Cognitive and emotional behavioural changes associated with methylphenidate treatment: a review of preclinical studies

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Abstract
There is evidence from animal studies that repeated exposure to methylphenidate (MPH), a widely used psychostimulant for the treatment of attention deficit hyperactivity disorder (ADHD), produces behavioural, structural and neurochemical changes that persist long after drug administration has ended. However, the translational utility of much of this work is compromised by the use of drug doses and routes of administration that produce plasma and brain MPH levels that fall outside the clinical range, i.e. experimental parameters more relevant to drug abuse than ADHD. We used PubMed to identify pre-clinical studies that employed repeated MPH administration at low doses in young rodents and examined long-term effects on cognition, emotion, and brain structure and function. A review of this work suggests that repeated MPH treatment during early development can modify a number of cognitive, behavioural and brain processes, but these are reduced when low therapeutic doses are employed. Moreover, MPH sites of action extend beyond those implicated in ADHD. Studies that combined neurobiological and behavioural approaches provide important insights into the mechanisms underlying MPH-produced effects on cognitive and behavioural processes, which may be relevant to MPH therapeutic efficacy. There is an emerging consensus that pharmacological treatment of childhood psychiatric disorders produces persistent neuroadaptations, highlighting the need for studies that assess long-term effects of early developmental pharmacotherapy. In this regard, studies that mimic clinical therapy with rodents are useful experimental approaches for defining the behavioural and neural plasticity associated with stimulant therapy in paediatric populations.

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Introduction
The psychostimulant methylphenidate (MPH) is a first-line pharmacological treatment for attention deficit hyperactivity disorder (ADHD) (Biederman et al. 2004), a neurodevelopmental disorder characterized by inattentiveness, impulsiveness and hyperactivity (Swanson et al. 1998). The prescribed use of MPH is widespread and has increased in recent years in developed (Pastor & Reuben, 2008) and developing (Scheffler et al. 2007) countries. Clinical evidence supports pharmacotherapy as the most effective treatment for ADHD throughout the duration of the disorder (Biederman et al. 2004; Mézsáros et al. 2009), which can begin in early childhood (Zito et al. 2000) and, in many cases, continues into adulthood (Spencer et al. 1996). Despite the therapeutic efficacy of MPH, the mechanisms underlying its cognitive and behavioural effects are less clear. Moreover, there is limited data regarding the long-term consequences of MPH exposure over extended periods.

The ability of MPH to improve ADHD symptoms is believed to occur as a consequence of its actions on catecholaminergic neurotransmission in fronto-subcortical circuits involved in the coordination of a variety of cognitive processes. The prefrontal cortex (PFC) circuitry directs goal-driven behaviours, regulates attentional processes, and inhibits inappropriate responses, functions that are impaired in ADHD and are improved with MPH (Arnsten, 2006). Because
dopamine (DA) and norepinephrine (NE) systems are the primary targets of MPH and undergo pronounced early developmental changes (Giedd et al., 2004). MPH effects during development are potentially greatest on functions that are mediated by catecholaminergic systems, such as cognition, motivation and emotion (Rosso et al., 2004). There is evidence from animal studies that repeated exposure to MPH during early development produces behavioural, structural and neurochemical changes associated with catecholaminergic processes that persist long after drug administration has ended (Bolaños et al., 1998, 2003; Brandon & Steiner, 2003; Brandon et al., 2003; Britton et al., 2007; Carlezon et al., 2003; LeBlanc-Duchin & Taukulis, 2007; Moll et al., 2001; Thanos et al., 2007). Additionally, a substantial body of pre-clinical work established associations between the effects of repeated exposure to stimulants on the brain’s reward circuitry and an increased risk for substance abuse in adulthood (White & Kalivas, 1998).

Currently, there are no reports of adverse long-term effects of MPH treatment in clinical populations, and psychostimulant therapy continues to be endorsed as the most effective approach to treating ADHD symptoms (Swanson et al., 2010). However, considering that MPH is prescribed at increasingly younger ages (Zito et al., 2000), it is essential to consider potential effects on cognition and behaviour that may go unnoticed in clinical populations. There are currently several proposed animal models of ADHD, but the insights gained from these models with respect to ADHD pharmacotherapy remain inconclusive (see Sontag et al., 2010 for review). Moreover, converging evidence indicates that MPH effects on behaviour and cognition are not unique to ADHD, and occur in both human and animal normal subjects. Therefore, repeated administration of psychostimulants in normal animals represents the best available experimental approach for understanding the mechanisms underlying MPH effects on neurotransmission and behaviour, as well as long-term neurobehavioural adaptations, associated with early developmental exposure to MPH in clinical populations.

