Age-dependent effects of methylphenidate in the prefrontal cortex: evidence from electrophysiological and Arc gene expression measurements

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Abstract
Methylphenidate, a drug widely used for attention deficit hyperactivity disorder in children, may affect neuronal function differently in young and adult subjects, particularly in the prefrontal cortex, a brain structure that does not fully develop until adulthood. We compared the impact of development on the effects of methylphenidate on single unit electrical activity and mRNA expression of the effector immediate gene activity-regulated cytoskeletal-associated protein (Arc) following methylphenidate in the prefrontal cortex in adult (more than 60 days old) and juvenile (25–35 days old) rats. Methylphenidate, administered under urethane anaesthesia to adult rats, at doses ranging from 1 mg/kg to 3 mg/kg intravenously, exerts a progressive activation of firing of prefrontal cortex neurones (30% to 84% from baseline). This activation was significantly lower in the juvenile rats, reaching only 37% of baseline levels at the highest dose (3 mg/kg, intravenous). In adults, methylphenidate (4 mg/kg intraperitoneal) produced marked increases in Arc mRNA levels compared with saline controls by 123% and 164% in cingulated and orbital cortex, respectively. Corresponding values for the juvenile rats were significantly lower (42% and 79%). In summary, this multi-approach investigation showed that the reactivity of prefrontal cortex neurones to methylphenidate differs markedly in juvenile and adult rats.

Keywords
Arc gene expression, juvenile rats, methylphenidate, prefrontal cortex, single unit extracellular recording

Introduction
Attention deficit hyperactivity disorder (ADHD) is the most commonly diagnosed psychiatric disorder in children and adolescents (with a prevalence of 3–7%). It is characterized by hyperactivity and deficits in sustained attention and impulsivity. Paradoxically, psychostimulants such as methylphenidate (MPH) are used as first line treatment for this disorder and prescriptions have increased dramatically during the last decade. Evidence from brain imaging studies indicates that structural abnormalities in the prefrontal cortex (PFC) and associated subcortical structures are consistently observed in ADHD (Kieling et al., 2008). The PFC is considered as one of the main brain regions involved in the behavioural-calming and cognition-enhancing effects of MPH (Arnsten, 2006). It plays a critical role in the control of higher cognitive function such as vigilance, attention, impulsivity and behavioural inhibition (Arnsten, 2006; Kieling et al., 2008). MPH potently blocks the synaptic reuptake of both dopamine and noradrenaline, resulting in increased levels of the two catecholamines in the extracellular space. Interestingly, this action is particularly pronounced in the PFC following therapeutic doses of the drug (Berridge et al., 2006).

MPH treatment has a powerful calming action in both ADHD children and healthy children (Rapoport and Inoff-Germain, 2002). At present, little is known about the mechanisms underlying this calming effect of psychostimulant drugs in children. The PFC is a late maturing part of the brain and several neurotransmitter systems in the PFC, including the monoamines, are known to undergo profound development during the postnatal period (Benes et al., 2000; Berridge et al., 2008; Murrin et al., 2007; Spear, 2000).

Animal studies show clear age-dependent effects of psychostimulants. Drug-induced locomotion is substantially more pronounced in adult rats compared with juveniles (Banerjee et al., 2009). In addition, juvenile and adolescent rats, unlike adult rats, do not develop behavioural sensitization following chronic exposure to psychostimulants, an effect that is prevented by PFC lesions in adults (Andersen et al., 2002; Lee et al., 2008).

MPH may have different effects on PFC neuronal function in juvenile and adult rats. Modification of the response of PFC neurones to MPH as a function of age may reflect critical developmental modifications occurring in the PFC and particularly catecholaminergic transmission, which reaches maturity later in the PFC (Murrin et al., 2007). Further evidence of differential effects by MPH in juvenile and adult rats is the recent findings that the drug induces strikingly different actions on gene expression throughout the rat brain (Banerjee et al., 2009). In the present study, we examined in vivo the impact of development on two types of functional response of...
PFC neurones to MPH: the baseline electrical extracellular activity of prefrontal cortex neurones and the expression of the effector immediate early gene (IEG) activity-regulated cytoskeletal-associated protein (Arc), a key regulator of neuronal plasticity and a marker of neuronal activity. We recorded single cell firing activity and assessed gene expression of Arc following MPH in two different postnatal age groups: 25–35 days old (P25, juveniles), and more than 60 days old (P60, adults).

