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**Regular Article**

# Maternal neglect and the serotonin system are associated with daytime sleep in infant rhesus monkeys

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**Abstract**

Environmental and biological factors contribute to sleep development during infancy. Parenting plays a particularly important role in modulating infant sleep, potentially via the serotonin system, which is itself involved in regulating infant sleep. We hypothesized that maternal neglect and serotonin system dysregulation would be associated with daytime sleep in infant rhesus monkeys. Subjects were nursery-reared infant rhesus macaques ($n = 287$). During the first month of life, daytime sleep-wake states were rated bihourly (0800–2100). Infants were considered neglected ($n = 16$) if before nursery-rearing, their mother repeatedly failed to retrieve them. Serotonin transporter genotype and concentrations of cerebrospinal fluid 5-hydroxyindoleacetic acid (*5-HIAA*) were used as markers of central serotonin system functioning. *t* tests showed that neglected infants were observed sleeping less frequently, weighed less, and had higher *5-HIAA* than non-neglected nursery-reared infants. Regression revealed that serotonin transporter genotype moderated the relationship between *5-HIAA* and daytime sleep: in subjects possessing the *Ls* genotype, there was a positive correlation between *5-HIAA* and daytime sleep, whereas in subjects possessing the *LL* genotype there was no association. These results highlight the pivotal roles that parents and the serotonin system play in sleep development. Daytime sleep alterations observed in neglected infants may partially derive from serotonin system dysregulation.

**Keywords:** development, 5-hydroxyindoleacetic acid, infant sleep, maternal neglect, serotonin transporter gene

Sleep is an important part of early-life development, and is crucial for normative neurological development and functioning (Ednick, Cohen, McPhail, Beebe, & Simakajornboon, 2009; Graven & Browne, 2008), as well as psychiatric health. At birth, infants typically spend 15–16 hrs per day sleeping, although there are substantial individual differences centered on this mean. As they develop, their nighttime sleep consolidates, and they gradually sleep less during daylight hours (Parmelee, Wenner, & Schulz, 1964). Although many changes in sleep occur in the first 6 months of life, individual differences in daytime sleep duration remain stable across this period (Figueiredo, Dias, Pinto, & Field, 2016) and are thought to be windows to the developing brain (Kohyama, 1998). Studies suggest that neonatal sleep patterns are endogenously mediated and consequently relatively immune to disruptions from family routines (McGraw, Hoffmann, Harker, & Herman, 1999). Its daily rhythm shows a 4-hr ultraradian sleep-wake, light-initiated sequence that extends to both nighttime sleep and daytime naps (Scher & Loparo, 2009), likely an evolutionary adaptation to feeding. Studies show that during the first month, infants sleep as much during the day as they do at night (Figueiredo et al., 2016), possibly reflecting the endogenous nature of neonatal regulation. Daytime sleep (napping) appears to be an extension of overall sleep, because studies have shown that during the first month of life, the amount of time infants spend sleeping during the day and during the night is positively correlated (Figueiredo et al., 2016; Iwata et al., 2017), as is the amount of time it takes for them to fall asleep during the day and at night (Iwata et al., 2017). Daytime sleep is a useful marker of infant development because it is easily measured and readily observable; for full-term infants, it shows a similar architecture to night sleep (Biagioni et al., 2005). Studies suggest that during infancy, it is equally as important for brain development as is nighttime sleep (Fagioli & Salzarulo, 1982; Spruyt et al., 2008), largely because neonatal and early infant sleep patterns are not fully consolidated into adult-like diurnal patterns (Ednick et al., 2009). Hence, aberrations in neonatal daytime sleep patterns likely reflect general sleep disturbances to the same degree as aberrations in nighttime sleep patterns during early infancy.

Although these studies suggest that sleep is largely immune to typical environmental influences during the neonatal period, sleep problems later in infancy and childhood are risk factors for future psychopathology, including anxiety and mood disorders (Gregory et al., 2005; Lam, Hiscock, & Wake, 2003; Ong, Wickramaratne, Tang, & Weissman, 2006). Aberrant sleep patterns in infancy often correlate with other physical and mental impairments (Ednick et al., 2009); hence, research devoted to understanding

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neonatal daytime sleep development may have therapeutic applications. To this end, translational research investigating daytime sleep in neonatal rhesus macaques (*Macaca mulatta*), a primate species with similar sleep-wake patterns as humans (Bowman, Wolf, & Sackett, 1970; Reite, Rhodes, Kavan, & Adey, 1965) and parallels in their underlying central nervous system (CNS) and developmental sequences (Gibbs et al., 2007), has shed light on the environmental and biological factors associated with daytime sleep in infants.

Although neonates are relatively immune to parental sleep-entrainment with consolidation occurring later in infancy, caregiving during the neonatal period is especially important because neonates show inefficient thermoregulation (Knobel, 2014; Waldron & MacKinnon, 2007); without access to their parents as a source of warmth, infants may suffer cold stress, eliciting cortisol release, and a cascade of other stress markers (Scaramuzzo et al., 2015), which may be life-threatening when severe (McCall, Alderdice, Halliday, Jenkins, & Vohra, 2006). In hospital settings, studies show that human neonates who are not kept warm exhibit increased levels of epinephrine and norepinephrine, as well as dysregulated metabolism (Knobel, 2014). Hunger and dehydration are also major stressors for neonates, leading to changes in energy expenditure and cortisol regulation (see Welberg & Seckl, 2001). Although these stressors lead to aberrant homeostatic states, once normal care returns, the aberrant states are thought to dissipate. Nevertheless, studies have not longitudinally followed neonates that experienced hypothermia and hunger during the neonatal period; thus, little is known about the long-term effects of neonatal hypothermia on the development of the brain and its long-term effect on sleep development.

