis ($F_{3,25} = 1.033$, $p = 0.394$).

EPM

A total of 31 rats (7–8 rats per group) that received chronic Amph or vehicle injections during adolescence were tested in the EPM under drug-free conditions during adulthood. One-way ANOVA revealed a main effect of adolescent Amph exposure on the percentage of time spent in the open arms ($F_{3,27} = 3.007$, $p = 0.05$). *Post-hoc* analyses further showed that rats administered 0.5 mg/kg and 5.0 mg/kg Amph spent significantly more time in the open arms compared to vehicle-treated rats ($p = 0.03$ and $p = 0.01$, respectively), while rats administered 1.5 mg/kg Amph displayed a modest increase in time spent exploring the open arms

($p = 0.12$) (Fig. 4a). Accordingly, there was also a main effect of adolescent exposure on the percentage of time spent in the closed arms ($F_{3,27} = 7.937$, $p = 0.001$). *Post-hoc* analyses revealed that vehicle-treated rats spent significantly more time in the closed arms relative to all three Amph treatment groups (all $p < 0.005$) (Fig. 4a). Moreover, there was a main effect of adolescent Amph treatment on the percentage of time spent in the central platform of the maze ($F_{3,27} = 3.515$, $p = 0.03$). *Post-hoc* analyses showed that vehicle-treated rats spent significantly less time in the central platform compared to all three Amph treatment groups (all $p < 0.025$) (Fig. 4a). There were no significant differences across groups according to the number of open-arm entries ($F_{3,27} = 2.006$, $p = 0.135$) (Fig. 4b). However, there was a main effect of Amph treatment on the number of stretch-attend postures made in the EPM ($F_{3,27} = 5.065$, $p = 0.005$), such that all three groups treated with Amph during adolescence exhibited an increase in this form of risk assessment relative to vehicle-treated animals (all $p < 0.03$) (Fig. 4c). When locomotor activity in the EPM was compared across treatment groups, there was no effect of treatment on the distance travelled in the closed arms ($F_{3,27} = 0.655$, $p = 0.587$), open arms ($F_{3,27} = 0.788$, $p = 0.511$), central platform ($F_{3,27} = 1.993$, $p = 0.138$), or total overall distance travelled in the EPM ($F_{3,27} = 0.315$, $p = 0.814$).

Discussion

Neurophysiological effects of adolescent Amph exposure

The results described herein are the first to demonstrate that repeated Amph exposure during adolescence has a prolonged impact on the firing rates of midbrain monoaminergic nuclei when tested in adulthood. Moreover, it appears that the different
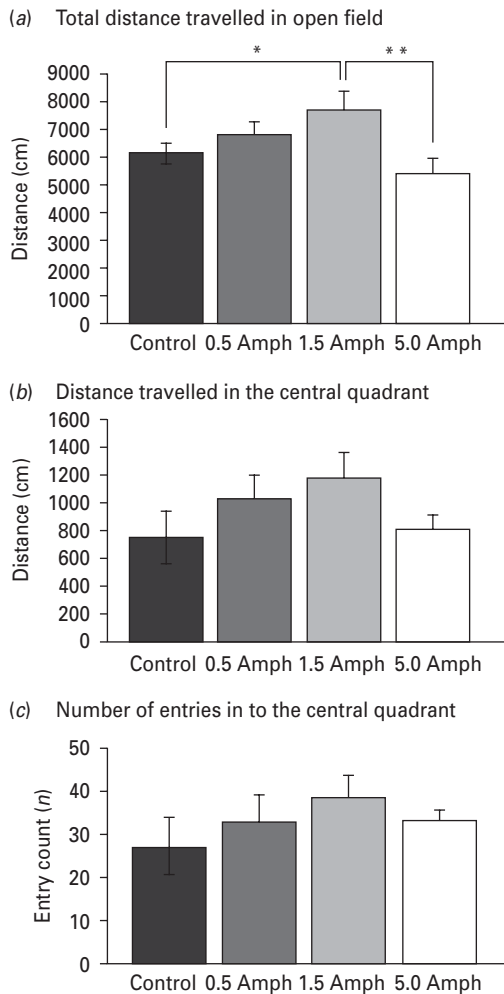
(a) Total distance travelled in open field