Material and methods

Subjects

Experiments were performed on juvenile (postnatal day [PND] 25–35, 90–120 g) and adult (PND >60, 260–350 g) male Sprague-Dawley rats (Charles River, UK) were housed (2–6/group) in separate age-matched groups, at constant temperature and humidity under a 24 h light/dark cycle (lights on from 6 a.m. to 6 p.m.) with free access to food and water. All experiments were carried out in accordance with the UK Animals Scientific Procedures Act (1986) and Home Office guidelines.

Following acclimatization to our animal facilities (3–7 days), separate groups of rats were used in electrophysiological or in situ hybridization type experiments.

Electrophysiological experiments

In vivo extracellular recordings were performed in both juvenile and young adult rats. Animals were initially deeply anaesthetized with urethane (1.2–1.7 g/kg intraperitoneal [i.p.]), with additional doses administered if necessary), secured to a stereotaxic frame and maintained at 36–37°C with a heating pad. An incision was made across the top of the head and the edges of the skin drawn back to reveal the cranium. Bregma was marked and a hole was drilled through the bone at the coordinates of the prefrontal cortex according to the atlas of Paxinos and Watson (1997).

Electrodes were manufactured in house from borosilicate capillaries (1.5 mm, Harvard Apparatus Ltd, UK) pulled on a PP-830 electrode puller (Narishige, Japan) and filled by hand with an electrolyte solution (in mM: NaCl 147, KCl 4, adjusted to pH 6). The tip of the electrode was broken down with an electrolyte solution (in mM: NaCl 147, KCl 4, adjusted to pH 6). The tip of the electrode was broken down under microscope to an external diameter of 1–1.5 μm. Typical resistance was in the range 4–8 MegOhm. Outputs from the electrode were sent to a Neurolog AC pre-amplifier and amplifiers (Digitimer, UK). Signals were filtered and sent to an audio amplifier, a Tektronix 2201 digital storage oscilloscope, and a 1401 interface connected to a computer running Spike 2 (CED, UK) for data capture and analysis. Descent of the electrode was carried out using a hydraulic micromanipulator (Narishige) in the prefrontal cortex at anteroposterior 2.5–3.5 mm, lateral 0.3–1 mm, dorsoventral 1.5–4 mm below the cortical surface.

According to previous criteria (Tseng and O’Donnell, 2007; Tseng et al., 2006), neurones in the PFC can be considered as pyramidal neurones if they exhibit action potential duration of >1 ms. Neurones with shorter action potential duration (<0.75 ms) can be considered as interneurones and were not included in the present study. More than 90% of the neurones tested had large action potential (>2 mV) starting with a positive inflection of relatively long duration (1.1–2 ms (Figure 2(a)) and a firing mode either in a single spike or burst. A minority of the neurones tested had lower amplitude action potentials (approximately 1 mV), but also of relatively long duration (>1.1 ms) starting with a negative inflection, and nearly always firing in burst. MPH (Sigma) was administered intravenously (i.v.) 7–15 min after stable recording. Three doses of 1 mg/kg were subsequently administered generally at 7–10 min intervals. In addition, the effect of a lower dose of MPH (0.3 mg/kg, i.v.) was also tested in another group of adult rats (n = 10).

Arc expression study

For measurements of Arc expression groups of adult and juvenile rats were administered with 4 mg/kg MPH. MPH (obtained from Sigma, UK) was dissolved in saline and corresponding age-matched control groups were injected with saline alone (1 ml/kg, i.p.). Rats were killed by rapid dislocation of the neck 2 h post-drug, and the brains were removed, frozen in isopentane and stored in −80°C prior to sectioning using a cryostat.