## Sleep and serotonin: A complex relationship

Serotonin has long been held as a CNS modulator of sleep, and dysregulation of central serotonin functioning can have profound effects on sleep patterns (Jouvet, 1999). By interacting with other neurotransmitters, serotonin has indirect effects on circadian rhythmicity, sleep patterns, and sleep development (Monti & Jantos, 2008). More specifically, however, serotonin can act directly on the reticular activating system to modulate wakefulness and sleep (Jones, 2003). CNS serotonin functioning is commonly measured by assaying cerebrospinal fluid (CSF) for concentrations of the serotonin metabolite 5-hydroxyindoleacetic acid (*5-HIAA*), a proxy measure of CNS serotonin turnover. This measure of serotonin function is especially useful because in humans and in monkeys, it can be directly measured from the cistern at the base of the brain (Higley et al., 1998; Williams et al., 2003). In a laboratory-based investigation of juvenile rhesus monkeys, Zajicek, Higley, Suomi, and Linnoila (1997) found that low concentrations of CSF *5-HIAA* were related to increased sleep disturbances (i.e., falling asleep later at night). In another study investigating free-ranging adult male monkeys, low CSF *5-HIAA* was associated with less nighttime sleep and more daytime naps (Mehlman et al., 2000). Although serotonin serves as a prenatal neurodevelopmental guide (Kepser & Homberg, 2015) and is one of the first monoaminergic systems to develop in the CNS (Daws & Gould, 2011), relatively little is known concerning the role of serotonin in the development of infant sleep. To our knowledge, no studies have assessed the relationship between serotonin and neonatal sleep, although studies of human neonates suggest serotonin can affect sleep development: as early as 2 weeks after birth, neonates prenatally exposed to selective serotonin reuptake inhibitors that their mothers used while pregnant show sleep aberrations (see Olivier et al., 2013), spending disproportionately more sleep time in the rapid eye movement stage and waking up more often when compared with healthy controls (Zeskind & Stephens, 2004). Developmentally, CSF *5-HIAA* shows a large decline from the neonatal period to middle infancy, although individual differences remain highly stable over this early period (Shannon et al., 2005), the time when infant sleep patterns begin to consolidate and circadian sleep patterns emerge (Bathory & Tomopoulos, 2017). To the degree that sleep is modulated by CNS serotonin during these formative months, this stability may be reflected in the stability of sleeping patterns; to our knowledge, however, this has not been investigated. This study will explore the nature of this association in infant monkeys.

As discussed previously, the conditions of the sleep environment and early experiences play a role in sleep development. Genetics may also play an important role in the development of sleep by shaping underlying CNS mechanisms, such as the serotonin system. The serotonin transporter (*5HTT*) gene is believed to be the sole gene encoding serotonin reuptake transporters (Daws & Gould, 2011; Lesch et al., 1994). Consequently, variations in this gene can affect CNS serotonin transport and functioning (Williams et al., 2003). The *5HTT* gene has two common allelic variants: the ancestral long (*L*) allele, and the low-expressing short (*s*) allele, a 44-bp insertion or deletion in the gene's promoter region (Heils et al., 1996). In early investigations of *5HTT* and sleep, adults possessing at least one copy of the *s* allele were more likely to suffer from insomnia (Deuschle et al., 2010). As observed in many other studies of the *5HTT* gene (see Caspi, Hariri, Holmes, Uher, & Moffitt, 2010; Suomi, 2006), its effects on sleep appear to be modulated by gene-by-environment interactions, with the *s* allele modulating sleep, but only in subjects experiencing chronic stress. In one study, researchers showed that chronically stressed caregivers carrying the *s* allele experienced poorer sleep quality than individuals in the same stressful condition that were homozygous for the *L* allele (Brummett et al., 2007). Those that were not under the stress of being a caregiver were undifferentiated by genotype. Not all studies have found this relationship, however (e.g., see Barclay et al., 2011). Other studies suggest that possession of the *s* allele is linked with sleeping longer at night and going to bed earlier (Koga, Fukushima, Sakuma, & Kagawa, 2016). To our knowledge, no studies have investigated the relationship between neonatal and infant sleep and the *5HTT* gene. Suggestive of the role of *5HTT* genotype in infant sleep development are studies linking variation in *5HTT* genotype with sudden infant death syndrome; the *L* allele is found more frequently in infants that die in their sleep from this disorder (Filonzi et al., 2012; Lavezzi, Casale, Oneda, Weese-Mayer, & Matturri, 2009). Research investigating the association between *5HTT* genotype and sleep may inform future research aimed at developing personalized medicine to prevent and treat developmental disorders.

## Hypotheses

A central purpose of this study was to investigate the association between serotonin functioning and sleep development. We were also interested in the association between early life experiences and sleep development, in particular the consequences of neonatal maternal neglect. Rhesus monkeys are commonly used to investigate the role that mothers play in infant development because maternal parenting behaviors are similar to humans (Hinde & Spencer-Booth, 1967; McCormack et al., 2015). For

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example, rhesus mothers can profoundly affect their infant's developing serotonin system (Shannon et al., 2005), and they play a primary role in regulating their infant's arousal and internal temperature (Harlow, 1958; Reite et al., 1978). Rhesus monkeys generally give birth in the early spring (March and April), when nights are longer and cooler than during summer months. At the National Institute of Child Health and Development nonhuman primate facility where data were collected, the average nighttime temperature in March and April is between 0.5° and 6.7°C. At birth, rhesus monkey neonates are cradled and held tightly as they nurse, preventing hypothermia. A mother's failure to exhibit normative maternal care can be life-threatening to the infant. Consequently, those few infants that do not receive proper maternal care are typically placed in an incubator and raised in the neonatal nursery.

