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Motherhood and infant contact regulate neuroplasticity in the serotonergic midbrain dorsal raphe

M. Allie Holschbach*, Joseph S. Lonstein

Neuroscience Program, 108 Giltner Hall, Michigan State University, East Lansing, MI 48824, USA

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ABSTRACT

The adult brain shows remarkable neuroplasticity in response to hormones and the socioemotional modifications that they influence. In females with reproductive and maternal experience, this neuroplasticity includes the birth and death of cells in several forebrain regions involved in maternal caregiving and postpartum affective state. Such plasticity in midbrain sites critical for these behavioral and emotional processes has never been examined, though. By visualizing bromodeoxyuridine (BrdU) to label mitotic cells, NeuroD for neuronal precursors, and TUNEL to identify dying cells, we found that the midbrain dorsal raphe nucleus (DR, the source of most ascending serotoninergic projections) exhibited significant neuroplasticity in response to motherhood. Specifically, BrdU analyses revealed that DR newborn cell survival (but not proliferation) was regulated by reproductive state, such that cells born early postpartum were less likely to survive 12 days to reach the late postpartum period compared to cells born during late pregnancy that survived 12 days to reach the early postpartum period. Many of the surviving cells in the DR were NeuN immunoreactive, suggesting a neuronal phenotype. Consistent with these findings, late postpartum rats had fewer NeuroD-immunoreactive DR cells than early postpartum rats. Maternal experience contributed to the late postpartum reduction in DR newborn cell survival because removing the litter at parturition increased cell survival as well as reduced cell death. Unlike cytogenesis in the maternal hippocampus, which is reduced by circulating glucocorticoids, DR newborn cell survival was unaffected by postpartum adrenalectomy. These effects of reproductive state and motherhood on DR plasticity were associated with concurrent changes in DR levels of serotonin's precursor, 5-HTP, and its metabolite, 5-HIAA. Our results demonstrate for the first time that cytogenesis occurs in the midbrain DR of any adult mammal, that DR plasticity is influenced by female reproductive state and maternal experience, and that this plasticity is accompanied by changes in DR serotonergic function. Because serotonin is critical for postpartum caregiving behaviors and maternal affective state, plasticity in the DR may contribute to the neurochemical changes necessary for successful motherhood.

1. Introduction

Circulating hormones act during numerous periods of the lifespan to help drive neurobehavioral change and do so, in part, by modifying central nervous system structure and function (for reviews see Sisk et al., 2012; Juraska and Willing, 2016). In adult females, the transition to motherhood involves dramatic fluctuations in steroid and other hormones, resulting in a suite of behavioral, emotional, and physiological adaptations that are required for the successful rearing of offspring (Lonstein et al., 2014; Numan et al., 2006). These modifications include sensitive caregiving of the young, maternal aggression toward threats, reduced anxiety, milk production and letdown, and blunted endocrine responses to many stressors. All of these changes revert back to a prepartum state around the time of weaning the offspring (Lonstein et al., 2014; Numan et al., 2006).

To support such changes in behavior and physiology, the maternal brain undergoes equally remarkable neuroplasticity (Lévy et al., 2011). The birth and death of new cells are two of the mechanisms through which hormones modify the structure, connectivity, and chemical function of the developing and adult nervous systems (Pawluski et al., 2009; Del Pino Sans et al., 2015; Juraska and Willing, 2016), and numerous studies have found that motherhood alters both forebrain cytogenesis and cell death (Bridges and Grattan, 2003; Erbil et al., 2014). For example, pregnant and early postpartum laboratory rodents have a prolactin-mediated increase in cytogenesis in the subventricular zone (SVZ) that gives

* Corresponding author at: Department of Biomedical Sciences 1617 Campus Delivery W327 Anatomy/Zoology Colorado State University Fort Collins, CO 80523, USA.
E-mail address: maholsch@colostate.edu (M.A. Holschbach).

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rise to new olfactory neurons (Furuta and Bridges, 2005; Bridges and Grattan, 2003; Shingo et al., 2003). Furthermore, early postpartum maternal experience increases newborn cell survival in other sites involved in maternal behavior including the nucleus accumbens and bed nucleus of the stria terminalis (Akbari et al., 2007). On the other hand, early postpartum rats also experience a corticosterone-mediated decrease in cytogenesis in the hippocampal dentate gyrus (DG) (Pawluski et al., 2009; Pawluski and Galea, 2007; Leuner et al., 2007) and pregnant mice have more cell death in the DG and prefrontal cortex compared to nulliparae (Erbil et al., 2014).

Motherhood-associated neuroplasticity has never been reported in the midbrain, though, perhaps because studies of adult cytogenesis almost invariably focus on forebrain sites directly apposing or close to the forebrain ventricular system – where cytogenesis is traditionally thought to occur (Kokoeva et al., 2007; Migaud et al., 2010). However, the lining of the midbrain cerebral aqueduct is known to be an important proliferative niche from early development through adulthood and aging (Zhao and Lang, 2009; Zhao et al., 2003). One brain site that is both adjacent to the proliferative cerebral aqueduct and involved in postpartum behavior and physiology is the dorsal raphe nucleus (DR). The DR contains 80% of forebrain-projecting serotonergic neurons (Lowry et al., 2008) and selectively lesioning DR serotonergic neurons in postpartum rats increases pup mortality (Barofsky et al., 1983). Furthermore, maternal behaviors are severely impaired by knocking out *Pet-1* (transcription factor for serotonin neuron differentiation) or *TPH2* (enzyme necessary for neuronal serotonin synthesis) (Angoa-Pérez et al., 2014; Lerch et al., 2008), or after acute pharmacological manipulation of serotonin signaling (Veiga et al., 2010; Zhao and Li, 2009). Postpartum physiological processes, including the suckling-induced prolactin and oxytocin surges necessary for milk production and release, also rely on DR serotonin (Barofsky et al., 1983). Given these roles for serotonin in postpartum behavior and physiology, one might predict that reproduction and motherhood are associated with neuroplastic changes in the DR.

