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MOLECULAR AND DEVELOPMENTAL NEUROSCIENCE

Differential regulation of psychostimulant-induced gene expression of brain derived neurotrophic factor and the immediate-early gene *Arc* in the juvenile and adult brain

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Abstract

Psychostimulant drugs are widely used in children for the treatment of attention-deficit/hyperactivity disorder. Recent animal studies have suggested that exposure to these agents in early life could be detrimental to brain development. Here, for the first time, the effect of methylphenidate (MPH) and D-amphetamine (AMPH) on the expression of two key genes for neuronal development and plasticity, brain-derived neurotrophic factor (*bdnf*) and the effector immediate early gene activity-regulated, cytoskeletal-associated protein (*Arc*), was examined in both juvenile and adult rats. Both MPH [2 mg/kg, intraperitoneal (i.p.)] and AMPH (0.5 mg/kg, i.p.) induced marked decreases of *bdnf* mRNA in hippocampal and cortical brain regions of juveniles, whereas effects in adults were significantly less (hippocampus) or opposite (frontal cortex). In comparison, *Arc* mRNA was decreased (hippocampus and parietal cortex), largely unaffected (frontal cortex) or increased (striatum) in juveniles, whereas in adults, *Arc* mRNA increased in most brain regions. MPH-induced locomotion was also measured, and showed a much smaller increase in juveniles than in adults. In summary, our data show that the effects of MPH and AMPH on expression of the neurodevelopmentally important genes, *bdnf* and *Arc*, differ markedly in juvenile and adult rats, with juveniles showing evidence of brain region-specific decreases in both genes. These age-dependent effects on gene expression may be linked with the reported long-term harmful effects of psychostimulants in animal models.

Introduction

Reported prevalence rates of childhood attention-deficit/hyperactivity disorder (ADHD) vary from 2% to as high as 18%, depending on the diagnostic criteria used (Faraone *et al.*, 2003). As a result, it has been suggested that not only ADHD sufferers but also large numbers of children who do not meet the full diagnostic criteria are subjected to psychostimulant drug therapy for ADHD (Angold *et al.*, 2000). Indeed, it is now established that the psychostimulants methylphenidate (MPH) and D-amphetamine (AMPH) have a calming effect in children regardless of ADHD diagnosis (Rapoport & Inoff-Germain, 2002; Andersen, 2005).

Given the widespread use of these drugs throughout one of the most plastic and sensitive phases of neurodevelopment, surprisingly little is known about the actions of these agents on the young brain. Indeed, there is increasing concern regarding the long-term effects of early life exposure to psychostimulants (Robbins, 2002; Volkow & Insel, 2003; Andersen, 2005; Grund *et al.*, 2006).

Previous studies, in adult animals and humans, have shown that both MPH and AMPH target the brain monoamines noradrenaline and dopamine, and it is assumed that similar effects occur in juveniles. Specifically, these agents block monoamine re-uptake (MPH) and increase vesicular release (AMPH), and increase extracellular brain concentrations of both noradrenaline and dopamine (Kuczenski & Segal, 2001; Volkow *et al.*, 2001). In adult and adolescent rats, psychostimulants also evoke the expression of monoamine-regulated genes, including the immediate early gene (IEG) *c-fos*, in brain regions that receive major dopaminergic inputs, such as the striatum and nucleus accumbens (Brandon & Steiner, 2003; Adriani *et al.*, 2006; Yano *et al.*, 2006; Cotterly *et al.*, 2007). However, much less is known about psychostimulant action on gene expression in juveniles, particularly in regions with less dense monoaminergic inputs, such as the hippocampus and cortex, which are important for key cognitive and emotional functions.

Importantly, monoaminergic systems undergo pronounced neurodevelopmental changes during childhood and adolescence (Spear, 2000; Andersen, 2005). For example, the central dopamine system shows substantial re-organization during development, including changes in dopamine fibre density, release, transporter levels and receptor numbers (Kalsbeek *et al.*, 1988; Stamford, 1989; Teicher & Andersen, 1995; Tarazi *et al.*, 1998; Moll *et al.*, 2000).

Previous experiments conducted in young rodents have provided evidence that exposure to MPH in early life may disrupt brain

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maturation, including developmental changes of D3 dopamine receptor expression in the prefrontal cortex (Andersen, 2005; Andersen *et al.*, 2008). Brain-derived neurotrophic factor (BDNF) and the effector IEG activity-regulated, cytoskeletal-associated protein (Arc) are crucial for neural development and plasticity, as well as cognition and emotional behaviours (Altar *et al.*, 1997; Schwartz *et al.*, 1997; Guzowski *et al.*, 2000; Lu *et al.*, 2005; Tzingounis & Nicoll, 2006). In the current study, we assessed the effects of MPH and AMPH on mRNA expression of *bdnf* and *Arc* in both juvenile and adult rats, and found evidence for striking decreases in the expression of both genes in the juvenile but not adult brain.

Materials and methods

Animals and drug treatment

Juvenile [postnatal day (PND) 20] and adult (PND > 60) male Sprague–Dawley rats (Harlan Olac, Bicester, UK) were housed (six per group) in separate age-matched groups at constant temperature and humidity under a 24 h light/dark cycle (lights on from 06.00 h to 18.00 h), 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 the animal facilities (5 days), groups (six rats per treatment group) of juvenile (PND 25, 80–95 g) and adult (PND > 60 days, 260–285 g) rats were given either 2 mg/kg MPH or 0.5 mg/kg AMPH. Similar doses of MPH administered chronically to young rats have been reported to produce impairments of *bdnf*-dependent and *Arc*-dependent processes in adulthood (Lagace *et al.*, 2006; Gray *et al.*, 2007). AMPH is normally used clinically at doses approximately four times lower than those of MPH (Kuczenski & Segal, 2001). Drugs (obtained from Sigma-Aldrich, York, UK) were dissolved in saline, and corresponding age-matched control groups were injected with saline alone [1 mL/kg, intraperitoneal (i.p.)]. Rats were killed by rapid dislocation of the neck 2 h after drug administration, and brains were removed, frozen in isopentane, and stored at −80°C prior to sectioning using a cryostat. The rationale behind the chosen time period of 2 h after drug admistration for killing the animals is based on previous studies by ourselves and others, measuring changes of *bdnf* and *Arc* mRNA expression after acute administration of various centrally acting drugs, including MPH, at this time point (Zetterström *et al.*, 1999; Pei *et al.*, 2000; Chase *et al.*, 2007). Separate groups of juvenile and adult rats were given MPH (2 mg/kg, i.p.) and tested for locomotor activity changes together with their age-matched controls given saline (for details see below).

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, Berlin, Germany), and pre-treated using a standard protocol as described previously (Pei *et al.*, 1996). Oligonucleotides complementary to mRNA were as follows: (i) a 36 mer (5′-GGTCTCGTAGAA-ATATTGCTTCAGTTGGCC TTTTGA-3′) complementary to bases 878–913 of rat *bdnf* cDNA (Genbank accession number: BC087634.1), which encode all isoforms of the *bdnf* gene; and (ii) a 45 mer (5′-CTTGGTTGCCCATCCTCACCTGGCACCCAAG-ACTGGTATTGCTGA-3′) complementary to bases 789–833 of rat *Arc* cDNA (Genbank accession number: NM019361.1). Oligonucleotides were 3′-tail labelled with [^{35}S]dATP by terminal deoxynucleotide transferase, and labelled probes (specific activity > 10^9 cpm/μg) were added to each section (2×10^6 cpm per section) in hybridization buffer as previously described (Pei *et al.*, 1996). After incubation in humidity chambers containing 50% formamide in 4 × saline sodium citrate at 35 °C (*bdnf* probe) or 36 °C (*Arc* probe) for 14–16 h, slides were washed in 1 × saline sodium citrate buffer at 55 °C for 3 × 20 min and for 2 × 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.