The conditions in the National Institute of Child Health and Development neonatal nursery allow a homogeneous environment for the subjects, and variables such as maternal neglect can be opportunistically studied by comparing neglected infants with those randomly removed from their mother for experimental purposes (hereafter referred to as non-neglected nursery-reared infants). The mother- and nursery-reared subjects were on the same 24-hr day-night cycle, with exposure to the same ambient sunlight and human activity schedule. Neonates were handled routinely for feeding, changing of soiled blankets, and weighing. Except for the time spent with their neglecting mothers, the experiences of the neglected infants were identical to those of the non-neglected nursery-reared infants once they were placed in the neonatal nursery. Hence, any differences in daytime sleep between these two groups of nursery-reared infants are likely due to maternal neglect.

In the nursery, sleep-wake states were collected every 2 hr during the day across infants' first month of life (approximately equivalent to the first 3–4 months of life for human infants in terms of development and maturation). Although nighttime sleep measures were not collected, Meier and Berger (1965) investigated sleep in five neonatal nursery-reared monkeys during their first month of life and found little evidence of circadian rhythmicity in sleep-wake patterns, a finding that parallels research in human infants cited previously (McGraw et al., 1999). To the extent that daytime sleep reflects overall sleep in infant rhesus monkeys, we hypothesized that neonatal rhesus monkeys experiencing early maternal neglect would exhibit differences in the frequency of observed daytime sleep compared to non-neglected nursery-reared infants.

Although nursery-rearing has been shown to alter older monkeys' sleep patterns (Barrett et al., 2009; Kaemingk & Reite, 1987), little is known concerning neonatal sleep in nonhuman primates. In the nursery, all neonates are given the same treatment, and, although they are reared without their mother, many components of maternal care can be artificially supplemented with heating pads to keep them warm, consistent access to milk, and a soft surrogate mother that rocks or moves for cuddling and comfort (Lubach & Coe, 2006). Hence, the controlled environment in the neonatal nursery was ideal for assessing the effects of maternal neglect and the developing serotonin system on daytime sleep without the variability of maternal care and social group influences that occurs when monkeys are raised by their mothers. CSF can also be removed repeatedly at different developmental time points with little to no adverse medical effects (Shannon et al., 2005). Using this technique, a study from our laboratory showed that the serotonin system of infants reared in the neonatal nursery is modulated by the *5HTT* genotype, as measured by concentrations of *5-HIAA* in cisternal CSF (Bennett et al., 2002). Because the precise mechanism by which *5HTT* genotype affects sleep as well as the direction of the effect are still unclear, another purpose of this study was to explore the complex relationship between *5HTT* genotype, CNS serotonin functioning, and sleep. Rhesus monkeys possess a human *5HTT* ortholog (Rogers et al., 2006), have similar sleep architecture to humans (Deley, Turner, Freeman, Bliwise, & Rye, 2006; Reite et al., 1965), and, unlike in human infants, it is possible to safely collect CSF samples during infancy. We hypothesized that for infant rhesus monkeys, dysregulation of the serotonin system would be associated with dysregulated sleep. Specifically, we hypothesized that an interaction between the *5HTT* genotype and CSF *5-HIAA* would predict daytime sleep occurrence.

## Methods and Materials

### Subjects and rearing procedures

Subjects were 287 infant rhesus monkeys in a nursery-rearing program at the Laboratory of Comparative Ethology, National Institute of Child Health and Human Development. Data were collected between 1994 and 2006 for each birth-year cohort. Because of technical problems and project availability, some subjects were missing data, leaving $n = 116$ subjects with a complete battery of data (see Appendix A in the online supplementary material for a summary of subjects with available data).

Details of housing conditions and nursery protocols are explained in detail elsewhere (see Shannon, Champoux, & Suomi, 1998). Briefly, randomly selected infants were separated from their mothers between 1 and 3 days postpartum and relocated to the neonatal nursery where they were individually housed with a heated "surrogate mother" that had a bottle attached for feeding (see Shannon et al., 2005, for a detailed description of the assignment procedure). Subjects were housed in a room maintained at approximately 27°C, where they could see and hear other infants but could not physically contact them. Infants were hand-fed a 50–50 mixture of Similac (Ross Laboratories, Columbus, OH) and Primilac (Bio-Serv, Frenchtown, NJ), enriched with docosahexaenoic acid formula *ad libitum* until they could feed themselves from the bottle fastened to the surrogate. When subjects were about 2 weeks of age (between 11 and 17 days old), infants were removed from their neonatal cage and administered a developmental assessment, following which blood and cisternal CSF samples were obtained.

Following testing, infants were relocated to a different room and housed individually in larger nursery cages. Subjects retained their surrogate mother and blanket, but the electric heating pad was removed. The room was maintained at 22–26°C with the lights on from 0700 to 2100. Because the room was connected to a central room with windows open to the outside environment, subjects were also exposed to outside light and noise. When subjects were approximately 4 weeks of age (between 26 and 31 days old), they were administered another developmental assessment, following which additional blood and cisternal CSF samples were obtained. All procedures conformed to standards set forth in the National Guidelines for the Care and Use of Laboratory Animals.

