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Rearing condition may alter neonatal development of captive Bolivian squirrel monkeys (*Saimiri boliviensis boliviensis*)

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Abstract

Nursery rearing has well-known consequences for primate species. Relative to some other primate species, research has indicated a reduced impact of nursery rearing on squirrel monkeys, particularly in terms of rates, severity, and persistence of abnormal behavior. We administered the Primate Neonatal Neurobehavioral Assessment to 29 dam-reared and 13 nursery-reared squirrel monkeys (*Saimiri boliviensis boliviensis*) at two and six weeks of age. Mixed model ANOVAs comparing composite scores and individual assessment items across age, rearing status, and sex revealed a number of developmental differences. Dam-reared infants scored higher on all four composite measures compared to nursery-reared infants ($p < .05$) indicating that nursery-reared animals had slower motor development, were less active and attentive, and were more passive than their dam-reared counterparts. Consistent with infant rhesus macaques, nursery-reared squirrel monkeys showed an increased sensitivity to tactile stimulation ($p < .05$). Altogether, these results suggest a disruption of species-typical development when squirrel monkey infants are reared in a nursery setting, with activity, orientation, and state control areas most effected, though experimental research is needed to determine if this is a causal relationship. Contrary to previous behavioral research, there are likely developmental differences between dam-reared infant squirrel monkeys and those reared in a nursery setting.

Keywords

primates; *Saimiri*; squirrel monkeys; rearing; development

1 Introduction

Nursery rearing is essential for certain biomedical research protocols involving primates and is necessary in cases of inadequate maternal attention. However, nursery rearing has certain well-documented negative consequences for behavior, development, and health. Though nursery rearing protocols and husbandry techniques have improved over several decades,

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nursery rearing today still includes inanimate surrogates, bottle holders, and at least partial isolation in the early postnatal period followed by socialization with same-age peers (Novak & Sackett, 2006). Unfortunately, these are not comparable replacements for natural parent-offspring interaction, and research has demonstrated that nursery rearing has many negative consequences (e.g. Berkson & Mason, 1964; Suomi et al., 1971; Mason & Capitanio, 1988; Novak & Sackett, 2006).

Most of our knowledge about the behavior of nursery-reared primates comes from the study of rhesus macaques (*Macaca mulatta*) and chimpanzees (*Pan troglodytes*). Increases in abnormal behaviors, such as rocking, self-stimulation, clinging, and other stereotypies have been well-documented (e.g. Berkson & Mason, 1964; Suomi et al., 1971; Mason & Capitanio, 1988; Gottlieb et al., 2013; Bauer & Baker, 2016; Bloomsmith et al., 2017; Capitanio, 2017). Nursery rearing also impacts social behavior, with surrogate-peer rearing of rhesus macaques associated with lower rank at age 3 compared to dam-reared and peer-only-reared counterparts. These differences persist into adulthood (age 3–11; Bastian et al., 2003; Dettmer et al., 2017).

Physiological effects of nursery rearing have also been documented. Nursery rearing has immunological and health consequences for rhesus macaques such as heightened proliferation responses, lower natural killer cell activity, increased CD4+ /CD8+ ratios that result in increased risk for gastrointestinal infections and later treatment with antibiotics, and higher prevalence and frequency of illness as adults (Coe et al., 1992; Lubach & Coe, 2006; Conti et al., 2012). The early rearing environment has also been shown to interact with genotype to affect central nervous system functioning. Among macaques who were separated from their mothers, those that were heterozygous for the rh5-HTTLPR allele exhibited lower CSF serotonin concentrations than those who were homozygous; however, dam-reared individuals showed no such difference as a function of genotype (Bennett et al., 2002). In addition, brain imaging of chimpanzees has revealed important neurological consequences of nursery rearing, including lesser global white-to-grey matter volume, less cortical folding, and atypical grey matter covariation in areas associated with motor, cognitive, socio-emotional, and motivational systems in nursery-reared chimpanzees compared to their dam-reared counterparts (Bogart et al., 2014; Bard & Hopkins, 2018).

Substantial efforts to refine nursery-rearing strategies have been made by those studying infant rhesus macaques (e.g. Burbacher et al., 2013; Sackett et al., 2002; Kaburu et al., 2015; Dettmer et al., 2016). For example, utilizing a hanging mobile surrogate results in better coordination, balance, and grasping, as well as increased exploration and lower stress than stationary rocking surrogates (Dettmer et al., 2008). More complex surrogates which have multiple textures, pacifiers, side handles, and resting bases, or that incorporate mechanical feedback (e.g., heartbeat sounds, vibrations, vocalizations) improved exploration, and foraging behavior over a simple stuffed animal “surrogate” (Brunelli et al., 2014). In addition, increased human handling and face-to-face interaction have been shown to improve locomotion, working memory, and social interest, and decrease fear during social introductions, and stress-related behaviors (Dettmer et al., 2016; Simpson et al., 2017). In chimpanzees, increased human interaction even prevented changes in gray matter volume

(particularly in the basal forebrain) compared to standard nursery rearing (Bard & Hopkins, 2018).