Locomotor activity measurements

Each animal was taken from the home cage, weighed, injected with MPH or saline, and transferred with no prior habituation to a standard laboratory cage (43 × 43 × 35 cm with sawdust bedding) placed between 15 horizontal infrared photocells (2.54 cm apart; Opto-Varimex-Mini, Columbus Instruments, Ohio, USA). Spontaneous horizontal activity was registered as a locomotor count each time that animal movement caused a beam break. Locomotor counts were recorded every fifth minute over 2 h after MPH or saline administration. All activity measurements were recorded between 10.00 h and 12.00 h.

Data analysis and statistics

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 ^{35}S tissue equivalents using ^{14}C microscales (Amersham). After establishment of standard curves, densitometric measurements were obtained from regions of interest in one of two ways. In the case of hippocampal areas [i.e. CA1, CA3 and dentate gyrus (DG)], the piriform cortex (at the level of the medial prefrontal cortex) (see Fig. 3B) and the caudate nucleus (dorsal striatum; see Fig. 6C), a series of non-overlapping point densitometric measurements was obtained bilaterally in three sections. Alternatively, in the case of the parietal cortex (see Fig. 3A), cingulate cortex, prelimbic cortex (see Fig. 3B), orbital cortex (see Fig. 6B) and caudate nucleus (see Fig. 6C), densitometric measurements were made bilaterally in three sections using a fixed area quadrant. Bilateral densitometric values from three sections (i.e. six measurements per region per rat) were averaged to provide one measurement per region per rat. Data are presented as group mean ± SEM values (six rats per group) and expressed as either nCi/g tissue or percentage changes of corresponding saline-treated controls. For details of stereotaxic landmarks, see legends for Figs 3 and 6 (Paxinos & Watson, 2005).

Raw data were analysed statistically (GRAPH-PAD PRISM, version 4.00, La Jolla, California, USA) prior to conversion to percentage values. Data from the *in situ* experiments were analysed statistically on a region-by-region basis, using Student's unpaired two-tailed *t*-test. If data groups failed tests for normality or equal variance (conducted automatically by statistical software), the level of significance was tested with the Mann–Whitney rank sum test (this occurred in four of 76 comparisons). The multiple groups of locomotor data were analysed statistically by analysis of variance (two-way ANOVA, with age and MPH treatment as independent measures), followed by the Bonferroni *post hoc* test. In all tests, $P < 0.05$ was taken as the level of statistical significance.

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Results

Regional distribution of bdnf and Arc mRNA in juvenile and adult rat brain

The regional distribution of the *bdnf* and *Arc* mRNA was determined by densitometric quantification of autoradiograms from saline-treated control groups, in juvenile and adult rats (Tables 1 and 2). *Bdnf* mRNA abundance was unevenly distributed in the brain regions examined in both age groups, as previously described (Conner *et al.*, 1997; Zetterström *et al.*, 1999). The abundance of *bdnf* mRNA was, however, significantly greater in the CA3 and DG in juvenile than in adult rats, ($t_{10} = 3.38$, $P < 0.01$, Student's *t*-test, and $T = 57$, $P = 0.002$, Mann–Whitney rank sum test) (Table 1).

Arc mRNA abundance did not differ between juvenile and adult rats in areas of the frontal cortex. However, there were noticeable differences between the two age groups in other regions. As with *bdnf*, *Arc* mRNA abundance was significantly higher in juvenile than in adult rats in hippocampal regions: CA1, $t_{10} = 23.46$, $P < 0.001$; CA3, $t_{10} = 10.91$, $P < 0.001$; and DG, $t_{10} = 17.37$, $P < 0.001$. In contrast, *Arc* mRNA abundance in the dorsal striatum was significantly lower in juvenile than in adult rats ($t_{10} = -5.97$, $P < 0.001$) (Table 2).

Age-dependent effect of MPH and AMPH on bdnf mRNA

The effects of MPH and AMPH on *bdnf* mRNA abundance are illustrated in Figs 1–3. Both drugs evoked striking age-dependent effects on *bdnf* mRNA throughout the brain regions examined. Statistical analysis revealed significant differences in drug-evoked changes in *bdnf* mRNA between juvenile and adult rats, in the following brain regions: CA1 (MPH, $t_{10} = -8.86$, $P < 0.001$; and AMPH, $t_{10} = -0.79$, $P < 0.001$), CA3 ($t_{10} = -10.69$, $P < 0.001$, and $t_{10} = -18.95$, $P < 0.001$), DG ($t_{10} = -14.34$, $P < 0.001$, and $t_{10} = -22.23$, $P < 0.001$), and parietal cortex ($t_{10} = -9.91$, $P < 0.001$, and $t_{10} = -2.43$, $P < 0.05$) (Figs 1A and 2A). *Bdnf* mRNA in regions of the frontal cortex of juvenile and adult rats was similarly different: respectively, cingulate cortex − MPH, $t_{10} = -12.63$, $P < 0.001$, and AMPH, $t_{10} = -4.79$, $P < 0.001$; prelimbic cortex, $t_{10} = -8.94$, $P < 0.001$, and $t_{10} = -14.59$, $P < 0.001$; and piriform cortex, $t_{10} = -13.85$, $P < 0.001$, and $t_{10} = -13.86$, $P < 0.001$ (Figs 1B and 2B).

In juvenile rats, as compared to saline controls, MPH induced marked reductions in *bdnf* mRNA in hippocampal sub-regions (CA1, $t_{10} = 6.24$, $P < 0.001$; CA3, $t_{10} = 8.61$, $P < 0.001$; DG, $t_{10} = 18.62$, $P < 0.001$) and the parietal cortex ($t_{10} = 7.79$, $P < 0.001$) (Fig. 1A). Similar reductions in *bdnf* mRNA were detected in hippocampal sub-regions of juvenile rats after AMPH admistration (Fig. 2A). In comparison, in adult rats, MPH and AMPH either evoked small reductions or did not affect *bdnf* mRNA in the hippocampus and parietal cortex (Figs 1A, 2A and 3A).

This age-dependent effect of MPH and AMPH on *bdnf* mRNA was even more pronounced in regions of the frontal cortex, Thus, in these regions, MPH and AMPH reduced *bdnf* mRNA in juvenile rats, but increased *bdnf* mRNA in adult rats (Figs 1B, 2B and 3B). Statistically significant differences for the action of MPH in juvenile vs. adult rats were detected in the cingulate cortex ($t_{10} = -12.62$, $P < 0.001$), prelimbic cortex ($t_{10} = -8.94$, $P < 0.001$), and piriform cortex ($t_{10} = -13.85$, $P < 0.001$) (Fig. 1B). Corresponding results for AMPH were as follows: cingulate cortex, $t_{10} = -4.80$, $P < 0.001$; prelimbic cortex, $t_{10} = -14.60$, $P < 0.001$; and piriform cortex, $t_{10} = -13.87$, $P < 0.001$ (Fig. 2B).