Modelling MPH therapy in pre-clinical studies

Several researchers have noted that the translational utility of pre-clinical studies of early developmental MPH treatment is limited by a number of methodological factors, namely drug dosage and route of drug administration (Gerasimov et al., 2000; Kuczenski & Segal, 2002, 2005). In most animal studies MPH treatment is achieved through a subcutaneous (s.c.) or intraperitoneal (i.p.) injection across a wide range of doses (0.5–80.0 mg/kg) that exceed the relatively low recommended oral doses (0.3–2.0 mg/kg) in humans (Biederman et al., 2004). Repeated high-dose MPH injections have been shown to produce behavioural sensitization, a process that has been implicated in drug abuse liability and addiction (Robinson & Berridge, 1993; White & Kalivas, 1998), whereas low therapeutic doses are rarely associated with reinforcing effects (Berridge et al., 2006; Devilbiss & Berridge, 2008). In addition, the route of drug administration appears to be an important determining factor in consequent behaviour and neurochemistry. Orally administered MPH in rodents and humans produces a slower rate of absorption and lower peak concentrations, as well as a distinct neurochemical response, relative to MPH injections at comparable doses; thus, the same MPH dose applied through different routes produces widely different behavioural and neurochemical effects (Dafny & Yang, 2006; Gerasimov et al., 2000; Kuczenski & Segal, 2001, 2002; Volkow et al., 1998). In all likelihood, differences among pre-clinical studies in dosing parameters account for the varying reports derived from animal studies regarding the neurobehavioural effects and potential risks associated with MPH treatment in clinical populations.

Inclusion criteria

The aim of this review was to examine the effects of repeated MPH administration on cognition, emotional behaviours, and brain systems in healthy rodent subjects. Rodents are especially useful for examining pharmacological effects on developmental factors for a number of reasons. The juvenile rodent brain resembles the developing human brain in many ways, including the alterations in catecholaminergic systems and PFC circuitry that take place during adolescence (Moll et al., 2000; Spear, 2000). Moreover, several paradigms for assessing simple and complex behaviours in rodents have been established, along with a large body of evidence regarding the associated neural circuitry. Importantly, studies with rodent subjects are critical for establishing links between MPH-produced changes in neural processes and MPH-produced changes in behaviour, an approach that may reveal therapeutic mechanisms as well as potential risk factors related to MPH therapy in humans.

In order to identify relevant studies for this review, a search was conducted in PubMed in the last week of May 2010 using the terms ‘methylphenidate’, ‘cognition’, ‘emotion’, ‘rats’, ‘mice’, and their variants. Studies were included if they assessed MPH effects on
cognition, emotional behaviours and/or brain structure and function, and met the following criteria: (1) employed juvenile or adolescent normal rodent subjects (see McCutcheon & Marinelli, 2009 and Verma et al. 2004, for discussions of rodent developmental age); (2) administered doses of MPH ≤5 mg/kg, regardless of route of administration, and (3) administered MPH for at least 7 d during these developmental periods. The latter criterion was established in order to assess the effects of repeated MPH exposure, a topic that has received less attention than the effects of single MPH administrations (see Askenasy et al. 2007 and Dafny & Yang, 2006, for reviews of acute MPH treatment effects). The effects of acute MPH were included for discussion purposes only. Long-term treatment was arbitrarily established as 1-wk treatment in a young rodent’s life, which is roughly equivalent to 1–2 years of human adolescence (Spear, 2000). Selection of the dose and route of administration criteria were based on estimates indicating that oral doses ≤3 mg/kg and i.p. injections ≤2 mg/kg produce plasma drug levels in rodents that fall within the clinical range of 8–40 ng/ml (Aoyama et al. 1990; Gerasimov et al. 2000) and below threshold values for locomotor activation, and thus may be considered close approximations to clinical MPH therapy (Dowell-Edwards et al. 2008; Kuczenski & Segal, 2002, 2005). Moreover, by including doses of MPH ≤5 mg/kg, effects of therapeutic MPH doses could be contrasted with those exceeding clinical levels. The studies that met the above criteria are summarized in Table 1.

**MPH effects on neurochemistry, cognition and emotion**

**Drug targets and drug dose: relevance to therapeutic outcome**

The effects of MPH on catecholaminergic systems have been studied extensively. MPH functions in a manner similar to other stimulants by increasing extracellular DA and NE, but differs in that it has negligible effects on serotonin levels (Kuczenski & Segal, 1997). MPH enhances catecholaminergic levels by blocking reuptake through the dopamine transporter (DAT) and norepinephrine transporter (NET) (Arnsten & Dudley, 2005; Volkow et al. 1998, 2001). Although previous research focused on MPH-produced enhancements of striatal and nucleus accumbens DA, recent studies using low doses of MPH show significant effects in areas beyond motor and brain reward pathways (Kuczenski & Segal, 2002; Volkow et al. 1997, 2007). Low doses of MPH (≤3 mg/kg p.o., ≤1.0 mg/kg i.p., 2.5 mg/kg s.c.) increase hippocampal NE without appreciable effects on striatal or nucleus accumbens DA, and increase both DA and NE release in PFC (Berridge et al. 2006; Kuczenski & Segal, 2002; Weikop et al. 2007) primarily through NET inhibition (Arnsten & Dudley, 2005). In PFC, MPH-produced increases in catecholaminergic release promote improvements in cognitive functions through DA actions on D1 receptors and NE actions on α2A receptors (Arnsten & Dudley, 2005). MPH also transiently increases cortical and hippocampal acetylcholine release (Tzavara et al. 2006). Importantly, converging evidence suggests that increases in catecholaminergic signalling in PFC are more relevant to the therapeutic efficacy of MPH (Arnsten & Li, 2005; Berridge et al. 2006; Levy, 2008), as both NE and DA have critical influences on PFC cognitive processes such as working memory and sustained attention.

