



Published in final edited form as:

Dev Psychobiol. 2014 December ; 56(8): 1613–1625. doi:10.1002/dev.21241.

Neonatal Handling: An Overview of the Positive and Negative Effects

Charlis Raineke¹, Aldo B. Lucion², and Joanne Weinberg¹

Charlis Raineke: craineke@mail.ubc.ca

¹Department of Cellular and Physiological Sciences, University of British Columbia, 2350 Health Sciences Mall, Vancouver, BC, Canada, V6T 1Z3

²Departamento de Fisiologia, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

Abstract

As one of the first rodent models designed to investigate the effects of early-life experiences, the neonatal handling paradigm has helped us better understand how subtle changes in the infant environment can powerfully drive neurodevelopment of the immature brain in typical or atypical trajectories. Here, we review data from more than 50 years demonstrating the compelling effects of neonatal handling on behavior, physiology, and neural function across the lifespan. Moreover, we present data that challenge the classical view of neonatal handling as an animal model that results only in positive/beneficial outcomes. Indeed, the overall goal of this review is to offer the suggestion that the effects of early-life experiences—including neonatal handling—are nuanced rather than unidirectional. Both beneficial and negative outcomes may occur, depending on the parameters of testing, sex of the subject, and neurobehavioral system analyzed.

Keywords

neonatal handling; rat; early-life experience; stress; anxiety; reproduction; social behavior; food consumption; learning; memory; renal function

One of the major questions driving the field of developmental psychobiology is “How do we become who we are?” This question is one of the most intriguing, and incidentally, one of the most complicated to address. Part of the complication arises from the fact that each individual encounters unique experiences throughout his or her life course that can have profound impacts on the brain and, consequently, behavior. Additionally, windows of vulnerability exist during different periods of development, when the environment can exert particular influence in modulating the development of brain structure and function (Andersen & Teicher, 2008; Fox, Levitt, & Nelson, 2010; Knudsen, 2004). One such window of vulnerability occurs during the early postnatal period, a time of considerable brain plasticity, when the brain can undergo long-term programming (Cirulli, Berry, & Alleva, 2003; Davidson & McEwen, 2012; Roth & Sweatt, 2011). Early-life experiences

play a critical role in steering the development of the immature brain in normal or pathological directions. Indeed, negative experiences during this time are highly associated with increased vulnerability to stressors and compromised physical and mental health later in life (Andersen & Teicher, 2008; Bale et al., 2010; Davidson & McEwen, 2012; Heim & Binder, 2012; McEwen, 2008; Rainekei, Rincón-Cortés, Belnoue, & Sullivan, 2012), whereas positive early-life experiences are associated with resilience in the face of later-life challenges and good physical and mental health (Baldini et al., 2013; Bowlby, 1969).

As one of the pioneers in the field of developmental psychobiology, Seymour Levine developed one of the first rodent models to evaluate the long-lasting effects of early-life trauma (Levine, Chevalier, & Korchin, 1956). Levine's model was influenced by Sigmund Freud's theory that traumatic experiences during infancy can contribute to the development of later-life psychopathologies (Freud, 1936). Accordingly, Levine utilized daily, 3-min sessions of mild shock from postnatal day (PN) 1 until PN20 as a model of early-life trauma. Controls included placing animals in the shock chamber for the same daily 3-min sessions without shock, the "neonatal handled" group; and leaving animals undisturbed until PN20, the "non-handled" group. When tested in adulthood, the results revealed, surprisingly, that *both* mild shock and neonatal handling resulted in a "more adaptive" animal (Levine, 1956, 1962; Levine et al., 1956). Contrary to predictions, mild shock had positive rather than negative effects on development, and handling, meant to be the control for mild shock, was actually an experimental treatment in itself, with beneficial effects similar to those of mild shock. Moreover, the non-handled group, meant to be a positive control group, showed adverse outcomes. Thus, mild stimulation in infancy, whether through mild shock or simple handling, resulted in more adaptive development and responsiveness, whereas rearing in relatively unstimulated conditions appeared to have negative effects on behavioral and physiological development. Since these initial experiments, the effects of early-life experiences have been a major focus in developmental neuroscience.

The current review will provide an overview of the impact of neonatal handling on neurobehavioral development, describe the outcomes of the different neonatal handling protocols used over the years, and present data that challenge the classical view of neonatal handling as an animal model resulting only in positive outcomes. We ultimately hope to stimulate discussion around how best to interpret results from different models of early-life experience, and in particular, how these models induce beneficial and/or harmful effects depending on the parameters of the test situation, sex of the subject, and neurobehavioral system analyzed. In the case of the neonatal handling model, protective benefits in some neurobehavioral systems, but adverse effects on other neurobehavioral systems may be observed. Our review will focus primarily on studies employing rat models without accounting for different strains.