### Neglected infants

In some cases, an infant originally assigned to remain with its mother was reassigned to the nursery because of maternal neglect ($n = 16$). Decisions to reassign infant rearing conditions were

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made by nursery and veterinary staff, who observed the animals daily to assure that the mother-reared infants were on their mothers' ventrum, suckling, and otherwise healthy. If the staff discovered an infant left alone on the floor, they approached the cage to determine if the mother would actively retrieve the infant. If the mother consistently failed to retrieve the infant, the infant was considered neglected. Before neglected infants were placed in the nursery, the mother and neglected infant were temporarily separated from the larger social group and placed together in a small cage where they could be more closely observed to determine lactation and patterns of maternal behavior. If there were no other lactating females to which the neglected infant could be fostered, it was removed from its mother and reassigned to the neonatal nursery. Infants that had been relocated to the neonatal nursery because they or their mothers were sick ($n = 6$) were not considered neglected and were not used in analyses. None of the neglected infants included in analyses showed subsequent severe pathology over the next year.

Before being placed in the neonatal nursery, neglected infants were housed with their mothers in connected indoor-outdoor enclosures with 8–10 adults and 2–5 other infants (see description of mother-reared monkeys in Shannon et al., 1998). Similar to the nursery, the indoor pens were maintained on a 12-hr light-dark schedule, with lights on from 0700 to 1900. In both the neonatal nursery and the indoor pens, external windows or wire mesh allowed ambient light from sunrise to sunset. Unless temperatures were below freezing, mother-infant pairs were allowed to sleep outside as they pleased. Mothers were provided with monkey chow *ad libitum*. About one-half of the neglected infants were identified within the first 48 hr after birth and remained with their mothers for the same amount of time as non-neglected infants randomly assigned to the nursery. In other cases, some neglected infants remained with their mother for a longer period. In these cases, mothers retrieved their infant when observed by animal care staff, but later would drop the infant when the staff left. These infants were typically discovered as being neglected because they either appeared ill or dehydrated, lethargic, and unresponsive. Of the 16 neglected infants, 7 were determined to be neglected and relocated on the first or second day of life and 9 were determined to be neglected and relocated after the first week of life (7–19 days old). Preliminary analyses showed no differences between neglected infants placed in the nursery on the first or second day of life when compared with neglected infants placed in the nursery after the first week of life in *5HTT* genotype or sex, nor in sleep, weight, or CSF *5-HIAA* at either the week 2 assessment, the week 4 assessment, or when the average of week 2 and week 4 was used ($p > .42$). For this reason, all neglected infants were combined into a single group and compared with the non-neglected nursery-reared infants ($n = 271$).

### Daytime sleep measurements

While infants were in the nursery, trained observers recorded daily sleep states 7 times a day, every 2 hr, between 0800 and 2000 hr. Scores were initially recorded on a 6-point Likert-type scale: 0 = *quiet sleep*, 1 = *rapid eye movement sleep*, 2 = *drowsy* (transitional state between sleep and wake), 3 = *quiet wake*, 4 = *active wake*, 5 = *distressed awake*. To improve face validity of measuring sleep-wake states based on observation, this scale was recoded by combining quiet sleep with rapid eye movement sleep, and combining quiet awake with active awake and distressed awake. These values were then reverse-scored, resulting in a 3-point scale: 1 = *awake*, 2 = *drowsy*, 3 = *sleep*. To determine average sleep during the first month of life unbiased by neonatal assessment and anesthesia for CSF sampling, each subject's sleep scores for the 7 days before their week 2 and week 4 neonatal assessments were aggregated. These time points were selected to create corresponding measures of daytime sleep that were just before and temporally associated with CSF sampling. Sleep data for days on which infants were tested as part of other projects were excluded from this average. The average sleep score showed good overall reliability across the 14 days (Cronbach reliability $\alpha = .71$).

### CSF 5-HIAA metabolite assay

CSF *5-HIAA* assays were completed for a subset of 176 subjects (see Appendix A). Procedures for CSF extraction and assays are explained in detail elsewhere (see Shannon et al., 2005). Briefly, subjects were anesthetized using 10 mg/kg of ketamine hydrochloride, delivered intramuscularly. Within 15 min of administering the anesthesia, a 22-gauge needle and 5-mL syringe were inserted into the cisterna magna, and 1 mL of CSF was obtained. CSF samples were assayed for *5-HIAA* with high-performance liquid chromatography and electrochemical detection (see Scheinin, Chang, Kirk, & Linnoila, 1983; Seppala, Scheinin, Capone, & Linnoila, 1984). Inter- and intra-assay variability was <10%.

### 5HTT Genotyping

Genotyping for the *5HTT* gene was completed for a subset of 220 subjects (see Appendix A). The procedures for *5HTT* genotyping are explained in detail elsewhere (see Bennett et al., 2002). DNA was extracted from blood samples obtained during testing at weeks 2 and 4 of life, and segments were amplified using standard polymerase chain reaction methods. Allelic sizes and identities for the *L* allele (419 bp) and *s* allele (398 bp) were confirmed using electrophoresis visualized by ethidium bromide staining. Three subjects were excluded from analyses of *5HTT* genotype because of their genotype's infrequent occurrence: two subjects were homozygous for the *s* allele and one subject had a copy of the *L* allele and a copy of an extra-long allele. For the remaining 217 subjects with available *5HTT* genotype data, there were 174 that were homozygous for the *L* allele and 43 that were heterozygous with the *Ls* genotype. Subjects from the neglected group were not different in *5HTT* genotype frequency compared with non-neglected nursery-reared subjects (see Appendix A). Within the 116 subjects that had no missing data, the genotyping found 91 subjects were homozygous for the *L* allele and 25 subjects were heterozygous.