The role of catecholamines in PFC function and its importance in cognitive processes is supported by both basic and clinical research. In humans, acute administration of MPH at low therapeutic doses produces improvements in PFC-dependent tasks that involve working memory in normal (Elliott et al. 1997; Mehta et al. 2000) and ADHD (Mehta et al. 2004) subjects. Similarly, in rodent studies, acute administration at low doses improves memory and correct performance in a number of tasks in juvenile (3 mg/kg p.o.; Zhu et al. 2007, 2010) and young adult (≤2 mg/kg p.o. and i.p.; Arnsten & Dudley, 2005; Paine et al. 2007) rats. The effects of low-dose MPH on NE levels in the PFC (Berridge et al. 2006; Kuczenski & Segal, 2002) are believed to underlie the capacity of MPH to reduce hyperactivity and improve various forms of academic performance in ADHD children (see Pietrzak et al. 2006 for review). In contrast to the beneficial effects of low-dose MPH, administration at higher doses impairs cognitive performance in both rodents (10 mg/kg p.o.; 5 mg/kg i.p.; Chuhan & Taubulis, 2006; Heyser et al. 2004) and humans (Arnsten, 2006; Douglas et al. 1995). High doses may impair cognitive function through excessive catecholaminergic activity in PFC, just as high levels of catecholamines released during stress produce impaired PFC function (Arnsten & Li, 2005). Altogether, findings from human and animal research suggest that MPH achieves its therapeutic effects on cognitive processes when administered at low doses that promote optimal catecholaminergic release within PFC circuitry.

**MPH effects on cognitive processes**

Surprisingly few studies have examined the long-term effects of chronic MPH exposure on cognition using
### Table 1. Early developmental methylphenidate (MPH) treatment (≤5 mg/kg) effects on cognition, emotional behaviours, and neural systems