(b) Distance travelled in the central quadrant

(c) Number of entries in to the central quadrant

Fig. 3. The effect of repeated adolescent amphetamine (Amph) exposure on behaviours in the open-field test (OFT) in adulthood. (*a*) Adolescent Amph exposure at a moderate dose of 1.5 mg/kg significantly increases the mean distance travelled in the OFT relative to vehicle-treated and 5.0 mg/kg Amph-treated rats when tested in adulthood. (*b*) Amph exposure during adolescence did not substantially modify the mean distance travelled in the centre quadrant of the open field, although visual inspection of the data suggests that rats treated with the intermediate (1.5 mg/kg) dose of Amph exhibit a non-significant elevation in distance travelled in the central quadrant relative to vehicle-treated and 5.0 mg/kg Amph-treated rats. This dose-related pattern closely matches the effect of Amph on the total distance travelled in the OFT depicted in (*a*). (*c*) Similarly, adolescent Amph exposure did not significantly modify the mean number of entries in to the central quadrant of the open field, although there does appear to be a trend of increased central quadrant entries in the group treated with the intermediate (1.5 mg/kg) dose of Amph relative to vehicle-treated and 5.0 mg/kg Amph-treated animals in adulthood. All data are presented as the mean±s.e.m. * $p<0.05$, ** $p<0.01$; two-way ANOVA, followed by Fisher's LSD *post-hoc* test.

Amph dosing regimens have dissociable effects. Whereas adolescent exposure to the low dose of Amph (0.5 mg/kg) had one effect only, modestly increasing DRN 5-HT cell firing rate, the intermediate dose regimen (1.5 mg/kg) markedly increased both 5-HT and VTA DA cell firing rates, and the highest dose (5.0 mg/kg) increased LC NE firing rates with no effect on DA cell firing and only weak effects on 5-HT firing rates.

Although some of these increases may be understood in terms of enduring effects of extended monoamine transporter blockade (Blier & de Montigny, 1994), the distinct combinations of effects may be better accounted for by reciprocal interactions between the monoaminergic systems. For instance, DA projections from the VTA have been shown to exert an excitatory influence on DRN 5-HT firing activity (Guiard *et al.* 2008*b*), while 5-HT projections from the DRN have been shown to either decrease (Guiard *et al.* 2008*b*) or increase VTA firing (Gervais & Rouillard, 2000; Tassin, 2008). The findings from the present study show the presence of a reciprocal firing activity enhancement at the level of DRN and VTA, particularly when rats were administered the moderate (1.5 mg/kg) dose of Amph. This dose resulted in a marked increase in both DRN 5-HT and VTA DA firing in adulthood, suggesting that Amph administration during adolescence may act to enhance positive feedback between these two systems.

