Physiology & Behavior 147 (2015) 149–156

Contents lists available at ScienceDirect

Physiology & Behavior

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

Early rearing experience is associated with vasopressin immunoreactivity but not reactivity to an acute non-social stressor in the prairie vole

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HIGHLIGHTS

- The relationship between early experience and non-social stress response is examined.
- CRH-*ir* was increased after a swim stressor but was not impacted by early experience.
- AVP-*ir* was impacted by early care but not by a swim stressor in a sex-dependent way.
- High-contact females had increased AVP-*ir* in the SON compared to low-contact females.
- High-contact males had decreased AVP-*ir* in the PVN compared to low-contact males.

ARTICLE INFO

Article history:
Received 30 January 2015
Received in revised form 6 March 2015
Accepted 12 April 2015
Available online 15 April 2015

Keywords:
Prairie vole
Biparental care
Natural variation
Forced swim test
Vasopressin
Corticotropin releasing hormone

ABSTRACT

The early life experiences of an organism have the potential to alter its developmental trajectories. Perhaps one of the most powerful influences during this period is the parent–offspring relationship. Previous work in several mammalian species has demonstrated that parental care in early life and specifically maternal behavior can influence several adult outcomes in offspring, including affiliative and aggressive behavior, parental behavior, hypothalamic–pituitary–adrenal (HPA) functioning and risk of psychopathology. We have previously demonstrated that naturally occurring variation in the type and amount of care given to offspring in a biparental species, the prairie vole (*Microtus ochrogaster*), is related to social, anxiety-like, aggressive behaviors as well as HPA response to chronic and acute social stressors. Here we aim to determine the effects of early biparental care on HPA functioning and the interaction between early care and later reactivity to a forced swim test, an acute non-social stressor. Behavior during the swim test as well as several indicators of HPA activity, including plasma corticosterone (CORT), corticotropin releasing hormone immunoreactivity (CRH-*ir*), and vasopressin immunoreactivity (AVP-*ir*) were measured. Results here indicate an effect of early experience on AVP-*ir* but not CRH-*ir* or plasma CORT. There were no differences in CORT levels between high-contact (HC) and low-contact (LC) males or females for either control animals or after a swim stressor. CRH-*ir* was higher in the central amygdala following a swim test but was not influenced by early care. However, AVP-*ir* was not influenced by exposure to a swim stressor but was affected by early parental care in a sex-dependent manner. Female HC offspring had increased AVP-*ir* in the SON while HC male offspring had decreased AVP-*ir* in the PVN compared to their LC counterparts. The differential response of CRH and AVP to early experience and later stress, and the lack of an interaction between early care rearing and later adult stress, suggest an independence in response of some components of the HPA system. In addition, these findings expand our understanding of the relationship between naturally occurring variation in early biparental care and sexual dimorphisms in adult outcomes.

1. Introduction

Early life experiences have the potential to shape numerous outcomes in later life and its impact on response to stressors has been extensively studied in humans [1–4] as well as several animal models [5–9]. Models of early life experience in a variety of species have shown that suboptimal early rearing environments can lead to changes in the regulation of the hypothalamic–pituitary–adrenal (HPA) axis in response to later-life stressors. Naturally occurring infant abuse in rhesus monkeys in early life results in an increased cortisol and adrenocorticotropic hormone (ACTH) response in corticotropin-releasing hormone (CRH) stimulation [10] while offspring that experience increased

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http://dx.doi.org/10.1016/j.physbeh.2015.04.024
0031-9384/

rates of rejection in the first 2 months of life show increased cortisol responses to social separation at 6, 12, and 18 months of age in the cooperatively breeding marmoset [11]. Deprivations in the social environment in rodents, particularly in parental care, have similar consequences. Repeated long-term maternal separation in early life in rats leads to an increase in basal corticosterone (CORT) levels and increased CRH mRNA in the paraventricular nucleus of the hypothalamus (PVN) [12] as well as an increase in ACTH release following a brief restraint stress in adulthood [13]. Early weaning of offspring also leads to increased basal CORT and increased CRH mRNA after a stressor [14]. Taken together, these findings indicate that disruption of the early care environment has lasting implications for HPA activity, both at a basal level and in response to a stressor.

In response to an acute stressor, the PVN releases both CRH and vasopressin (AVP), which in turn stimulates corticotroph cells in the anterior pituitary to release ACTH into the peripheral blood supply. ACTH then acts on the adrenal glands, promoting the release of glucocorticoids, including CORT. Each point in this hormonal cascade has been found to be sensitive to increased amounts of tactile stimulation in the early environment of offspring. Early handling manipulations in which the offspring is briefly separated from the mother each day for approximately 2 weeks results in an increase in maternal care of pups upon their return to the nest [15–18]. This handling, and therefore increased maternal stimulation, leads to decreases in CRH production and secretion [13,19], decreased basal levels of AVP in the median eminence [19], decreased ACTH release following restraint stress [19,20], and a decreased CORT response to stressors [13,21]. These same experiences in early life also impact the response of adult offspring to various stressors. For example, there are decreases in depressive-like behavior as measured by a forced swim test in animals experiencing early handling compared to those experiencing maternal separation [12,22,23].