<table>
<thead>
<tr>
<th>Reference</th>
<th>Age*, species</th>
<th>Dosingb and duration</th>
<th>Time between MPH exposure and assessment</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bethancourt et al. (2009)</td>
<td>PND 27, rats</td>
<td>2 or 5 mg/kg p.o., b.i.d., 7 wk</td>
<td>18 d</td>
<td>No effects on delayed (20–30 min) object recognition or contextual fear memory</td>
</tr>
<tr>
<td>Bolaños et al. (2003)</td>
<td>PND 20, rats</td>
<td>2 mg/kg i.p., b.i.d., 16 d</td>
<td>5 d for play behaviour; 6 wk all other tests</td>
<td>Decreased exploration in novel environment; increased anxiety in elevated plus-maze; increased depressive-like symptoms in forced swim test; no effect on social interaction or play behaviour</td>
</tr>
<tr>
<td>Brandon et al. (2003)</td>
<td>PND 35, rats</td>
<td>2 mg/kg i.p., 7 d</td>
<td>1–3 d or 14–21 d</td>
<td>Altered midbrain DA neural activity: increased after early withdrawal (1–3 d), decreased after late withdrawal (14–21 d); no change in autoreceptor sensitivity.</td>
</tr>
<tr>
<td>Britton et al. (2007)</td>
<td>PND 25, rats</td>
<td>2 mg/kg i.p., b.i.d., 15 d</td>
<td>42 d</td>
<td>Enhanced contextual (but not cued) fear memory at 24 h post-training; no effect on fear acquisition</td>
</tr>
<tr>
<td>Britton &amp; Bethancourt (2009)</td>
<td>PND 27, rats</td>
<td>2 or 3 mg/kg p.o., b.i.d., 4 or 7 wk</td>
<td>18 d</td>
<td>No effects on anxiety-related behaviours in open field, light-dark, elevated plus-maze, or contextual fear-conditioning tests</td>
</tr>
<tr>
<td>Carlezon et al. (2003)</td>
<td>PND 20, rats</td>
<td>2 mg/kg i.p., b.i.d., 16 d</td>
<td>25 d</td>
<td>Increased depressive-like symptoms in forced swim test; reduced habituation</td>
</tr>
<tr>
<td>Chase et al. (2003)</td>
<td>PND 25, rats</td>
<td>1 or 2 mg/kg s.c., 14 d</td>
<td>2 h</td>
<td>Decreased c-fos expression (2 mg/kg) relative to acute MPH injection</td>
</tr>
<tr>
<td>Gomes et al. (2010)</td>
<td>PND 25, rats</td>
<td>2 mg/kg i.p., 28 d</td>
<td>2 h</td>
<td>No effects on acquisition or short-term retention of inhibitory avoidance in rats treated at night, but impaired long-term retention; impaired long-term memory for rats treated during the day</td>
</tr>
<tr>
<td>Gray et al. (2007)</td>
<td>PND 7, rats</td>
<td>5 mg/kg i.p., b.i.d., 28 d</td>
<td>95–100 d</td>
<td>No effects on open-field behaviours, decreased anxiety in elevated plus-maze; no neurochemical or cellular differences in brain regions associated with ADHD</td>
</tr>
<tr>
<td>Heyser et al. (2004)</td>
<td>PND 15 and PND 28, rats</td>
<td>2 or 5 mg/kg i.p., b.i.d., 7 d</td>
<td>30 min</td>
<td>Increased locomotor activity and impaired novel object exploration at high (5 mg/kg) but not low (2 mg/kg) dose</td>
</tr>
<tr>
<td>Koda et al. (2010)</td>
<td>PND 35, mice</td>
<td>3 mg/kg i.p., 21 d</td>
<td>24 h</td>
<td>No effect on monoamine levels or patterns of c-fos expression in striatum or prefrontal cortex; no effect on locomotor activity</td>
</tr>
<tr>
<td>Study</td>
<td>Age</td>
<td>Treatment</td>
<td>Duration</td>
<td>Behavioral Changes</td>
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<tr>
<td>Lagace et al. (2006)</td>
<td>PND 20, rats</td>
<td>2 mg/kg i.p., 16 d</td>
<td>12–78 d</td>
<td>Decreased hippocampal neurogenesis at adulthood; increased plasma corticosterone but no effect on cell proliferation; no effect on locomotion</td>
</tr>
<tr>
<td>LeBlanc-Duchin &amp; Taukulis (2007)</td>
<td>PND 35–39, rats</td>
<td>2, 3 or 5 mg/kg p.o., b.i.d., 11 or 21 d</td>
<td>14, 28, or 42 d</td>
<td>Impaired memory (but not acquisition) in delayed (3 h) object recognition test (3 and 5 mg/kg for 21 d) at all test intervals</td>
</tr>
<tr>
<td>McFadyen et al. (2002)</td>
<td>PND 26, mice</td>
<td>2.5 mg/kg s.c., 7 d</td>
<td>24 h</td>
<td>No effects on exploration or anxiety-related behaviour in elevated plus-maze testing; no effect on water maze learning</td>
</tr>
<tr>
<td>Moll et al. (2001)</td>
<td>PND 25, rats</td>
<td>2 mg/kg p.o., 2 wk</td>
<td>6 d or 4.5 wk</td>
<td>Decreased density in striatal DAT (25% after 6 d, 50% in adulthood)</td>
</tr>
<tr>
<td>Penner et al. (2002)</td>
<td>PND 3, mice</td>
<td>5 mg/kg s.c., 9 d</td>
<td>30 min, tested every 2 d</td>
<td>No effect on anxiety levels (ultrasonic vocalizations) on any test day</td>
</tr>
<tr>
<td>Scherer et al. (2010)</td>
<td>PND 15, rats</td>
<td>2 mg/kg i.p., 30 d</td>
<td>24 h</td>
<td>Impaired spatial learning, memory and navigation in Morris water maze; decreased brain-derived neurotrophic factor content and increased acetylcholinesterase activity in prefrontal cortex but not hippocampus</td>
</tr>
<tr>
<td>Thanos et al. (2007)</td>
<td>PND 30, rats</td>
<td>1 or 2 mg/kg p.o., 2 or 8 months</td>
<td>24 h</td>
<td>Decreased striatal DA receptor availability after 2 months, increased after 8 months</td>
</tr>
<tr>
<td>Wiley et al. (2009)</td>
<td>PND 20, rats</td>
<td>2 mg/kg i.p., b.i.d., 16 d</td>
<td>55 d</td>
<td>Increased anxiety in elevated plus-maze; increased depressive-like symptoms in forced swim test</td>
</tr>
<tr>
<td>Zeise et al. (2007)</td>
<td>PND 28, rats</td>
<td>1 mg/kg p.o., t.i.d., 17 wk</td>
<td>1 h</td>
<td>No effect on spatial learning or re-learning in water maze task with invisible platform; improved with visible platform</td>
</tr>
<tr>
<td>Zeile et al. (2007)</td>
<td>PND 22, degus</td>
<td>1 or 5 mg/kg i.p., 24 d</td>
<td>24 h</td>
<td>Increased dendritic length and complexity of pyramidal neurons in anterior cingulate cortex; low dose (1 mg/kg) produced most extensive dendritic growth</td>
</tr>
<tr>
<td>Zhu et al. (2007)</td>
<td>PND 22, rats</td>
<td>3 mg/kg p.o., 19 d</td>
<td>30 min, repeated testing</td>
<td>Enhanced spatial learning and memory in water maze task (PND 22–39), no effect on locomotion (PND 40)</td>
</tr>
</tbody>
</table>