In situ hybridization

Brain sections (20 μm) were thaw-mounted on to pre-cleaned, ready to use super-frost glass slides (Menzel GmbH & Co. KG, Braunschweig, Germany) and pre-treated using a standard protocol as described previously (Pei et al., 1996). An oligonucleotide (5’TCT GTT GCC CCA TCC TCA CCT GCC ACC CAA GAC TGG TAT TGC TGA 3’) complementary to bases 789–833 of rat Arc cDNA (Genbank accession number: NM019361.1) were used in the experiments. The oligonucleotide was 3’-tail labelled with 35S-dATP by terminal deoxynucleotide transferase and the labelled probe (specific activity >106 cpm/μg) was added to each section (2 x 106 cpm/section) in hybridization buffer as previously described (Pei et al., 1996). After incubation in humidity chambers (containing 50% formamide in 4 x saline-sodium citrate (SSC) at 36°C for 14–16 h, slides were washed in 1 x SSC buffer at 55°C for 3 x 20 min followed by 2 x 60 min at room temperature, and then air-dried and exposed to autoradiography film ( Biomax, Amersham, UK) for 5–7 days at room temperature. Controls included the use of oligonucleotides in the sense orientation and displacement with unlabelled probes. Searches with the Genbank database using the BLAST program revealed no significant homology of the nucleotide sequences with other previously characterized rodent genes.

The relative abundance of mRNA in areas of interest was determined by densitometric quantification of signals measured from autoradiograms using MCID™ core autoradiography software (InterFocus Imaging Ltd, Cambridge, UK). Optical density values were calibrated to 35S tissue equivalents using 14C microscales. After establishing standard curves, density values were obtained from PFC regions of measurable Arc mRNA distribution in saline-treated control animals (i.e. cingulate cortex and orbital cortex).
Densitometric measurements were made bilaterally in three sections using a fixed area rectangle. Bilateral densitometric values from three sections (i.e. six measurements/region/rat) were averaged to provide one measurement/region/rat. Data are presented as group mean±SEM values (4–5 rats/group) and expressed as either nCi/g tissue or percentage changes of corresponding saline-treated controls. For details of stereotaxic landmarks see legends for Figure 6 (Paxinos and Watson, 1997).

Calculations and statistical analysis

Values are expressed as the mean±SEM. Mean basal firing activity was evaluated after the neurone has attained a stable firing rate, after at least 5 min of recording. Pre-drug values of firing rate were obtained by averaging the firing rate over a period of at least 4 min immediately prior to the i.v. administration, post-drug values were obtained by averaging firing over a period of at least 5 min, immediately after the third minute following administration. In general, subsequent i.v. administrations were separated by 7–10 min intervals. Changes in firing rate or Arc mRNA abundance induced by MPH were assessed by analysis of variance (ANOVA) or Student’s t-test, as appropriate.

Neuronal burst activity was also evaluated as the percentage of spikes occurring in bursts versus the total number of spikes. Burst was defined according to established criteria on PFC neurones (Labonte et al., 2009), as a train of at least two spikes with an initial interspike interval of 45 ms or less, and a termination interval greater than or equal to 90 ms. Burst parameters were calculated by a script program included in Spike 2 and analysed over a period of 4–500 consecutive interspike time intervals.

Results

Effects of MPH in adult rats

A total of 31 neurones were studied, but only 16 neurones were successfully recorded during the complete sequence of MPH cumulative administration (3 mg/kg, i.v.). Most of the neurones tested (26 out of 31) exhibited large action potentials of at least 2 mV amplitude starting with a positive inflection, and relatively long duration (>1.1 ms) (Figure 1(a)), the remaining neurones (5 out of 31) had a biphasic wave-form, of approximately 1 mV amplitude, characterized by initial negative-going deflections (>90%), with an action potential duration between 1.1 and 2 ms. According to recent in vivo electrophysiological studies combining electrophysiology and labelling techniques of gamma-aminobutyric acid (GABA) and glutamate neurones, neurones with an action potential duration longer than 0.75 ms and relatively low firing activity (<60 spikes/10 s) can be considered as pyramidal neurones (Tseng et al., 2006). The basal firing activity of the neurones recorded was variable, from 0.5 to 70 spikes/10 s (average 22.7 ± 4 spikes/10 s). All neurones included in the present data were recorded in the dorsal part of the medial PFC in subregions which include the cingulated (areas 1 and 3, sometimes called the prelimbic cortex; lateral 0.5–0.7 mm, ventral 1.8–4 mm and anterior to the bregma 2.7–3.7 mm) and the very medial part of frontal cortex area 2 (lateral 0.8–1 mm, ventral 1500–2000), according to Paxinos and Watson (1997). There were apparently no relationships between the location of the neurones tested and their electrophysiological characteristics or response to MPH, in both juvenile and adult rats.