NE and 5-HT systems are also mutually regulated by each other. Indeed, the LC and DRN are under a mutual inhibitory/excitatory tone: α_1-adrenergic receptors in the DRN excite 5-HT neurons (Baraban & Aghajanian, 1980; Bortolozzi & Artigas, 2003; Pudovkina *et al.* 2003) and α_2-adrenergic receptors decrease 5-HT release (Haddjeri *et al.* 1997; Pudovkina *et al.* 2003) while 5-HT$_{2A}$ receptors located on GABAergic interneurons in the LC inhibit NE firing (Szabo & Blier, 2002). However, following repeated exposure to psychostimulants, this mutual inhibition vanishes, resulting in an uncoupling of NE and 5-HT systems and a resultant increase in extracellular levels of these monoamines (Salomon *et al.* 2006; see Tassin, 2008 for a review of this literature). This psychostimulant-induced NE/5-HT uncoupling may partially explain the present results, since we also observed elevations in 5-HT firing following moderate doses of Amph and a marked elevation in NE firing following the highest dose of Amph exposure. The precise mechanism responsible for this drug-induced 5-HT/NE uncoupling has yet to be fully elucidated. However, it can be speculated that this may be the consequence of dendritic regression or perhaps a
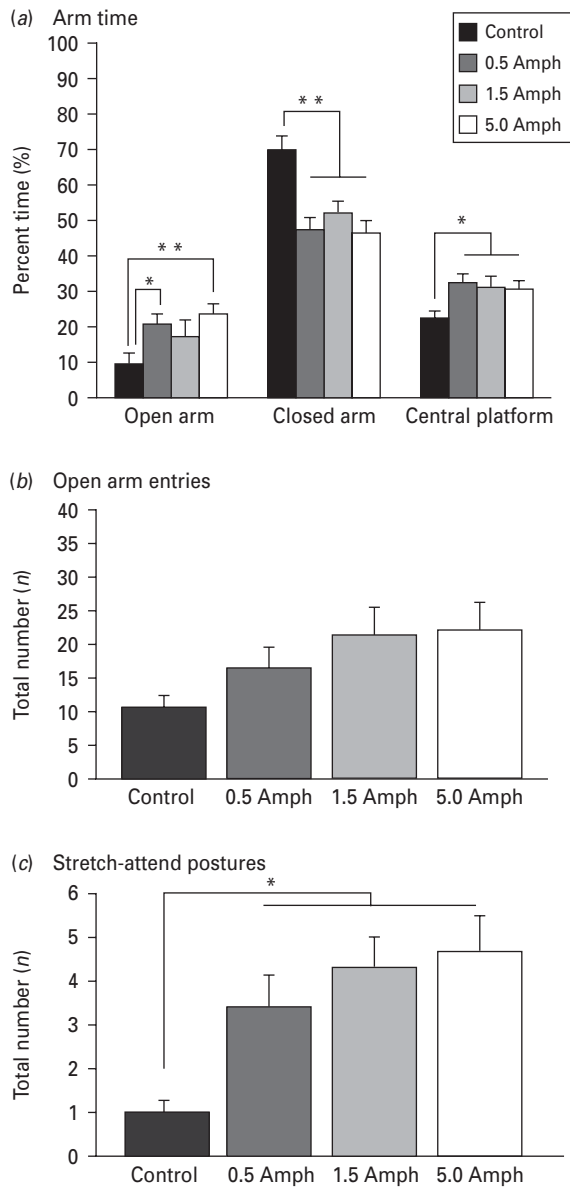
Fig. 4. The effect of repeated adolescent amphetamine (Amph) exposure on behaviours in the elevated plus maze (EPM) in adulthood. (*a*) Adolescent Amph exposure significantly modulates behaviour in the EPM by increasing the percentage of time spent in the open arms, decreasing the percentage of time spent in the closed arms, and increasing the percentage of time spent in the central platform at all three doses of Amph relative to vehicle-treated animals in adulthood. (*b*) Adolescent Amph exposure did not significantly modify the total number of open-arm entries in the EPM in adulthood. (*c*) Adolescent Amph exposure at all three doses significantly increased the total number of stretch-attend postures in the EPM relative to vehicle-treated rats in adulthood, suggestive of enhanced risk assessment in Amph-treated animals. All data are presented as the mean ± S.E.M. * $p < 0.05$, ** $p < 0.01$; two-way ANOVA, followed by Fisher's LSD *post-hoc* test.

delayed maturation of dendrites within the DRN and LC of Amph-exposed adolescent animals, as Amph and other drugs of abuse are known to induce robust morphological modifications within monoaminergic neural circuits (Robinson & Kolb, 2004).

Reciprocal interactions also exist between NE cells in the LC and DA cells in the VTA. For instance, iontophoretic application of NE in the VTA inhibits the activity of DA neurons (Guiard *et al.* 2008*a*). Additionally, enhanced VTA DA firing has been reported in response to a selective pharmacological lesion of the LC (Guiard *et al.* 2008*b*). These findings suggest that NE input from the LC exerts a net suppression on VTA DA firing, which probably contributes to the reduction in VTA DA firing observed in rats treated with the 5.0 mg/kg dose in the present study, as this is the only dose that produced a substantial increase in NE firing. Moreover, this dose-specific enhancement of LC activity is supported by another study reporting increased neuronal activation in the LC of rats treated with a high (10 mg/kg), but not low (2.0 mg/kg) dose of Amph during adolescence (McPherson & Lawrence, 2006). Notably, there was also blunted neuronal activation in the VTA following this high-dose regimen, suggesting that at high doses Amph preferentially activates LC neurons, which is accompanied by a lack of VTA activity.