PND, Postnatal day; p.o., oral; b.i.d., twice daily; t.i.d., three times daily; i.p., intraperitoneal; s.c., subcutaneous; DA, dopamine; DAT, dopamine transporter.

a Age at first MPH exposure.

b Only dose values that meet inclusion criteria (≤5 mg/kg) are included.

c Indicates behavioural testing took place during active phase of animals’ circadian cycle.
clinically relevant doses. Recent studies have produced mixed results regarding MPH effects on spatial learning, namely, impairments (2 mg/kg i.p.), enhancements (3 mg/kg p.o.) or no effects (1 mg/kg p.o.) have been reported following repeated MPH treatment (Scherer et al. 2010; Zeise et al. 2007; Zhu et al. 2007) (see Table 1). Impairments in spatial reference and working memory were linked to decreased BDNF levels and increased acetylcholinesterase activity in PFC, but not hippocampus (Scherer et al. 2010). In contrast, repeated MPH exposure has produced more consistent dose-dependent effects on object recognition memory, a task widely employed to examine memory processes by assessing animals’ ability to discriminate a previously encountered stimulus from a novel stimulus (Ennaceur & Delacour, 1988). Repeated MPH treatment produced impaired memory for objects in weanling and peri-adolescent rats at a high (5 mg/kg i.p.) but not a low (2 mg/kg) dose (Heyser et al. 2004) (see Table 1). Similarly, an acute high dose (10 mg/kg p.o.) impaired memory in adult rats (Chuhan & Taukulis, 2006). Animals treated with oral MPH (2, 3 or 5 mg/kg) twice daily for 11 d or 21 d and tested 2, 4, and 6 wk post-treatment (LeBlanc-Duchin & Taukulis, 2007) (see Table 1) showed impairments at the two highest doses, but not the lower dose, at each of the three test sessions. Similarly, no enduring effects of 2 mg/kg oral MPH were found in rats treated for 7 wk and tested 18 d following the last drug administration (Bethancourt et al. 2009) (see Table 1). A high oral MPH dose (5 mg/kg) produced impairments when rats were treated for 3 wk (LeBlanc-Duchin & Taukulis, 2007) but not for 7 wk (Bethancourt et al. 2009), which may be due to a several factors. The timing of treatment onset, as well as treatment duration, produced different effects on object recognition memory, and there is evidence that both factors influence the effects of MPH during development (Andersen et al. 2002; Thanos et al. 2007). Moreover, impairments were found when animals were treated and tested during the inactive period (light cycle) of the light/dark cycle (LeBlanc-Duchin & Taukulis, 2007), but not during the active period (Bethancourt et al. 2009). Several studies have established that MPH administration in rodents at different points during the circadian cycle produces widely different behavioural responses (Gaytan et al. 1996, 2000; Gomes et al. 2010) (see Table 1), and further, that testing animals during the inactive period limits the extent to which behavioural results can be extrapolated to clinical populations (Kuczenski & Segal, 2002). Last, MPH produced deficits when the interval between acquisition and retention was 3 h (LeBlanc-Duchin & Taukulis, 2007) but not 30 min (Bethancourt et al. 2009), and under normal circumstances, longer delays (>30 min) have been shown to decrease the preference for novelty (Ennaceur & Delacour, 1988). In sum, while the effects of MPH dose on spatial memory vary across studies, the majority of research assessing MPH effects on object recognition memory indicates that low doses do not produce impairments.

**MPH effects on emotional processes**

Catecholamine signalling systems also mediate a complex of emotional and motivational processes. DA neurotransmission is implicated in signalling the emotional salience of events (Gray et al. 1997), and NE activation in the amygdala is associated with enhanced consolidation of emotional memories (McGaugh, 2000). Clinical research focusing on the influence of MPH on emotional processes has produced mixed results (Brignell et al. 2007; Tannock et al. 1995; Williams et al. 2008), but there is some evidence of limbic abnormalities associated with ADHD that may account for motivational aspects of ADHD pathology. Positron emission tomography studies have provided preliminary evidence of disrupted DA release in hippocampus and amygdala of ADHD adults (Volkow et al. 2007), and morphological abnormalities in amygdala and hippocampus have been detected in ADHD children and adolescents (Plessen et al. 2006). Based on the roles of amygdala and hippocampus in mediating motivational and memory functions (Broadbent et al. 2004; Gray et al. 1997), it is possible that brain abnormalities underlie ADHD impairments in attention and academic performance. However, whether structural abnormalities in ADHD brains reflect specific behavioural deficits remains unknown.