### Weight, cohort, and sex variables

Subjects were weighed each morning as a part of veterinary rounds. Because weight is a proxy measure for gestational age and corresponding brain development (Vohr et al., 2000), and because human infants born preterm exhibit sleep impairments (Huang, Paiva, Hsu, Kuo, & Guilleminault, 2014), weight was included as a covariate in all analyses to control for age since conception. Because preliminary *t* tests showed that neglected infants' weight did not differ from non-neglected infants' weight during the first week of life ($p > .14$), average weight was used in analyses, which was computed using the day before subjects' week 2 assessment and subjects' week 4 assessment.

Sleep and weight data were recorded for 13 different birth-year cohorts. CSF *5-HIAA* and *5HTT* genotype data were not available

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for the last two cohort years, and were collected for only 11 birth-year cohorts. As is commonly done in studies spanning multiple years, cohort was considered in analyses (Zajicek et al., 1997). Preliminary one-way analyses of variance revealed cohort-differences in sleep, $F_{(12,\ 256)} = 6.36$, $p < .001$; and CSF *5-HIAA*, $F_{(10,\ 166)} = 2.85$, $p = .003$. Dummy codes for cohort were included in subsequent analyses, with the cohort of infants born in 1994 (the first year that data were collected) set as the reference group.

Although there was not a significant sex difference in daytime sleep ($p = .15$), because some studies indicate a sex difference in sleep in human infants (Moss, 1967) and nonhuman primates (Champoux & Suomi, 1988; Sackett, Fahrenbruch, & Ruppenthal, 1979), sex was included as a covariate in analyses. A *t* test revealed sex differences in average weight, such that males weighed more than females, $M = 601$ g and 579 g, respectively, $t_{(260)} = -2.44$, $p = .02$. Although preliminary analyses did not reveal sex differences in CSF *5-HIAA* ($p = .50$), sex was also considered because other investigations commonly report sex differences in CSF *5-HIAA* (Williams et al., 2003; Young, Gauthier, Anderson, & Purdy, 1980).

### Analyses

All analyses were performed using SPSS (IBM, V.23). Kolmogorov-Smirnov tests of normality revealed that all variables were normally distributed, excluding subjects if their data included outliers >3 standard deviations from the group mean. To investigate the effect of maternal neglect, independent *t* tests were performed, with neglect status as the independent variable and separate tests performed for the dependent variables of sleep, CSF *5-HIAA*, and weight. For each comparison, Cohen's *d* was calculated as a measure of the effect size. Because there were few subjects considered neglected, and not all subjects had available data for all three dependent variables (see Appendix A), analyses were performed in a pair-wise fashion to maximize available data for these analyses. To verify that the amount of time that neglected infants spent with their mothers was not a confound, all analyses were repeated with number of days with mother as a covariate; however, it was not significant in any analysis, and the results are not reported.

To investigate the combined effects of maternal neglect, CSF *5-HIAA*, and *5HTT* genotype on daytime sleep, multiple regression was performed using the PROCESS add-in for SPSS (Hayes, 2013). Fifteen variables were used in base regression models, including CSF *5-HIAA*, *5HTT* genotype (coded as 0 = *LL*, 1 = *Ls*), the interaction between CSF *5-HIAA* and *5HTT* genotype (calculated by multiplying subjects' mean-centered CSF *5-HIAA* by their mean-centered *5HTT* code), neglect status (0 = non-neglected nursery-reared, 1 = neglected nursery-reared), weight, sex, and nine dummy codes for cohort. To investigate how interactional effects within the serotonin system affect daytime sleep, Model 1 of the PROCESS add-in was used to test for a moderation effect of *5HTT* genotype on CSF *5-HIAA*, with all other variables included as covariates. *A posteriori* simple slopes analyses were performed separately for subjects with the *LL* genotype and subjects with the *Ls* genotype.

## Results

### Effect of maternal neglect on daytime sleep, the serotonin system, and weight

Independent samples *t* tests showed that, when compared with the non-neglected nursery-reared infants, neglected infants slept less during the day, $t_{(265)} = 2.19$, $p = .030$, $d = 0.65$ (Figure 1), had higher concentrations of CSF *5-HIAA*, $t_{(174)} = -2.45$, $p = .015$, $d = 0.76$ (Figure 2), and, on average, weighed less, $t_{(260)} = 2.87$, $p = .004$, $d = 0.88$ (Figure 3).

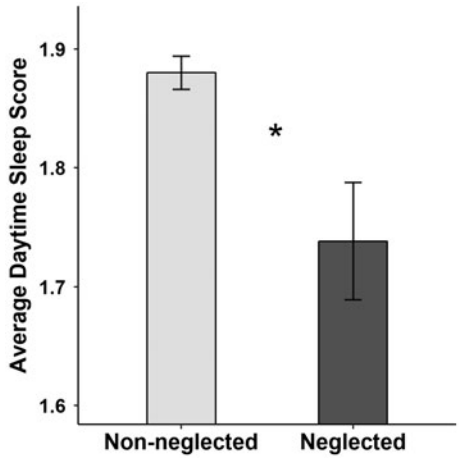


**Figure 1.** Effect of maternal neglect on daytime sleep. Compared to non-neglected nursery infants (gray bar, $n = 255$), neglected infants (black bar, $n = 12$) were less frequently observed sleeping during the day. Asterisk indicates $p < .05$. Error bars show ± 1 standard error.