Squirrel monkeys (*Saimiri* species) serve as a long-standing biomedical model in a wide range of human health-related research including cardiovascular diseases, vaccine development, central nervous system impairment, metabolic issues, and behavioral pharmacology (Middleton et al., 1967; Strickland & Clarkson, 1985; Barrett, 1985; Schätzl et al., 1995; Boinski & Cropp, 1999; Abee, 2000; Williams et al. 2010). Though nursery rearing is not common practice, it has been implemented in several captive squirrel monkey breeding and research facilities since the 1970s (King & King, 1970; Kaplan, 1974; Roy, 1981; Hennessey, 1985), and is still utilized if necessary for research protocols and in the event of poor maternal behavior. Considering the use of nursery-reared squirrel monkeys in biomedical research, there is far less published work on the effects of, and improvements to, nursery rearing for squirrel monkeys compared to other primate species.

There are inconsistencies across studies regarding the nursery effects on squirrel monkeys. For example, nursery-reared infants (*Saimiri sciurius* indigenous to Bolivia, Colombia, and Peru) showed increased abnormal behaviors including self-clasping/tail huddling, rocking, self-sucking, rolling in a ball, and other stereotypies (Roy, 1981), while other nursery-reared infants (*S. sciurius* indigenous to Colombia and Peru) did not display as many or as severe abnormal behaviors (King & King, 1970; Kaplan, 1974; Hennessey, 1985). Inconsistencies in rearing effects are likely due to differences in the early nursery environment including timing of separation from mothers, length of isolation period, type of surrogates, bottle access, peer socialization timing, etc. In early studies of captive squirrel monkeys, social behavior appeared to be largely unaffected by nursery rearing, though direct comparisons of dam- and nursery-reared individuals were rare (Hennessey, 1985). Unlike the case for rhesus macaques, research suggests that most of these abnormal behaviors are reduced in squirrel monkeys by adulthood and social behavior appears normal if infants are placed in peer groups before 12 months old (Roy, 1981); however, self-sucking appears to persist into adulthood (King & King, 1970; Kaplan, 1979; Roy, 1981; Hennessey, 1985).

Compared to behavior, physiological and health effects of nursery rearing appear to be more consistent in squirrel monkeys. For example, nursery-reared squirrel monkeys have a slower growth rate than dam-reared squirrel monkeys (Brady et al. 2000), though these rates differ among *Saimiri* species (Kaplan, 1974). Both brief (1–6 hour) and prolonged (weeks) separation of infant squirrel monkeys from their mothers resulted in reduced thermoregulation, elevated plasma cortisol, and adverse effects on the immune system (Coe et al., 1983; Coe et al., 1985). Infants separated from their mothers for 2 weeks showed elevated plasma cortisol, decreased complement proteins (C3 and C4; important in immune reactivity) and immunoglobulins (Coe et al., 1985), all of which could impact biomedical research.

Since there is potential for both nursery- and dam-reared squirrel monkeys to serve as subjects in biomedical studies, it is critical to expand our understanding of nursery rearing effects on each captive species. Assessments of neurobehavioral organization can aid in our understanding of rearing consequences and can help to better understand biomedical

research outcomes (Williams & Glasgow, 2000) by identifying those infants with developmental deficits or impaired immune functioning. Neurobehavioral assessments have been used for assessing high-risk and preterm infants, investigating cross-species comparisons, determining typical developmental trajectories, and examining the effects of stressors on development (Bayley, 1969; King & King, 1970; Brazelton, 1973; Hallock et al. 1989; Bard et al. 1992; Bayley 1993; Williams & Glasgow, 2000; Mulholland et al., 2019).

One such assessment, the Primate Neonatal Neurobehavioral Assessment (PNNA: Schneider, 1987; Schneider & Suomi, 1992; Schneider et al., 2006), was designed to assess neurobehavioral development in non-human primate infants. This standardized multi-item assessment measures characteristics that are not easily observable or are easily missed in later assessments and includes ratings of visual and auditory orientation, motor activity and maturity, state control, and reflexes. The PNNA and other measures of biobehavioral organization can be used to establish which infants would benefit from behavioral management interventions and closer monitoring, and identify naturally occurring models of human disorders (e.g., autism spectrum disorder; Capitanio, 2017). In addition, neurobehavioral measures are correlated with altered immune functioning, diarrhea, and other health-related outcomes (Capitanio, 2017; Schneider et al., 2006) which could impact biomedical research.

