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Full length article

MicroPET/CT assessment of FDG uptake in brain after long-term methylphenidate treatment in nonhuman primates☆

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ABSTRACT

Methylphenidate (MPH) is a psychostimulant commonly used for the treatment of Attention-Deficit Hyperactivity Disorder (ADHD). Since the long-term effects of this drug on the central nervous system (CNS) are not well understood, we conducted microPET/CT scans on young adult male rhesus monkeys (n = 4/group) to gather information on brain metabolism using the uptake of [^{18}F]Fluoro-2-deoxy-2-D-glucose (FDG) as a marker. Approximately two-year old, male rhesus monkeys were treated orally with MPH twice per day, five days per week (M-F) over a 6-year period. Subjects received MPH at either 2.5 or 12.5 mg/kg/dose or vehicle (Prang). To minimize the acute effects of MPH on FDG uptake, microPET/CT scans were scheduled on Mondays before their first daily dosing of the week (approximately 68 h since their last treatment). FDG (370 ± 8.88 MBq) was injected intravenously and 30 min later microPET/CT images were obtained over 60 min. Radiolabeled tracer accumulation in regions of interest (ROIs) in the prefrontal cortex, temporal cortex, striatum and cerebellum were converted into Standard Uptake Values (SUVs). Compared to the control group, the uptake of FDG in the cerebellum was significantly decreased in both the low and high dose groups. These preliminary data demonstrate that microPET imaging is capable of distinguishing differences in retention of FDG in the brains of NHPs treated chronically with MPH and suggests that this approach may provide a minimally invasive biomarker for exploring the effects of chronic MPH treatment on aspects of brain function.

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1. Introduction

Attention-deficit hyperactivity disorder (ADHD) is one of the most common neurobehavioral disorders of childhood that can last through adolescence and into adulthood (Dulcan, 1997; Katzman and Sternat, 2014; Kessler et al., 2006; Wender et al., 2001). This disease affects 5.9–7.1% of children and adolescents and 3.4–4.4% of adults (Katzman and Sternat, 2014; Modesto-Lowe et al., 2012; Willcutt, 2012). Multiple etiologies such as genetic predisposition, environmental disturbance, neurobiological and neurochemical dysfunction may be involved in the mechanism underlying ADHD (Akay et al., 2006; Wilens, 2008). ADHD is characterized by hyperactivity, inattention, and impulsivity. Untreated ADHD results in poor development, emotional and cognitive malfunctioning, and the increased risk of other psychiatry disorders, such as bipolar disorder and anxiety (Bolanos et al., 2003; Houghton et al., 2013; Katzman and Sternat, 2014; Nierenberg et al., 2005; Reus et al., 2013).

Psychostimulant medications such as methylphenidate (MPH) and amphetamine are typically the first-line pharmacotherapy in the treatment of ADHD. MPH has been used for approximately 5 decades and is able to reduce the primary symptoms in approximately 70% of children with ADHD. It is believed that MPH exerts its effects via blockade of the dopamine transporter (DAT) and the norepinephrine transporter (NET), thereby increasing monoamine signaling at the synapse (Katzman and Sternat, 2014; Reus et al., 2013; Sadasivan et al., 2012; Wilens, 2008). The high prevalence of ADHD, persisting symptoms in adolescents and adults and over-diagnosis of ADHD have led to an increased medical use of MPH. This has raised concerns regarding its long-term side effects and potential toxicity to the central nervous system (CNS) (Bruchmuller et al., 2012; Evans et al., 2010; Klein-Schwartz, 2003; Scaini et al., 2008; Simchon-Tenenbaum et al., 2015).

Noninvasive in vivo imaging methods, such as positron emission tomography (PET), have emerged as popular approaches for investigating biological, biochemical, pathological and pharmacological process in living tissue and organs. Three-dimensional molecular information from

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the intact brain of a variety of animals including nonhuman primates (NHP) can be collected using microPET imaging applications (Zhang et al., 2009; Zhang et al., 2011; Zhang et al., 2012; Zhang et al., 2013a; Zhang et al., 2013b; Zhang et al., 2013c). Because PET imaging is widely utilized in human patients, our utilization of animal (microPET) imaging approaches will provide quantitative, translational information concerning the status of regional brain activity associated with chronic psychostimulant drug exposure. To our knowledge, there has been no research into the effects of chronic MPH exposure on brain metabolism/activity. Therefore, the current study was designed to evaluate the long-term consequences of chronic, early life MPH exposure on CNS activity.

2. Materials and methods

2.1. Animals

All animal procedures were approved by the National Center for Toxicological Research (NCTR) Institutional Animal Care and Use Committee and conducted in full accordance with the Animal Welfare Act and the PHS Policy on Humane Care and Use of Laboratory Animals.

All monkeys were purchased as juveniles and housed at the FDA's National Center for Toxicological Research AAALAC International accredited nonhuman primate research facility. Animal procedures were designed to minimize the number of animals required and any pain or distress associated with the experimental procedures.