Given the findings described above, the following hypothetical model can be proposed. Following repeated low to moderate dose administration of Amph during adolescence, there may be a preferential enhancement of DRN 5-HT and VTA DA firing activity in adulthood due to increased positive feedback between these two structures, while the coupling between DRN 5-HT and LC NE systems appears to remain intact. Conversely, following administration of higher doses of Amph, this 5-HT/NE coupling becomes disrupted, leading to the preferential activation of the LC, resulting in a net inhibition of VTA DA firing. Thus, the electrophysiological findings from the present study are in line with previous research, and suggest that dysregulation of reciprocal monoamine interactions may be at the crux of the long-term neurobiological alterations induced by Amph exposure during adolescence. In turn, these disturbances may be relevant for deficits in reward processing and emotional behaviour that have been observed following chronic adolescent psychostimulant use (Carlezon *et al.* 2003; Mague *et al.* 2005).

Behavioural effects of adolescent Amph exposure

When the effects of adolescent Amph exposure on locomotor behaviours in the OFT were examined in

adulthood, a similar dose-specific pattern emerged. Rats that were administered low doses of Amph displayed a modest, non-significant increase in the total distance travelled in the open field, while rats treated with the moderate Amph dose regimen showed a robust increase in this measure. On the other hand, rats treated with high doses of Amph during adolescence displayed a significant reduction in the total distance travelled in the OFT relative to rats treated with the moderate dose of Amph. Thus, only moderate doses of Amph during adolescence appear to substantially augment locomotor activity in the OFT in adulthood. However, since adolescent Amph exposure failed to augment the distance travelled and percentage of time spent in the inner quadrant of the open field, it does not appear that these Amph treatment regimens specifically alter exploration of an anxiogenic environment.

Given the congruency between VTA and DRN firing activity (Gervais & Rouillard, 2000; Guiard *et al.* 2008*b*), and the known role of these systems in exploration and novelty seeking (Legault & Wise, 2001; Muller *et al.* 2007), it is tempting to speculate that the hyperlocomotion reported in the present study may be primarily mediated by the increase in DA and/or 5-HT firing rates. When higher doses of Amph were administered, this hyperlocomotor response was nullified, perhaps due to increased LC-mediated inhibition of VTA DA activity. In support of a role for DA, a recent study has shown that rats that had self-administered either cocaine or methylphenidate during adolescence displayed increased Amph-induced locomotion and neuronal activation within the nucleus accumbens (Burton *et al.* 2010). Moreover, acute fluoxetine administration, resulting in increased 5-HT function, has been shown to potentiate the hyperlocomotor effects induced by 0.5–1.0 mg/kg Amph, as well as the increased DA overflow in the nucleus accumbens of Amph-treated rodents (Sills *et al.* 1999). Hence, the dose-specific stimulatory effect of adolescent Amph administration on locomotor activity in the OFT in adulthood is probably influenced by feedforward activation between DA and 5-HT systems, which is in line with the effects of Amph on electrophysiological properties in the VTA and DRN described above.

In contrast to the effects observed in the OFT, all three adolescent Amph dosing regimens effectively decreased the percentage of time spent exploring the closed arms and increased time spent in the open arms of the maze, which is typically suggestive of an anxiolytic response. This finding is in agreement with a recent report demonstrating that an acute injection of methamphetamine increases the percentage of time spent in the open areas of the elevated zero maze in adult mice (Acevedo & Raber, 2011). However, these results are largely contrary to the majority of studies examining the effect of chronic Amph administration during adulthood, which typically depict an anxiogenic profile (Biala & Kruk, 2007; Cancela *et al.* 2001; Vuong *et al.* 2010). Moreover, studies to date examining the effect of adolescent psychostimulant exposure on anxiety-like behaviours in adulthood are scarce and largely equivocal (Estelles *et al.* 2007; Santucci & Rosario, 2010).