The PNNA has been used to examine the effects of prenatal stress on infant squirrel monkey development. The PNNA revealed that two week old *S. b. peruviansis* infants who were exposed to chronic stress (multiple housing changes and continuous unstable social groups) during gestation had poorer motor skills and balance, and shorter attention spans than infants exposed to mid-gestational stress or no stress (Schneider & Coe, 1993). Similarly, *S. b. boliviensis* infants exposed to episodic stress during gestation, such as high spatial density and dams being hand caught for ultrasounds, had poorer coordination, motor maturity and muscle strength, reduced visual orientation and resistance to restraint, and shorter attention spans than those exposed to a single stressor or no stress (Glasgow, 2000). Results also indicated differences in the timing of stress, with early prenatal stress resulting in the worst outcomes (Glasgow, 2000).

In the current study, we utilized the Primate Neonatal Neurobehavioral Assessment to investigate the effects of nursery rearing versus mother rearing on *S. b. boliviensis* neurobehavioral development. *S. b. boliviensis* can be commonly found in research facilities and there is potential for nursery rearing to continue in this type of setting due to either maternal rejection, clinical concerns, research protocols, or breeding purposes. To examine the time course of any differences, we assessed dam-reared and nursery-reared infants at 2 weeks and again at 6 weeks old. Based on the extensive rhesus macaque literature, as well as the known impact of prenatal stress on neurobehavioral development in squirrel monkeys (Schneider & Coe, 1993; Glasgow, 2000), we predicted that nursery-reared *S. b. boliviensis* would have lower composite scores on the PNNA than dam-reared infants. Specifically, we hypothesized that nursery-reared infants would have lower activity, state control, and motor maturity scores than dam-reared infants.

2 Methods

2.1 Subjects

We used archival data collected from 29 dam-reared (14 female, 15 male) and 13 nursery-reared (7 female, 6 male) squirrel monkey infants (*Saimiri boliviensis boliviensis*) between April and June, 2003. All subjects were housed indoors at the Squirrel Monkey Breeding and Research Resource Colony at the University of South Alabama in Mobile, Alabama. Dam-reared infants were housed in social groups (comprised of one adult male, 10–14 adult females, and differing numbers of yearlings and juveniles) in indoor cages (1.5 × 2.5 × 4.5 m) with up to three cages connected by staff-controllable access. Each cage consisted of aluminum bars front and back with solid sheet metal walls separating adjoining cages, and concrete floors covered with plastic dry-deck material. Cage interiors were equipped with PVC perches at varying heights, hide boxes, and enrichment devices. Food (Purina High Protein Monkey Chow 5045, along with fresh vegetables and fruits daily) and water was available *ad libitum*, though all dam-reared infants were still nursing during the course of this study. All dam-reared infants were born vaginally in the dams' social groups overnight and there was no evidence of complicated births.

Subjects were not randomly assigned to rearing condition and, instead, were placed in the nursery for colony management reasons. All nursery-reared infants were found not clinging to their dam in the morning after their birth, and were subsequently moved to the nursery, weighed, and fed. The attending veterinarian performed a physical on the infant. Those infants found clinically compromised were placed on treatments and moved into the nursery, and were not subjects in the current study. If the infant did not require treatment, an attempt was made to reunite the dam-infant pair. If this succeeded, the dam-infant pair was returned to their social group; if not, the infant was started on the hand rearing protocol. Only this second set of infants (not clinically compromised, able to cling, but unable to be reunited with their dam) were used in the study. Though these infants were deemed healthy during clinical exams, we recognize that there may be other unknown factors leading to maternal rejection, which introduces potential confounds. Our results should be interpreted with this in mind.

For the duration of the study, nursery-reared infants were housed in a separate room, away from the general colony, in small 0.5 meter square cages made of wire mesh. Each cage had a terry cloth surrogate (stitched together and placed on a 3-inch PVC pipe mounted at a 45 degree angle) and bars for perching. All nursery-reared infants had *ad libitum* access to water. For hand feedings, the infant was placed on an unmounted tube held by the technician and fed SMA® formula. Newborn infants were placed on an unmounted tube held by the technician and fed iron-fortified SMA® formula at hourly from 700 until 2000 hours daily from a syringe (see Brady et al., 2006 for detailed nursery procedures). Body weight and temperature were measured every morning in the nursery. As the infants grew, the feedings were reduced to every two hours and around day 5 weaning from hand feeding began. During this time a bottle with a lixit stopper was placed in the cage filled with formula. Once the infants had demonstrated the ability to self-feed (generally around day 10), hand feeding was discontinued and instead the cage-mounted bottle of formula was changed twice daily.

Over the next four months, bottle feeding was gradually reduced to once per day and the infant was introduced to solid foods (monkey biscuit soaked in formula). By the end of month four, the infant was no longer receiving formula and was on a solid diet. At six months of age, infants were introduced to same-aged peers in progressively larger numbers. At a year of age, females were kept together to form a new social group or introduced to an existing breeding group.