MPH was generally administered after 8–10 min of stable recording at 5–7 min intervals. Figure 2 shows that the subsequent administration of MPH of 1 mg/kg i.v. up to a cumulative dose of 3 mg/kg, progressively and significantly increased the firing activity of PFC neurones (p < 0.01, Newman–Keuls test after significant repeated-measure ANOVA). Neurones were found to respond differently from one another to the cumulative administration of MPH (see examples in Figure 1). In some neurones tested the interval between the first and second dose was extended in order to verify that the lowest dose does not have a delayed effect (Figure 1(c)). A slow and progressive dose-dependent increase in firing activity was observed in seven neurones out of the 16 tested (45% of the neurones tested) during the complete sequence of MPH administration. Four neurones (25%) responded to the first dose of MPH by an immediate and large increase in firing activity, which was sometimes short lasting. Four neurones (25%) were only significantly activated after the third dose of MPH and two neurones (12%) did not respond by an increase in firing activity. However, despite this apparent variability, the average mean firing activity of PFC neurones significantly increased by 84% after the administration of the third dose of MPH (3 mg/kg cumulative) compared with the baseline, with eight neurones (40%) showing large activation of firing activity by more than 20 spikes/10 s over their respective baseline levels. Individual changes in firing rate induced by MPH were not correlated with their respective baseline values. Neurones with a very low or high basal firing rate could be indifferentially responsive to the drug.

Cumulative doses of MPH administration were also found to moderately but significantly increase the percentage of spikes firing in burst (from 30 ± 6% at baseline, to 45 ± 6% at 3 mg/kg, n = 16, p < 0.001, Newman–Keuls test after significant repeated-measure ANOVA). Only three out of 16 neurones showed large changes in burst activity with only moderate or no change in firing.

The effect of a single administration of a low dose of MPH (0.3 mg/kg) was also tested in a group of 10 adult animals. MPH did not change significantly the firing activity of the PFC neurones tested (mean firing rates = 14.3 ± 5 and 15.2 ± 5 spikes/10 s, before and after MPH administration, respectively, NS). However, three neurones out of the 10 neurones tested showed an activation of their firing activity by more than 5 spikes/10 s, but a reduction of activity by more than 5 spikes/10 s was also observed in two neurones.

Subsequent administration of saline, administered over the same time intervals as MPH, did not affect significantly the firing activity of PFC neurones (Figures 1, 2 and 4). Only one neurone, out of the 11 tested (8%), was found to be activated by saline administration by more than 40% over its baseline level.
Effects of MPH in juvenile rats and comparison with adult rats

A total of 24 PFC neurones from juvenile rats were tested, of which 20 were successfully recorded during the complete sequence of MPH cumulative administration (3 mg/kg, i.v.). Most of the neurones tested (20 out of 24) exhibited, as for the adult rats, large action potentials with initial positive inflection. In juvenile rats, the mean basal firing activity (9.8 ± 1.4 spikes/10 s) was more constant (minimum: 0.5 spike/10 s, maximum: 27 spikes/10 s) and significantly lower than in the adult rats (p = 0.02, unpaired Student’s t-test). On the other hand, basal burst activity did not differ significantly between juvenile and adult rats.

Figure 3 shows that the subsequent administration of MPH of 1 mg/kg (i.v.) up to a cumulative dose of 3 mg/kg only slightly but significantly increased the firing activity of PFC neurones by 37% over baseline (p < 0.05, Newman–Keuls test after significant repeated-measure ANOVA). The large majority of the neurones tested (95%) were insensitive to the first dose. Only ten neurones (50% of the neurones tested) were activated after the third dose by more than 5 spikes/10 s (Figure 4) and large activation (more than 20 spikes/10 s) occurred in only one neurone (5% of the neurones tested).

