Neurotoxicology and Teratology 55 (2016) 1–7

Contents lists available at ScienceDirect

Neurotoxicology and Teratology

journal homepage: www.elsevier.com/locate/neutera

Sleep disturbance as detected by actigraphy in pre-pubertal juvenile monkeys receiving therapeutic doses of fluoxetine

Mari S. Golub [a,*], Casey E. Hogrefe [b]

[a] Department of Environmental Toxicology, University of California Davis, One Shields Ave, Davis, CA 95616, USA
[b] California National Primate Research Center, University of California Davis, One Shields Ave, Davis, CA 95616, USA

ARTICLE INFO

Article history:
Received 4 December 2015
Received in revised form 25 February 2016
Accepted 29 February 2016
Available online 5 March 2016

Keywords:
Sleep
Nonhuman primate
Juvenile fluoxetine exposure
MAOA genotype

ABSTRACT

Sleep disturbance is a reported side effect of antidepressant drugs in children. Using a nonhuman primate model of childhood selective serotonin reuptake inhibitor (SSRI) therapy, sleep was studied quantitatively with actigraphy. Two 48-h sessions were recorded in the home cage environment of juvenile male rhesus monkeys at two and three years of age, after one and two years of treatment with a therapeutic dose of the SSRI fluoxetine, and compared to vehicle treated controls. A third session was conducted one year after discontinuation of treatment at four years of age. During treatment, the fluoxetine group demonstrated sleep fragmentation as indexed by a greater number of rest–activity transitions compared to controls. In addition fluoxetine led to more inactivity during the day as indexed by longer duration of rest periods and the reduced activity during these periods. The fluoxetine effect on sleep fragmentation, but not on daytime rest, was modified by the monkey's genotype for polymorphisms of monoamine oxidase A (MAOA), an enzyme that metabolizes serotonin. After treatment, the fluoxetine effect on nighttime rest–activity transitions persisted, but daytime activity was not affected. The demonstration in this nonhuman primate model of sleep disturbance in connection with fluoxetine treatment and specific genetic polymorphisms, and in the absence of diagnosed psychopathology, can help inform use of this drug in children.

1. Introduction

Fluoxetine was introduced as an antipsychotic in 1987 (Perez-Caballero et al., 2014) under the trade name Prozac® and became the premier member of the selective serotonin reuptake inhibitor (SSRI) class of antidepressants. It is estimated that 10% of US adults (>12 years) are treated with antidepressants (Pratt et al., 2011). Fluoxetine was the third most prescribed antidepressant in a 2008 Medicare survey (Chen et al., 2008) and is widely used in children (O'Sullivan et al., 2015). The favorable efficacy and safety profile of fluoxetine in clinical practice has sustained high levels and expanded range of use of this drug. In addition to its competitive binding to the serotonin reuptake transporter, fluoxetine has been found to influence a number of biological processes important to brain development including neurogenesis, BDNF regulation, spine synapse formation and neurosteroid production (De Foubert et al., 2004; Norrholm and Ouimet, 2000; Pinna et al., 2006; Wu et al., 2014; Oberlander et al., 2009).

Drowsiness, insomnia and sleepiness are all symptoms commonly reported with SSRI therapy in adults. Early studies in depressed patients showed that fluoxetine increased nighttime awakenings, decreased rapid eye movement (REM) sleep, and increased leg movements and oculomotor activity (EOG) (Slater et al., 1978; Armitage et al., 1997a, b). In normal adult subjects, similar electroencephalography (EEG) and EOG findings were seen with short term fluoxetine administration (Vasar et al., 1994; Saletu et al., 1991; Feige et al., 2002). These studies indicate that fluoxetine affects biological pathways regulating sleep independent of underlying psychopathology.