Age-dependent effects of MPH and AMPH on Arc mRNA

The effects of MPH and AMPH on *Arc* mRNA abundance were also measured in juvenile and adult rats. Like *bdnf* mRNA, *Arc* mRNA showed clear evidence of age-dependent differences in sensitivity to MPH and AMPH. In the DG of juvenile rats, both drugs significantly reduced *Arc* mRNA (MPH, $t_{10} = 17.56$, $P < 0.001$; AMPH, $t_{10} = 17.00$, $P < 0.001$) (Figs 4A and 5A), and this reduction was greater in juveniles than in adults for both MPH ($t_{10} = -7.03$, $P < 0.001$) and AMPH ($t_{10} = -7.79$, $P < 0.001$) (Figs 4A and 5A). Moreover, in CA1, whereas MPH and AMPH decreased *Arc* mRNA in juveniles, both drugs increased *Arc* mRNA in adults (MPH, $t_{10} = -25.98$, $P < 0.001$; AMPH, $T = 21$, $P = 0.002$).

As with the hippocampus, *Arc* mRNA in other cortical regions responded differently between juvenile and adult rats. In the case of the parietal cortex, *Arc* mRNA showed no change after MPH

TABLE 1. Brain distribution of *bdnf* mRNA in areas of interest from saline-treated juvenile and adult control rats

	Hippocampus				Frontal cortex		
	CA1	CA3	DG	Parietal cortex	Cingulate	Prelimbic	Piriform
Juvenile	288 ± 22	661 ± 36*	548 ± 25*	231 ± 12	157 ± 3	195 ± 9	208 ± 10
Adult	251 ± 8	547 ± 6	435 ± 6	237 ± 7	147 ± 5	191 ± 11	155 ± 7

Values (nCi/g tissue weight, mean ± SEM, $n = 6$ rats/group) were determined by densitometric quantification of autoradiograms. *$P < 0.01$ (juvenile vs. adult group). DG, dentate gyrus.

TABLE 2. Brain distribution of *Arc* mRNA in areas of interest from saline-treated juvenile and adult control rats

	Hippocampus				Frontal cortex		
	CA1	CA3	DG	Parietal cortex	Cingulate	Prelimbic	Dorsal striatum
Juvenile	350 ± 8**	235 ± 8**	153 ± 3**	143 ± 7	246 ± 3	356 ± 30	188 ± 10**
Adult	179 ± 2	148 ± 4	56 ± 5	91 ± 2	272 ± 8	340 ± 5	272 ± 11

Values (nCi/g tissue weight, mean ± SEM, $n = 6$ rats/group) were determined by densitometric quantification of autoradiograms. **$P < 0.001$ (juvenile vs. adult group). DG, dentate gyrus.

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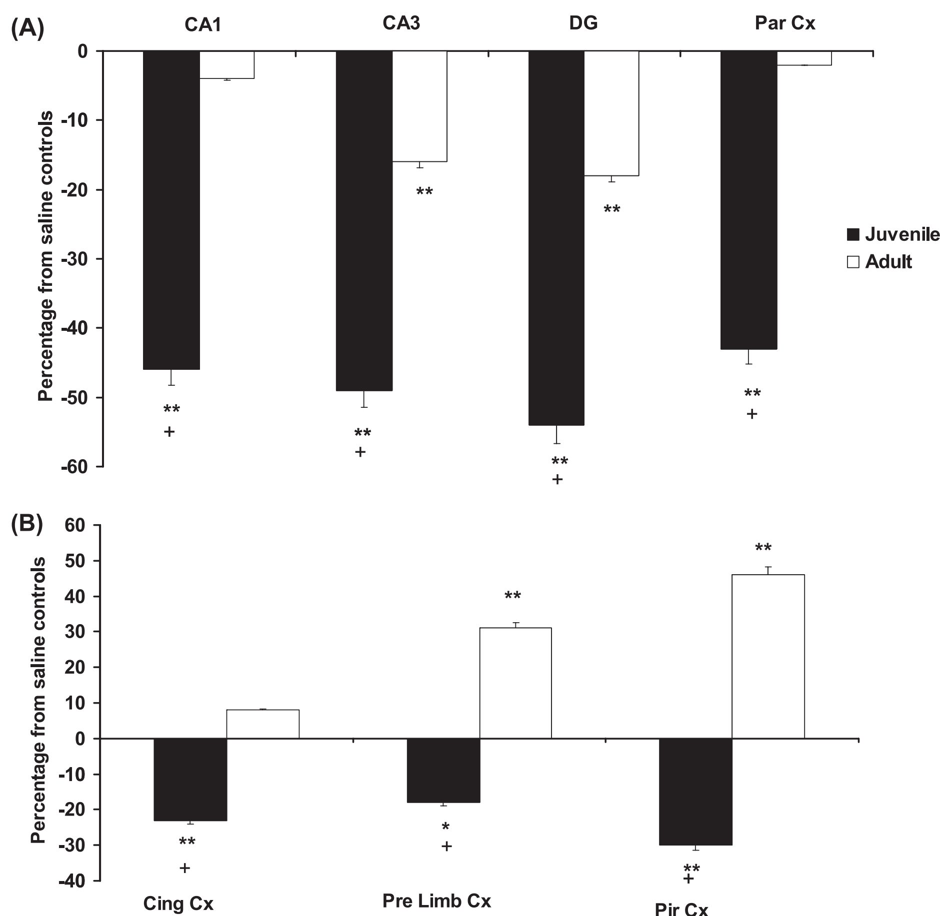

FIG. 1. Age-dependent effects of methylphenidate (MPH) [2 mg/kg, intraperitoneal (i.p.)] on *bdnf* mRNA in hippocampal sub-regions and parietal cortex (A), and regions of the frontal cortex (B). Measurements were taken 2 h after drug administration. Data are expressed as percentage of saline-treated controls (1 mL/kg, i.p.); each column represents mean ± SEM value from five or six rats. *$P < 0.05$ and **$P < 0.001$ as compared to corresponding age-matched saline-injected controls; $^{+}P < 0.001$ as compared to the adult MPH group. DG, dentate gyrus; Par Cx, parietal cortex; Cing Cx, cingulate cortex; Pre Limb Cx, prelimbic cortex; Pir Cx, piriform cortex.

administration and a small but significant decrease (−25%) after AMPH administration ($T = 56$, $P = 0.004$) in juvenile rats, whereas in adult rats both drugs evoked striking increases (+60%) in *Arc* mRNA (MPH, $t_{10} = -6.62$, $P < 0.001$; AMPH, $t_{10} = -10.54$, $P < 0.001$) (Figs 4A, 5A and 6A). Similarly, in both cingulate and orbital cortices, *Arc* mRNA showed a striking increase (+95–115%) in response to MPH ($T = 21$, $P = 0.002$) and AMPH ($t_{10} = -9.98$, $P < 0.001$) in adult rats, but much smaller changes were observed in juveniles (Figs 4B and 5B). Finally, in the caudate nucleus, both drugs increased *Arc* mRNA expression more in juvenile rats than in adult rats, and this difference was particularly pronounced for MPH (+80% and +30% for juvenile and adults, respectively, $t_{10} = 10.16$, $P < 0.001$) (Figs 4B and 5B).

