Hormones and Behavior 58 (2010) 575–581

Contents lists available at ScienceDirect

Hormones and Behavior

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

Maternal care effects on the development of a sexually dimorphic motor system: The role of spinal oxytocin

Kathryn M. Lenz [a,*], Dale R. Sengelaub [b]

[a] Department of Physiology and Program in Neuroscience, University of Maryland, Baltimore, Baltimore, MD, 21201, USA
[b] Department of Psychological and Brain Sciences and Program in Neuroscience, Indiana University, Bloomington, IN, 47405, USA

ARTICLE INFO

Article history:
Received 12 April 2010
Revised 9 July 2010
Accepted 25 July 2010
Available online 3 August 2010

Keywords:
Maternal care
Motoneuron
Sexual behavior
Development
Oxytocin
Dendrite
Experience
Hormone
Masculinization

ABSTRACT

Maternal licking in rats affects the development of the spinal nucleus of the bulbocavernosus (SNB), a sexually dimorphic motor nucleus that controls penile reflexes involved with copulation. Reduced maternal licking results in decreased motoneuron number, size, and dendritic length in the adult SNB, as well as deficits in adult male copulatory behavior. Our previous findings that licking-like tactile stimulation influences SNB dendritic development and upregulates Fos expression in the lumbosacral spinal cord suggest that afferent signaling is changed by differences in maternal stimulation. Oxytocin afferents from the hypothalamus are a possible candidate, given previous research that has shown oxytocin is released following sensory stimulation, oxytocin modulates excitability in the spinal cord, and is a pro-erectile modulator of male sex behavior. In this experiment, we used immunofluorescence and immediate early gene analysis to assess whether licking-like tactile stimulation of the perineum activated parvocellular oxytocinergic neurons in the hypothalamus in neonates. We also used enzyme immunoassay to determine whether this same stroking stimulation produced an increase in spinal oxytocin levels. We found that stroking increased Fos immunolabeling in small oxytocin-positive cells in the paraventricular nucleus of the hypothalamus, in comparison to unstroked or handled control pups. In addition, 60 s of licking-like perineal stimulation produced a transient 89% increase in oxytocin levels in the lumbosacral spinal cord. Together, these results suggest that oxytocin afferent activity may contribute to the effects of early maternal care on the masculinization of the SNB and resultant male copulatory behavior.

Introduction

Early social contact between mother and offspring shapes the neural and behavioral development of offspring (Hofer, 1978, 1994). In humans, parental care is a crucial factor in the development of offspring, with parental deprivation or loss predicting future mental health problems (Agid et al., 1999; Carter et al., 1999) and individual differences in maternal care influencing the physiological and psychological development of children (Hane and Fox, 2006). Rats and non-human primates also show substantial sensitivity to early maternal influences (e.g., Pryce et al., 2005), and as such serve as excellent model systems to manipulate maternal care. Natural variations in rodent maternal behavior produce offspring that differ on many neural and behavioral dimensions. Pups that receive higher levels of maternal licking, grooming, and arched-back nursing, for example, show greater hippocampal neuron density (Bredy et al., 2003), greater levels of neurotrophin expression throughout the brain (Liu et al., 2000), altered dopamine levels in the prefrontal cortex (Zhang et al., 2005), altered GABA receptor subunit expression in the amygdala (Caldji et al., 1998, 2000), altered hypothalamic-pituitary-adrenal axis development (Liu et al., 1997), altered oxytocin receptor expression (Francis et al., 2000), and consequent changes in the many behaviors mediated by these structures. Cross-fostering shows that these maternal effects are epigenetic, with the level of maternal care received predicting the offspring's phenotype.

Adult sexual behavior is also shaped by early maternal care. In female rats, natural variations in maternal licking contribute to partner preference, receptivity, and paced mating behavior (Cameron et al., 2008a,b). In males, experimental reductions in maternal licking produce behavioral deficits in adult copulatory behavior. These deficits include increased latency to ejaculation, increased latency to post-ejaculatory intromission, and increased inter-intromission intervals (Moore, 1984).

