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Targeted sensory enrichment interventions protect against behavioral and neuroendocrine consequences of early life stress

Amanda C. Kentner[a,*], Stephanie Scalia[b], Junyoung Shin[b], Mattia M. Migliore[b], Alejandro N. Rondón-Ortiz[b]

[a] School of Arts & Sciences, Health Psychology Program, Massachusetts College of Pharmacy and Health Sciences, Boston MA, 02115 United States
[b] School of Pharmacy, Massachusetts College of Pharmacy and Health Sciences, Boston MA, 02115, United States

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ABSTRACT

Both basic and clinical research support the use of tactile stimulation to rescue several neurobiobehavioral consequences that follow early life stress including. Here, using a translational rodent model of the neonatal intensive care unit (NICU), we tested the individual prophylactic potential of a variety of sensory interventions including tactile (brushing pups with a paint brush to mimic maternal licking), auditory (a simulated lactating rat dam heart beat), and olfactory (a series of aroma therapy scents) stimulation. The NICU model was developed to mimic not only the reduced parental contact that sick infants receive (by isolating rat pups from their litters), but also the nosocomial infections and medical manipulations associated with this experience (by utilizing a dual lipopolysaccharide injection schedule). Each of the neurobiobehavioral consequences observed were dissociable between isolation and inflammation, or required a combined presentation ('two hits') of the neonatal stressors. Sprague-Dawley rats exposed to these early life stressors presented with sex-specific disruptions in both separation-induced ultrasonic vocalization (USV) distress calls (males & females) and juvenile social play USVs (males only). All three sensory enhancement interventions were associated with the rescue of potentiated distress calls while olfactory stimulation was protective of social vocalizations. Female rats exposed to early life stress experienced precocious puberty and shifts in the hypothalamic GnRh axis; sensory enrichment counteracted the advanced pubertal onset. Animals that underwent the NICU protocol also displayed maturational acceleration in terms of the loss of the rooting reflex in addition to hyperalgesia, a reduced preference for a novel conspecific, blunted basal plasma corticosterone and reduced hippocampal glucocorticoid receptor expression. These alterations closely simulated the clinical effects of early life adversity in terms of disruptions in the hypothalamic pituitary "stress" axis, social communication and engagement, tactile system processing, and accelerated maturation. Moreover, sensory enrichment attenuated many of these behavioral and neurophysiological alterations, and even slowed maturation. Overall, this supports the translatability of our novel rodent model and its potential utility in understanding how brain maturation and quality of early life experiences may interact to shape the integrity of stress and sensory system development. Future work must determine the appropriate modalities and parameters (e.g. patterning, timing) for effective sensory enrichment interventions.

1. Introduction

In the neonatal intensive care unit (NICU), preterm infants undergo stressful and often painful procedures (Brummelte et al., 2012; Campbell-Yeo et al., 2014) and are at a heightened risk of acquiring nosocomial or other types of infections (Hornik et al., 2012; Stoll et al., 2004, 2010). In combination with parental separation, early life stressors such as these are associated with permanent alterations in neurobiological, hormonal, and adverse behavioral outcomes (see Mooney-Leber and Brummelte, 2017; Rand et al., 2016). For example, preterm infants with a confirmed neonatal infection were at increased risk for neurodevelopmental impairments including diminished motor ability, attention deficit hyperactivity disorder, and IQ delay at 9 years of age (Rand et al., 2016). Moreover, this population is significantly more likely to have cerebral palsy, a low Bayley Scales Infant Development II score, and sensory impairments (Stoll et al., 2004).

In the animal laboratory, early life stressors such as infection and poor maternal care lead to similar developmental deficits to that of the NICU experience. Specifically, maternally separating neonatal rats leads to disruptions in social and cognitive functioning, increased anxiety-

* Corresponding author.
E-mail address: amanda.kentner@mcphs.edu (A.C. Kentner).

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like behavior, alterations in the pain sensory system, and hyperactivity of the hypothalamic pituitary adrenal (HPA) axis in response to stressors; many of these disruptions emerge in a sex specific manner across later points of development (Holmes et al., 2005; Suri et al., 2013; Weaver et al., 2007; Lomanowska and Melo, 2016). In our own work, using a neonatal inflammatory model, we have observed reduced levels of social interaction (MacRae et al., 2015a, b), disrupted spatial discrimination (MacRae et al., 2015b), and changes in pain sensitivity (Yan and Kentner, 2017) that also manifest in a sex specific, and sometimes biphasic, manner across later maturational time points (MacRae et al., 2015a). Therefore, there is a period between when these neonatal 'insults' occur and when symptoms later emerge, highlighting a potential window of opportunity for intervention, which is rarely explored.

Here, we sought to establish a novel and translational rodent model of the NICU experience by mimicking not only the prolonged parental separation but also the infections and medical manipulations associated with this experience. While the artificial rearing model is an excellent method for deconstructing the specific effects of permenant maternal/sensory deprivation and replacement therapy on development (Lovic et al., 2006, 2013; Lomanowska and Melo, 2016), in the NICU clinicans, parents and guardians are often in contact with infants in terms of providing care, skin-to-skin contact, holding/cuddling, touching, in addition to talking, singing and reading (Cong et al., 2017). Therefore, we did not permenantly isolate rat pups from their dams or littermates during the neonatal period. Additionally, we wanted to develop a less technically challenging animal model that did not require gastric gavage, in order to make it more easily accessible to implement in the laboratory. By adapting an accepted litter isolation procedure (McCormick et al., 1998; Kehoe et al., 2000) and combining it with a neonatal infection model (Bilbo et al., 2007; Shanks et al., 1995; Sominsky et al., 2012) to mimic the NICU, male and female rats were exposed to both inflammatory challenges and an injection procedure, alongside reduced parental contact, which led to sex specific alterations that closely simulated the clinical effects of early life adversity.