2.2 Primate Neonatal Neurobehavioral Assessment

The Primate Neonatal Neurobehavioral Assessment (PNNA; Schneider et al., 2006; Schneider & Suomi, 1992; Schneider, 1987) was administered to each infant at two and six weeks old. These ages were based on previous work with rhesus macaques, the life history and locomotor development of *Saimiri* species, and coincided with self-feeding and independent movement in our captive colony (Williams & Glasgow, 2000; Hartwig, 1995; Young, 2009). Data collected during the second week of life are comparable to those collected in the macaque studies, while those data collected at six weeks of age are an extension of the original work designed to explore whether early findings continued to hold after the infants began to move and feed independently.

All infants were hand caught and placed on a cloth surrogate for testing. The dam-infant pairs were hand caught from the social housing by trained technicians who entered the group housing wearing appropriate protective equipment and leather capture gloves (Tardif et al., 2006). The dam-infant pair were hand caught by the technician as they moved to evade capture and the infants were gently separated from the dams. The dams were held, awake, in a separate room for the duration of the infant assessment. After the exam the infants were placed on the dams and the pair was returned to their social group (Note: all dam-infant reunions were successful, no dam refused to take her infant back). All testing was done by trained animal care technicians (IRR > 80%) and occurred in a quiet area outside of the colony rooms. The 50-item assessment took approximately 20 minutes, and included visual, auditory, motor, reflex, and temperament ratings during both hand-held testing and a 5-minute cage observation.

2.3 Data coding and analysis

Activity, motor maturity, orientation, and state control composite scores were created from individual PNNA items (as found in Schneider & Suomi, 1992; Schneider et al., 2006; see Table 1 and supplementary materials for factor analysis). In addition, we examined individual items including balance, coordination, response speed, total vocalizations, resistance to restraint, startle responses, as well as rooting (average of right and left), Moro (the degree to which the infants spread their extremities in response to sudden loss of support), and grasping reflexes (average of palmar and plantar ratings).

In order to determine that nursery- and dam-reared infants did not differ in birthweight (which could indicate premature birth, etc.), birthweight was examined using Factorial ANOVAs with rearing and sex as independent variables. The composite scores and select individual items were compared using mixed model ANCOVAs with rearing and sex as between-subjects variables, and age (2 and 6 weeks) as the within-subjects variable.

Omnibus tests were followed-up by pairwise comparisons (using a Bonferroni correction) when significant or to test specific *a priori* hypotheses. All analyses were conducted using SPSS version 24 (IBM Corp, 2016).

3 Results

3.1 Birth Weight and Composite Scores

A factorial (rearing $\times$ sex) ANOVA revealed no significant differences in birthweight between rearing conditions, sex, nor was there an interaction ($p > 0.05$). Dam-reared infants weighed an average of 112.24 g ($SE = 2.37$) and nursery-reared infants weighed an average of 111.87 g ($SE = 3.54$). We therefore proceeded with analyzing the PNNA data without controlling for birthweight. Mixed-model ANOVAs revealed significant main effects of age and rearing on activity, state control, and orientation (see Figure 1), and a main effect of rearing on motor maturity. There were no significant rearing by age interaction effects for any of the composite scores ($p > 0.05$). Nursery-reared infants had significantly lower state control scores (less agitation and easier to console; $M = 1.74$, $SE = 0.10$) compared to dam-reared infants ($M = 2.40$, $SE = 0.07$) [$F(1,38) = 9.90$, $p = 0.003$, $\eta^2 = 0.21$], and these scores increased with age [$F(1,38) = 24.05$, $p < 0.001$, $\eta^2 = 0.39$]. Similarly, nursery-reared infants ($M = 1.08$, $SE = 0.14$) had significantly reduced activity compared to dam-reared infants ($M = 1.44$, $SE = 0.09$) [$F(1,38) = 4.94$, $p = 0.032$, $\eta^2 = 0.12$] and significant increases in activity with age [$F(1,38) = 39.34$, $p < 0.001$, $\eta^2 = 0.51$]. The same pattern of results was seen with orientation scores. Orientation significantly increased with age [$F(1,35) = 28.10$, $p < 0.001$, $\eta^2 = 0.45$], and nursery-reared infants scored significantly lower in orientation ($M = 1.02$, $SE = 0.10$) compared to dam-reared infants ($M = 1.26$, $SE = 0.06$) [$F(1,35) = 4.30$, $p = 0.045$, $\eta^2 = 0.11$], indicating that nursery-reared infants were less likely to orient toward and pay attention to visual stimuli.

A mixed-model ANOVA also revealed a significant main effect of rearing on motor maturity composite scores [$F(1,36) = 6.79$, $p = 0.013$, $\eta^2 = 0.16$], with nursery-reared infants ($M = 1.14$, $SE = 0.10$) having reduced coordination and muscle control compared to dam-reared infants ($M = 1.45$, $SE = 0.07$). Unlike the other composite scores, there were no significant differences in motor maturity between 2 and 6 weeks of age [$F(1,36) = 1.33$, $p > 0.05$, $\eta^2 = 0.04$].

