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Chronic oral methylphenidate induces post-treatment impairment in recognition and spatial memory in adult rats

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ABSTRACT

Recent pre-clinical research has indicated that chronic treatment with methylphenidate (Ritalin®) in young animals can result in lasting and potentially detrimental alterations in brain function that can persist into adulthood. Chronic methylphenidate-induced neuronal alterations may result in behavior and cognitive deficits that include increases in behavioral responses and impairment in recognition memory. This study compared the cognitive consequences following chronic treatment with two doses (5 and 10 mg/kg) of methylphenidate on recognition and spatial memory in adult male Long-Evans rats using an established oral dosing procedure. The animals were then tested in the Object Recognition test at 14 days post treatment and the Object Placement test at 21 days post treatment. The results indicate that repeated exposure to oral methylphenidate impaired the performance of rats in these tests. The current findings add to recent research demonstrating negative consequences in rats pre-treated with methylphenidate, and extend previous findings to include deficits in spatial recognition memory.

1. Introduction

Chronic stimulant administration can result in behavioral sensitization and cognitive deficits in clinical populations that are typically greater than those seen with other classes of drugs and persist beyond the period of initial withdrawal (Block, Erwin, & Ghoneim, 2002; Hoff et al., 1996; Horner, Harvey, & Denier, 1999; Laruelle, 2000; Rogers & Robbins, 2001; Rogers et al., 1999; Sax & Strakowski, 2001; Verdejo-García, Lopez-Tórrecillas, Giménez, & Pérez-García, 2004). Animal studies provide converging evidence in support of stimulant-induced cognitive deficits. For example, impairment in recognition memory in rats has been reported following chronic treatment with methamphetamine (Bisagno, Ferguson, & Luine, 2002), MDMA (ecstasy) (Piper & Meyer, 2004), D-amphetamine (Bisagno, Ferguson, & Luine, 2003), and methylphenidate (Heyser, Pelletier, & Ferris, 2004; LeBlanc-Duchin & Taukulis, 2007).

Methylphenidate (MPD) and amphetamine are two of the most widely prescribed stimulants used in the treatment of attention deficit hyperactivity disorder (ADHD) (Solanto, 1998). Amphetamine has pharmacodynamics similar to those of MPD (Solanto, 2000) and essentially, both drugs increase extracellular norepinephrine and dopamine by blocking the reuptake of these neurotransmitters (Kuczenski & Segal, 2001). Therefore, it is not surprising that chronic administration of methylphenidate may bring about similar lasting and potentially detrimental alterations in brain function as seen with other stimulants.

Since MPD is commonly administered to children, most preclinical research investigating the long-term negative consequences of chronic exposure to this drug has understandably focused on treatment of adolescent or juvenile rats (Bolaños, Barrot, Berton, Wallace-Black, & Nestler, 2003; Carlezon, Mague, & Andersen, 2003; Kuczenski & Segal, 2002). Results from recent neurochemical studies indicated that repeated administration of MPD to juvenile rats attenuated hippocampal neurogenesis in adulthood (Lagace, Yee, Bolaños, & Eisch, 2006) and induced increases in monoamine fiber density in the medial prefrontal cortex and decreases in norepinephrine fiber density in the hippocampus (Gray et al., 2007). These changes in neurogenesis and in neurotransmitter systems suggest that repeated exposure to MPD will alter neurochemistry in brain areas important in cognition. The behavioral consequences of MPD-induced neuronal alteration in these brain areas may be expressed as enduring deficits in cognitive function. Indeed, pre-adolescent rats exposed to oral MPD (3 and 5 mg/kg) for a total of 21 days demonstrated impairment in recognition memory that lasted into adulthood (LeBlanc-Duchin & Taukulis, 2007). In this earlier study, the animals were treated chronically during a period of substantial neuronal growth and pruning in young animals, resulting in behavioral effects that suggested long-term consequences for the development and function of cognitive processes (LeBlanc-Duchin & Taukulis, 2007).

Many adults are also treated with MPD and recent research indicates an increase in abuse of this prescription medication

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(Caplan, Epstein, Quinn, Stevens, & Stern, 2007; Kessler et al., 2006; Wilens et al., 2008). Certainly, it is possible that the mature neuronal systems may compensate for drug-induced structural and functional changes that could result in more resilience to chronic MPD-induced memory deficits. However, in addiction, it is suggested that long-term exposure to stimulants may change the same neuronal and molecular systems involved in the synaptic plasticity of learning (such as the nucleus accumbens and the prefrontal cortex) and induce changes in brain function that can alter future beneficial experienced–induced neuronal plasticity (Kolb, Gibb, & Robinson, 2003; Robinson & Kolb, 2004). As a result, drug abuse may limit future synaptic plasticity that can lead to long-term behavioral and cognitive deficits. Although this has never been investigated in clinical populations, a similar cognitive deficit may be seen in adults following long-term treatment or abuse of methylphenidate. These possible chronic stimulant-induced neuronal changes may be detected using animal models that challenge memory processes.

The purpose of the present study was to evaluate post-treatment cognitive integrity in adult rats chronically exposed to two oral doses (5 and 10 mg/kg) of MPD for a total of 21 days. Testing began 14 days after the last MPD treatment, using paradigms for the analysis of non-spatial and spatial recognition memory namely the Object Recognition (OR) test and the Object Placement (OP) test (Ennaceur & Meliani, 1992). Object recognition is a valid measure of memory function that exploits the rat's natural tendency to investigate novel rather than familiar objects (Ennaceur & Delacour, 1988). The OP test of spatial recognition memory is adapted from the OR test, in which an animal investigates a familiar object in a novel location (Ennaceur & Meliani, 1992).

