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The effects of amphetamine exposure on juvenile rats on the neuronal morphology of the limbic system at prepubertal, pubertal and postpubertal ages

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ABSTRACT

Amphetamines (AMPH) are psychostimulants widely used for therapy as well as for recreational purposes. Previous results of our group showed that AMPH exposure in pregnant rats induces physiological and behavioral changes in the offspring at prepubertal and postpubertal ages. In addition, several reports have shown that AMPH are capable of modifying the morphology of neurons in some regions of the limbic system. These modifications can cause some psychiatric conditions. However, it is still unclear if there are changes to behavioral and morphological levels when low doses of AMPH are administered at a juvenile age. The aim of this study was to assess the effect of AMPH administration (1 mg/kg) in Sprague-Dawley rats (postnatal day, PD21-PD35) on locomotor activity in a novel environment and compare the neuronal morphology of limbic system areas at three different ages: prepubertal (PD 36), pubertal (PD50) and postpubertal (PD 62). We found that AMPH altered locomotor activity in the prepubertal group, but did not have an effect on the other two age groups. The Golgi-Cox staining method was used to describe the neural morphology of five limbic regions: (Layers 3 and 5) the medial prefrontal cortex (mPFC), the dorsal and ventral hippocampus, the nucleus accumbens and the amygdala, showing that AMPH induced changes at pubertal ages in arborization and spine density of these neurons, but interestingly these changes did not persist at postpubertal ages. Our findings suggest that even early-life AMPH exposure does not induce long-term behavioral and morphological changes, however it causes alterations at pubertal ages in the limbic system networks, a stage of life strongly associated with the development of substance abuse behaviors.

1. Introduction

Repeated administration of psychostimulants induce behavioral alterations like increased arousal and hyperactivity (Hutson et al., 2014), as well as persistent effects on structural plasticity in the central nervous system (CNS) (Robinson and Kolb, 2004) a phenomenon known as experience-dependent plasticity (Kolb et al., 2014). Amphetamine (AMPH) is a psychostimulant that enhance dopamine, norepinephrine and serotonin synapse availability by targeting monoamine transporters, and they are widely prescribed for the treatment of attention deficit hyperactive disorder (ADHD) in children (Heal et al., 2013). Despite contradictory information about their long term effects (Sharma and Couture, 2014), some studies indicate that AMPH treatment in

Abbreviations: ADHD, attention deficit hyperactive disorder; AMPH, amphetamine; BDNF, brain derived neurotrophic factor; BLA, basolateral amygdala; CA1, Cornus ammonis area 1; DA, Dopamine; DG, Dentate gyrus; DH, Dorsal hippocampus; NAcc, nucleus accumbens; mPFC, medial part of the prefrontal cortex; PD, postnatal day; VH, ventral hippocampus; VTA, ventral tegmental area; ROS, reactive oxygen species; TDL, total dendritic length.

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ADHD pediatric patients induces a potential risk for abuse and substance dependency behavior in adolescence and adulthood (Advokat, 2007; Berman et al., 2009). Other researchers also suggest that AMPH not only increase the risk for substance disorders, but also have a protective effect on the development of these behaviors (Biederman, 2003; Faraone and Wilens, 2003). Animal studies have demonstrated experience-dependent plasticity by AMPH exposure with chronic and rising dosage protocols in the medial prefrontal cortex (mPFC) and nucleus accumbens (NAcc), increasing spine density and the dendritic length of neurons in these areas, which are relevant in drug addiction issues (Robinson and Kolb, 1997, 1999; Singer et al., 2009). However, there is a lack of information about the effects of low dose AMPH exposure, such as some of the protocols used in ADHD therapeutics.

Thus, in this study we assessed the effect of AMPH treatment, with a similar dose to those used in clinical practice with children, on the locomotor activity of healthy juvenile rats in a novel environment at three different ages: prepubertal (postnatal day [PD 35]), pubertal (PD50) and postpubertal (PD 62). Moreover, we evaluated the effect of AMPH on neuronal morphology of five areas of the limbic system: the mPFC, the NAcc, CA1 of the ventral hippocampus (VH), CA1 of the dorsal hippocampus (DH) and the basolateral amygdala (BLA) by using the Golgi-Cox technique, with the aim of describing if juvenile AMPH exposure induces long-lasting morphological and behavioral changes.

2. Materials and methods

2.1. Animals

Sprague-Dawley female rats and their pups were obtained from our facilities (University of Puebla). Rats were kept in an environment with a controlled temperature (20–23 °C), humidity (40–50%), and a 12–12 h light-dark cycle with free access to food and water. Considering the day of birth (postnatal day [PD0]), PD21 pups were weaned and randomly assigned to either the vehicle or the AMPH treated group. Only male rats were used for all the experiments. All procedures described in the present study were performed in accordance with the "Guide for Care and Use of Laboratory Animals" of the Mexican Council for Animal Care (Norma Oficial Mexicana NOM-062-ZOO-1999). Every effort was made to minimize the number of animals used and to ensure minimal pain and discomfort.