In animal studies, the influence of MPH on emotional behaviours has been examined with paradigms that assess spontaneous fear responses such as the elevated plus-maze, a behavioural task based on rodents’ innate aversion to open and elevated spaces (Lister, 1987). An acute oral dose of MPH (3 mg/kg) reduced anxiety in juvenile rats tested in the elevated plus-maze (Zhu et al. 2010). A different measure of anxiety, ultrasonic vocalizations, was not affected in neonatal mice treated with an acute high dose (5 or 20 mg/kg) (Penner et al. 2002) (see Table 1). Research employing learned-fear paradigms revealed enhancing effects of acute MPH (1 and 5 mg/kg) exposure on associative fear conditioning in adult rats (Horsley & Cassaday, 2007). Namely, learned-fear
responding in adult rats to both discrete and contextual cues was enhanced by acute MPH exposure, suggesting that MPH actions are capable of modulating amygdala- and hippocampus-dependent forms of learning (Davis, 2000; Phillips & LeDoux, 1992).

Repeated administration of MPH during early development has produced mixed results in regard to emotional behaviours, but experimental parameters varied greatly across studies. Prenatal exposure (5 mg/kg s.c. for 9 d) decreased anxiety in mice tested in adulthood (McFadyen-Leussis et al. 2004) (see Table 1), and the same dose administered chronically (i.p.) at ages when catecholaminergic systems are still developing decreased anxiety in the plus-maze at adulthood (Gray et al. 2007) (see Table 1). Adolescent mice exposed to MPH for 1 wk (2.5 mg/kg s.c.) were not impaired in elevated plus-maze testing or spatial learning (McFadyen et al. 2002) (see Table 1), although 1-wk treatment with a high dose (40 mg/kg s.c.) reduced anxious behaviours (Carrey et al. 2000). Variations in MPH doses, routes of administration and testing procedures among these studies limit the degree to which conclusions can be drawn regarding MPH effects. Notably, the study that employed a low dose of MPH and behavioural testing during active hours in mice (McFadyen et al. 2002), reported no effects on behavioural responses.

Four studies that employed similar regimens of MPH dosing (2 mg/kg i.p.) and treatment duration (15–20 d) in peri-adolescent rats produced consistent increases in emotional behaviours. Chronic MPH exposure decreased exploration of a novel environment, increased anxiety in the elevated plus-maze, and increased depressive-like symptoms in the forced swim test (Bolaños et al. 2003; Carlezon et al. 2003; Wiley et al. 2009) (see Table 1), a behavioural assay in which increased latencies to exhibit immobility in a cylinder filled with water are interpreted as evidence of depressive-like behaviour (Petit-Demouliere et al. 2005). The same treatment enhanced learned fear behaviours using the fear-conditioning paradigm (Britton et al. 2007). Specifically, MPH increased responding to learned contextual cues, an amygdala- and hippocampal-dependent process, but not to a discrete auditory cue, a form of amygdala-dependent learning (Davis, 2000; Phillips & LeDoux, 1992). This is in contrast to the effects of acute MPH effects on fear conditioning (Horsley & Cassaday, 2007), which produced effects on both forms of fear learning. Together, the results of fear-conditioning studies point to age, treatment duration, and/or dose-dependent effects of long-term MPH treatment on limbic regions, and suggest that MPH actions on brain memory systems beyond those implicated in ADHD pathology should be considered in research addressing its therapeutic effects.

When MPH treatment is designed to mimic clinical therapy more closely (i.e. oral administration at low therapeutic doses during periods of normal activity), no enduring effects on anxiety behaviours are observed. Adolescent rats exposed to low doses (2 or 3 mg/kg p.o.) of MPH for extended periods spanning adolescence through early adulthood exhibited no behavioural impairments under various experimental conditions that elicit a complex of learned and unlearned fear responses (Bethencourt et al. 2009; Britton & Bethencourt, 2009) (see Table 1). In a battery of behavioural tests, only a high dose (5 mg/kg p.o.) produced transient increases in fear responding to an aversive context, and this effect was attenuated with repeated exposure to the context (Bethencourt et al. 2009). Interestingly, although MPH had no effect on fear behaviours, one aspect of this work suggests that a more prolonged period of MPH exposure (7 wk) produced lower levels of anxiety relative to a shorter period (4 wk) (Bethencourt et al. 2009). To the extent that the immature nervous system of the adolescent rat resembles that of a young child, these findings suggest that clinically relevant oral doses do not produce significant enduring effects on emotional behaviours. Nevertheless, because MPH is used widely in clinical practice, even subtle effects on emotional processes can have important implications for the drug’s actions on emotional cognitive function in ADHD. Importantly, the consistent pattern of altered emotional behaviours in rats following 2-wk treatment with 2 mg/kg (Bolaños et al. 2003; Britton et al. 2007; Carlezon et al. 2003; Wiley et al. 2009), underscores the importance of assessing MPH effects on emotional brain systems more closely.