Reductions in licking influence neural development relevant to male sexual behavior as well. One of the neural structures that controls male copulatory behavior is the spinal nucleus of the bulbocavernosus (SNB). The SNB (also known as the dorsomedial nucleus, Schroder, 1980) is a sexually dimorphic population of motoneurons in the lumbar spinal cord, which innervates the anal sphincter of both males and females and additionally in males, the

* Corresponding author. Department of Physiology, 655 W. Baltimore St, BRB 5-014, Baltimore, MD 21201, USA. Fax: +1 410 706 8341.
E-mail address: kmlenz@umaryland.edu (K.M. Lenz).

0018-506X/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.yhbeh.2010.07.010

bulbocavernosus (BC) and levator ani (LA) muscles of the perineum (Breedlove and Arnold, 1980; Schroder, 1980; McKenna and Nadelhaft, 1986). The BC and LA muscles encircle the base of the penis and their fast, robust contractions produce an intense penile erection with flaring of the glans that allows seminal plug formation and removal (Sachs, 1982; Hart and Melese-D'Hospital, 1983). The development of SNB motoneurons extends well into the early postnatal period (Nordeen et al., 1985; Goldstein et al., 1990) and, interestingly, is sensitive to maternal care. Specifically, reductions in maternal licking produce decreased motoneuron number (Moore et al., 1992), motoneuron size, and dendritic length in the SNB (Lenz and Sengelaub, 2006).

The tactile stimulation conferred by maternal licking appears to be a critical component of the behavior, with supplemental stimulation offsetting the negative behavioral and physiological effects of maternal deprivation (Suchecki et al., 1993; Levy et al., 2003; Chatterjee et al., 2007). We have previously found licking-like tactile stimulation to be an important modulator of neural development in the SNB, with pups that received low levels of stimulation showing deficits in ex copula penile reflexes in adulthood as well as reduced dendritic length in the SNB relative to animals receiving high levels of stimulation (Lenz et al., 2008). We have also found that licking-like tactile stimulation of the perineum produces transient increases in spinal Fos expression in the area of the SNB dendritic field, suggesting that tactile stimulation may regulate SNB dendritic growth through an activity-dependent mechanism (Lenz and Sengelaub, 2009).

Supraspinal afferent input also regulates dendritic development in the SNB, with thoracic spinal transection causing localized redistributions of the dendritic arbor (Hebbeler and Sengelaub, 2003). The SNB receives input from many supraspinal afferent populations that have been shown to influence penile reflex behavior, including serotonergic, noradrenergic, dopaminergic, and oxytocinergic afferents (Giuliano and Rampin, 2000). Although any or several of these populations could be candidate mechanisms, we have chosen to first focus on the role of oxytocin inputs from the paraventricular nucleus (PVN) of the hypothalamus, for reasons elaborated below.

Oxytocinergic afferents from the PVN innervate the lumbosacral spinal cord (Swanson and McKellar, 1979; Cechetto and Saper, 1988; Wagner and Clemens, 1993; Tang et al., 1998; Veronneau-Longueville et al., 1999; Hallbeck et al., 2001). Interestingly, oxytocin is centrally released following sensory stimulation (Stock and Uvnas-Moberg, 1988; Uvnas-Moberg et al., 1993), and electrically stimulating the dorsal penile nerve or stroking the perineum activates oxytocin neurons in the PVN (Yanagimoto et al., 1996; Caba et al., 2003). Oxytocin has also been shown to be a proerectile modulator of male copulatory behavior (Argiolas et al., 1986; Giuliano et al., 2001), altering glutamatergic signaling to increase the excitability of dorsal horn neurons (Jo et al., 1998). In addition, the development of the oxytocinergic system is known to be sensitive to early life experience (Francis et al., 2000; Todeschin et al., 2009). These findings together suggest that oxytocin signaling in the spinal cord may mediate the effects of maternal licking or licking-like tactile stimulation on the morphology of SNB motoneurons and resultant penile reflexes. The goal of the current research was to determine whether early maternal licking-like stimulation regulates oxytocin signaling in the developing spinal cord.