Age-dependent effects of MPH on locomotor activity

The effects of MPH on locomotor activity in juvenile and adult rats are shown in Fig. 7. This effect was different in juvenile and adult rats, and there was a highly significant interaction of age and MPH treatment on locomotor activity over the total 2 h post-injection period ($F_{1,20} = 34.23$, $P < 0.0001$, two-way ANOVA). Whereas MPH-treated juvenile rats showed a trend for an increase in locomotor activity as compared to saline controls, statistical analysis with Bonferroni *post hoc* tests revealed that MPH did not significantly increase locomotor activity in the juvenile rats, either over the total 2 h time period or at any 15 min period illustrated in Fig. 7 (saline vs. MPH, $P > 0.05$). In comparison, adult rats treated with MPH showed significantly more locomotor counts over the total 2 h time period than their corresponding saline-treated control group (saline vs. MPH, $P < 0.001$, Bonferroni *post hoc* test), and this difference was apparent at each 15 min period of the 2 h time course, with the greatest difference occurring 30–75 min after drug administration (Fig. 7).

Discussion

The psychostimulant drugs MPH and AMPH are widely used in children for the treatment of ADHD, but recent data suggest that exposure to these agents in early life could be detrimental to brain development. The present study sought for the first time to compare the effects of MPH and AMPH on adult and juvenile rats in terms of changes in expression of two key genes for brain development and plasticity, *bdnf* and *Arc*. The data show striking age-dependent effects of MPH and AMPH on *bdnf* and *Arc* mRNA expression in brain regions implicated in the mechanisms of action of psychostimulants in the treatment of ADHD (Solanto, 1998; Volkow *et al.*, 2001; Andersen, 2005).

Both MPH and AMPH induced marked reductions in *bdnf* mRNA abundance throughout the hippocampus and parietal cortex of juvenile

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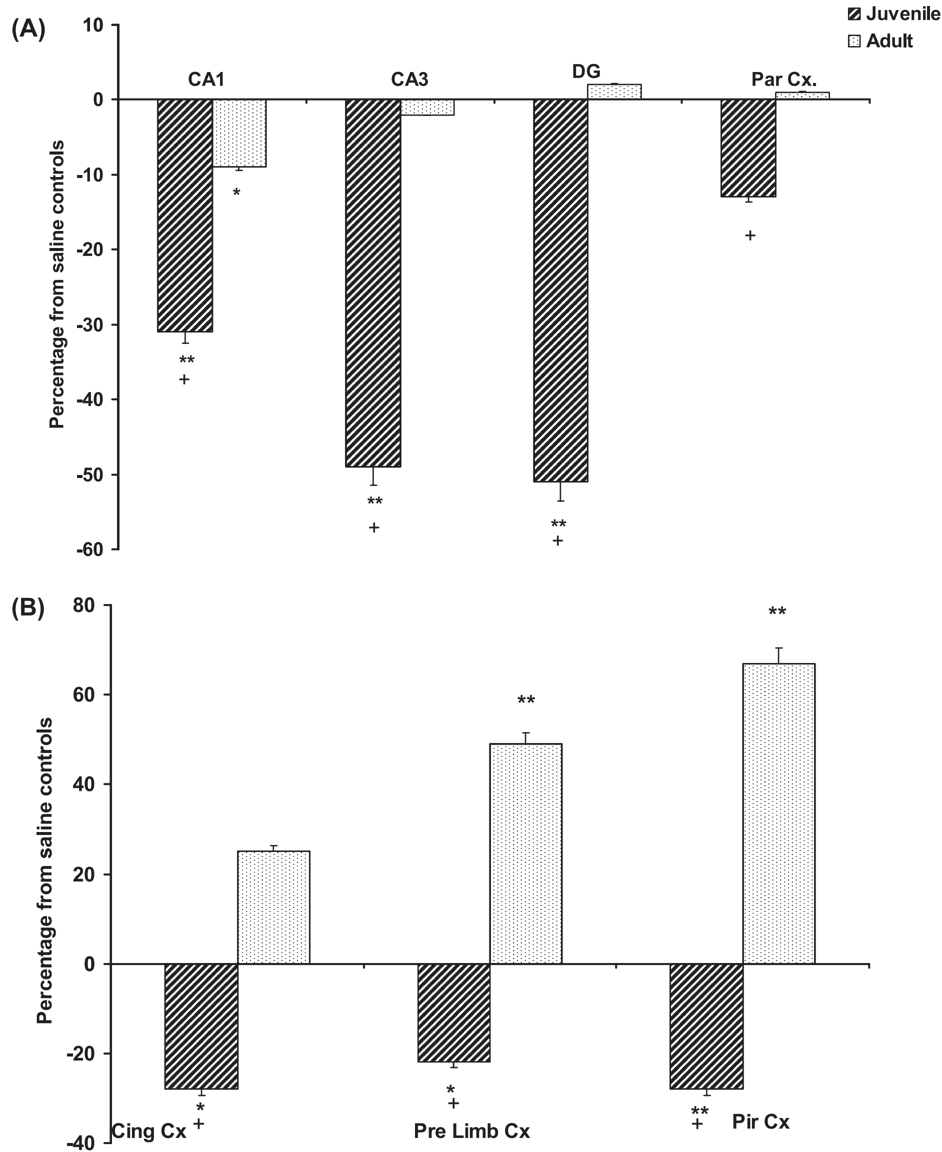

FIG. 2. Age-dependent effects of D-amphetamine (AMPH) [0.5 mg/kg, intraperitoneal (i.p.)] on *bdnf* mRNA in hippocampal sub-regions and the parietal cortex (A), and in regions of the frontal cortex (B). Measurements were taken 2 h after drug administration. Data are expressed as percentage of saline-treated controls (1 mL/kg, i.p.); each column represents mean ± SEM value from five or six rats. *$P < 0.05$ and **$P < 0.001$ as compared to corresponding age-matched saline-injected controls; $^{+}P < 0.001$ as compared to the adult AMPH group. DG, dentate gyrus; Par Cx, parietal cortex; Cing Cx, cingulate cortex; Pre Limb Cx, prelimbic cortex; Pir Cx, piriform cortex.

rats, whereas in the adult rats, decreases in these regions were minor in comparison. In areas of the frontal cortex, the age-dependent effects were even more dramatic, with the drugs having opposite effects in adult and juveniles. Specifically, the data show that in these regions, both MPH and AMPH evoked significant reductions of *bdnf* mRNA in juvenile rats, whereas in adult rats the drugs increased *bdnf* gene expression (see Figs 1–3).

In common with their effects on *bdnf* gene expression, MPH and AMPH also showed marked age-dependent effects on *Arc* gene expression. For example, in the adult rats, both psychostimulants significantly increased *Arc* mRNA in the CA1 region of the hippocampus and cortical areas, whereas in juvenile rats the drugs had little effect (frontal cortex) or the opposite effect (hippocampus and parietal cortex). The finding of a lack of effect of MPH and AMPH on *Arc* gene expression in specific regions of the frontal cortex in juvenile rats agrees with a recent study demonstrating that MPH did not change *Arc* mRNA levels in the whole frontal cortex of juvenile rats (Chase *et al.*, 2007).