Although it is possible that adolescent Amph administration reduces anxiety-like behaviour in adulthood, perhaps a more parsimonious explanation is that Amph is inducing alterations in the neural circuitry subserving motor impulsivity, risk assessment, and risk taking. For instance, in addition to increased open-arm exploration, all three Amph doses increased the percentage of time spent in the central platform, a neutral area where open and closed arms intersect, and also increased the number of stretch-attend postures made. Previous studies have interpreted these responses in the EPM to be reliable indexes of risk-taking behaviour (Cortese *et al.* 2010; Toledo-Rodriguez & Sandi, 2011; van Heerden *et al.* 2010; Weiss *et al.* 1998). Moreover, open-arm exploration has been shown to correspond to risk-taking behaviour when the latency to approach an appetitive reinforcer is examined in the presence of an aversive stimulus such as predator odour (Roybal *et al.* 2007). Therefore, it could be argued that exploration of the open arms is a form of risky behaviour, and thus adolescent Amph exposure may be augmenting motor impulsivity and consequently disinhibiting exploration of the aversive environment in adulthood (i.e. hastening the transition from risk 'assessment' in the neutral central platform to risk 'taking' in the aversive open arms). Moreover, it should be noted that the increase in time spent in the open arms following chronic adolescent Amph administration is not simply due to increased locomotor activity, as the distance travelled in the EPM was not significantly different across groups. Increased risk taking is also a known behavioural correlate of chronic exposure to drugs of abuse (Pattij & Vanderschuren, 2008), and is known to be particularly susceptible to alterations in 5-HT functionality (Fletcher *et al.* 2007). Thus, this increased propensity to engage in risky behaviour following adolescent Amph exposure may also manifest as increased drug seeking and vulnerability to addiction in adulthood. This is especially relevant given that adolescence is a highly sensitive and plastic period characterized by

extensive remodelling within neuronal networks implicated in behavioural inhibition and, moreover, is particularly susceptible to drug-induced modifications.

Concluding remarks

The goal of the present study was to characterize the effects of different doses of chronic Amph during adolescence on monoaminergic activity and behavioural responding in adulthood. These results suggest that different doses of Amph have dissociable effects on monoaminergic systems in adulthood. Low to moderate dose Amph treatment was shown to preferentially augment DRN 5-HT and VTA DA neuronal firing in adulthood, possibly via potentiated positive feedback between these two structures. In contrast, a higher dose of Amph enhanced LC NE firing only, which could be a consequence of impaired mutual inhibition between the 5-HT and NE systems, leading to enhanced LC-mediated suppression of VTA DA firing. Although the theoretical framework described herein is speculative, congruent findings from the literature do provide initial support for this model. However, additional research is needed to validate this framework and further clarify the long-term effects of chronic adolescent Amph exposure on midbrain monoamine systems.

In addition to these neurophysiological effects, chronic adolescent Amph also induced a hyperlocomotor response in the OFT, but only in rats treated with the 1.5 mg/kg dose. This dose-specific effect is in agreement with our finding that VTA DA firing is also increased with this dose, but remains largely unaffected by low and high Amph doses. Last, all three Amph doses increased open-arm exploration in the EPM, which was accompanied by an increase in time spent in the central platform of the maze as well as the number of stretch-attend postures made, suggesting that adolescent Amph exposure engages more risk assessment and risk taking in the EPM in adulthood, regardless of the dose administered. Collectively, these findings raise the possibility that Amph may be promoting hyperlocomotion as documented in the OFT, thus increasing the propensity to engage in risky behaviours, such as exploring the open arms of the EPM. Indeed, alterations in impulsivity and response inhibition are core symptoms of prolonged Amph use that perpetuates further drug seeking and addiction, and may be affected more so in adolescence given the considerable synaptic plasticity and remodelling occurring during this sensitive developmental stage.