### Interaction between 5HTT genotype and levels of CSF 5-HIAA predicts sleep

Regression analyses with daytime sleep as the dependent variable showed that the interaction between *5HTT* genotype and CSF *5-HIAA* significantly predicted daytime sleep ($p = .002$; Table 1). Simple slopes analyses revealed that there was a positive association between daytime sleep and CSF *5-HIAA*, but only among infants that were heterozygous with the *Ls* genotype ($p = .019$, $\beta = .56$), an association not present among infants that were homozygous for the *L* allele ($p = .63$; Figure 4). Analyses also showed that daytime sleep varied negatively with weight ($p < .001$, $\beta = -.44$), and occurred less frequently in neglected nursery-reared infants compared with non-neglected nursery-reared infants ($p = .001$, $\beta = -.27$). Three of the nine cohort dummy codes were significant

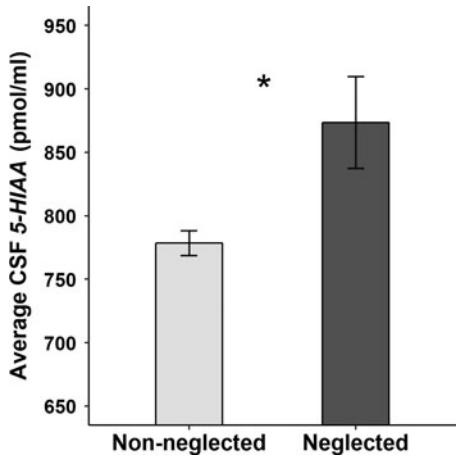


**Figure 2.** Effect of maternal neglect on cerebral serotonin turnover. Compared to non-neglected nursery infants (gray bar, $n = 165$), neglected infants (black bar, $n = 11$) exhibited higher cerebrospinal fluid 5-hydroxyindoleacetic acid (CSF *5-HIAA*). Asterisk indicates $p < .05$. Error bars show ± 1 standard error.

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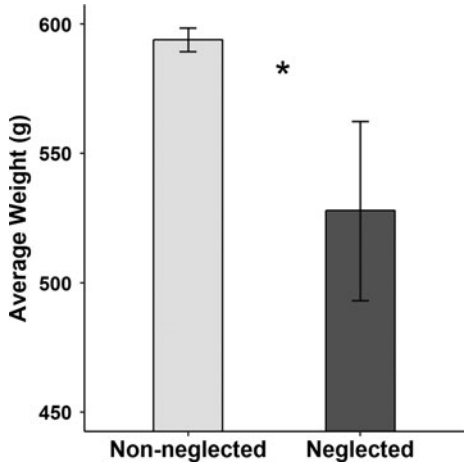


**Figure 3.** Effect of maternal neglect on maturational weight. Compared to non-neglected nursery infants (gray bar, $n$ = 251), neglected infants (black bar, $n$ = 11) on average weighed less during the first month of life. Asterisk indicates $p < .05$. Error bars show ± 1 standard error.

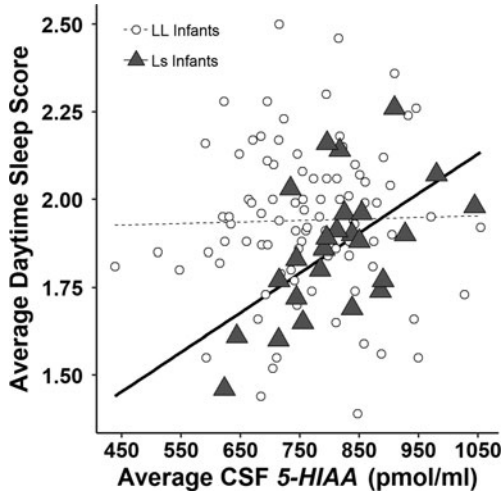


**Figure 4.** Concentrations of cerebrospinal fluid 5-hydroxyindoleacetic acid (CSF *5-HIAA*) predict daytime sleep in infants heterozygous with the *Ls* genotype. The *x*-axis represents concentrations of CSF *5-HIAA*, and the *y*-axis represents average daytime sleep. The white dots represent subjects that were homozygous for the serotonin transporter gene (*5HTT*) *L* allele ($n$ = 91), and the black triangles represent subjects that were heterozygous for the *5HTT Ls* genotype ($n$ = 25). The dotted line is the line of best fit for subjects that were homozygous for the *L* allele, and the solid black line is the line of best fit for subjects that were heterozygous for the *Ls* genotype. There was a positive correlation between concentrations of CSF *5-HIAA* and daytime sleep among infants that were heterozygous with the *Ls* genotype. There was no association between concentrations of CSF *5-HIAA* and daytime sleep among infants that were homozygous for the *L* allele.

in the model ($p < .04$; $\beta_{1996} = .25$; $\beta_{2002} = .33$; $\beta_{2003} = -.18$). Although sex did not achieve significance ($p = .072$, $\beta = .14$), it was retained in the model because it approached significance and because of its association with weight and CSF *5-HIAA*.

## Discussion

### *Maternal neglect, neonatal stress, and sleep patterns*

We found that early maternal neglect was associated with decreased daytime sleep and serotonin system functioning in infant rhesus monkeys. These findings add to a growing literature suggesting that early experiences have profound consequences for neurological and behavioral development (Bennett et al., 2002; Fisher et al., 1997; Spinelli et al., 2009) and that these early experiences interact with genotype to produce differences in developmental outcome. As hypothesized, neglected infants exhibited dysregulated daytime sleep: whereas infants typically slept frequently during the daytime, neglected infants were observed sleeping less often during the day than the non-neglected nursery-reared infants. Although it is widely held that serotonin plays a primary function in sleep (Jouvet, 1999), to our knowledge, this is the first study to show that aberrations in the CNS serotonin system are correlated with variation in infant sleep development. Our study also showed that infants possessing a copy of the *s* allele exhibited a stronger coupling of CNS serotonin functioning with sleep when compared with infants with the *LL* genotype, as measured by the positive correlation between CSF *5-HIAA* concentrations and daytime sleep. This finding is of interest because some theoretical models suggest that the *s* allele of the *5HTT* gene may be more sensitive to experiences and settings that are outside the range of the species-typical environment (Belsky et al., 2009).