We here tested the hypothesis that motherhood involves DR neuroplasticity by conducting a series of experiments in which we identified mitotic cells using 5-bromo-2′-deoxyuridine (BrdU), young neurons using immunohistochemical detection of NeuroD, and dying cells using Terminal dUTP-Nick End Labeling (TUNEL). We measured cytogenesis in female rats across different reproductive states, in postpartum rats with or without maternal experience, and in adrenalectomized mothers to examine if corticosterone affects DR cytogenesis (as it does in the hippocampus; Pawluski et al., 2009; Pawluski and Galea, 2007; Leuner et al., 2007). To gain insight into the phenotype of the mitotic cells, we colocalized BrdU and Neuronal Nuclei antigen (NeuN), a common neuronal marker. We also examined if reproductive state alters DR expression of NeuroD, a neurogenic transcription factor expressed by neuroblasts and terminally differentiating neurons (Gao et al., 2009). Lastly, we measured numerous aspects of the DR serotonin system across reproductive states to evaluate whether changes in DR plasticity occur contemporaneously with local neurochemical modifications. We found that reproduction and motherhood alter DR newborn cell survival and cell death, as well as the serotonin synthetic and metabolic pathway.

2. Methods

Subjects

Subjects were female Long-Evans rats born and raised in our breeding colony and maintained as previously described (Smith et al., 2013). Pregnant females were singly housed approximately five days before parturition, and litters culled to contain four males and four females within 24 h after birth. The day of parturition was considered PPD 0. All procedures were conducted in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and the Institutional Animal Care and Use Committee at Michigan State University.

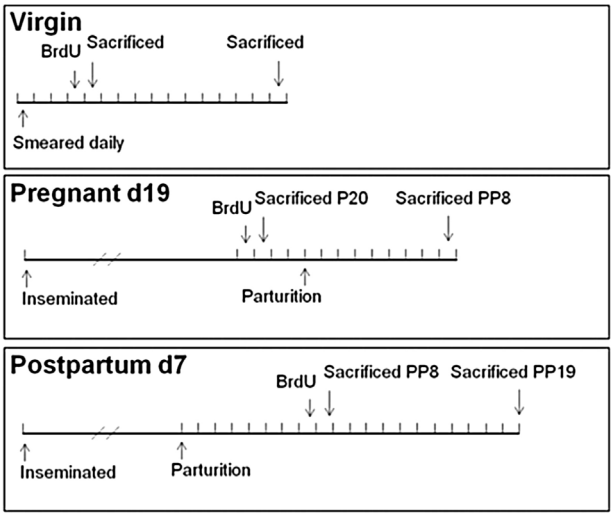

Fig. 1. Experimental time course to determine the effects of reproductive state on cell proliferation and survival. Adult female rats were injected with the mitotic marker BrdU as diestrus virgins, on day 19 of pregnancy (PD 19), or on day 7 postpartum (PPD 7). Females were sacrificed one day later to measure cell proliferation, or 12 days later to measure cell survival.

2.1. Experiment 1: effects of reproductive state on DR cytogenesis and cell death in adult female rats

2.1.1. BrdU injections

To measure cytogenesis in the DR and elsewhere in the brain, female rats were injected with BrdU, a thymidine analogue permanently incorporated into the DNA of dividing cells (Taupin, 2007). A dose of 150 mg/kg bodyweight was chosen to approach a saturating dose that does not have deleterious effects on fetuses in the pregnant dams (Taupin, 2007; Cameron and McKay, 2001). Rats were injected once with BrdU dissolved in 0.9% sterile saline (IP; 10 mg/mL; Sigma-Aldrich, St. Louis, MO) either on a day of diestrus (determined by cytology in vaginal smears), on pregnancy day (PD) 19, or on postpartum day (PPD) 7 (Fig. 1). Subjects were sacrificed one day after BrdU injection to examine cell proliferation ($n = 8$ per group), or 12 days after injection to examine short-term cell survival ($n = 10$ per group). The 12-day survival period was chosen for several reasons. First, this survival period allowed us to measure cell survival during the onset and early maintenance of postpartum behaviors and physiology (from PD 19 to PPD 8) and then have another discrete measure as these events wane (from PPD 7 to PPD 19), thus allowing us to evaluate cell survival during the two most salient periods of behavioral change in mothers. In addition to these behavioral changes, the physiological events of parturition may be expected to affect survival of cells in the maternal brain, which could further contribute to differences between these two groups. Second, because newborn cells migrate from the cerebral aqueduct to the substantia nigra (SN) within 10 days (Zhao et al., 2003), newborn cells still residing within the even closer DR 12 days after BrdU injection were unlikely to be migrating through the structure but instead have probably reached their final destination there. Third, 12 days after mitosis is sufficient time for expression of the neu-