3.2 Individual PNNA Items

Similar to the composite score results, a mixed-model ANOVA revealed significant main effects of age and rearing on balance, coordination, and response speed, in addition to a rearing by sex interaction for response speed. Balance, coordination, and response speed all significantly improved with age ($p < 0.05$; see Figure 2) and nursery-reared infants had significantly poorer balance [$F(1,38) = 12.51$, $p = 0.001$, $\eta^2 = 0.25$] and coordination [$F(1,38) = 7.53$, $p = 0.009$, $\eta^2 = 0.17$] compared to dam-reared infants. In addition, nursery-reared infants had slower response speeds than dam-reared infants [$F(1,36) = 6.78$, $p = 0.013$, $\eta^2 = 0.16$]; however, this effect is misleading as the rearing by sex interaction [$F(1,36) = 4.65$, $p = 0.038$, $\eta^2 = 0.11$] revealed that this reduction in response speed was only seen in nursery-reared males (see Figure 2).

There was also a significant age by rearing interaction effect on the number of vocalizations emitted during a cage observation [$F(1,38) = 9.19, p = 0.004, \eta^2 = 0.20$]. At 2 weeks old, nursery-reared infants vocalized less than dam-reared infants; however, the number of calls increased with age for nursery-reared infants and decreased with age for dam-reared infants (see Figure 3). A mixed-model ANOVA also revealed a significant main effect of rearing on the infants' response to restraint [$F(1,38) = 15.72, p < 0.001, \eta^2 = 0.29$]. Nursery-reared infants were less resistant to a brief restraint compared to dam-reared infants. There was no significant effect of age nor any interaction effects ($p > 0.05$). Examination of the infants' response to a sudden auditory stimulus revealed a significant effect of age [$F(1,38) = 8.87, p = 0.005, \eta^2 = 0.19$], with a greater startle response at 6 weeks of age, but no effects of rearing, sex, nor interactions ($p > 0.05$).

A mixed-model ANOVA examining aversion to tactile stimuli revealed significant main effects of age and rearing, as well as interactions between age and rearing, age and sex, and rearing and sex. Overall, nursery-reared infants were more sensitive to tactile stimulation ($M = 1.011, SE = 0.08$) compared to dam-reared infants ($M = 0.67, SE = 0.05$) [$F(1,37) = 15.872, p < 0.001, \eta^2 = 0.30$]; however, the significant interaction revealed that this difference was only significant at 2 weeks of age. The high sensitivity in nursery-reared infants remained consistent as the infants aged (2 weeks: $M = 1.03, SE = 0.11$; 6 weeks: $M = 1.00, SE = 0.08$), while dam-reared infants increased as they aged reaching levels similar to nursery-reared infants by 6 weeks old (2 weeks: $M = 0.39, SE = 0.08$; 6 weeks: $M = 0.89, SE = 0.06$) [$F(1,37) = 10.61, p = 0.002, \eta^2 = 0.22$]. In addition, sensitivity differed between the sexes in the nursery-reared condition; females had higher sensitivity to tactile stimulation than males [$F(1,37) = 7.32, p = 0.010, \eta^2 = 0.17$; Figure 4].

3.3 Reflexes

A mixed-model ANOVA revealed a significant main effect of age on the rooting reflex [$F(1,38) = 6.60, p = 0.014, \eta^2 = 0.15$], as well as a significant interaction between age and rearing [$F(1,38) = 5.49, p = 0.025, \eta^2 = 0.13$]. Nursery-reared infants had a consistently weak rooting reflex at both 2 weeks ($M = 0.27, SE = 0.26$) and 6 weeks of age ($M = 0.31, SE = 0.22$), while dam-reared infants had a stronger rooting reflex that got stronger as they aged (2 weeks: $M = 1.47, SE = 0.17$; 6 weeks: $M = 2.38, SE = 0.14$). When examining the Moro reflex, there was also a significant interaction between age and rearing [$F(1,38) = 4.95, p = 0.032, \eta^2 = 0.12$]. Dam-reared infants had more exaggerated abduction (spreading of the extremities) at 2 weeks old ($M = 1.48, SE = 0.12$) which decreased at 6 weeks ($M = 0.93, SE = 0.15$), while nursery-reared infants had only slight abduction at 2 weeks ($M = 0.99, SE = 0.17$) which increased at 6 weeks ($M = 1.21, SE = 0.23$). Grasping reflex, on the other hand, did not differ across age, rearing condition, or sex ($p > 0.05$).

