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Predator odor exposure of rat pups has opposite effects on play by juvenile males and females

Sara L. Stockman *, Margaret M. McCarthy

University of Maryland School of Medicine, 655 West Baltimore Street, Bressler Research Building 5-014, Baltimore, MD 21201, United States

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ABSTRACT

Juvenile social play behavior is one of the earliest sexually differentiated behaviors to emerge. In rats, as with most other species that play, males engage in more rough-and-tumble play compared to females. Exposure to early life adversity is a major driver of adult health and can manifest differently in males and females. However, the effects of adverse early life exposure on play behavior in the juvenile period are poorly understood. To address this, male and female neonatal rats were exposed to predator odor (PO), for 5 min/day on PN1-PN3. At the time of exposure to PO, both male and female pups suppressed ultrasonic vocalization and displayed more freezing behavior. Circulating corticosterone increased in males immediately following PO exposure but not in females. The enduring effects of PO exposure were opposite in males compared to females in that PO exposed males decreased social play, while PO exposed females increased play behavior compared to same sex controls. PO exposure did not significantly affect cell genesis in the neonatal dentate gyrus of either sex. PO exposure did not affect anxiety-like behavior assessed in the juvenile period or in adulthood, nor did it affect social interactions in adulthood. This work provides new insight into how sex may interact with adverse early life events to contribute to development of the social consequences of such exposures.

1. Introduction

In most species, early social interactions occur in the context of same-aged conspecific social play behavior. In rats, this play behavior is most commonly manifested as rough-and-tumble play or play fighting (Pellis and Pellis, 1997, 1998). Play-fighting is goal-directed towards play-biting an opposing participant and frequently involves wrestling-like behaviors, as animals attempt to dominate one another (Aldis, 1975). Rough-and-tumble play in the juvenile rat is sexually differentiated, as males exhibit a higher frequency of play events compared to females, and this is determined by neonatal testosterone (Meaney and Stewart, 1981; Olioff and Stewart, 1978; Thor and Holloway, 1986). The sex difference in juvenile social play behavior provides a route to assess how external factors may interact with sex to effect social behavior in a non-reproductive context.

Adverse early life events greatly influence subsequent development and can have lasting implications for adult mental and physical health (Anda et al., 2006; Felitti et al., 1998; Heim and Nemeroff, 2001; Schilling et al., 2007). Stressful environments in early life harmfully influence behavior and broad physiological functions. Frequently these consequences persist as permanent pathology. Early life adversity is particularly important in mediating the risk for neuropsychiatric disorders in adulthood including many which have significant social contributions (Kendler et al., 2002; Morgan et al., 2007). A number of factors interact to shape how early life adversity may manifest as differential vulnerabilities to later-life pathologies. The nature of the early life adverse event, an individual's genetic background and sex are among these factors (Kundakovic et al., 2013).

Investigations into the consequences of early life adversity have left both social and sex-specific sequelae understudied. Among the limited studies to probe these areas, results are contradictory. Adversity in early life, modeled through maternal separation in male pups, induces increased evasion of play contacts, suggesting feminized patterns of play (Arnold and Siviy, 2002), as well as a shift towards more aggressive play (Veenema and Neumann, 2009). Others find few sex-specific effects as a result of early stressful exposures (Zimmerberg and Sageser, 2011). Thus no consensus has emerged on the ramifications of early life adversity on juvenile social play and the role of sex in mediating potential differences has rarely been considered.

Multiple brain areas contribute to execution of juvenile social play behavior. Lesion of the cortex, nucleus accumbens, hypothalamus and amygdala all decrease social play (Vanderschuren et al., 1997). The amygdala is also crucial for developmental organization, as local

Abbreviations: AR, androgen receptor; DHT, dihydrotestosterone; EPM, elevated plus maze; L/D box, light/dark box; OFT, open field test; PN, postnatal day; PO, predator odor; SIT, social interaction test; USV, ultrasonic vocalization.

* Corresponding author.
E-mail addresses: sara.stockman@som.umaryland.edu (S.L. Stockman), mmccarthy@som.umaryland.edu (M.M. McCarthy).

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implant of steroids increase masculinized play (Meaney and McEwen, 1986; Tönjes et al., 1989). The amygdala continues to contribute to social behaviors throughout life including social recognition and interaction (Kling and Brothers, 1992), aggression (De Vries and Buijs, 1983; Davidson, 2000), sexual behavior (Newman, 1999) and maternal behavior (Nephew and Bridges, 2008; Bosch and Neumann, 2010; Nephew et al., 2010). Other brain regions are important for development of appropriate social behaviors in later life as well, including the hippocampus. The hippocampus is comprised of distinct regions including Ammon's horn and the dentate gyrus. The principle cells of the dentate gyrus, the granule cells, are primarily produced during the first two weeks of postnatal life (Schlessinger et al., 1975). The abundant period of postnatal neurogenesis of this region allows for early life experiences to modify granule cell production. Exposure of rat pups to stressful experiences early in life suppresses the production of granule neurons in the developing dentate gyrus (Tanapat et al., 1998). Pharmacologic lesions of the ventral hippocampus during this vulnerable postnatal period produce deficits in social interaction and social memory and increase displays of aggression in the juvenile period and adulthood (Becker et al., 1999; Sams-Dodd et al., 1997; Becker and Grecksch, 2000). These findings suggest that environmental exposures during periods of hippocampal development could influence granule cell formation and affect the outcome of a behavior dependent on this brain region.