**MPH effects on brain structure, neurochemistry and gene expression**

In agreement with the results of behavioural studies, the effects of long-term MPH treatment on brain systems are complex and varied, and have been shown to depend on a number of factors. Age determines the magnitude of PFC activation following MPH administration (Benjamin et al. 2010; Devilbiss & Berridge, 2008), probably due to maturational differences in catecholaminergic systems (Spear, 2000). The duration of drug treatment also determines the effects of MPH exposure on brain function. Oral MPH treatment at
low doses (1 and 2 mg/kg) beginning in adolescence and lasting for 2 or 8 months produced decreases and increases, respectively, in DA receptor availability (Thanos et al. 2007) (see Table 1). Acute MPH (3 mg/kg i.p.) treatment in adolescent mice [postnatal day (PND) 35] produced increases in extracellular levels of NE and DA in PFC as expected, whereas long-term treatment (21 d) did not produce enduring changes in either neurotransmitter or in locomotor activity (Koda et al. 2010) (see Table 1). The period of drug withdrawal also has been shown to influence the effects of MPH administration. Four weeks of treatment (5 mg/kg i.p.) in young rats produced immediate structural changes in various brain regions that were absent in animals examined several months later (Gray et al. 2007) (see Table 1). Similarly, the activity of midbrain DA neurons was shown to be affected differentially by the period of withdrawal following 1-wk treatment with MPH (2 mg/kg i.p.; Brandon et al. 2003) (see Table 1). Additional evidence of long-lasting changes in catecholaminergic neurotransmission following early MPH administration indicated that low-dose oral treatment (2 mg/kg) for 14 d in adolescent rats produced a decrease in DAT levels in striatum following short and long withdrawal periods (Moll et al. 2001) (see Table 1). Whether these drug-produced effects on striatal DA function are related to cognitive-emotional function has not been established; however, modifications in DA processes could potentially influence cognitive processes such as working memory and cognitive flexibility through extensive projections to PFC (Rosso et al. 2004).

Studies employing genetic and molecular approaches have identified MPH-produced changes in gene expression and neural plasticity, both when applied acutely and chronically. Immunodetection of the immediate early gene c-fos, believed to reflect increased neuronal activity (Hughes & Draganow, 1995), was modified by acute and chronic treatment with MPH in rodent striatum in a dose (Brandon & Steiner, 2003; Chase et al. 2003), treatment duration (Koda et al. 2010), and age-dependent (Penner et al. 2002) manner. Additionally, acute and chronic MPH (2 mg/kg i.p.) produced decreased expression of BDNF, a gene associated with neuronal development and plasticity, in PFC and hippocampus of juvenile rats (Banerjee et al. 2009; Scherer et al. 2010), an effect that was linked to MPH-produced impairments in spatial learning and memory (Scherer et al. 2010) (see Table 1). Recent studies have revealed also the potential of MPH treatment to modify neural plasticity. Hippocampal neurogenesis, a BDNF-dependent process, was decreased by long-term MPH treatment (2 mg/kg i.p.) (Lagace et al. 2006) (see Table 1). At a lower dose, long-term MPH treatment (1 mg/kg i.p.) increased dendritic length and complexity in anterior cingulate cortex (Zehle et al. 2007) (see Table 1), a region known to play a key role in attention and executive functions (Swanson et al. 2010). These findings are consistent with reports that other DA stimulants administered at therapeutic doses promote long-term dendritic growth (Diaz Heijtz et al. 2003), and have interesting implications for enduring psychostimulant effects on synaptic organization. In sum, MPH produces a number of neuroadaptations that vary depending on dose, treatment pattern and duration, among other factors, that jointly provide evidence of the variety of cellular and molecular modifications that may be associated with drug-induced changes in complex behaviours. Future work combining neurobiological and behavioural approaches will clarify this possibility.

While most information regarding the therapeutic effects of low-dose MPH treatment has been derived from studies focused on drug-related effects on catecholaminergic signalling in PFC and PFC-dependent behaviours (Arnsten & Dudley, 2005; Arnsten & Li, 2005; Berrett et al. 2006), examples of MPH-induced neural plasticity outside the PFC make novel contributions to an understanding of the cognitive enhancing effects of low-dose MPH treatment. In one study, low-dose MPH administration directly into the amygdala was shown to improve performance in cue-reward learning and to facilitate amygdala plasticity (Tye et al. 2010). Importantly, enhanced learning and improved focus, two therapeutic effects of MPH in clinical populations (Pietrzak et al. 2006), were linked to amygdala D1 and D2 receptor activation, respectively (Tye et al. 2010), implicating MPH modulation of amygdala in mediating emotional and motivational aspects of MPH therapy. An earlier study showed that a low-dose acute application of MPH enhanced long-term plasticity in the hippocampus (Dommett et al. 2008), that coupled with previous work showing MPH-produced increases in hippocampal NE (Kuczynski & Segal, 2002), suggests that the drug acts directly at brain sites and on molecular mechanisms implicated in cognition through its actions on hippocampal NE signalling (Arnsten & Li, 2005). Taken together, these studies provide preliminary evidence that low-dose MPH treatment achieves its effects on cognitive processes by promoting neural plasticity mediated by catecholaminergic systems not only in PFC but also in limbic areas directly implicated in learning and memory processes.
Pre-clinical studies of MPH pharmacotherapy: clinical implications and limitations

