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EFFECTS OF LONG-TERM METHYLPHENIDATE TREATMENT IN ADOLESCENT AND ADULT RATS ON HIPPOCAMPAL SHAPE, FUNCTIONAL CONNECTIVITY AND ADULT NEUROGENESIS

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Abstract—Methylphenidate (MPH) is a widely prescribed stimulant drug for the treatment of attention deficit hyperactivity disorder (ADHD) in children and adolescents. Its use in this age group raises concerns regarding the potential interference with ongoing neurodevelopmental processes. Particularly the hippocampus is a highly plastic brain region that continues to develop postnatally and is involved in cognition and emotional behavior, functions known to be affected by MPH. In this study, we assessed whether hippocampal structure and function were affected by chronic oral MPH treatment and whether its effects were different in adolescent or adult rats. Using behavioral testing, resting-state functional MRI, post-mortem structural magnetic resonance imaging (MRI), and immunohistochemistry, we assessed MPH's effects on recognition memory, depressive-like behavior, topological features of functional connectivity networks, hippocampal shape and markers for hippocampal neurogenesis and proliferation. Object recognition memory was transiently impaired in adolescent treated rats, while in animals treated during adulthood, increased depressive-like behavior was observed. Neurogenesis was increased in adolescent treated rats, whereas cell proliferation was decreased following adult treatment. Adolescent treated rats showed inward shape deformations adjacent to ventral parahippocampal regions known to be involved in recognition memory, whereas such deformations were not observed in adult treated animals. Irrespective of the age of treatment, MPH affected topological features of ventral hippocampal functional networks. Thus, chronic oral treatment with a therapeutically relevant dose of MPH preferentially affected the ventral part of the hippocampus and induced contrasting effects in adolescent and adult rats. The differences in behavior were paralleled by opposite effects on adult neurogenesis and granule cell proliferation.

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Key words: methylphenidate, hippocampus, neurogenesis, MRI, memory, depressive-like behavior.

INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) is a neurodevelopmental disorder that is nowadays commonly diagnosed in children and adolescents and often persists into adulthood (Kessler et al., 2006; Pastor and Reuben, 2008). Widely prescribed psychostimulants such as methylphenidate (MPH) provide effective treatment, but their potential interference with normal brain development is unclear and raises concerns regarding its possible long-term consequences (Andersen and Navalta, 2004; Marco et al., 2011). In rodents, early-life exposure to MPH has been associated with an increased anxiety and depressive-like behavior (Bolaños et al., 2003; Carlezon et al., 2003; Wiley et al., 2009) and with impaired learning and memory performance in hippocampal dependent tasks (Gomes et al., 2010). Since the hippocampus is critically involved in learning and memory (Squire, 1992; Cohen and Eichenbaum, 1993; Bannerman et al., 2004) and implicated in aspects of depression (De Kloet et al., 2005) and anxiety disorders (Revest et al., 2009; Canteras et al., 2010) and displays considerable ongoing plasticity (Lucassen et al., 2010), psychostimulant-induced hippocampal alterations may

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Abbreviations: ANOVA, analysis of variance; CA, cornu ammonis; DCX, doublecortin; FDR, false discovery rate; fMRI, functional MRI; FOV, field of view; FST, forced swimming test; GE, gradient-echo; MANOVA, multivariate analysis of variance; MPH, methylphenidate; MRI, magnetic resonance imaging; NOR, novel object recognition; PBS, phosphate-buffered saline; SE, standard errors of the mean; TBS, tris-buffered saline; TE, echo time; TR, repetition time.

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be implicated in the therapeutic potential as well as the long-term risks of this type of medication (Britton and Bethancourt, 2009).

Therapeutic-level doses of MPH have been shown to increase hippocampal noradrenaline levels (Kuczenski and Segal, 2002) and noradrenergic fiber density in the dentate gyrus (Gray et al., 2007), while juvenile treatment was found to attenuate aspects of neurogenesis during specific stages of adulthood (Lagace et al., 2006). However, it remains unclear to what extent hippocampal alterations arise from MPH's interference with ongoing neurodevelopmental processes during adolescence. Adult neurogenesis is a novel form of structural plasticity that decreases with age (Heine et al., 2004) and has been implicated in hippocampal function (Trouche et al., 2009; Deng et al., 2010). Also, cocaine, D-amphetamine and methamphetamine were shown to modulate cell proliferation in the hippocampus (Eisch and Harburg, 2006).