Increased maternal stimulation also occurs in more naturalistic settings, such as communal rearing of offspring. Being reared in a communal nest, with multiple mothers and multiple litters, results in offspring that display decreases in anxiety-like behaviors in a novel environment [24], decreases in plasma CORT after social stress [25], and a faster habituation of the CORT response following repeated stress [26]. As with the handling paradigm in rats, communal rearing also produces changes in depressive-like behaviors in a sex-specific manner. The direction of these changes, however, is less clear than in the handling paradigm in rats, with reports of both increased "depression" behavior [27] and increased resilience to depressive-like behaviors [28]. Naturally occurring increases in maternal licking and grooming (LG) behavior in rats also have similar effects to early handling, where offspring reared by high-LG dams display decreased ACTH and CORT responses to restraint stress as well as increased glucocorticoid feedback sensitivity which allows the system to return to baseline faster [29]. These results in rats are due to epigenetic changes following varying early care rather than being strictly a genomic transmission of traits [30]. There is also evidence in California mice, a biparental rodent species, that increases in paternal grooming of offspring are associated with decreases in CORT levels in male offspring [31], similar to the effects of maternal grooming in rats.

The study of parental care in rodents and its influences on adult offspring outcomes has primarily focused on maternal behavior, likely because active care from the father is relatively rare in mammalian species. One exception is the prairie vole (*Microtus ochrogaster*), a socially monogamous rodent native to the midwestern United States that displays biparental care of offspring, the father typically displaying the same range of parental behaviors as the mother aside from lactation and nursing postures [32,33]. This species has also repeatedly been shown to be sensitive to changes in early experiences, which results in differences in later parental behavior [34,35], partner preference formation [35,36], aggression [37,38], and neuropeptide systems [39–41]. In addition, HPA functioning is sensitive to early handling [42] as well as early oxytocin (OT) manipulation [43]. Taken together, these studies provide ample evidence that several systems, including HPA functioning, are vulnerable to early life experiences in the prairie vole.

Here we examined the long-term effects of early life rearing environment on HPA functioning as well as the potential interaction between early rearing experience and later response to a non-social stressor in the prairie vole. In this species, the father is heavily involved in care of offspring, which provides an opportunity to study the effects of early biparental care. We have previously shown that this species displays naturally occurring variation in biparental care and that this variation is associated with an increase in pro-social behavior in high-contact (HC) adolescent males [44] as well as an increase in aggression in low-contact (LC) adult males and an increase in plasma CORT and vasopressin immunoreactivity (AVP-*ir*) in the supraoptic nucleus of the hypothalamus (SON) in HC adult females following chronic and acute social stress [45]. In addition, cross-fostering has shown that behavioral outcomes, in particular alloparenting, are transmitted from parent to offspring through non-genomic routes while OT and AVP V1a receptor binding densities may be transmitted through genetic means [88]. In this study we investigated the relationship between the early rearing environment and adult responsiveness to a physical, non-social stressor, a forced swim test. We examined parental behavior received in the first days after birth, as well as behavior during the swim test. Plasma CORT levels were taken and CRH-*ir* and AVP-*ir* were quantified as indicators of HPA axis activity in response to the swim stressor. We predicted that LC offspring would display increased depressive-like behavior in the forced swim test compared to HC offspring. We also expected to see increases in plasma CORT levels, AVP-*ir* in the PVN and SON, and CRH-*ir* in the PVN and CeAmy in animals after a forced swim test compared to undisturbed controls, and for this post-swim test increase to be greater in LC compared to HC offspring. Because female prairie voles have previously shown increased HPA reactivity in response to stressors [45–47], we expected differences in these HPA outcome measures to be more pronounced in females compared to males. In particular, we anticipated that any increases in plasma CORT, AVP-*ir* or CRH-*ir* would be greatest in LC females.

2. Methods and materials

2.1. Subjects

Subjects were laboratory-bred prairie voles (*Microtus ochrogaster*), descendants of a stock wild-caught near Champaign, IL. Animals were housed on a 14:10 light dark cycle with lights on at 0600. Food (Purina high-fiber rabbit chow) and water were available in the home cage ad libitum. Breeder pairs and pre-weaning offspring were housed in large polycarbonate cages (44 × 22 × 16 cm) and were given cotton nestlets for bedding. Offspring were weaned on postnatal (PND) 20 and housed with an age-matched same-sex unrelated animal in a small polycarbonate cage (27 × 16 × 16 cm) until testing. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the University of California, Davis.

2.2. Parental care quantification and ranking

Following methods previously described [44], the type and amount of parental care given to offspring was observed for two separate litters for 40 different breeder pairs. Each parent was observed for 20 min in the morning (0800–1200) and 20 min in the afternoon (1300–1700) twice from PND 1–3 for a total of 4 maternal care and 4 paternal care observations per litter for two separate litters. Observations were done with the animals in their home cage; animals were not disturbed during the observations. Behaviors were recorded in real time by a trained observer using Behavior Tracker software (www.behaviortracker.com).