2.2. Amphetamine administration

On PD21 rats were injected (s.c.) every day for 15 days with either saline vehicle (NaCl 0.9%) for the vehicle group or 1 mg/kg free base of d-amphetamine sulfate (Sigma, St. Louis, MO, USA) for the treated group, subsequently rats were left undisturbed until PD38, PD50 or PD62. Six groups of animals were formed (n = 10 animals per group): (a) vehicle-treated PD38 (Veh38); (b) AMPH-treated PD38 (AMPH38); (c) vehicle-treated PD50 (Veh50); (d) AMPH-treated PD50 (AMPH50); (d) vehicle-treated PD62 (Veh62); and (e) AMPH-treated PD62 (AMPH62). The AMPH dose was selected based on the juvenile rat pharmacokinetic data described by Diaz et al. (Diaz Heijtz et al., 2003).

2.3. Locomotor activity

This probe was made as previously described in detail (Flores et al., 1996; Flores-Tochihuitl et al., 2008; Morales-Medina et al., 2008). Locomotor activity was monitored in individual cages (20 × 40 × 30 cm), each one equipped with 8-photo-beam detectors connected to a computer counter (Tecnología Digital, México). After either vehicle or AMPH treatment at PD38, PD50 or PD62, each rat was placed in an activity box for 120 min and the locomotor activity score was recorded.

2.4. Morphological analysis

2.4.1. Golgi-Cox stain method

Immediately after the locomotor activity test, rats were deeply anesthetized with sodium pentobarbital (60 mg/kg body weight, i.p.) and perfused intracardially with 0.9% saline solution. Brains were removed and stained using a modified Golgi-Cox method described previously (Flores et al., 2005). Coronal sections of 200-μm thickness at the level of the PFC, the NAcc, the hippocampus and the amygdala were obtained using a vibratome (Campden Instruments, MA752, Leicester, UK). These sections were collected on clean gelatin-coated microscope slides and submerged in ammonium hydroxide for 30 min, then for 30 min in Kodak Film Fixer and afterwards washed with distilled water, dehydrated and whitened in successive baths of 50% (1 min), 75% (1 min), 90% (1 min) and 100% (2 × 5 min) ethanol, followed by 15 min in a xylene solution. Finally slides were mounted with a resinous medium (Gibb and Kolb, 1998).

2.5. Microscopic observation and Sholl analysis

Pyramidal neurons from layers 3 and 5 of the mPFC (area Cg 1 and prelimbic cortex; Plate 7–9), medium spiny neurons from the NAcc (Plate 10–13), pyramidal neurons from the dorsal (CA 1, Plates 27–33) and the ventral hippocampus (CA 1, Plates 37–42) and pyramidal neurons from the BLA (Plates 27–31) were selected for study, in accordance with the (Paxinos and Watson, 1986). For each animal, five neurons from each hemisphere of the PFC, the NAcc, the HV, the HD and the BLA were drawn using a camera lucida at 400X magnification (DMLS Leica Microscope) by a trained observer who was blind to the experimental conditions (Kolb et al., 1998). Pyramidal neurons were identified by their characteristic triangular soma-shape, apical dendrites extending toward the pial surface and many dendritic spines. Medium spiny neurons of the NAcc were recognized by their soma size and dendritic extensions. Only completely impregnated neurons were included in our analyses. Two-dimensional reconstruction of the entire dendritic tree was generated for each neuron, and the dendritic tracings were quantified by Sholl analysis (Juarez et al., 2008; Morales-Medina et al., 2009; Sholl and Uttley, 1953; Vazquez-Roque et al., 2014). A transparent grid with equidistant (10 μm) concentric rings was centered over the dendritic tree tracings, and the number of ring intersections was used to estimate the total dendritic length and dendritic arborization (Silva-Gomez et al., 2003; Vazquez-Roque et al., 2012; Vega et al., 2004). Another estimate of dendritic arborization, the total number of dendritic branches (branching indicated by Y bifurcation), was counted at each order away from the cell body or dendritic shaft. To calculate the spine density, a length of dendrite (at least 10 μm long) was traced (at 1000×), the exact length of the dendritic segment was calculated, and the number of spines along the length were counted (to yield spines/10 μm) (Bringas et al., 2013).

2.6. Statistical analysis

Mean values from each brain region of each animal were treated as a single measurement for the data analysis. Data on locomotor activity, spine density and dendritic length were analyzed by two-way ANOVA, followed by the Newman-Keuls test for post hoc comparisons, with age and treatment as independent factors ($P < 0.05$ was considered significant). Data on the length per branch order were also analyzed by two-way ANOVA, followed by