Children and adults prescribed MPD typically take the drug orally, just before or with a meal, in a familiar environment. Oral ingestion of MPD results in a much slower, gradual absorption rate compared to IP injection (Gerasimov et al., 2000; Kuczenski & Segal, 2005) and delivery-dependent differences in peak concentration and kinetics may have fundamental implications for a drug's long-term effects. Therefore, in the present study, MPD was mixed with powdered, moistened rat chow and delivered to the animals in their home cages at approximately the same time each day. This oral drug delivery procedure, described in LeBlanc-Duchin and Taukulis (2004), ensured that the animals willingly consumed their allotted portion quickly, reliably, and completely.

2. Materials and methods

2.1. Subjects and housing

Thirty adult male Long-Evans rats were bred from stock obtained from Charles River Canada, St. Constant, Quebec. The animals were approximately 5 months old at the start of treatment with an average weight of 580 g. All the animals were housed individually in polycarbonate cages ($43 \times 21 \times 20$ cm, $l \times w \times h$) throughout the experiment. The animal holding room had a 14/10 h light/dark cycle and a temperature range of 22–24 °C. Water was available *ad libitum*. Food was restricted as described below. All aspects of the protocol were approved by the Animal Care Committee of the University of New Brunswick, Saint John, and adhered to the standards established by the Canadian Council on Animal Care.

2.2. Drugs

Tablets of methylphenidate were pulverized and suspended in distilled water at a concentration of 10 mg/2 ml. One drop of Tween 80 was included in each 2 ml of suspension to aid in equal dispersal of the drug. The drug was added to moistened powdered rat chow ('wet mash') via pipette at a dose of 5 and 10 mg/kg/day of active drug for oral ingestion.

2.2.1. Rationale for selection of doses

In this study, two oral doses (5 and 10 mg/kg) were chosen based on the resultant comparative blood plasma levels of MPD in rats and humans. That is, greater doses of MPD are required in rats to achieve comparable human plasma concentrations. Recently, Wheeler, Eppolito, Smith, Huff, and Smith (2007) determined that single oral doses between 1 and 5 mg/kg of MPD in young male rats would result in peak plasma levels of approximately 10–37 ng/ml. Yet, in children, comparative blood serum concentrations of 5–40 ng/ml are reached following oral doses between 0.2 and 1.0 mg/kg (Kuczenski & Segal, 2005; Swanson & Volkow, 2003; Wargin et al., 1983). Therefore, young rats require drug doses that are five times greater than human drug doses to achieve similar plasma concentrations.

In addition to the differences in peak plasma levels of comparative drug doses, there are also species differences in rate of metabolism. That is, metabolism of the drug in rats, when compared to that of humans, is relatively fast. Oral administration of MPD to rats results in peak maximal concentration in blood plasma in 15 min with a half-life of 1 h (Aoyama, Kotaki, Sawada, & Iga, 1996). Human maximal concentration of the drug in blood plasma peaks in 1.5–2 h with a half-life of 2–3 h (Swanson & Volkow, 2003). Therefore, on a strictly mg/kg basis, rodents require higher doses than humans due to differences in metabolism and oral doses of up to 10 mg/kg are considered moderate (Brandon, Marinelli, Baker, & White, 2001; Schenk & Izenwasser, 2002).

2.2.2. Treatment procedure

Before the start of drug treatment, the animals were given approximately 7–8 g of wet mash in a small, shallow, glass dish, secured to a porcelain dish, and suspended above the bedding in their home cages. The animals were habituated to receiving the wet mash in this manner for a total of 14 days to ensure complete and rapid consumption. Indeed, once habituation to the feeding procedure was complete, the animals would consistently ingest 100% of the wet mash and the wet mash/MPD mixture, without spillage, in 3 min or less of presentation of the food. Speed of administration was therefore only slightly slower than oral administration by gavage.

The wet mash consisted of approximately one part powdered rat meal (Purina #5012) and two parts distilled water. The wet mash was supplemented with 3–4 pellets of chow (approximately 12–16 g) each evening. On weekends (Friday evening until Sunday at 1700 h), the animals had free access to chow pellets. During the subsequent treatment period, the same feeding schedule continued. However, one third of the rats ($N = 10$) received 10 mg/kg of MPD in the wet mash and another third of the rats ($N = 10$) received 5 mg/kg of MPD in the wet mash. The remaining one third ($N = 10$) received unadulterated wet mash. The treatment schedule was staggered so that each animal was treated for a total of 21 days. During the post-treatment period, and for the remainder of the experiment, all of the rats were fed chow pellets *ad libitum* with no further drugs introduced into their daily diets.

It is important to note that the animals were not food-deprived during the feeding habituation or treatment periods. They were simply limited to eating at fixed times every day. All the animals maintained their weight throughout the treatment period in a steady manner consistent with the average weight for the age, gender, and strain of rat. Indeed, previous research has shown that therapeutic doses of MPD in rats will have no significant effect on weight (Bolaños et al., 2003; Teo et al., 2002; Zhu, Weedon, & Dow-Edwards, 2007).