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Research report

Effects of social deprivation on social and depressive-like behaviors and the numbers of oxytocin expressing neurons in rats

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ABSTRACT

Social isolation is a known stressor that negatively impacts the well-being of social species. In rodents, social deprivation experienced either before or after weaning profoundly impacts adult behavioral and neuroendocrine profiles. This study compared the effects of post-natal and post-weaning social deprivation on behavioral profiles and hypothalamic oxytocin (OT) neurons. Male and female Sprague–Dawley rats were assigned to two post-natal groups, maternally separated (MS) or non-MS. MS pups were separated from their mothers for 4 h daily during post-natal days 2–21 while non-MS litters remained undisturbed. Animals were then weaned and assigned to single or group housing conditions (SH/GH). Social behaviors were evaluated two weeks later and at 2–3 months of age, depressive-like behavioral profiles were assessed using the forced swim and sucrose preference tests. Animals were euthanized, and hypothalamic OT neurons were quantified. Post-weaning isolation significantly impacted behavioral profiles, with SH animals displaying more social behaviors than GH animals. SH animals also exhibited more immobility behavior in the forced swim test and a decreased sucrose preference. Effects of sex and MS were relatively limited. Correlation analyses revealed an inverse relationship between the display of antagonistic social behaviors and the numbers of OT cells in the anterior parvicellular division of the paraventricular nucleus (PVNap). There were no correlations between numbers of OT neurons and prosocial or depressive-like behaviors. Our results demonstrate a rapid and persistent disruption of behaviors in SH animals and suggest that some of these effects may be associated with numbers of OT neurons in the PVNap.

1. Introduction

Affiliative relationships are vital to the well-being of animals belonging to social species, and they depend on interactive relationships throughout their lifespans. Initial child–parent relationships are essential for offspring survival and normal development, and the need for integral relationships persists throughout adulthood. As individuals age, social relationships have as much impact on physical health as smoking, exercise, blood pressure and obesity [1]. Social support derived from primary relationships leads to increased productivity and a decrease in the prevalence of mental disorders [2], and individuals who are isolated from social support are more likely to develop mental illness and live shorter lives [3].

1.1. Developmental social deprivation

Neonatal and early post-natal social experiences are derived primarily through receipt of maternal care, and it is well established that maternal deprivation during early life impairs the development of normal social and emotional profiles. Studies in rodents that employ maternal separation (MS) as a neonatal stressor demonstrate that MS disrupts normal social development resulting in the increased display of aggressive behaviors [4,5]. In both humans and rats, abnormal social behavioral profiles associated with MS appear to be associated with the disruption of physiological stress responses. Early life stress caused by interruptions in maternal care alters functioning of the hypothalamic–pituitary–adrenal (HPA) stress axis [6–8]. MS increases HPA responses to stress, lowers stress resiliency, and is associated with the display of anxiety and depressive-like behaviors in both primates and rodents [9–12].

1.2. Adult social deprivation

Similar adverse effects are seen when rodents are subjected to social isolation in adulthood. Rats are highly social animals that normally display a rich variety of interactive behaviors. As rat pups gain independent mobility toward the end of the second week of life, they begin to engage in playful interactions with one another. These social interactions increase in complexity during the post-

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weaning period, with juvenile rats displaying a broad array of both agonistic and antagonistic social play behaviors (for review, see [13,14]). Rats continue to engage in social activities into adulthood, and display a significant preference for social contact over other forms of environmental enrichment [15]. They live and sleep in groups, establish dominance hierarchies, and exhibit cooperative behaviors [16]. These social relationships are important for the functioning of the colony, but also appear to be important to the wellbeing of individual animals [17]).

Social isolation in rats is a known stressor that causes hypersensitivity of the hypothalamic–pituitary–adrenal (HPA) axis and alters neurotransmitter systems related to stress, anxiety and depression [6,14,17]. Single housing of rats and mice has been shown to increase aggressive social behaviors [4,18] and cause long-term changes in behavior and physiology consistent with stress hyper-reactivity [19–22]. Individual housing of rodents in the laboratory environment is now considered to be such a significant stressor that current guidelines of the National Research Council of the National Academies require it to be a justifiable necessity [23].

1.3. Oxytocin and sociality

A broad body of research has investigated the neurobiology of social interactions in rodents. The display of social behaviors likely reflects the combined actions of monoaminergic and peptidergic neurotransmitters in cortical and limbic brain areas that process rewarding and/or negative experiences. In recent years, attention has focused increasingly on the role of oxytocin (OT) as a mediator of sociality. OT is a hypothalamic nonapeptide that is most widely recognized for its peripheral effects related to reproductive function. These peripheral actions are attributable to magnocellular OT neurons in the paraventricular and supraoptic nuclei of the hypothalamus (PVN, SON) that project to the posterior pituitary gland. However, a growing body of literature demonstrates an important role for centrally projecting parvicellular neurons of the PVN in mediating the behavioral effects of OT. OT receptors are widely distributed throughout the forebrain [24–26], and studies in both rodents and humans suggest an important role for centrally released OT in regulating reproductive and maternal behaviors, social interactions and the formation of social bonds (see [27,28] for review).