As shown in Figure 3(a), the increase in firing activity of PFC neurones obtained after the cumulative administration of MPH appears much higher in the adult group than in the juveniles. In this figure, we have also excluded the neurones with high firing activity (>30 spikes/10 s), to allow comparison between groups with similar baseline values. As a matter of fact, individual change in firing activity induced by MPH (1 or 3 mg/kg) between juvenile and adult rats (with or without the high firing cells removed) were significantly different (p < 0.015, unpaired Student’s t-test) (Figure 5). In addition, two-way repeated-measure ANOVA analysis revealed a significant interaction of age and MPH administration on PFC firing activity in all neurones (F3,34 = 4.7, p < 0.005) or in low-firing neurones (F3,29 = 4.3, p < 0.007). In addition, juvenile rats did not show any significant change in burst activity following MPH administration (from 36/0.05% at baseline to 40/0.04% after 3 mg/kg, n = 20, NS).

![Figure 1](image-url)
Age-dependent effect of MPH on Arc expression in the PFC

As shown previously, Arc mRNA abundance did not differ significantly between juvenile and adult saline-treated rats in PFC areas (Banerjee et al., 2009). Values in the cingulate cortex for juveniles were 603 ± 33 (nCi/g tissue weight, mean ± SEM, n = 4–5 rats/group) and for adults (660 ± 55); corresponding values for the orbital cortex were 627 ± 97 and 682 ± 35. The effect of MPH (4 mg/kg, i.p.) on Arc

![Graph showing the effect of MPH on Arc expression in the PFC](image)

Figure 2. Mean firing activity of prefrontal cortex neurones subsequently treated with three doses of saline (1 ml/kg, i.v.) or methylphenidate (1 mg/kg, i.v.) in adult rats. The same neurones were recorded in the complete sequence. The number shown in the top of each figure represents the number of neurones tested. The figure shows that methylphenidate, but not saline, induces a significant increase in firing activity. **p < 0.01, compared with respective pre-treatment values, Newman–Keuls test after significant repeated-measure ANOVA.

![Graph showing the effect of MPH on saline treatment](image)

Figure 4. Proportion of the electrophysiological response of prefrontal cortex neurones (expressed in percentage) to methylphenidate in adult and juvenile rats. Neurones were considered responding if their firing activity changed by more than five spikes/10 s. Only cells with a low baseline activity (<30 spikes/10 s) were included in the present figure (a figure with a very similar profile could be obtained when all neurones were included).

![Graph showing the effect of MPH on juvenile and adult rats](image)

Figure 3. (a) Mean firing activity of prefrontal cortex neurones treated with three subsequent doses of methylphenidate (1 mg/kg, i.v.) in juvenile rats (left) and adult rats having a low baseline activity (<30 spikes/10 s) for comparison. The same neurones were recorded in the complete sequence. The number shown in the top of each figure represents the number of neurones tested. The figure shows that the effect of methylphenidate, although significant in both groups, is more robust in adult rats than in juvenile rats. *p < 0.05, **p < 0.01, compared with respective pre-treatment values, Newman–Keuls test after significant repeated-measure ANOVA. (b) Representative example of the individual electrophysiological response of a prefrontal cortex neurone from juvenile rats to the administration of methylphenidate.
mRNA expression is illustrated in Figures 6 and 7. As in the electrophysiological recording experiments in PFC, the effect of MPH on Arc abundance was also clearly different in juvenile and adult rats. Statistical analysis revealed a significant interaction of age and MPH administration on Arc expression in cingulate ($F_{1,14} = 37.2, p < 0.0001$, two-way ANOVA) and orbital cortices ($F_{1,14} = 42.8, p < 0.0001$). MPH (4 mg/kg, i.p.) increased levels of Arc mRNA in both age groups compared with the corresponding saline-injected controls. This effect was however significantly ($p < 0.001$, Student’s $t$-test) more marked in both the cingulate and orbital PFC of adult rats as compared with juveniles. In the adult rats, MPH increased levels of Arc mRNA by 123% and 164% compared with saline-injected controls in cingulate and orbital PFC, respectively, while corresponding percentage increases in the juveniles were 42% and 79% (Figures 6 and 7).