Early-life exposure to MPH has complex effects on cognition, emotional behaviours and related brain systems in rodents, and may have similar effects in children. Pre-clinical research has identified a number of MPH-induced alterations in brain structure and function that suggest that early pharmacotherapy can produce neuroadaptations that persist long after drug treatment has ended, but with few exceptions, the behavioural relevance of these changes has not been established. Similarly, results from behavioural studies suggest that cognitive and emotional behaviours are modified by repeated MPH exposure, but results have not always been consistent. Nevertheless, pre-clinical studies with rodents are helpful in advancing an understanding of MPH effects on brain and behavioural processes, especially when clinically relevant parameters are employed. As described earlier, evidence from animal behaviour studies has revealed several factors that influence the long-term effects of MPH exposure during early development, including drug dose and developmental age. Thus, an important consideration that emerges from the literature is the influence of employing clinically relevant parameters in pre-clinical studies aimed at determining the effects of pharmacotherapy on paediatric populations. In this review, the majority of studies that employed low doses of MPH, as well as all of the studies that conducted behavioural testing during rodents’ active hours, reported the absence of MPH effects on a variety of cognitive and behavioural tasks (see Table 1). Although this should not be taken as unequivocal evidence of a lack of MPH effects on the behaviours tested, the results are consistent with clinical observations of no adverse effects of repeated low-dose MPH treatment in children and adolescents (Greenhill et al. 2006; Shaw et al. 2009), and underscore the influence of employing clinically relevant parameters in behavioural pharmacological studies of MPH.

On the other hand, pre-clinical evidence of MPH effectiveness in enhancing cognitive function has been less consistent. Overall, animal studies report improvements, impairments or no changes associated with low-dose MPH treatment, presumably due to variations in experimental parameters and cognitive behavioural models (Carlezon & Konradi, 2004). Improvements in pre-clinical approaches to the question of MPH effectiveness should promote a better understanding of the short- and long-term therapeutic effects of MPH treatment. The studies reviewed here indicate that the application of a low-dose MPH regimen that has been shown to improve cognitive function without producing hyperactivity provides the opportunity to investigate the neural mechanisms underlying MPH therapy and its long-term effects on cognition and emotion. This view is supported by clinical evidence that shows that the benefits of low-dose MPH therapy are greater for behavioural (i.e. reducing hyperactivity) than for cognitive (i.e. enhancing focus and attention) domains (Swanson et al. 2010).

Last, there are a number of limitations regarding pre-clinical studies employing rodent models that should be clarified. Although considerable similarities exist between human and rodent species, the degree of generalizability from pre-clinical to clinical contexts is limited by differences in stages of brain development and the complexity of cognitive processes that can be modelled in rodents. A recent study conducted with juvenile rhesus monkeys showed that long-term (16 months) oral MPH administration with escalating doses (0.15–2.5 mg/kg) produced no long-term impairments in the performance in an operant test battery, whereas higher doses (0.15–12.5 mg/kg) produced notable performance deficits (Rodriguez et al. 2010). These results, which confirm the findings that low MPH doses do not produce impairments in cognitive performance, are particularly intriguing because of the high degree of generalizability between the monkey and human brain regarding catecholaminergic systems. Nevertheless, a second limitation that pertains to these results and others reviewed here, involves the use of normal animal subjects and not animal models of ADHD. In this regard, studies with healthy animals primarily address the question of how MPH affects normal brain functioning, not necessarily ADHD brains. Because clinical evidence points to a dysfunctional DA system as the underlying cause of ADHD and the widely held assumption that the brains of ADHD and normal children are distinctly different in regard to catecholaminergic neurotransmission (Prince, 2008), it remains to be determined whether repeated exposure to MPH has different effects in ADHD brains. An effective approach to this question will require improvements in animal models, comparative cognitive-behavioural tests, and non-invasive methods for detecting drug-produced pharmacological effects in humans.

Conclusions and future directions

Pre-clinical findings indicate that MPH treatment during early development may cause enduring effects
on behavioural and brain processes under a variety of conditions, although the functional significance of these findings is largely unknown. Currently, non-invasive methods for use in human subjects are insufficiently sensitive to verify neurobiological evidence derived from pre-clinical research. As such, although there are limitations regarding the generalizability of results obtained from non-primate subjects, studies with rodents continue to be useful approaches for deriving working hypotheses regarding MPH effects in human brains. Researchers agree that there is a need to develop pre-clinical and clinical approaches to the study of MPH therapy that can provide information on different aspects of cognitive, emotional and motivational processes (Carlezon & Konradi, 2004), especially in light of clinical evidence that the magnitude of MPH-produced effects on cognition varies across a range of cognitive functions (Pietrzak et al. 2006; Swanson et al. 2010). The existing research in basic and human developmental pharmacology continues to stimulate the search for mechanisms underlying psychostimulant-produced behavioural and neural plasticity. Ultimately, this work will provide a better understanding of pharmacological therapies aimed at child and adolescent populations.

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Statement of Interest

None.

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