Less is known about fluoxetine effects on sleep in children. Fluoxetine is approved by the FDA for treating depression (MDD) and obsessive compulsive disorder (OCD) in children (FDA, 2003) and is also widely used to treat a number of more common childhood behavior disorders including autism, Down's syndrome, conduct disorder, separation anxiety, anorexia, and social anxiety (Dorks et al., 2013; Williams et al., 2013; Costa and Scott-McKean, 2013; El-Chammas et al., 2013; Masi, 2004; Connor and Steingard, 1996; Markowitz, 1992; Riddle et al., 1990). It is valuable to know whether this widely used drug (O'Sullivan et al., 2015) influences sleep regulation in children. Sleep disturbances in children are associated with poorer performance on standardized tests, specifically cognitive function and hyperactivity–impulsivity (Touchette et al., 2007). More importantly, developmental sleep disturbance may predict long-term impairment

* Corresponding author at: CNPRC, BMB, University of California Davis, One Shields Ave, Davis, CA 95616, USA.
E-mail addresses: msgolub@ucdavis.edu (M.S. Golub), cehogrefe@ucdavis.edu (C.E. Hogrefe).

http://dx.doi.org/10.1016/j.ntt.2016.02.006
0892-0362/

of sleep regulation and executive function (Turnbull et al., 2013). Long-term changes in the brain's serotonin system, a well known regulator of sleep (Ursin, 2002), were recently reported in rhesus monkeys 1.5 years after the end of a one year juvenile treatment with fluoxetine (Shrestha et al., 2014).

A limited number of studies of children diagnosed with MDD or OCD have addressed the effects of fluoxetine on sleep. A small study (N = 31) reported 13% incidence of insomnia in depressed children treated with fluoxetine (Jain et al., 1992). Later research showed that fluoxetine in six children with depression increased nighttime arousals and oculomotor activity and also dramatically increased leg movement (Armitage et al., 1997a,b). In a chart review of 82 children and adolescents treated with SSRIs, 35% reported sleep disturbance (Wilens et al., 2003). No studies of sleep in children not treated for depression were located in the published literature. When children are treated "off-label", side effects may vary depending on the underlying biology of the disorder. A detailed study of fluoxetine and sleep disturbance in an appropriate animal model of childhood not specific to one of these disorders would allow identification of the undesirable side effects associated with this drug in the absence of childhood psychopathology. To address the question of whether fluoxetine treatment affects sleep regulation during childhood stages of brain maturation, we employed a juvenile nonhuman primate model.

Rhesus monkeys provide a valuable nonhuman primate model of both juvenile brain development and sleep architecture. Nonhuman primates, like children, have a prolonged period of brain development after infancy and prior to puberty that is not seen in rodent models (Pagel and Harvey, 2002). The consolidated pattern of nighttime sleep seen in humans is also seen in nonhuman primates but not in rodents (Lesku et al., 2006; Balzamo et al., 1977). Rhesus monkeys are particularly well studied as a model for human sleep (Balzamo et al., 1998; Benca et al., 2000; Daley et al., 2006; Barrett et al., 2009; Hsieh et al., 2008; Andersen et al., 2013; Andersen et al., 2010; Balzamo, 1995; Balzamo, 1997; Weitzman et al., 1968). Although most studies use adults, juvenile rhesus have also been studied (Barrett et al., 2009; Benca et al., 2000; Pryce et al., 2011).

In the present study, actimeters were used to measure sleep disturbance. In clinical studies questionnaire and symptom report data are important indices of sleep disturbance. Both EEG and actimeter studies are used to quantify sleep disturbance in nonhuman primates and humans. The EEG provides a record of brain electrical activity but requires head restraint or implantation of sensors. The actimeter, or accelerometer, is noninvasive, records movement, and is widely used in children (Veatch et al., 2015; De Crescenzo et al., 2015; Markovich et al., 2014; Meltzer et al., 2012). The primary indices of sleep disturbance derived from actimeter data are duration of nighttime rest (insomnia), time to onset of rest at night (sleep onset insomnia), the number of nighttime awakenings (sleep maintenance insomnia) and the number of nighttime awakenings and daytime sleep episodes (sleep fragmentation) (American Academy of Sleep Medicine, 2005; Morgenthaler et al., 2007). Sleep fragmentation, a measure of the disruption of the consolidated sleep pattern, is a major index of sleep disturbance in humans (Haba-Rubio et al., 2004; American Academy of Sleep Medicine, 2005; Balzamo et al., 1977).