**Discussion**

The psychostimulant MPH is widely used in young children for the treatment of ADHD and the PFC is one of the suggested main target areas for its calming action in children. Given the wide use of MPH in young children little is known about the drug’s effect in the developing PFC. Here, for the first time, we have directly compared the action of MPH on in vivo electrophysiological responses in adult and juvenile PFC. Our data provide clear evidence of an age-dependent effect in the PFC by MPH on electrical activity, as well as on gene expression of the Arc protein, a marker of neuronal activity.

Our electrophysiological investigation shows that MPH administered systemically dose-dependently increased the excitability of PFC neurones in adult rats. It is anticipated that the types of neurones recorded in the present study are pyramidal neurones, as GABA interneurones in the PFC exhibit different action potentials characteristics (Tseng et al., 2006) and pyramidal neurones are by far the most easily detectable group of neurones in the PFC (more than 80% of PFC neurones are pyramidal). However, it could not be ruled out that a small minority of interneurones was misclassified as pyramidal neurones in our study, as the recorded neurones were not labelled with glutamatergic or
The most important finding of the present study involves evidence from both electrophysiological and Arc gene expression measurements that MPH induces an age-dependent effect on PFC neuronal activity. In our electrophysiological study in juvenile rats, PFC neurones appear to be far less sensitive to the different doses of MPH compared with the adult rats, as MPH could only elicit a slight stimulatory effect at the highest dose tested (3 mg/kg). To our knowledge, our study is the first study to demonstrate that MPH exerts a PFC neurones by approximately 25%. However, unlike Devilbiss and Berridge (2008), we found a potent dose-dependent increase in PFC neuronal activity induced by MPH, at least over the range of 0.3–3 mg/kg (i.v.). Few other electrophysiological studies have attempted to determine the electrophysiological effects in the PFC of other drugs having pharmacological similarities with MPH. Interestingly, it has been found that low doses (0.5 mg/kg, i.p.) of amphetamine (Homayoun and Moghaddam, 2007), and to a lesser extent methamphetamine (4 mg/kg, i.p.) (Jang et al., 2007), elicit preferentially excitatory responses to PFC neurones in awake animals, while higher dose of amphetamine (2 mg/kg) tends to reduce their activity. Amphetamine, like MPH, blocks the synaptic re-uptake of dopamine and noradrenaline, but is also capable, unlike MPH, of facilitating the release of serotonin, which may explain its depressing effects at higher doses. In addition, these previous in vivo studies on MPH or amphetamines used multi-channel recording techniques in freely moving animals. Therefore, methodological differences related to recording conditions (such as electrode characteristics, including electrode impedance, or presence/absence of anaesthetics) may contribute to explain the partial result discrepancy between the former in vivo electrophysiological studies and our data.

As already mentioned, MPH blocks both the dopamine and noradrenaline transporters and previous in vivo microdialysis studies showed that MPH potently increases extracellular monoamine levels in the PFC (Berridge et al., 2006; Kuczenski and Segal, 2002). Consequently, the stimulatory effect of MPH in the PFC on firing activity, as well as on Arc gene expression, is likely to be triggered by an enhancement of catecholaminergic tone. Whether this MPH-induced enhancement of neuronal activity is caused by activation of noradrenergic and/or dopamine receptors present on the PFC neurones themselves (whether pyramidal or interneurones), or induced by excitatory inputs from regions outside the PFC is not currently known. The PFC contains a wide range of different dopamine and noradrenergic receptor subtypes, which are distributed on interneurones, pyramidal neurones and afferent terminals (Steketee, 2003); in particular, alpha1, alpha2, beta1 and D1/2 receptors are potential targets for the mechanism of action by MPH (Arnsten et al., 2006; Pascoli et al., 2005). Interestingly, one in vitro intracellular electrophysiological study on PFC slices has demonstrated that bath application of MPH increases the excitability of pyramidal neurones through an action of noradrenaline on alpha2 adrenoceptors, likely located on adjacent interneurones (Andrews and Lavin, 2006). More in vivo studies need to be undertaken to understand better the pharmacological mechanism underlying these excitatory effects.