The mechanisms through which OT influences sociality are not fully understood but may involve its role in neuroendocrine responses to stress. Central release of OT leads to a reduction in anxiety and stress reactivity that appears to be mediated through the HPA axis [29]. OT inhibits CRH responses to stress and diminishes stress-induced activity of the HPA axis in both humans [30], and rats [29]. These effects may serve to increase resiliency to stress, supporting the possibility that OT may also exert anti-depressive effects [31–33]. Perturbations in the HPA axis are believed to be a fundamental neurobiological irregularity of depression [34–36]. Consequently, OT may play a compelling role in the etiology of depression because of OT's pivotal role in mammalian social behavior and its ability to attenuate the HPA axis' stress response.

1.4. Experimental aims

The aim of the present studies was to compare the effects of post-natal and post-weaning social deprivation on the subsequent display of social play and depressive-like behaviors in rats and to investigate possible correlations between behavioral effects and numbers of hypothalamic OT neurons. MS and non-MS pups were weaned into either a single housed (SH) or group housed condition (GH) using a cross balanced design. Animals were tested for a wide array of agonistic and antagonistic social behaviors using a standard social play testing paradigm. Animals were then tested for depressive-like behaviors in the forced swim test and sucrose preference test. Brain sections containing the PVN and SON were immunostained for OT immunoreactivity (OT-ir), and numbers of OT-ir cells in the PVN and SON were quantified stereologically.

2. Materials and methods

2.1. Experimental overview

A schematic diagram depicting the timeline of experimental procedures is presented in Fig. 1. The day of birth for each litter was designated as post-natal day (PND) 0. As shown, newborn offspring were cross-fostered on PND 1 to generate 8 experimental litters of 10 pups each ($n = 80$), with each litter comprising 5 male pups and 5 female pups. Maternal separation (MS) or controlled non-separation (non-MS) procedures occurred daily from PND 2 to weaning on PND 21. Animals were weaned on PND 21 and using a cross balanced design, were housed either singly or in groups for the remainder of the experiment. All experimental animals were tested once for social play behaviors between PND 33 and PND 38. Animals were then tested once in the forced swim test between PND 60 and PND 64, followed by a sucrose preference test between PND 79 and 81. All animals were euthanized between PND 103 and 107.

2.2. Animals

Experimental animals were the offspring of 12 timed pregnant Sprague–Dawley rats (Harlan Laboratories, Indianapolis, IN). Upon arrival on gestational day (GD) 15, dams were housed in pairs until GD 18 and were then single housed from GD 19 until parturition. The animals were housed on a 12 h light cycle (lights on at 0800 h) in standard cages (43 cm × 20.5 cm × 20 cm) with 1/4 in. corn cob bedding (Harlan Laboratories) with access to food (Teklad LM-485 mouse/rat serializable diet, Harlan Laboratories) and water *ad libitum*. The animal housing room was maintained at 20–26 °C with 50% controlled humidity throughout the experiment. Animal care and maintenance was in accord with the Animal Welfare Act and the U.S. Department of Health and Human Services "Guide for the Care and Use of Laboratory Animals" [23], and all experimental procedures were consistent with National Institute of Health guidelines and approved by the Howard University Institutional Animal Care and Use Committee.

On PND 1, litters were culled and pups were cross-fostered so that each dam had a litter of five males and five females. Culled pups were euthanized by lethal injection with the euthanasia drug SomnaSol (active ingredients: 390 mg/ml sodium pentobarbital and 50 mg/ml phenytoin; Henry Schein Animal Health, Dublin, Ohio) and were then rapidly decapitated to ensure death. Pups from eight of the litters ($n = 80$) were included in the experiment, and eight additional pups (four males and four females) were housed in same sex groups to be used as conspecific stimulus animals for social play testing.

2.3. Post-natal and post-weaning social deprivation

Four of the eight experimental litters ($n = 40$ pups) were randomly assigned to undergo post-natal social deprivation through maternal separation (MS). MS was performed from PND 2 through PND 21 for 4 h daily (0800–1200 h) based on the protocol of Leussis et al. [10]. During MS treatment, the dam was gently removed from the home cage and placed in an individual cage in a separate room while the pups remained in their home cages with their littermates. The other four litters (non-MS, $n = 40$ pups) remained unseparated from their mothers until weaning at three weeks of age.