4 Discussion

Overall, we found several developmental differences between nursery- and dam-reared *S. b. boliviensis* infants on the PNNA. We note that these results need to be interpreted with caution, as a cause and effect relationship between rearing condition and neurobehavioral development cannot be determined from this non-experimental design and lack of random

assignment to rearing condition. With that in mind, nursery-reared infants were less active, more passive and withdrawn, and responded less to visual and auditory stimulation through 6 weeks of age compared to dam-reared infants. Motor maturity was also reduced at 2 weeks, but by 6 weeks of age nursery- and dam-reared infants were similar. State control scores were also lower for nursery-reared infants, indicating that the infants were more passive, less irritable and fearful, and less likely to struggle during testing. Further examination of individual PNNA items revealed nursery-reared infants had poorer balance and coordination, slower response speed, fewer vocalizations, and less resistance to a brief restraint. When examining reflexes, nursery-reared infants had a weaker rooting reflex and less pronounced Moro reflex than dam-reared infants, but there was no difference in the grasping reflex.

These results are similar to those reported in studies of rhesus macaque neurobehavioral development. At two weeks old, nursery-reared macaque infants had decreased activity and increased passivity compared to dam-reared infants (Schneider, 1987; Schneider & Suomi, 1992). Infants whose mothers were exposed to stress during pregnancy, developed similar deficits including decreased activity, motor maturity and muscle tone, slower response speeds, and poorer balance and coordination than those not exposed to stress during gestation (Schneider, 1992; Schneider et al., 1999). Similarly, after exposure to chronic or early prenatal stress, *S. b. peruviansis* infants had poorer motor maturity, balance, and shorter attention spans (Schneider & Coe, 1993), and *S. b. boliviensis* infants had decreased motor maturity, coordination, shorter attention spans, as well as reduced visual orientation and resistance to movement (Glasgow, 2000) compared to non-prenatally stressed control infants. Glasgow (2000) also found that *S. b. boliviensis* infants who were exposed to early prenatal stress had lower state control scores compared to control infants and those exposed to late prenatal stress.

This pattern of behavioral inhibition (reduced activity, vocalizations, and resistance) as a result of prenatal stress or nursery rearing may indicate a reduced reactivity to stressors. In squirrel monkeys, infants respond to even brief maternal separation with elevated cortisol and increased vocalizations. Repeated separation, however, can result in inconsistencies between behavioral and physiological markers of stress, with elevated cortisol responses persisting but vocalizations decreasing over the course of repeated separation (Coe et al., 1983; Coe et al., 1985). Physiological responses to this repeated separation and reunification may be different than the permanent separation and rearing with a surrogate used in the current study. Nursery-reared macaques had significantly lower cortisol in response to a relocation for testing at 3–4 months than dam-reared infants, and cortisol was reduced in response to injection of ACTH (which normally causes release of corticosteroids) (Capitanio et al., 2005). Furthermore, prenatal stress of macaques was associated with behavioral inhibition lasting into adulthood and increased hypothalamic-pituitary-adrenal (HPA) system activation (Schneider et al., 2001). This indicates dysregulation of the HPA system in response to prenatal stress and nursery rearing.

At two weeks old, infants reared in the nursery were more sensitive to tactile stimulation, with the highest sensitivity found in nursery-reared females. Macaques showed a similar heightened sensitivity to tactile stimulation when nursery reared (Schneider, 1987; Schneider

& Suomi, 1992). This aversion to sensory stimuli may be due to the lack of species-typical stimulation in a nursery setting. Dam-reared infants receive tactile and vestibular-proprioceptive stimulation from body contact, grooming, and body movements of their mothers, all of which are lacking in the nursery environment (Schneider et al., 2006). Recent research modifying the nursery rearing experience (e.g., increased human handling, modified surrogate mobility) have been successful in mitigating some of these negative consequences of nursery rearing in rhesus macaques (Simpson et al., 2017; Dettmer et al., 2008). As nursery rearing is likely to continue in response to poor maternal behavior in captive populations of squirrel monkeys, it is important to further develop and empirically test interventions or modifications to the nursery environment.

In contrast to the severe behavioral consequences of nursery rearing reported for rhesus macaques (e.g. Suomi et al., 1971; Mason & Capitanio, 1988; Gottlieb et al., 2013; Bauer & Baker, 2016; Capitanio, 2017), previous research reported minimal impact of nursery rearing on squirrel monkey behavior (King & King, 1970; Kaplan, 1979; Roy, 1981; Hennessy, 1985). However, most studies of squirrel monkey rearing did not have direct comparisons between nursery- and dam-reared infants. Only one study described severe abnormal behavior in squirrel monkeys reared in a nursery, which researchers attributed to different nursery practices such as surrogate type, bottle access, etc. (Roy, 1981; see review in Hennessy, 1985). However, the current study reveals several neurobehavioral deficits potentially associated with nursery rearing of *S. b. boliviensis*. This disparity demonstrates the advantages of using the PNNA; the assessment tells us about the early neonatal period, measuring behaviors and characteristics that are easily missed during behavioral observations. As such, the PNNA may help to identify at-risk infants earlier and more accurately than observations of abnormal behavior, particularly in species, like squirrel monkeys, that display fewer or less severe abnormal behavior in response to nursery rearing compared to rhesus macaques.