Statement of Interest

None.

References

Acevedo SF, Raber J (2011). Histamine-dependent behavioral response to methamphetamine in 12-month-old male mice. *Brain Research* **1393**, 23–30.

Aghajanian GK, Vandermaelen CP (1982). Intracellular identification of central noradrenergic and serotonergic neurons by a new double labeling procedure. *Journal of Neuroscience* **2**, 1786–1792.

Allers KA, Sharp T (2003). Neurochemical and anatomical identification of fast- and slow-firing neurones in the rat dorsal raphe nucleus using juxtacellular labelling methods *in vivo*. *Neuroscience* **122**, 193–204.

Bambico FR, Nguyen NT, Katz N, Gobbi G (2010). Chronic exposure to cannabinoids during adolescence but not during adulthood impairs emotional behaviour and monoaminergic neurotransmission. *Neurobiology of Disease* **37**, 641–655.

Baraban JM, Aghajanian GK (1980). Suppression of serotonergic neuronal firing by alpha-adrenoceptor antagonists: evidence against GABA mediation. *European Journal of Pharmacology* **66**, 287–294.

Biała G, Kruk M (2007). Amphetamine-induced anxiety-related behavior in animal models. *Pharmacological Reports* **59**, 636–644.

Blier P, de Montigny C (1994). Current advances and trends in the treatment of depression. *Trends in Pharmacological Sciences* **15**, 220–226.

Bortolozzi A, Artigas F (2003). Control of 5-hydroxytryptamine release in the dorsal raphe nucleus by the noradrenergic system in rat brain. Role of alpha-adrenoceptors. *Neuropsychopharmacology* **28**, 421–434.

Burton CL, Nobrega JN, Fletcher PJ (2010). The effects of adolescent methylphenidate self-administration on responding for a conditioned reward,

amphetamine-induced locomotor activity, and neuronal activation. *Psychopharmacology* (*Berlin*) **208**, 455–468.

Cancela LM, Basso AM, Martijena ID, Capriles NR, *et al.* (2001). A dopaminergic mechanism is involved in the 'anxiogenic-like' response induced by chronic amphetamine treatment: a behavioral and neurochemical study. *Brain Research* **909**, 179–186.

Carlezon Jr. WA, Mague SD, Andersen SL (2003). Enduring behavioral effects of early exposure to methylphenidate in rats. *Biological Psychiatry* **54**, 1330–1337.

Caster JM, Walker QD, Kuhn CM (2005). Enhanced behavioral response to repeated-dose cocaine in adolescent rats. *Psychopharmacology* (*Berlin*) **183**, 218–225.

Chambers RA, Taylor JR, Potenza MN (2003). Developmental neurocircuitry of motivation in adolescence: a critical period of addiction vulnerability. *American Journal of Psychiatry* **160**, 1041–1052.

Chen CY, Storr CL, Anthony JC (2009). Early-onset drug use and risk for drug dependence problems. *Addictive Behaviors* **34**, 319–322.

Cortese BM, Mitchell TR, Galloway MP, Prevost KE, *et al.* (2010). Region-specific alteration in brain glutamate: possible relationship to risk-taking behavior. *Physiology and Behavior* **99**, 445–450.

Creese I, Iversen SD (1974). The role of forebrain dopamine systems in amphetamine induced stereotyped behavior in the rat. *Psychopharmacologia* **39**, 345–357.

Estelles J, Lluch J, Rodriguez-Arias M, Aguilar MA, *et al.* (2007). Cocaine exposure during adolescence affects anxiety in adult mice. *Brain Research Bulletin* **71**, 393–403.

Fletcher PJ, Tampakeras M, Sinyard J, Higgins GA (2007). Opposing effects of 5-HT(2A) and 5-HT(2C) receptor antagonists in the rat and mouse on premature responding in the five-choice serial reaction time test. *Psychopharmacology* (*Berlin*) **195**, 223–234.

Gervais J, Rouillard C (2000). Dorsal raphe stimulation differentially modulates dopaminergic neurons in the ventral tegmental area and substantia nigra. *Synapse* **35**, 281–291.