In addition to the above, our data showing that MPH induced much less locomotion in juvenile than in adult rats is consistent with previous studies investigating the age-dependent behavioural action of psychomotor stimulants in rodents (Bolaños *et al.*, 1998; Spear, 2000; Andersen, 2005). Behavioural activation (including increased locomotion) is known to enhance hippocampal and cortical *bdnf* and *Arc* gene expression (Russo-Neustadt *et al.*, 2000; Vazdarjanova *et al.*, 2006). Therefore, it is possible that the greater locomotor effect of MPH seen in adults than in juveniles is related to the findings that MPH generally induced larger increases (frontal cortex) or smaller decreases (hippocampus) in *bdnf* and *Arc* expression in the adults than in the juveniles.

The age-dependent effects observed in the present study are unlikely to be due to differences in the pharmacokinetic profile of

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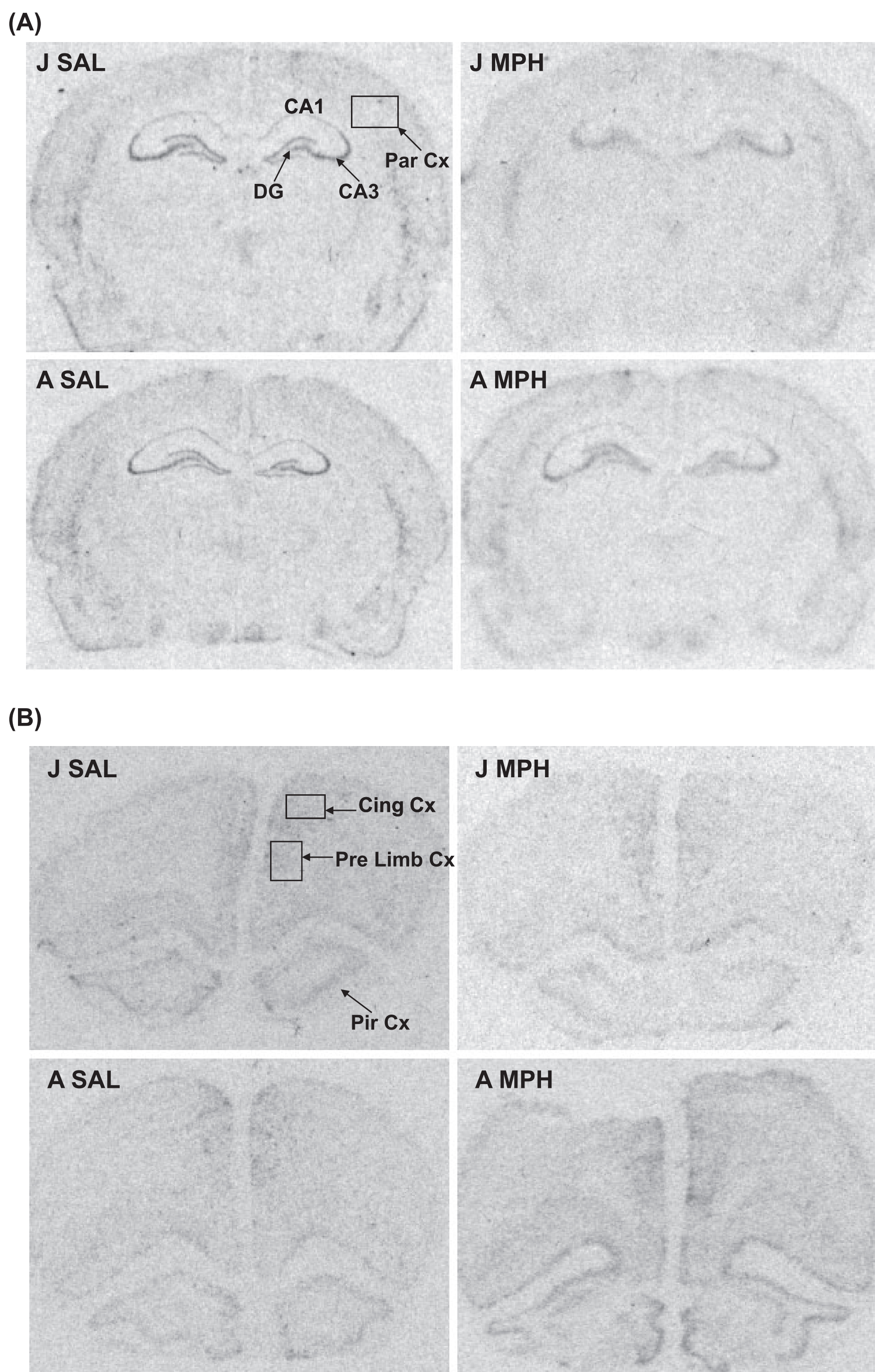

FIG. 3. Representative autoradiograms showing the age-dependent effect of methylphenidate (MPH) (2 mg/kg, intraperitoneal) on *bdnf* mRNA expression in hippocampal sub-regions and the parietal cortex (A), and in regions of the frontal cortex (B). SAL, saline. DG, dentate gyrus; Par Cx, parietal cortex; Cing Cx, cingulate cortex; Pre Limb Cx, prelimbic cortex; Pir Cx, piriform cortex. Stereotaxic landmarks from bregma (Paxinos & Watson, 2005) were: −3.48 to −3.60 mm (A) and +3.24 to +3.72 mm (B).

MPH and AMPH in juvenile and adult rats, because psychostimulant-evoked gene responses did not follow a consistent pattern. For example, the psychostimulant-induced reduction in hippocampal *bdnf* was greater in juvenile than in adult rats, whereas the psychostimulant-induced increase in cortical *Arc* expression was smaller in juvenile than in adult rats. Therefore, it seems more likely that the age-related effects reflect developmental differences in brain responsiveness to the psychostimulants. Indeed, marked maturation continues between adolescence and adulthood (Spear, 2000) specifically in regions such as the prefrontal cortex and hippocampus, which showed clear-cut age-dependent responses to the psychostimulants.

Importantly, both the dopamine and noradrenaline systems, which are main targets for MPH and AMPH, undergo pronounced