It is important to keep in mind that we cannot infer a causal relationship between nursery rearing (in this manner, with immobile surrogates and limited interaction with peers) and the developmental differences reported in the current study. The infants were not randomly assigned to rearing condition, and were nursery reared due to maternal rejection on their day of birth rather than for the purposes of this study. It is possible that both the maternal rejection (leading to nursery rearing) and the developmental differences revealed herein were caused by another variable not accounted for in the current study, or that the developmental differences (e.g. poor grasping or decreased activity) could have led to maternal rejection. However, the infants in the nursery were found abandoned by their mother the morning after delivery, were otherwise deemed normal and healthy by a veterinarian, and were able to cling to the surrogate on their own. The infants showed no gross size or health differences from those raised by their dams, other than the differences in development noted here. Additionally, the results are consistent with those seen in nursery-reared macaques that were randomly assigned to rearing condition and squirrel monkeys randomly assigned to prenatal stress conditions (Schneider, 1987; Schneider & Suomi, 1992; Schneider, 1992; Schneider & Coe, 1993; Schneider et al., 1992; Glasgow, 2000). The differences seen in the current study, and consistencies with previous experimental research, indicate the importance of further studying the potential effects of nursery rearing on squirrel monkeys.

The differences between dam- and nursery-reared squirrel monkeys found in the current study, no matter the cause, can have consequences for biomedical research. Older infants (3–6 months) with brief or prolonged separation from their mothers had reductions in thermoregulation, elevated plasma cortisol, and decreased immunoglobulins and proteins that facilitate the immune response (Coe et al., 1983; Coe et al., 1985). If nursery-reared squirrel monkeys with impaired neurobehaviors also have compromised immune systems and reduced reactivity to stressors, this may influence biomedical research outcomes or make it more difficult to interpret findings. Some biobehavioral measures are associated with altered immune function, diarrhea, and other health-related outcomes in rhesus macaques (Capitanio, 2017; Schneider et al., 2006); for example, those high in nervousness show evidence of glucocorticoid resistance (Capitanio, 2017), which has implications for disease processes involving inflammation (e.g., increased incidence of coronary heart disease in humans; Nabi et al., 2008). Assessments of neurobehavior can also help to identify naturally occurring models of human disorders (e.g., autism spectrum disorder; Sclafani et al., 2006; Capitanio, 2017; Bauman & Schumann, 2018).

In summary, this was the first study to use the Primate Neonatal Neurobehavioral Assessment to compare nursery- and dam-reared *S. b. boliviensis*, revealing a number of developmental differences. Regardless of the causal relationship, nursery-reared infants scored lower on all four composite measures compared to dam-reared infants, indicating that nursery-reared animals had poorer motor maturity, were less active and attentive, and more passive than their dam-reared counterparts. Consistent with infant rhesus macaques, nursery-reared squirrel monkeys showed an increased sensitivity to tactile stimulation possibly due to the lack of species-typical sensory experiences. In addition, rooting and Moro reflexes differed between dam- and nursery-reared squirrel monkeys, with weaker reflexes seen in nursery-reared infants. Altogether, these demonstrate a potential disruption of species-typical development when *S. b. boliviensis* infants are reared in a nursery setting in the manner reported here (i.e. maternal rejection, immobile surrogates and limited peer interaction) which needs to be further explored.

Future research should further investigate the impacts of nursery rearing on squirrel monkeys. First, the current study examined archival data and infants were not separated from their dams for experimental reasons or randomly assigned to rearing condition; therefore, we cannot determine if rearing is the cause of the observed developmental disruptions. There may be other factors that lead to the initial maternal rejection and affected development. In addition, the rearing strategies reported here are historical (e.g., immobile surrogates) and have since been updated. However, these preliminary findings reveal the importance of studying the impact of current nursery-rearing protocols using random assignment of infants to rearing condition. Second, longitudinal studies could elucidate when/if nursery-reared infants “catch up” to dam-reared infants on measures of neurobehavior. In the current study, nursery-reared infants had delayed motor development, with lower motor maturity scores at two weeks old but similar motor maturity to dam-reared infants at six weeks old. By extending the age of testing, we have shown how the effects seen earlier in life change as the infants develop independence from their dam. Assessing neurobehavioral measures after 6 weeks will provide additional developmental data, and may explain some discrepancies with the previous observational research (King & King, 1970; Kaplan, 1979; Roy, 1981;

Hennessey, 1985). Based on extensive research with rhesus macaques and squirrel monkeys (Schneider, 1987; Schneider & Suomi, 1992; Schneider & Coe, 1993; Glasgow, 2000; Schneider et al., 2006), we tested the infants with the PNNA at 2 weeks old (Williams & Glasgow, 2000). However, due to differences in developmental trajectories and socioecological factors (e.g., precocial development and more arboreal compared to rhesus macaques) this may not be the optimal age for testing squirrel monkeys. This, as well as long-term effects of rearing, can be determined by using repeated weekly testing over the first months of life and following the PNNA with other assessments of neurobehavior designed for use with older infants (e.g., the BioBehavioral Assessment; Capitanio, 2017).