Developing brains may be vulnerable to the non-specific programming effects of pharmacotherapies (Rodriguez-Porcel et al., 2011), highlighting an advantage of environmental interventions and an underlying motivation for their increased use and acceptability by many parents/guardians. One type of environmental intervention used in pediatric settings is sensory enrichment; there are many examples of sensory enrichment leading to positive developmental outcomes, both in preclinical and clinical settings (Chatterjee et al., 2007; Lomanowska and Melo, 2016; Tessier et al., 2003; Bayley and AAP Committee on Fetus and Newborn, 2015; Charpak et al., 2017; Gonzalez et al., 2001; Pineda et al., 2017). In humans, the most widely recognized is Kangaroo care (e.g. skin-to-skin tactile contact of a preterm newborn with an adult) which results in improved hearing and speech as well as executive functioning at 5 and 10 years old, compared to standard incubator care (Tessier et al., 2003; Bayley and AAP Committee on Fetus and Newborn, 2015). Importantly, the neurodevelopmental, cognitive, and transgenerational parental care benefits appear to be enduring (Charpak et al., 2017). Tactile stimulation of artificially reared rat pups also leads to modest improvements in behavior, including maternal care, when artificially reared animals have their own litters to care for (Gonzalez et al., 2001).

Less understood and utilized are visual, auditory and olfactory/gustatory interventions. Preliminary work has demonstrated the safety and feasibility of audio systems in NICU incubators (Panagiotidis and Lahav, 2010) and exposure to maternal voice and heartbeat sounds reportedly lead to fetal auditory plasticity (Webb et al., 2015), lower infant heart rate, and potentially improved autonomic stability (Rand and Lahav, 2014). A Family Nurture Intervention including touch, encouragement of parent-infant vocal communication, and olfactory stimulation in the form of mother-infant scent exchange (Welch et al., 2015) was associated with improved cognitive and language scores, attention, and social-relatedness on the Modified Checklist for Autism in Toddlers. In randomized controlled clinical trials, sensory motor-enrichment, including olfactory and tactile stimulation, has demonstrated success as a clinical therapy for autism (Woo and Leon, 2013; Woo et al., 2015), in addition to feasibility as parents have accepted these sensory enrichment protocols (Aronoff et al., 2016). However, in these multimodal interventions, the individual contribution of each element (e.g. tactile, olfactory, auditory) of the procedure is unknown. Moreover, the benefits of such interventions, and the neural mechanisms that underlie them, are poorly understood. In the present study, we evaluated individual sensory enrichment elements and their ability to prevent disruptions in behavioral and hypothalamic pituitary "stress" axis (HPA) functioning following a combination of neonatal inflammatory challenges and a litter isolation procedure, modeling the reduced parental care that sick infants receive in the NICU.

2. Materials and methods

2.1. Animals and housing

Virgin female and male Sprague–Dawley rats from Charles River (Wilmington, MA) were pair-housed in standard cages (27 × 48 × 20 cm; with tube, a Nylabone® chew toy, and Nestlets® from Ancare) and maintained at 20 °C on a 12-h light/dark cycle (0700–1900 light) with ad libitum access to food and water. Following a one-week acclimatization period, animals were bred and pregnancy confirmed by continued weight gain and visible teats during the later phase of gestation. Approximately two days prior to parturition, female dams (N = 20) were separated into individual standard cages (containing Nestlets® from Ancare) and were maintained in this condition until weaning on postnatal day (P)22. A flowchart of the study procedures can be found in Fig. 1. Experimental procedures were approved by the Massachusetts College of Pharmacy and Health Sciences Institutional Animal Care and Use Committee and were carried out in compliance with the recommendations outlined by the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

2.2. Procedures

2.2.1. Litter isolation and inflammatory challenge

Day of birth was designated as postnatal day (P)1. On P2 litters were adjusted to 12 pups, with balanced sex ratios. Between P2–P10, offspring from 12 litters were physically isolated (ISO) from both their dams and littermates for 180 min (9:00am–12:00pm) daily and individually housed in clear plastic ice cream cups, to mimic a NICU incubator. Cups were positioned side-by-side inside a larger Plexiglas cage, placed on top of a heating pad to maintain the body temperature of the pups. During the isolation period, a subset of matched siblings from each litter were exposed to one of three types of sensory enrichment, described below. In terms of ISO timing, P2-7 is thought to correspond to the human third trimester, and human birth to P12-13 in rat (Clancy et al., 2007), equating this period to preterm delivery and exposure to the NICU. A subset of 8 control (CON) litters were not isolated but instead were maintained in the home cage with their dams. Between P2-P15, developmental milestones were evaluated (described below), therefore all ISO and CON pups received similar amounts of handling. After P10, all pups remained with their dams until weaning on P22, at which point offspring were housed in same-sex pairs in standard cages. Between this period, and after weaning, cage changes took place twice per week.

In order to model a neonatal (n) infection in the NICU, offspring from all ISO and CON offspring were administered either 50 μg/kg of the inflammatory endotoxin, lipopolysaccharide (nLPS; *Escherichia coli*, serotype 026:B6; L-3755, Sigma, St. Louis, MO, USA) or pyrogen-free saline (nSaline) on P3 and P5, as described previously (MacRae et al., 2015a; Yan and Kentner, 2017) to model late onset sepsis (nosocomial).