Gobbi G, Bambico FR, Mangieri R, Bortolato M, *et al.* (2005). Antidepressant-like activity and modulation of brain monoaminergic transmission by blockade of anandamide hydrolysis. *Proceedings of the National Academy of Sciences USA* **102**, 18620–18625.

Grace AA, Bunney BS (1983). Intracellular and extracellular electrophysiology of nigral dopaminergic neurons – 1. Identification and characterization. *Neuroscience* **10**, 301–315.

Guiard BP, El Mansari M, Blier P (2008*a*). Cross-talk between dopaminergic and noradrenergic systems in the rat ventral tegmental area, locus ceruleus, and dorsal hippocampus. *Molecular Pharmacology* **74**, 1463–1475.

Guiard BP, El Mansari M, Merali Z, Blier P (2008*b*). Functional interactions between dopamine, serotonin and norepinephrine neurons: an *in-vivo* electrophysiological study in rats with monoaminergic lesions. *International Journal of Neuropsychopharmacology* **11**, 625–639.

Haddjeri N, Blier P, de Montigny C (1997). Effects of long-term treatment with the alpha 2-adrenoceptor antagonist mirtazapine on 5-HT neurotransmission. *Naunyn Schmiedebergs Archives of Pharmacology* **355**, 20–29.

Kalivas PW, Stewart J (1991). Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. *Brain Research. Brain Research Reviews* **16**, 223–244.

Kuczenski R, Segal D (1989). Concomitant characterization of behavioral and striatal neurotransmitter response to amphetamine using *in vivo* microdialysis. *Journal of Neuroscience* **9**, 2051–2065.

Kuczenski R, Segal DS (1997). Effects of methylphenidate on extracellular dopamine, serotonin, and norepinephrine: comparison with amphetamine. *Journal of Neurochemistry* **68**, 2032–2037.

Kuczenski R, Segal DS, Cho AK, Melega W (1995). Hippocampus norepinephrine, caudate dopamine and serotonin, and behavioral responses to the stereoisomers of amphetamine and methamphetamine. *Journal of Neuroscience* **15**, 1308–1317.

Laviola G, Adriani W, Terranova ML, Gerra G (1999). Psychobiological risk factors for vulnerability to psychostimulants in human adolescents and animal models. *Neuroscience and Biobehavioral Reviews* **23**, 993–1010.

Legault M, Wise RA (2001). Novelty-evoked elevations of nucleus accumbens dopamine: dependence on impulse flow from the ventral subiculum and glutamatergic neurotransmission in the ventral tegmental area. *European Journal of Neuroscience* **13**, 819–828.

Mague SD, Andersen SL, Carlezon Jr. WA (2005). Early developmental exposure to methylphenidate reduces cocaine-induced potentiation of brain stimulation reward in rats. *Biological Psychiatry* **57**, 120–125.

McPherson CS, Lawrence AJ (2006). Exposure to amphetamine in rats during periadolescence establishes behavioural and extrastriatal neural sensitization in adulthood. *International Journal of Neuropsychopharmacology* **9**, 377–392.

Muller CP, Carey RJ, Huston JP, De Souza Silva MA (2007). Serotonin and psychostimulant addiction: focus on 5-HT1A-receptors. *Progress in Neurobiology* **81**, 133–178.

Pattij T, Vanderschuren LJ (2008). The neuropharmacology of impulsive behaviour. *Trends in Pharmacological Sciences* **29**, 192–199.

Paxinos G, Watson C (1986). *The Rat Brain in Stereotaxic Coordinates*, 2nd edn. San Diego, CA, USA: Academic Press Inc.

Pudovkina OL, Cremers TI, Westerink BH (2003). Regulation of the release of serotonin in the dorsal raphe nucleus by alpha1 and alpha2 adrenoceptors. *Synapse* **50**, 77–82.

Robinson TE, Kolb B (2004). Structural plasticity associated with exposure to drugs of abuse. *Neuropharmacology* **47** (Suppl. 1), 33–46.