**Table 1.** Multiple regression model showing dysregulation in the serotonin system is associated with daytime sleep

| Variable | *B* | SE *B* | *β* | *p* |
|---|---|---|---|---|
| Weight | **<−.01** | **< .01** | **−.44** | **<.001** |
| Neglected by mother | **−.26** | **.07** | **−.27** | **.001** |
| Male | −.06 | .03 | .14 | .072 |
| *5HTT* genotype (heterozygous *Ls*)[a] | −.08 | .04 | −.15 | .060 |
| CSF *5-HIAA*[a] | <.01 | <.01 | .08 | .321 |
| Interaction (CSF *5-HIAA* × *5HTT* genotype) | **<.01** | **<.03** | **.25** | **.002** |
| Overall model: $R^2 = .71$, $F_{(15,\ 100)} = 6.75$, $p < .001$. | | | | |

*Note:* Bold indicates variables that were significant in the model at $p < .05$. Nine dummy codes for cohort were also included in the model, but are not reported. CSF *5-HIAA*, cerebrospinal fluid concentrations of the serotonin metabolite 5-hydroxyindoleacetic acid; *5HTT*, serotonin transporter; *Ls*, heterozygous genotype with one long allele and one short allele.
[a]CSF *5-HIAA* and *5HTT* genotype were mean centered.

These findings suggest that the early experiences neglected neonates had before they were placed in the nursery contributed to dysregulated sleep. Infants are born with a specific set of needs that must be met for normative development to occur, something often referred to as experience-expectant brain development. One possible explanation for our findings is that the neglected neonates did not receive the adequate "experience-expectant" mothering necessary for proper brain development, resulting in decreased daytime sleep during their first month of life, potentially because of hypothermia resulting from poor maternal care immediately following birth. In humans, stressful events, such as circumcision, acutely increase sleep duration and depth in neonates, a process attributed to adaptations allowing recovery from stress (Emde, Harmon, Metcalf, Koenig, & Wagonfeld, 1971; Gunnar, Malone, Vance, & Fisch, 1985). Although all infants in this study eventually received identical nursery-rearing, the neglected infants experienced the additional stress of a neglecting mother in the critical first few days of life. Mothers are the primary source of heat for neonatal rhesus monkeys (Harlow, 1958), and without a mother to hold an infant close, hypothermia

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can result, which is a major stressor for a newborn and can lead to elevated cortisol (Scaramuzzo et al., 2015) and dysregulate the hypothalamic-pituitary-adrenal axis (Knobel, 2014). The non-neglected nursery-reared infants had constant access to a heat source from their first day of life. Although we could not observe the neglected infants constantly, it is likely that they were left on the cold floor and not held by their mothers for extended periods, potentially impeding brain development and resulting in decreased daytime sleep and weight. Our findings suggest that the lack of maternal care and hypothermia during the first days of life had a lasting effect on the developing infant across the first month of life.

Although one alternative explanation for the differences between the neglected and non-neglected nursery-reared infants is that the time with the neglecting mothers entrained the sleep differences seen in the neglected infants (see Lubach, Kittrell, & Coe, 1992), there are reasons to think this unlikely. First, when the early-removed infants (within the first 2 days of life) and late-removed infants (after the first week of life) were compared, there were no differences in sleep between the two groups; when the number of days infants spent with their mother before being placed in the nursery was considered in the analyses, days on mother was not statistically related or trending. Second, because of the natural light that entered the pens and the nursery, the light-dark cycle was identical for all infants. Also, as noted in the introduction, in humans and macaques, neonatal sleep-wake rhythms show ultraradian patterns that are relatively robust to routine disturbances (in humans: McGraw et al., 1999; in macaques: Meier & Berger, 1965; Sackett et al., 1979), and sleep does not fully consolidate until later in infancy (in humans: Ednick et al., 2009; in macaques: Sackett et al., 1979). It is clear that the early neglect had deleterious effects, and although it is possible that maternal entrainment contributed to the sleep differences, its effects were likely secondary to the traumatic effects of neglect, hypothermia, and potential dehydration and malnutrition.

Besides maternal entrainment, it could be argued that compared with the mothers' breast milk, the formula the nursery-reared monkeys were fed affected their sleep (Ball, 2003) and weight (Dewey, 1998; Kelleher, Chatterton, Nielsen, & Lönnerdal, 2003). Weight measurements from normally reared infants raised by their biological mother were available (see Shannon et al., 1998) and could be used as reference data. Although the neglected infants' birth weights were identical to the mother-reared infants, by the time the neglected infants were placed in the nursery, they weighed less than mother-reared infants of the same age and sex. This analysis suggests that the differences in maternal treatment were responsible for the neglected infants' low weight, which was low even when compared with non-neglected infants that had never consumed formula. Although sleep data were not collected for mother-reared infants, considering the extreme effects of maternal neglect on neonates' weight, it is likely that formula alone does not explain the differences in sleep between the neglected and non-neglected nursery-reared infants, and maternal neglect likely contributed. Because infant weight is considered a window to postconception age and maturational brain development (Vohr et al., 2000), this finding suggests that neonatal maternal neglect can have consequences for physical and neurological development. Nonetheless, further investigation of neonatal daytime sleep in mother-reared monkeys is needed to confirm this interpretation. Regardless of the etiological reason for the differences between the neglected infants and the nursery controls, it is clear that maternal neglect is associated with dysregulated sleep in infants. In further support of this interpretation, neglected infants had impaired CNS serotonin functioning (i.e., higher concentrations of CSF *5-HIAA*), suggesting that neglect-mediated impairments in central serotonin functioning may be, at least in part, a potential biological mechanism by which the neonates' sleep was dysregulated. Taken together, the findings of this study illustrate the critical role that primary caregivers play in shaping their infants' sleep and physiological development.