Social deficits exist at the core of several neuropsychiatric disorders (Couture, 2006; Pelcovitz et al., 1994; Segrin, 2000). Among these disorders the prevalence, presentation, or a combination thereof, is greatly influenced by the biological variable of sex (Bao and Swaab, 2010). Natural variations in juvenile social play behavior between males and females provide a spectrum upon which to assess the impact of adverse early life event exposure. Correlates of early life stress are significantly influenced by the type of exposure (Zimmerberg and Sageser, 2011). Both neonatal isolation from the dam and predator odor exposure are commonly used models of stressful exposures (Zovkic and Sweatt, 2013). Exposure to live predators or cues indicative of the threat of predation impact a wide range of species. While responses are largely species-specific, nearly all consist of increased stress reactivity (St-Cyr and McGowan, 2015). Rodents primarily depend on olfaction to detect predators (Takahashi, 2014). Exposure of rodents to predator odor elicits an unconditioned fear response (Takahashi et al., 2008). Early life exposure of rat pups to predator odor significantly affects later life behaviors including fear responding (Hacquemand et al., 2010; Ayers et al., 2016). The impact of this early postnatal exposure to predator odor can also differentially affect male and female offspring (Mashoodh et al., 2009). To date, the consequences of early postnatal predator odor on later juvenile social play has not been examined. This study seeks to explore the sex specific consequences of early predator odor exposure on later juvenile social play in order to gain insight into the etiology of disrupted social behavior in neuropsychiatric disease.

2. Methods and materials

2.1. Animals

Timed pregnant Sprague-Dawley rats (Harlan) mated in our facility were allowed to deliver normally under standard laboratory conditions. The morning pups were found in the nest was designated as the day of birth (PN0). Pups were individually identified on PN0 by injection into the footpad with India ink. On PN21 animals were weaned and housed in groups consisting of two to three individuals of the same sex and exposure in polycarbonate cages ($20 \times 40 \times 20$ cm) with corncob bedding under a reverse 12:12-h light/dark cycle. Food and water were ad libitum. All breeding and experimental procedures were approved by the Institutional Care and Use Committee at the University of Maryland, Baltimore and performed in accordance with national animal care and use guidelines.

2.1.1. Pup condition distribution

Each litter was culled to 10–12 pups and divided to contain equal numbers of male and female pups exposed to either control or predator odor conditions. Pups were assigned in this manner to distribute and thus control for differences in maternal care between dams. For assessment of freezing behavior and USV detection, 3 male and 3 female pups were exposed to maternal control bedding and 3 male and 2 female pups were exposed to predator bedding from a single litter. To determine plasma corticosterone following exposure, from two litters, 8 males and 6 females were exposed to maternal control bedding, while 7 males and 6 females were exposed to predator bedding. For BrdU analysis, from four litters, 10 males and 12 females were assigned to control conditions and 11 males and 11 females were exposed to predator odor. To identify effects of predator odor exposure on later life behaviors, 10 males and 10 females underwent exposure to maternal control bedding and 12 males and 12 females were exposed to predator odor. (See Fig. 1.)

2.2. Predator odor (PO) exposure

PO exposures occurred during the first three full days of life (PN1–3) for 5 min each day. Individually, newborn animals were placed in a chamber constructed from a perforated base (16.5×12.7 cm) raised 2.5 cm from the ground with 6.35 cm walls contained within a

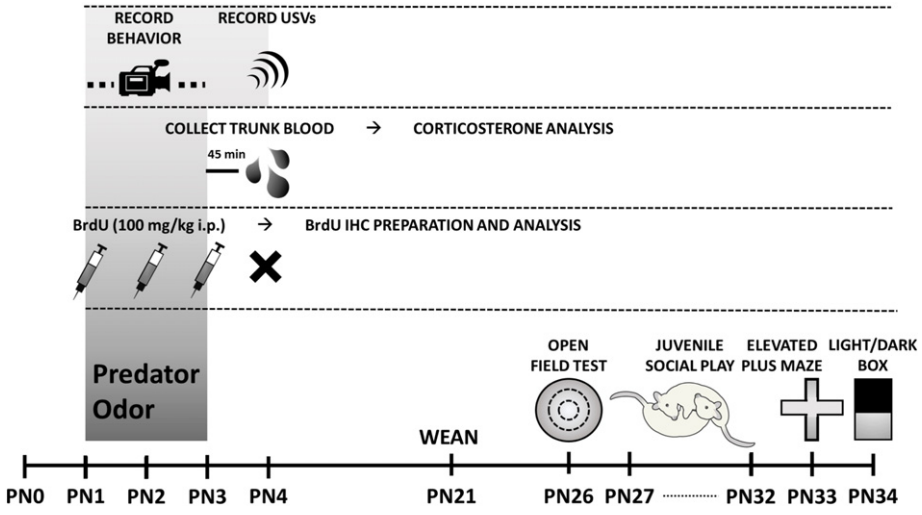

Fig. 1. Timeline of the sequence of exposures and assessments for each cohort of animals.