The design of the current study included genotyping for high and low transcription monoamine oxidase A (MAOA) polymorphisms. Monoamine oxidase (MAO) metabolizes monoamine neurotransmitters and MAOA has high selectivity for serotonin. MAOA genotype interacts with SSRI therapeutic effects (Peters et al., 2004; Yu et al., 2005) but has not been studied for interaction with side effects. MAOA genotype is emerging as an important factor interacting with environmental influences on brain development in both human and nonhuman primates. We recently found that fluoxetine and MAOA genotype interact to influence metabolomic profiles in plasma and CSF of juvenile rhesus (He et al., 2014). Also, in studies of nutritional effects on brain development, we found that MAOA genotype interacted with prenatal iron deficiency in affecting sleep fragmentation in juvenile rhesus monkeys (Golub and Hogrefe, 2014b).

2. Materials and methods

2.1. Assurance of compliance with animal codes

All animal procedures followed the Guide for the Care and Use of Laboratory Animals of the US National Research Council and were approved by the UC Davis Institutional Animal Care and Use Committee.

2.2. Subjects and dosing

Thirty-two male rhesus monkeys (*Macaca mulatta*) born and raised in outdoor social groups at the California National Primate Research Center (CNPRC) were enrolled at one year of age in a four year study of fluoxetine effects on growth, social interaction, emotional responsiveness, impulsivity, attention, and cognition (Supplementary Table 1). A two year dosing period was used to cover chronic treatment and also several brain developmental stages that might be differentially sensitive to disruption. The sleep assessments were first conducted after one year of dosing, repeated at the end of the two year dosing period, and repeated again one year after the end of dosing, at four years of age. Male rhesus typically reach puberty in the breeding season of the fourth year of life.

The subjects were housed together in the same indoor caging room as described previously (He et al., 2014). Each monkey lived with a compatible peer in the same dosing group in a double cage with a connecting door. The caging partners were separated for activity assessment by closing the door of the double cage. This was necessary to prevent damage to the Actitrac monitor by the cagemate. Although the monitored animal could not reach the monitor, it could readily be manipulated and damaged by another animal in the same cage. Caging partners also were separated by closing the connecting door several times per week for behavioral assessments during the day, and also for longer periods for husbandry, veterinary, and experimental procedures. It is important to note that monitoring was conducted in the usual social environment of the cage room where peers were available for auditory and visual interactions and the vocal and locomotor activity of the cagemate was also accessible. A monitoring session was minimally stressful to the social environment because different groups of four cagemate pairs were monitored regularly on successive weekends.

Monkeys were trained with successive approximations and positive reinforcement to come forward to the front of the cage and place their mouths around the end of a 3 or 6 cm^3 syringe to receive flavored syrup (Torani®) or liquefied baby food (Gerber®). Daily oral dosing with fluoxetine (Webster Veterinary Supply, Devens, MA) dissolved in a favored vehicle was then conducted at doses which resulted in plasma levels in the range of therapeutic doses in children as determined in preliminary pharmacokinetic studies (Golub and Hogrefe, 2014a). Control animals received daily dosing with the vehicle (flavored syrup or baby food) only. Syrup and food flavors were changed throughout the study to maintain interest. Plasma fluoxetine plus norfluoxetine concentrations in samples taken 23 h after a daily dosing with 2.4 mg/kg at 3 years of age were 273 ± 31 ng/mL (mean ± s.e.m.) as compared to 363 ng/mL measured 8–12 h after dosing in a pharmacokinetic study of pediatric patients treated with fluoxetine at therapeutic doses (Wilens et al., 2002). All but two of the animals consumed 99 +% of the scheduled dose over the 110 weeks of treatment; the other two consumed 85% and 97%.

Subjects were genotyped for behaviorally relevant polymorphisms of the serotonin transporter (SERT, 5HTTLPR VNTR polymorphisms) and serotonin metabolizing enzyme monoamine oxidase A (MAOA, uVNTR polymorphisms) (Kinnally et al., 2008; Capitanio et al., 2012). Treatment groups were balanced for polymorphisms resulting in different transcription rates for these genes (5HTTLPR: SS, SL, LL groups;