Rothman RB, Baumann MH (2006). Balance between dopamine and serotonin release modulates behavioral

effects of amphetamine-type drugs. *Annals of the New York Academy of Sciences* **1074**, 245–260.

Rothman RB, Baumann MH, Dersch CM, Romero DV, *et al.* (2001). Amphetamine-type central nervous system stimulants release norepinephrine more potently than they release dopamine and serotonin. *Synapse* **39**, 32–41.

Roybal K, Theobold D, Graham A, DiNieri JA, *et al.* (2007). Mania-like behavior induced by disruption of CLOCK. *Proceedings of the National Academy of Sciences USA* **104**, 6406–6411.

Salomon L, Lanteri C, Glowinski J, Tassin JP (2006). Behavioral sensitization to amphetamine results from an uncoupling between noradrenergic and serotonergic neurons. *Proceedings of the National Academy of Sciences USA* **103**, 7476–7481.

Santucci AC, Rosario A (2010). Anxiety-like responses in adolescent rats following a 10–11-day withdrawal period from repeated cocaine administration. *Brain Research Bulletin* **81**, 441–444.

Seager MA, Huff KD, Barth VN, Phebus LA, *et al.* (2004). Fluoxetine administration potentiates the effect of olanzapine on locus coeruleus neuronal activity. *Biological Psychiatry* **55**, 1103–1109.

Sessions GR, Meyerhoff JL, Kant GJ, Koob GF (1980). Effects of lesions of the ventral medial tegmentum on locomotor activity, biogenic amines and responses to amphetamine in rats. *Pharmacology, Biochemistry and Behavior* **12**, 603–608.

Sills TL, Greenshaw AJ, Baker GB, Fletcher PJ (1999). Acute fluoxetine treatment potentiates amphetamine hyperactivity and amphetamine-induced nucleus accumbens dopamine release: possible pharmacokinetic interaction. *Psychopharmacology* (*Berlin*) **141**, 421–427.

Spear LP (2000). The adolescent brain and age-related behavioral manifestations. *Neuroscience and Biobehavioral Reviews* **24**, 417–463.

Spear LP, File SE (1996). Methodological considerations in neurobehavioral teratology. *Pharmacology, Biochemistry and Behavior* **55**, 455–457.

SAaMHSAOoA (2009). The NSDUH Report: Nonmedical use of Adderall® among full-time college students. Substance Abuse and Mental Health Services Administration, Office of Applied Studies, Rockville, MD.

Szabo ST, Blier P (2002). Effects of serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibition plus 5-HT(2A) receptor antagonism on the firing activity of norepinephrine neurons. *Journal of Pharmacology and Experimental Therapeutics* **302**, 983–991.

Tassin JP (2008). Uncoupling between noradrenergic and serotonergic neurons as a molecular basis of stable changes in behavior induced by repeated drugs of abuse. *Biochemical Pharmacology* **75**, 85–97.

Toledo-Rodriguez M, Sandi C (2011). Stress during adolescence increases novelty seeking and risk-taking behavior in male and female rats. *Frontiers in Behavioral Neuroscience* **5**, 1–10.

UNODC (2010). World drug report (http://www.unodc.org/documents/data-and-analysis/WDR2010/Youth-school-amphetamines.pdf). United Nations Office on Drugs and Crime. Accessed 19 May 2011.

Weiss SM, Wadsworth G, Fletcher A, Dourish CT (1998). Utility of ethological analysis to overcome locomotor confounds in elevated maze models of anxiety. *Neuroscience and Biobehavioral Reviews* **23**, 265–271.

van Heerden JH, Russell V, Korff A, Stein DJ, *et al.* (2010). Evaluating the behavioural consequences of early maternal separation in adult C57BL/6 mice; the importance of time. *Behavioral Brain Research* **207**, 332–342.

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* (*Berlin*) **151**, 99–120.

Vuong SM, Oliver HA, Scholl JL, Oliver KM, *et al.* (2010). Increased anxiety-like behavior of rats during amphetamine withdrawal is reversed by CRF2 receptor antagonism. *Behavioral Brain Research* **208**, 278–281.