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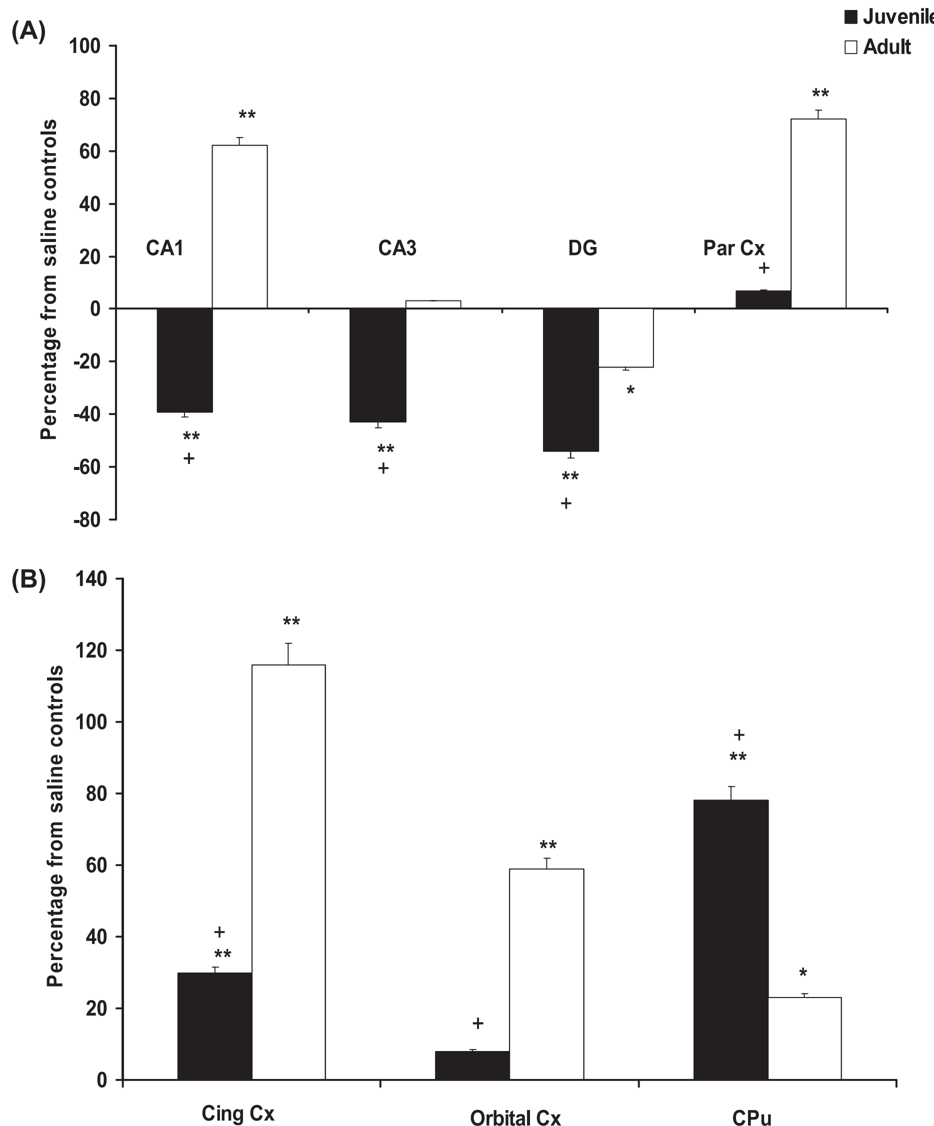

FIG. 4. Age-dependent effects of methylphenidate (MPH) [2 mg/kg, intraperitoneal (i.p.)] on *Arc* mRNA in hippocampal sub-regions and the parietal cortex (A), and in regions of the frontal cortex and caudate–putamen (B). Measurements were taken 2 h after drug administration. Data are expressed as percentage of saline-treated controls (1 mL/kg, i.p.); each column represents mean ± SEM value from five or six rats. $*P < 0.05$ and $**P < 0.001$ as compared to corresponding age-matched saline-injected controls; $^{+}P < 0.001$ as compared to the adult MPH group. DG, dentate gyrus; Par Cx, parietal cortex; Cing Cx, cingulate cortex; Orbital Cx, orbital cortex; CPu, caudate–putamen (dorsomedial striatum).

neurodevelopmental changes throughout juvenile and adolescent life. For example, previous studies indicate that, in comparison to adults, juvenile rats have a higher dopamine 'tone', including a lower density of dopamine transporters and increased depolarization-induced dopamine release *in vitro* (Stachowiak *et al.*, 1987; Stamford, 1989; Coulter *et al.*, 1996; Moll *et al.*, 2000). Age-dependent shifts in dopamine D1/D2 receptor density, and redistribution of the vesicular-mediated transporter 2, have also been reported (Andersen, 2002; Hanson *et al.*, 2004). In the case of the noradrenaline system, juveniles are different from adult rats in terms of shifts in noradrenaline turnover, increased *in vitro* release in response to blockade of the noradrenaline transporter, and reduced α_2 autoreceptor function (Choi *et al.*, 1997; Sanders *et al.*, 2005).

It is possible that the age-dependent effects on gene expression observed here are due to differences between adults and juveniles in psychostimulant-induced increases in extracellular monoamines. *In vivo* microdialysis studies show that MPH and AMPH increase extracellular monoamine levels in the brains of both juvenile and adult rats (Zetterström *et al.*, 1983; Hurd & Ungerstedt, 1989; Kuczenski & Segal, 2001; Berridge *et al.*, 2006). However, experiments directly comparing the effects of MPH and/or AMPH in adult and juvenile rats have, to our knowledge, not been conducted.

In the present study, measurements of *bdnf* and *Arc* expression were performed at 2 h after injections of MPH or AMPH. Previous studies in adult rats have shown that psychostimulants induce a short-lasting (0.5–3 h) increase followed by a delayed (6–24 h) fall in expression of some but not all *IEG* mRNAs (Yano & Steiner, 2005). The dynamics of psychostimulant-induced *IEG* mRNA changes in juvenile rats are currently unknown, and it cannot be fully excluded that these are different in the two age groups, that is, a faster onset in fall of gene expression in juveniles than in adults. If this is the case, it could explain the differences between the two age groups in *Arc* gene expression in the hippocampus and cortex (more psychostimulant-induced inhibition of mRNA expression in juveniles

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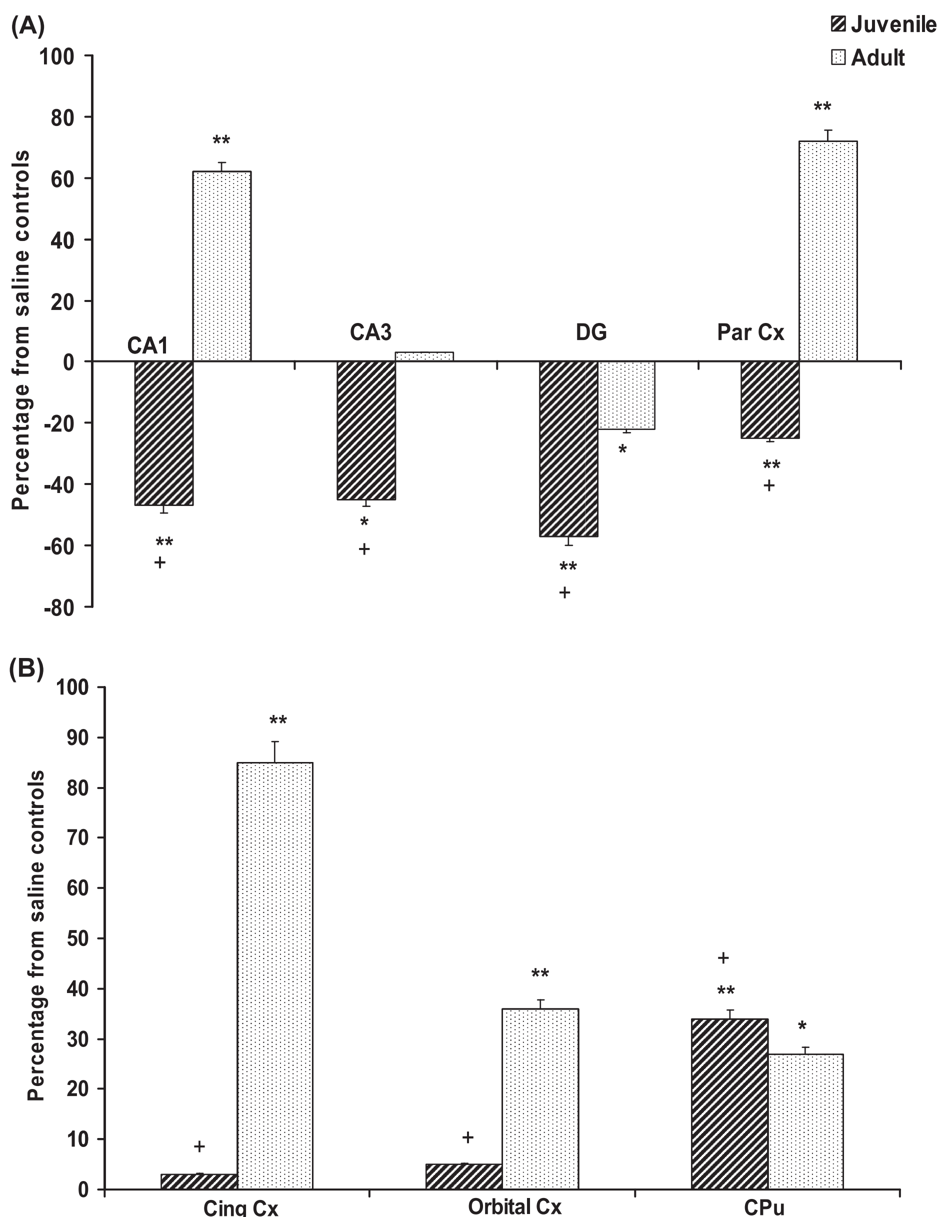