GABAergic markers. Nevertheless, the effect of MPH was found to be unidirectional and consistent. More than 80% of the neurones investigated in the adult rats responded to MPH, within the dose range tested, by an increase in firing activity. There were, however, some interindividual differences between neurones regarding the amplitude of their response and sensitivity to the dose administered, which were apparently not related to their electrophysiological characteristics (e.g. length of action potential, basal activity, etc.) or their location within the subregions of the medial PFC tested. There is one other study that has examined the in vivo electrophysiological effect of MPH in the PFC of adult rats (Devilbiss and Berridge, 2008), using multi-channel recording techniques in freely moving animals. The authors found that low doses of MPH (0.25–2 mg/kg, i.p.) modestly increased the spontaneous discharge rate of PFC neurones (by approximately 20%), while intermediate (5 mg/kg, i.p.) or high doses (15 mg/kg, i.p.) did not alter or reduced the discharge rate. This is in partial agreement with our finding that a dose of 1 mg/kg (i.v.) increased the firing activity of

**Figure 7.** Age-dependent effects of methylphenidate (4 mg/kg, intraperitoneal) in regions of the prefrontal cortex: cingulate cortex (a) and orbital cortex (b), measurements were taken 2 h post-drug. Data are expressed as percentage of saline-treated controls (1 ml/kg, intraperitoneal); each column represents mean ± SEM value from 4–5 rats. *p < 0.05 and **p < 0.001 compared with corresponding age-matched saline-injected controls; *p < 0.001 compared with juvenile methylphenidate group (Student’s t-test). MPH: methylphenidate.

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\[ \text{Arc mRNA % from controls} \]

- **Figure 7.** Age-dependent effects of methylphenidate (4 mg/kg, intraperitoneal) in regions of the prefrontal cortex: cingulate cortex (a) and orbital cortex (b), measurements were taken 2 h post-drug. Data are expressed as percentage of saline-treated controls (1 ml/kg, intraperitoneal); each column represents mean ± SEM value from 4–5 rats. *p < 0.05 and **p < 0.001 compared with corresponding age-matched saline-injected controls; *p < 0.001 compared with juvenile methylphenidate group (Student’s t-test). MPH: methylphenidate.
differential electrophysiological effect on PFC neurones in adult and juvenile rats. Similarly, as shown previously using a lower dose of MPH (Banerjee et al., 2009), the present study also demonstrated that MPH had a more robust effect on PFC Arc mRNA expression in the adult than in the juvenile rats. This reduced sensitivity to the stimulatory action of MPH is likely to be associated with the immature state of the catecholaminergic system in juvenile rats. The PFC is a late-maturing part of the brain and several neurotransmitter systems in the PFC, including the monoamines, are known to undergo profound development during the postnatal period (Spear, 2000). Anatomical studies have shown a progressive in-growth of dopamine fibres into the PFC that continues until the early adult period (Benes et al., 2000). In addition, dopamine receptor expression also increased steadily to reach a stable level only in adulthood (Tarasi and Baldessarini, 2000). There is also evidence for functional differences in dopamine receptor function in different age groups. For example, D1 receptor-induced increase in cAMP levels is reduced in juvenile compared with adult PFC (Andersen, 2002). This is relevant to the present study since Arc gene expression is known to be triggered by increased formation of cAMP (Moró et al., 2007), and MPH had significantly lower effect on Arc mRNA levels in juvenile PFC compared with adults. In the case of the noradrenergic system, in vitro release and autoradiographic studies have demonstrated an increase in noradrenaline turnover and changes in noradrenaline transporter and alpha2 receptor densities in frontal areas during development (Choi et al., 1997; Happe et al., 2004; Sanders et al., 2005).