Methods

Animals

Untimed pregnant Sprague–Dawley rat dams (Harlan, Indianapolis, IN) were maintained on a 12:12 h light/dark cycle, with unlimited access to food and water. On postnatal day (P) 1, pups were sexed and culled to litters of eight with even sex ratios where possible. All experimental manipulations were performed on P10, during the period when the maternal licking behavior is most robust. All experimental procedures were approved by the Institutional Care and Use Committee at Indiana University and in accordance with national animal care and use guidelines.

Immediate early gene analysis

Pups were removed from the dam and placed on a heating pad in a novel plastic container. Pups were handled individually and stimulated with a moist paintbrush in the anogenital region for 60 s. Control groups consisted of an undisturbed control group, which was left with the dam and not stroked, and a handled control group, which was held by the experimenter in the same manner as the stroked animals, but not stroked, for 60 s.

Pups were sacrificed 30 min following the stroking manipulation, a period that has been previously shown to achieve maximum expression of the immediate early gene, Fos, in the brain and spinal cord following maternal care or licking-like stimulation (Fenoglio et al., 2006; Lenz and Sengelaub, 2009). All animals were weighed, overdosed with urethane (approximately 0.25 g/100 of body weight), and transcardially perfused with saline followed by cold 4% paraformaldehyde. Brains were removed, postfixed in the same fixative overnight, and then transferred to sucrose phosphate buffer (10% w/v, pH 7.4) for cryoprotection until tissue sank. Brains were then frozen-sectioned horizontally at 30 microns into PBS into three alternate series. One series was counterstained with thionin to locate the PVN. The other two series were processed immunohistochemically to visualize the immediate early gene product, Fos, and the peptide hormone, oxytocin.

Immunohistochemistry

Immunohistochemistry series underwent a procedure similar to Bharati and Goodson (2006). Briefly, sections were rinsed in PBS in free-floating wells, incubated for 1 hr in PBS + 5% bovine serum albumin (BSA) + 0.3% Triton-X for blocking, and incubated for 24 h in rabbit anti-Fos (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA) and guinea pig anti-oxytocin (1:1000; Bachem, Torrance, CA) in PBS + 2.5% BSA + 0.3% Triton-X + 0.05% sodium azide at 4 °C. Following primary antibody incubation, sections were rinsed and incubated for 2 hr in goat anti-rabbit secondary antibody conjugated to Alexa Fluor 488 (3 μl/ml; Invitrogen, Eugene, OR) and goat anti-guinea pig secondary antibody conjugated to Alexa Fluor 594 (5 μl/ml; Invitrogen) in PBS + 2.5% BSA + 0.3% Triton-X + 0.05% sodium azide at RT. Sections were rinsed in PBS mounted onto gelatin-coated slides, and coverslipped with Vectashield Hard Set (Vector Laboratories, Burlingame, CA).

Data collection

Cell counts were performed under epifluorescent illumination using a Nikon Eclipse 80i microscope (Nikon Instruments, Melville, NY) interfaced with a camera (Microfire; Optronics; Santa Barbara, CA). We have previously performed pilot studies using a DAPI counterstain, which showed Fos labeling in neonates to be primarily nuclear. Some nonspecific vascular labeling of epithelial cells also occurs (see Fig. 1b), but this nonspecific labeling is morphologically distinguishable from the clear, spherical nuclear labeling in neurons. To ensure accuracy, cell counts were performed at higher magnification where the Fos label is more distinguishable from background (20×; see Fig 1c), and cells were only counted as double labeled when there was clear co-localization between the oxytocin immunopositive cell body and a spherical Fos-positive nucleus, often with a visible nucleolus.

The number of Fos and oxytocin double-labeled cells was quantified unilaterally across the entire extent of the PVN, in one of three alternate series. The total number of oxytocin single-labeled