### *Effect of 5HTT genotype on neonatal sleep and its relationship with CNS serotonin*

We hypothesized that dysregulation of the serotonin system would be associated with daytime sleep. In support of this hypothesis, we found that for infant monkeys with the *s* allele, there was a positive correlation between average frequency of daytime sleep and concentrations of CSF *5-HIAA*. There was no association between CSF *5-HIAA* and daytime sleep among infants that were homozygous for the *L* allele. Studies of adult rhesus monkeys tend to show negative correlations between CSF *5-HIAA* and measures of nighttime sleep, including sleep onset (Zajicek et al., 1997), nighttime activity, and daytime naps (Mehlman et al., 2000). Unlike those studies of adult monkeys, we found a positive correlation between CSF *5-HIAA* and average frequency of daytime sleep in this study. These studies, however, did not assess CNS serotonin functioning in the context of *5HTT* genotype, and in this case the association was only seen in subjects with the *s* allele. Moreover, our subjects were neonates reared without mothers, and early rearing conditions can profoundly shape the expression of the serotonin system (Maestripieri et al., 2006; Shannon et al., 2005). The discrepancy between our serotonin-mediated sleep findings and previous research could also be due to differences in the age or environmental conditions of the subjects in these studies. Although Zajicek et al. (1997) investigated laboratory-housed juvenile monkeys and Mehlman et al. (2000) investigated free-ranging adult males, the current study investigated laboratory-housed male and female infants. Thus, the positive correlation between CSF *5-HIAA* and daytime sleep reported in this study suggests that there may be developmental changes in the relationship between serotonin system functioning and daytime sleep, meriting further investigation.

As briefly noted, the moderating effects of *5HTT* genotype found in this study coincide with reports that the *s* allele confers greater individual sensitivity to the effects of the environment on a variety of outcomes (see Belsky et al., 2009), including sleep (Brummett et al., 2007), possibly leading to a closer coupling of CNS serotonin with sleep behavior, at least as the *s* allele genotype is phenotypically expressed in this environment. It is possible that in this study, the infants with the *s* allele were biologically more sensitive to the effects of their own serotonin system functioning on daytime sleep. The positive correlation between sleep and CSF *5-HIAA* may be explained by serotonin's inhibitory function in the reticular activating system, which is primarily involved in arousal (Jones, 2003) and is one of the first systems in the brain to develop (Barkovich, Kjos, Jackson, & Norman, 1988). Infants with the *s* allele and lower CSF *5-HIAA* may have had insufficient serotonin binding in their reticular formation to inhibit arousal, resulting in reduced daytime sleep. This interpretation remains speculative, because it is not possible to determine region-specific serotonin binding from concentrations of CSF *5-HIAA*, a measure of total CNS serotonin functioning and turnover. Further research

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integrating neuroimaging is needed to confirm this interpretation. Nonetheless, the results of this study are consistent with research in humans demonstrating that variation in *5HTT* genotype can moderate differences in sleeping patterns (Barclay et al., 2011; Koga et al., 2016) and concentrations of CSF *5-HIAA* (Williams et al., 2003). Ultimately, the results of this study suggest that the serotonin system has a complex association with sleep during early development.

## Limitations and conclusion

Sleep was assessed behaviorally (bihourly) during the day only, and although a variety of studies show that systematic behavioral observation of sleep-wake states are a viable measure of sleep development (Cohen-Mansfield, Waldhorn, Werner, & Billig, 1990; Papailiou, Sullivan, & Cameron, 2008), 24-hr monitoring would allow a better assessment of the relationship between daytime and nighttime sleep. Although nursery-rearing closely controls the environment, leading to homogenous treatments, considering the important role that mothers play in regulating their infant's development and physiology (Hofer, 1983), having a mother-reared control group would facilitate our understanding of how mothers affect neonatal sleep, which should be considered in future studies.

The results of this study are the first to study the role of serotonin in the development of sleep patterns in neonatal primates and to link maternal neglect and *5HTT* genotype effects on the functioning of the serotonin system, specifically because the serotonin system is related to infant daytime sleep. We found that early stressful experiences profoundly affect infants' sleep and physiological development, a finding highlighting the pivotal role that caregivers play in infant sleep development. The results of this study also illustrate the complex role that the serotonin system plays in sleep and highlight the need for more research to elucidate how serotonin affects sleep and sleep development. The strong coupling of CSF *5-HIAA* concentrations to daytime sleep in the subjects with the *s* allele but not in the subjects with the *LL* genotype further suggests that the possession of the *s* allele may lead to increased sensitivity of the serotonin system to aberrations in the brain, resulting in a close link between CNS serotonin functioning and sleep behaviors. This may have clinical implications when considering individual differences in the serotonin system when conducting sleep interventions in early development.

**Supplementary material.** To view the supplementary material for this article, please visit https://doi.org/10.1017/S0954579418001359

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