A total of twelve (9–10 year old) adult male rhesus monkeys (*Macaca mulatta*) were utilized. All animals were part of a larger study (Rodriguez et al., 2010) in which each of three groups initially contained 10 animals. Here, animals were randomly assigned to control (n = 4, vehicle), high MPH dose (n = 4, 12.5 mg/kg/dose), and low MPH dose (n = 4, 2.5 mg/kg/dose) groups. The doses of MPH were selected based upon attained plasma levels reported in our previous studies (Mattison et al., 2011; Morris et al., 2009; Rodriguez et al., 2010). Briefly, the serum concentration of MPH and its major metabolite were assessed monthly: doses in the low-dose groups achieved mean plasma levels of MPH within the clinical range observed in humans (4.05–14.03 ng/mL) and the dose of the high-dose group produced a concentration 5- to 10-fold higher than clinically observed, ranging from 28.75 to 270.85 ng/mL (Mattison et al., 2011).

All animals were treated orally, twice a day, five days per week (M–F). For at least 6 h prior to the start of microPET imaging (the time of FDG injection) animals were not allowed to consume any food. To minimize the acute effects of MPH, the microPET/CT scans were scheduled on Mondays before the start of their weekly dosing regimen. Thus, at this time, all animals were drug free for approximately 68 h.

2.2. Living conditions of animals

All animals were fed and watered according to the standard operating procedures of the NCTR's Primate Research Facility covering feeding of a manufacturer-formulated solid food (monkey chow, Ralston-Purina, Inc., St. Louis, MO) supplemented 3–7 times per week with fresh fruit and multivitamins. All animals (Rhesus monkey/Macaca mulatta) were singly housed throughout the study. The number of biscuits offered to the subjects were determined weekly with a goal of maintaining the current body weights.

2.3. Experimental procedures

Immediately prior to the initiation of anesthesia for the microPET/CT scans, animals were removed from their home cage and transferred to a holding cage in a procedure room where they were injected intramuscularly with ketamine (10 mg/kg) to induce anesthesia. The animal was then placed on the microPET scanning bed (Focus 220, Siemens Preclinical Solution, Knoxville, USA) in a supine position and intubated to allow delivery of maintenance inhalation anesthesia (a 1.5–2% isoflurane and oxygen mixture) for the duration of the microPET and CT scans. Body temperature was maintained at approximately 37 °C throughout the experiment via an electronic heating pad. Waste anesthetic gas was scavenged using an attached canister containing activated charcoal. Glycopyrrolate (0.01 mg/kg, American Reagent, Shirley, NY, USA) was administered intramuscularly prior to anesthesia to reduce airway secretions.

2.4. Monitoring of physiological parameters

During the microPET/CT scans, physiological parameters were monitored as described previously (Hotchkiss et al., 2007; Slikker et al., 2007) Briefly, non-invasive pulse oximetry (N-395 Pulse Oximeter, Nellcor, Pleasanton, CA; MouseO_X Plus Vital Sign Monitor, Starr™ Life Sciences, Oakmont, PA), and rectal temperature monitoring were used to verify the physiological status of subjects. Heart and respiration rates, arterial blood O_2 saturation levels, expired CO_2 concentrations, and rectal temperatures, were also recorded every 15 min.

2.5. MicroPET

A commercial high resolution small animal PET scanner (Focus 220, Siemens Preclinical Solution, Knoxville, USA) was used to quantitatively acquire images of the monkey brain. The scanner has 96 lutetium oxyortho-silicate detectors and provides a transaxial resolution of 1.35 mm full-width at half-maximum. Data were collected in a $128 \times 128 \times 95$ matrix with a pixel width of 0.475 mm and a slice thickness of 0.815 mm.

2.6. Computed tomography

Monkeys were also imaged in a newly developed mobile neurological CereTom CT scanner (Neurologica Corps. Danvers, MA, USA). The CT gantry of the CereTom scanner moves while the subject remains externally supported and fixed in space and therefore allows it to be physically connected to the microPET scanner. Exposure settings for each CT scan were 120 kVp, 5 mAs, scan time = 120 s. Data were collected in a 512×512 matrix with a pixel width of 0.49 mm and a slice thickness of 1.25 mm.

2.7. MicroPET/CT image acquisition

To assess resting cerebral glucose utilization, each monkey underwent microPET and CT scans following the intravenous injection of [^{18}F] Fluoro-2-deoxy-2-D-glucose (FDG). Animals were positioned head first, supine on a modified external bed controlled by the microPET unit. The bed replaced the standard microPET bed and allowed for sufficient travel to move animals through the microPET and CT fields of view (over an axial range of >50 cm). A transmission scan (10 min) with a rotating ^{57}Co point source was performed prior to injection of the radioisotope to be imaged using software supplied by the scanner manufacture. An attenuation correction sinogram file was created using a blank dataset and the acquired transmission dataset. This histogram file can be specified when reconstructing emission sinogram data and multiplied with the emission data thereby performing attenuation correction. The scatter correction requires a normalization file, an attenuation file, and an emission file. It was enabled in reconstruction configuration as well.

For each imaging session, [^{18}F] FDG (370 ± 8.88 MBq) was injected into the lateral saphenous vein of anesthetized animals. The emission scan began 30 min after [^{18}F] FDG injection and microPET images were collected over 1 h to assess the influx of the tracer. Emission data were statically reconstructed as 3-D volumes including scatter and attenuation correction. Micro-computed tomography (microCT) coronal images were obtained immediately after microPET imaging for