As a result of a lower density of catecholaminergic fibres, MPH should exert less potent effects in juveniles. Therefore, age difference in the density of monoamine transporters/receptors is probably the determinant factor controlling the sensitivity of PFC neurones to MPH. Interestingly, in our study, baseline electrical activity of PFC neurones was found to be lower in juvenile rats (see Figures 1 and 3). One can speculate that it may be the outcome of a reduced excitatory monoamine tone on to the PFC cells. While microdialysis studies have shown that MPH increases extracellular monoamine levels in the PFC of both juvenile and adult rats (Berridge et al., 2006; Kuczenski and Segal, 2002), experiments directly comparing the effects of MPH in adult and juvenile rats have to our knowledge not been conducted.

In common with our data, our previous study demonstrated a robust age-dependent effect of MPH on the gene expression of BDNF, a key gene for brain development, in the PFC, showing a marked increase in the adults and slight but significant decrease in the juveniles (Banerjee et al., 2009). Age-dependent effects of MPH (Banerjee et al., 2009; Spear, 2000) have also previously been observed on locomotor activity, which is strongly stimulated by MPH in the adult but not in the juvenile rat.

MPH exerts biphasic action on cognition in adult animals and human (either healthy volunteers or ADHD patients), with low doses inducing cognition-enhancing actions and higher doses (>2 mg/kg) impairing cognitive function (Arnsten, 2006; Berridge et al., 2006; Swanson and Wolkow, 2002; Wetzel et al., 1981). It is possible that the large stimulation of neuronal activity and Arc expression in the PFC of adult rats, observed in our study with relatively high doses (3–4 mg/kg), contributes to induce PFC-dependent cognitive impairment, as well as locomotor agitation (Arnsten, 2006). The dose range tested (1–3 mg/kg, i.v.) in our electrophysiological study likely far exceeds therapeutic levels of MPH when given orally at doses typically between 0.2 and 1 mg/kg to ADHD children, in part because MPH has a relatively low bioavailability (Wargin et al., 1983) and the i.v. route will produce a peak of plasma concentration with much shorter latency. The highest dose tested in our study (3 mg/kg, i.v.) should exceed the brain dopamine transporter blockade threshold of 60%, combined with an extremely rapid onset of action. Together, both factors are believed to be critical for inducing the positive reinforcement of psychostimulants (Gatley et al., 1999; Swanson and Wolkow, 2003).

Therefore, the large activation of PFC neuronal activity observed in our study at the highest dose may not only reflect the non-therapeutic effects of the drugs on cognitive deficit, but also reinforcing effects resulting in abuse. Importantly, this activation is less pronounced in juvenile animals in good agreement with most behavioural reports showing that peri-adolescent rats are less responsive than younger or older rats to psychomotor stimulants (Bolanos et al., 1998; Frantz et al., 2007; Laviola et al., 1995; Spear and Brake, 1983).

Even more interestingly, behavioural sensitization obtained after repeated administration of MPH at higher doses (2.5 mg/kg) does not occur in juvenile rats (Andersen et al., 2002) and this process is abolished following a PFC lesion (Lee et al., 2008). This further supports the assumption that over-activation of PFC neurones following MPH may contribute to the process of behavioural sensitization. It is also likely that this difference in developing behavioural sensitization between adult and juvenile rats following MPH is related to the poor ability of the drug to stimulate Arc mRNA expression in juvenile animals. An age-related difference of MPH on Arc mRNA expression has previously been detected after a lower dose of MPH (2 mg/kg, i.p.) as well (Banerjee et al., 2009).

Lower doses of MPH (0.3 mg/kg, i.v.), likely to reach plasma levels closer to therapeutic concentrations, were also tested in the present study resulting only in a moderate activation of a subset of PFC neurones.

In conclusion, the present study demonstrates evidence of clear-cut differences in the response of the adult and juvenile PFC to administration of MPH. Modification of the electrophysiological and Arc expression responses of PFC neurones to MPH with age may reflect critical developmental modifications occurring in the PFC and particularly in catecholaminergic neurotransmission systems. Consideration of such modifications may be of great significance in optimizing treatments for ADHD, evaluating the consequences of MPH administration and understanding its pharmacological actions.

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