In addition, research with several species (including humans, rodents, and non-human primates) have shown that adverse early life experiences have transgenerational effects (e.g., Maestripieri, 2005; Babb et al., 2014; Kundakovic & Champagne, 2015). For example, offspring of nursery-reared rhesus macaque males had elevated cortisol, lower immune cell counts, and increased emotionality compared to offspring of control males (Kinnally & Capitanio, 2015; Kinnally et al., 2018). Effects have been demonstrated in three generations, with paternal grandoffspring still showing these effects, and paternal great-grandoffspring showing only increased nervousness (Kinnally et al., 2018). Follow-up studies of the offspring of nursery-reared parents and their subsequent generations would tell us if nursery rearing has similar intergenerational effects in squirrel monkeys.

Also, the current study utilized the PNNA to assess nursery-reared *S. b. boliviensis*; however, other squirrel monkey species and subspecies (e.g., *S. b. peruviansis*, *S. s. sciureus*) also serve as biomedical models and have the possibility of being reared in a nursery setting. Earlier studies have described behavioral differences in nursery-reared *Saimiri* subspecies (Roy, 1981, Roy et al., 1981, Hennessey, 1985). Recent research has revealed developmental differences between dam-reared infants of these three subspecies (Mulholland et al., 2019), but no study to date has examined potential cross-species/subspecies differences in the developmental effects of nursery rearing. Utilizing the PNNA for such comparisons would be beneficial to better understand how these species and subspecies are affected by nursery rearing, and if differential response to nursery rearing could affect biomedical research outcomes utilizing these subspecies.

Lastly, modifications to squirrel monkey nursery-rearing protocols could ameliorate potential negative consequences of nursery rearing. Nursery modifications (e.g., mobile surrogates, earlier introduction to peers, increased human interaction and handling) have greatly benefitted rhesus macaques and chimpanzees reared in nursery settings (Dettmer et al., 2008; Burbacher et al., 2013; Sackett et al., 2002; Brunelli et al., 2014; Kaburu et al., 2015; Dettmer et al., 2016; Simpson et al., 2017; Bard & Hopkins, 2018). In the current study, nursery-reared infants had access to a stationary mounted terry cloth surrogate, transitioned from hand-feeding to self-feeding on a mounted bottle, and had limited social interaction with peers until six months of age. Refinements to squirrel monkey nursery protocols, such as introducing mobile or interactive surrogates and/or earlier, increased peer or human interaction, could reduce variability between nursery- and dam-reared infants, decrease potential negative effects, as well as improve research outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Nursery-reared infants (NR) had significantly a) lower state control, b) motor activity, c) orientation, and d) motor maturity scores compared to dam-reared (MR) infants. Error bars indicate $\pm$ S. E. and * indicates $p < 0.05$.

Figure 2.

Nursery-reared infants (NR) has a significantly worse a) balance, and b) motor maturity, but C) no difference in attention span compared to dam-reared (MR) infants. NR also had lower d) response speed than MR, though this is driven by reduced response speed in nursery-reared males. Error bars indicate $\pm$ S. E. and * indicates $p < 0.05$.

Figure 3.

Nursery-reared infants (NR) showed significantly a) less resistance to restraint compared to dam-reared (MR) infants across both weeks of testing, though NR infants became more resistant with age. b) Both NR and MR infants' aversion to sudden auditory stimuli increased with age. c) NR vocalized less than MR at 2 weeks, but did not differ at 6 weeks. Error bars indicate $\pm$ S. E. and * indicates $p < 0.05$.

Figure 4.

Nursery-reared (NR) infants were a) more sensitive to tactile stimulation than dam-reared infants, though MR increased in sensitivity by 6 weeks old. NR females were b) more sensitive than males, while there were no sex differences in MR infant sensitivity. Error bars indicate $\pm$ S. E. and * indicates $p < 0.05$.

Table 1.

Calculations used to create composite scores from individual PNNA items.

Composite Score	Calculation
Activity	Average of motor activity, spontaneous crawl, coordination, and passivity (inverse).
Motor Maturity	Average of coordination, head posture (prone and supine), response speed, and labyrinthian righting (left and right).
Orientation	Average of visual orientation (right, left, up, down), visual following (horizontal and vertical), duration of looking and attention span.
State Control	Average of amount of struggling, predominate state, irritability (inverse) and ability to console (inverse).