FIG. 5. Age-dependent effects of D-amphetamine (AMPH) (0.5 mg/kg, intraperitoneal) on *Arc* mRNA in hippocampal sub-regions and the parietal cortex (A), and in regions of the frontal cortex and caudate–putamen (B). Measurements were taken 2 h after drug administration. Data are expressed as percentage of saline-treated controls. Each column represents mean ± SEM value from five or six rats. *$P < 0.05$ and **$P < 0.001$ as compared to corresponding age-matched saline-injected controls; $^{+}P < 0.001$ as compared to the adult AMPH group. DG, dentate gyrus; Par Cx, parietal cortex; Cing Cx, cingulate cortex; Orbital Cx, orbital cortex; CPu, caudate–putamen (dorsomedial striatum).

than in adults) but not in the striatum, where the juvenile rats showed larger increases than the adults at 2 h after MPH or AMPH administration.

It is conceivable that the age-dependent changes in *bdnf* and *Arc* mRNA in response to psychostimulant administration reflect differences in the amino acid neurotransmitters γ-aminobutyric acid (GABA) and glutamate between juvenile and adult rats. Thus, the psychostimulants may evoke monoamine receptor-mediated changes in GABA and glutamate transmission, and this may have downstream effects on *bdnf* and *Arc* expression. In support of this hypothesis, GABA reduces *bdnf* mRNA expression and glutamate stimulates *Arc* mRNA expression (Zafra *et al.*, 1991; Heese *et al.*, 2000; Khundakar *et al.*, 2002), Moreover, age-related differences in these brain amino acid systems in cortical and hippocampal regions of the brain have been reported (Swann *et al.*, 1993; Liao & Malinow, 1996; Waters *et al.*, 1997; Sabau *et al.*, 1999; Potier *et al.*, 2006).

Numerous studies have shown that psychostimulant treatment evokes pronounced changes in expression of a variety of genes in the caudate nucleus (striatum) (Yano & Steiner, 2007). In the present study, basal levels of striatal *bdnf* mRNA were undetectable. This finding is consistent with previous studies demonstrating that BDNF protein in the striatum originates from prefrontal cortex projection neurons (Altar *et al.*, 1997; Conner *et al.*, 1997). In contrast, striatal neurons express readily detectable levels of *Arc* mRNA and the corresponding protein, and our finding of increased striatal *Arc* gene expression in both juvenile and adult rats following MPH and AMPH

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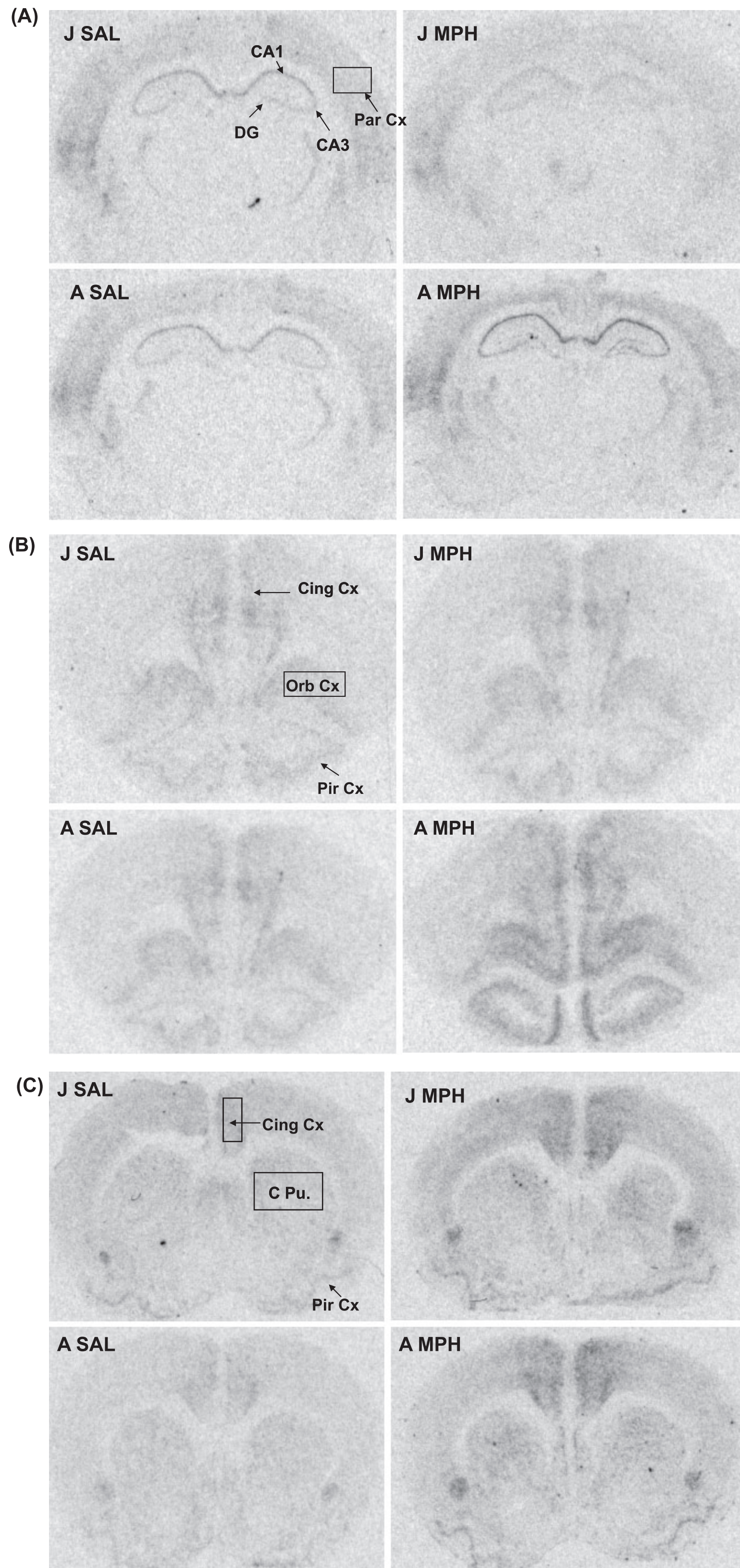

FIG. 6. Representative autoradiograms showing the age-dependent effect of methylphenidate (MPH) (2 mg/kg, intraperitoneal) on *Arc* mRNA expression in hippocampal sub-regions, the parietal cortex (A), regions of the frontal cortex (B) and the caudate–putamen (C). SAL, saline; DG, dentate gyrus; Par Cx, parietal cortex; Cing Cx, cingulate cortex; Orb Cx, orbital cortex; piriform cortex. Stereotaxic landmarks from bregma (Paxinos & Watson, 2005) were: −3.48 to −3.60 mm (A), +3.24 to +3.72 mm (B) and +0.36 to +0.48 mm (C).