NEONATAL HANDLING

Neonatal handling, also known as early or postnatal handling, is an experimental paradigm in which pups are briefly separated from the mother and exposed to a new environment. The classical neonatal handling procedure consists of removing the mother from the home cage to a holding cage, and then placing pups individually in small compartments for 3 min. This

procedure is repeated daily from PN1–20 (Levine, 1956, 1962). However, parameters of this model vary among laboratories. First, pups may spend from 1 to 15 min out of the home cage (*for 1 min*: Padoin, Cadore, Gomes, Barros, & Lucion, 2001; *for 3 min*: Denenberg & Karas, 1959; Levine et al., 1956; Weinberg, Smotherman, & Levine, 1978b; *for 15 min*: Fenoglio et al., 2005; Meaney, Aitken, Bodnoff, Iny, & Sapolsky, 1985; Plotsky & Meaney, 1993; Pryce, Bettschen, & Feldon, 2001). Second, the handling procedure may be repeated from 10 to 21 days (*for 10 days*: Padoin et al., 2001; *for 15 days*: Meaney, Aitken, Bodnoff, Iny, & Sapolsky, 1985, Weinberg et al., 1978b; *for 21 days*: Levine et al., 1956; Núñez et al., 1995; Pryce et al., 2001). Finally, pups can be handled individually (Levine, 1956; Meaney, Aitken, Bodnoff, Iny, & Sapolsky, 1985; Weinberg et al., 1978b) or as a litter (Fenoglio et al., 2005; Ladd, Thirivikraman, Huot, & Plotsky, 2005; Padoin et al., 2001). Despite these variations, the classical effects of neonatal handling—including reduced anxiety-like behaviors (also interpreted as reduced emotionality) and reduced stress responses—are robust and have been replicated across laboratories. However, divergent outcomes are not uncommon in the literature, making comparisons among results challenging, and limit the ability to generalize conclusions. Conversely, the use of different neonatal handling protocols has helped us appreciate the importance of how each variable within the model can differentially impact the infant's development.

CONTROL GROUP FOR THE NEONATAL HANDLING PARADIGM

Establishing an appropriate control for the neonatal handled group has been a matter of debate in the field (Pryce & Feldon, 2003; Macrì & Würbel, 2006). A common protocol is the non-handled condition, which consists of leaving animals completely undisturbed, including no routine cage changing over the days that neonatal handling occurs. Leaving the non-handled animals completely unstimulated during infancy may in itself have effects on development. An alternative control is the use of the *standard animal facility rearing condition*, in which animals undergo standard cage changing and other routine husbandry. However, this control presents its own set of challenges due to variation in standard procedures among facilities. For a more extensive discussion of this topic please see Pryce & Feldon (2003) and Macrì & Würbel (2006).

NEONATAL HANDLING AND THE STRESS RESPONSE

When confronted with a stressor (real or perceived), a series of neuroendocrine events begins to unfold in order to direct energy reserves to vital tissues, focus attention and memory, and suppress reproductive and immune functions in order to promote immediate survival and subsequent reinstatement of homeostasis. This is achieved by the activation of multiple interacting processes, involving behavioral, autonomic, endocrine, and immune systems, which produces a stress response (de Kloet, Vreugdenhil, Oitzl, & Joëls, 1998; Herman, 2013). Abnormal function of any of these systems may result in altered responses to stress that, if persistent, can increase vulnerability to later-life health problems. Indeed, either chronically high or chronically low levels of the stress hormones can impair physiological and behavioral functions, leading to pathology (Gold & Chrousos, 2007). The major endocrine system underlying the stress response is the hypothalamic–pituitary–adrenal (HPA) axis, which involves sequential secretion of corticotropin-releasing hormone

(CRH) and arginine vasopressin (AVP) from the hypothalamus, adrenocorticotrophic hormone (ACTH) from the anterior pituitary, and glucocorticoids (corticosterone in most rodents, cortisol in humans) from the adrenals (de Kloet et al., 1998, Herman, 2013). Importantly, the HPA axis belongs to a larger system that maintains homeostasis even in the absence of a potent stressor (Sapolsky, Romero, & Munck, 2000).