In this study we therefore assessed hippocampal structural and functional alterations in rats that received chronic oral MPH treatment either during adolescence or adulthood. We hypothesized that adult exposure to MPH would impair recognition memory and enhance depressive-like behavior, parallel to reduced neurogenesis and topological alterations of hippocampal functional networks, and that these changes would be more pronounced when animals were treated during adolescence.

Behavioral tests, functional and structural magnetic resonance imaging (MRI) and immunohistochemistry were combined to analyze MPH's lasting effects on; (1) hippocampus-mediated recognition memory, and depressive-like behavior in the forced swimming test (FST); (2) topological features of cerebral and hippocampal functional connectivity networks; (3) hippocampal shape, and (4) adult neurogenesis and cell proliferation in the dentate gyrus of the hippocampus.

EXPERIMENTAL PROCEDURES

Animals

All experiments were carried out in accordance with Dutch and French regulations governing animal welfare and protection, and all efforts were made to minimize animal suffering and to reduce the number of animals used.

Male Wistar rats (Harlan) were treated with methylphenidate HCl (MPH) (oral gavage, dissolved in 0.9% saline solution) or vehicle, daily around 12PM for 21 days from post-natal day (P)25 ± 0 (adolescent) or P65 ± 4 (adult) onward. Behavioral tests were performed after a 1- or 5-week washout period following treatment with 0, 2, or 5 mg/kg MPH in 120 animals (n = 10 per group) (Experiment A). Based upon our findings in behavioral tests, in a separate series of experiments, *in vivo* MRI was performed after a 1 week washout-period following treatment with 0 or 5 mg/kg MPH in 64 animals (n = 16 per group) (Experiment B1). A subset of dissected brains was subsequently used for postmortem MRI and immunohistochemical analysis of neurogenesis markers (Experiment B2).

The MPH dosing regimen used in the present study was chosen to mimic clinically relevant, therapeutic doses and route of administration in rats (Gerasimov et al., 2000; Grund et al., 2007) which had been shown before to induce approximately a four-fold increase in extracellular norepinephrine in the hippocampus (Kuczenski and Segal, 2002).

Experiment A: Behavior and cognition

Novel object recognition. The novel object recognition (NOR) test (Ennaceur and Delacour, 1988) was performed in a black wooden open-field box (100 × 100 × 60 cm) (Levallet et al., 2009). The objects to discriminate were a glass flask filled with sand and a small ceramic statue (10–15 cm high, available in quadruplicate). The first two days were devoted to habituation sessions to the apparatus and the procedure. The animals from the same cage (n = 3) were placed together in the testing box for ten min. From the third to the fifth day, each animal was placed in the box for 3 min, and an object (different from those used for the test) was placed in the center of the open field for the fourth and the last habituation session, in order to get the animal used to the presence of an object. The sixth day (corresponding to the first day after the end of the washout period) was devoted to the test itself, and comprised two sessions. The acquisition session (3 min) was performed by exposing the animal to two identical objects before being replaced in the home cage. Four hours later, a retention session (3 min) was performed with a familiar object (previously explored during the acquisition session) and a novel one. Object combinations (familiar vs. novel) were alternated between animals. All the sessions were video-taped and analyzed afterward. Exploration of each object at each session, defined as directing the nose to the object at a distance less than 2 cm and/or touching the object with the nose or forepaws, was collected. The exploration times of the familiar and the novel object were compared, and the recognition index (time spent exploring novel object – time spent exploring familiar object/total exploration time) at the retention session was calculated.

FST. Depressive-like behavior was assessed by a modified version of the FST (Castro et al., 2010; Bouet et al., 2011), initially designed by Porsolt et al. (1977). Briefly, it consisted of a single trial with the rat placed in a vase full of water (25 °C) for 6 min and measuring immobility, swimming, and climbing times from videotapes. The same rats were used that were exposed to object recognition before. Depth of the water was fixed at 40 cm and the diameter of the glass cylinder was 30 cm. Rats did not have the possibility to touch the bottom of the glass cylinder with their tail. Immobility was defined as the adoption by the animal of an oblique position (body angle of approximately 45° with regard to the horizontal plane of water) that was otherwise only associated with slow movements of the rat paws to remain on surface. It was performed on the third day after the end of the washout period.