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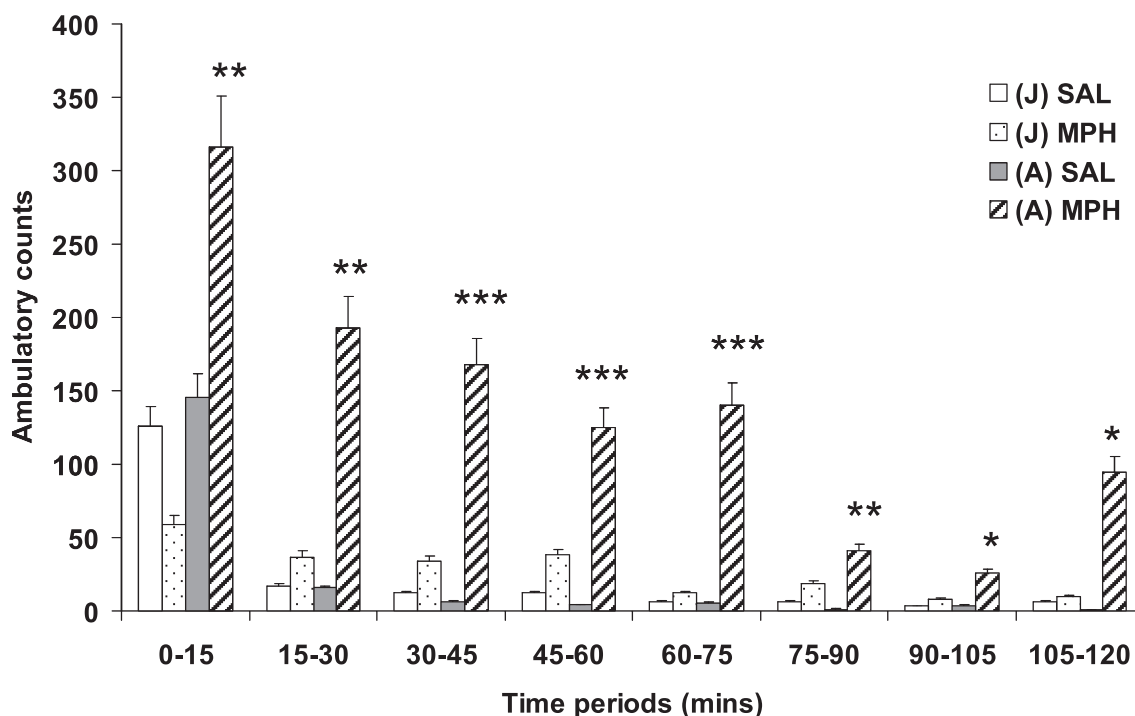

FIG. 7. Effects of methylphenidate (MPH) (2 mg/kg, intraperitoneal) on locomotion in juvenile and adult rats. Each bar shows cumulated counts/15 min. Rats were given saline or MPH, and placed without prior habituation in cages equipped with infrared photocells. Each point is mean ± SEM value (six rats per treatment group). $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$, as compared to the corresponding age-matched saline-treated control group (ANOVA followed by the Bonferroni *post hoc* test). SAL, saline; Cing Cx, cingulate cortex; CPu, caudate–putamen (dorsomedial striatum); Pir Cx, piriform cortex.

admistration is consistent with previous studies of *IEG* expression in rats of different ages (Brandon & Steiner, 2003; Adriani *et al.*, 2006). It is of particular relevance to the present study that it has recently been reported that both systemic and oral dosing of MPH increases striatal *Arc* mRNA and protein in juvenile rats (Chase *et al.*, 2007). The present study found that both MPH and AMPH increased *Arc* mRNA in the striatum and that this effect was more pronounced in the juveniles than in the adults. Interestingly, striatal *Arc* gene expression is triggered by a dopamine D1 receptor-induced increase in cAMP levels, and previous studies have reported enhanced D1 receptor expression and function in the juvenile striatum as compared to the adult striatum (Gelbard *et al.*, 1989; Andersen, 2002; Moro *et al.*, 2007).

The current finding of psychostimulant-induced decreases in *bdnf* expression in juvenile hippocampus and cerebral cortex, combined with the recognized importance of BDNF for normal neuronal development, survival and synaptic plasticity, highlights the possibility of harmful effects of psychostimulants on the developing brain. The potential seriousness of the reduced *bdnf* gene expression in young animals is underlined by our recent findings that the effect is dose-dependent, detected at half of the dose used in the present study, and sustained following chronic treatment (Banerjee & Zetterström, 2007, 2008). Monoamine systems rely on BDNF for normal development (Akbarian *et al.*, 2002; Dluzen *et al.*, 2002; Sokoloff *et al.*, 2002), and the present findings may therefore be linked to recent studies demonstrating that adult rats treated with MPH during early life (PND 20–35) show electrophysiological and behavioural evidence of decreased monoamine function in adulthood (Andersen *et al.*, 2002; Bolaños *et al.*, 2003; Brandon *et al.*, 2003; Carlezon *et al.*, 2003). Recent studies demonstrating impairments of BDNF-dependent and Arc-dependent processes such as hippocampal neurogenesis and memory in adult rats treated with MPH during development may also be related to the findings of the present study (Lagace *et al.*, 2006; LeBlanc-Duchin & Taukulis, 2007).

Conclusion

The present study demonstrates evidence of clear-cut differences in the responses of the juvenile and adult brain to administration of the psychostimulants MPH and AMPH. In the majority of brain areas examined, these drugs induced reductions in *bdnf* and *Arc* gene expression in the juveniles, whereas in adults the expression of these genes was generally increased. These results are highly relevant to recent studies in juvenile animals, using similar dosing regimens of MPH as those used in the present study, demonstrating alteration in behavioural models of depression, anxiety and cognition later in life. Determining how the present study and the majority of similar previous animal studies translate into the clinical situation will require further experiments, including those using oral doses of MPH, which produce plasma levels comparable with those seen in young children.

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Abbreviations

ADHD, attention-deficit/hyperactivity disorder; AMPH, D-amphetamine; BDNF, brain-derived neurotrophic factor; DG, dentate gyrus; GABA, γ-aminobutyric acid; i.p, intraperitoneal; IEG, immediate early gene; MPH, methylphenidate; PND, postnatal day.

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