Following birth, basal glucocorticoid levels rapidly decline and neonates enter a stress hyporesponsive period (SHRP), characterized by low basal glucocorticoid levels and a reduced response to stressors (Levine, 1994; Sapolsky & Meaney, 1986; Walker, Perrin, Vale, & Rivier, 1986). The SHRP, which in rats lasts from ~PN4 until PN14, represents a period during which the organism goes through rapid development that is dependent on consistent, low levels of glucocorticoids (Bohn, 1980; Erkine, Geller, & Yuwiler, 1979; Sapolsky & Meaney, 1986). Nevertheless, the neonate can show age-specific stress responses following maternal deprivation (Kuhn, Pauk, & Schanberg, 1990; Stanton, Gutierrez, & Levine, 1988; Suchecki, Nelson, van Oers, & Levine, 1995). Maternal behaviors such as nursing, contact, and tactile stimulation perform the dual functions of both inhibiting and suppressing HPA function in the neonate to maintain the characteristically low levels of glucocorticoids during the SHRP. Specifically, feeding seems to maintain adrenal insensitivity to ACTH, while tactile stimulation appears to alter the upstream neural ACTH secretagogues (Suchecki, Rosenfeld, & Levine, 1993).

Not surprisingly, altered mother–pup interactions during the SHRP can have a significant impact on the development of systems that regulate stress responses (Cirulli et al., 2003; Meaney et al., 1993). In general, although handled and non-handled animals do not differ in basal hormone levels (Hess, Denenberg, Zarrow, & Pfeifer, 1969; Liu, Caldji, Sharma, Plotsky, & Meaney, 2000), handled animals secrete less ACTH and corticosterone and show a faster return to basal levels following a wide variety of stressors, including restraint, open field, active avoidance learning, ether exposure, and re-exposure to a fear-conditioning chamber (Hess et al., 1969; Levine, Halmeier, Karas, & Denenberg, 1967; Liu et al., 2000; Meerlo, Horvath, Nagy, Bohus, & Koolhaas, 1999; Plotsky & Meaney, 1993; Weinberg & Levine, 1977). On the other hand, handled animals may secrete more corticosterone in response to more noxious stressors such as shock (Levine, 1962) but still show a faster recovery towards basal levels (Halmeier, Denenberg, & Zarrow, 1967). These findings suggest that neonatal handled rats show a “more appropriate” or better-modulated response to stress.

Central mechanisms that regulate stress responses are altered by neonatal handling in a manner consistent with better modulation of HPA activity. Studies have reported that handled animals have reduced CRH in the PVN, locus coeruleus (LC), central nucleus of the amygdala, and median eminence; with reduced CRH release from the median eminence following stress (Francis, Caldji, Champagne, Plotsky, & Meaney, 1999; Plotsky & Meaney, 1993; Plotsky et al., 2005; Viau, Sharma, Plotsky, & Meaney, 1993). Additionally, the LC of handled animals is less responsive to CRH (Swinney et al., 2010), which may be related to the decrease in total CRH binding (Plotsky et al., 2005), decreased dendritic length and branching in LC neurons (Swinney et al., 2010), and a reduction in the number of LC neurons (Lucion, Pereira, Winkelman, Sanvitto, & Anselmo-Franci, 2003).

Neonatal handling increases glucocorticoid receptor density and receptor binding in the hippocampus (Avishai-Eliner, Eghabal-Ahmadi, Tabachnik, Brunson, & Baram, 2001; Meaney & Aitken, 1985; Meaney, Aitken, Bodnoff, Iny, & Sapolsky, 1985; Meaney, Aitken, Bodnoff, Iny, Tatarewicz, et al., 1985; Meaney, Aitken, van Berkel, Bhatnagar, & Sapolsky, 1988; Meaney, Aitken, Viau, Sharma, & Sarrieau, 1989), which may be associated with the increase in neuronal density in the pyramidal cell layer of the hippocampus (Winkelmann-Duarte et al., 2011). Overall, the hippocampus has an inhibitory effect on the HPA (Myers, McKkveen, & Herman, 2012), and such structural and functional changes following neonatal handling seem to potentiate the efficacy of the negative feedback, reducing the ACTH and corticosterone response to stress (Meaney et al., 1993). The mPFC also has a predominantly inhibitory role on the HPA (Myers et al., 2012) and, similar to the hippocampus, handling increases glucocorticoid receptor density in the PFC (Meaney, Aitken, Bodnoff, Iny, Tatarewicz, et al., 1985), which may contribute to the increased HPA negative feedback efficacy observed in handled offspring. In contrast, the amygdala provides excitatory inputs to the HPA axis (Myers et al., 2012). Not surprisingly, rats handled during infancy show a reduction in glucocorticoid receptor expression in the central nucleus of the amygdala (Fenoglio, Brunson, Avishai-Eliner, Chen, & Baram, 2004). Additionally, neonatal handling also increases amygdala expression of benzodiazepine receptors (Caldji, Francis, Sharma, Plotsky, & Meaney, 2000), and decreases levels of serotonin, dopamine, and noradrenaline (Arbroelius & Eklund, 2007). Moreover, unpublished results from Lucion's laboratory suggest that neonatal handling reduces the number of neurons in the medial nucleus of the amygdala. All of these neonatal handling-related changes in amygdala structure and function can influence how the amygdala responds to stress and, most importantly, highlights the amygdala's vulnerability to early-life environmental experiences.

NEONATAL HANDLING AND ANXIETY-LIKE BEHAVIOR

One of the most well-characterized effects of neonatal handling is a reduction in anxiety-like behavior—commonly interpreted as reduced emotionality (Kosten, Kim, & Lee, 2012; Levine et al., 1967; Meerlo et al., 1999; Núñez et al., 1995; Severino et al., 2004). Neonatal handled rats show increased exploration of the open field, including more time in the center (Caldji et al., 2000; Levine et al., 1967; Madruga, Xavier, Achaval, Sanvito, & Lucion, 2006; Padoin et al., 2001), and spend more time in the open arm of the elevated plus maze (Kiosterakis, Stamatakis, Diamantopoulou, Fameli, & Stylianopoulou, 2009; Meerlo et al., 1999; Núñez et al., 1995; Severino et al., 2004; Vallée et al., 1997). These results suggest that neonatal handled animals show decreased fear and/or anxiety, and possibly, an enhanced ability to cope with stressful events due to better adaptation to the environment (Chapillon, Patin, Roy, Vincent, & Caston, 2002; Levine, 1956). Nevertheless, reductions in anxiety-like behavior are not always observed (Silveira, Portella, Clemente, Gamaro, & Dalmaz, 2005; Stevenson, Meredith, Spicer, Mason, & Marsden, 2009). Neonatal handling also differentially affects anxiety-like behavior in males and females (Weinberg, Krahn, & Levine, 1978a). In a hole-board apparatus, handled females showed greater head-dipping than non-handled females when no objects were present under the holes; however, when objects were placed under the holes, differences between handled and non-handled animals were eliminated. For males, non-handled and handled rats were similar in exploratory

behavior when no objects were present, but when objects were placed under the holes, handled males explored more than non-handled males.

NEONATAL HANDLING AND LEARNING AND MEMORY

Early-life environmental manipulations such as neonatal handling have been shown to induce long-lasting alterations in learning and memory; however, these effects appear to be task-dependent. Neonatal handling enhances spatial learning/memory but impairs aversive learning/memory, with the former seen as a positive effect and the latter as a negative effect (Kosten et al., 2012).

The majority of studies on spatial learning indicate that neonatal handled animals show improved performance in the Morris water maze and other spatial memory tasks, such as the radial arm maze (Vallée et al., 1999) and T-maze (Wong & Judd, 1973). In the Morris water maze, handling decreases latency to reach the platform and reduces distance traveled to reach the platform (Escorihuela, Tobeña, & Fernández-Teruel, 1995; Fenoglio et al., 2005; Lehmann et al., 2002; Pryce, Bettschen, Nanz-Bahr, & Feldon, 2003; Stamatakis et al., 2008). Additionally, handled animals spend more time in the quadrant where the platform was located during the acquisition phase (Garoflos et al., 2005; Stamatakis et al., 2008). Importantly, the beneficial effects of neonatal handling are even more evident when the animal reaches old age, as the memory deficits that emerged with age in the non-handled rats are almost absent in the handled rats (Meaney et al., 1988), suggesting that neonatal handling may attenuate the decline in learning and memory that typically occurs with aging.

With regards to aversive learning, neonatal handling has been shown to impair the expression (Guijarro et al., 2007; Kosten, Lee, & Kim, 2006; Madruga et al., 2006; Meerlo et al., 1999) and extinction (Madruga et al., 2006; Wilber, Southwood, & Wellman, 2009) of conditioned fear responses in both cue and context learning, without altering sensitivity to footshock (Kosten et al., 2006). Furthermore, neonatal handling impairs learning in other aversive conditioning tasks, such as inhibitory avoidance (Kosten, Kim, & Lee, 2007), eyeblink conditioning (Wilber et al., 2007), and conditioned taste aversion (Weinberg et al., 1978b). Interestingly, in avoidance learning tasks, handling was shown to have a greater impact on males than on females (Weinberg & Levine, 1977). Females, regardless of neonatal handling treatment, showed lower or better modulated HPA responsiveness, whereas only handled males showed such adaptive responses.

CAN NEONATAL HANDLING RESCUE PREVIOUS INSULT?

Because of its behavioral and physiological effects, neonatal handling has been suggested as a candidate for use as a potential intervention for ameliorating the adverse effects of earlier insults, such as prenatal alcohol exposure, prenatal stress, and perinatal malnutrition.

Prenatal Alcohol Exposure

Exposure to alcohol during gestation is associated with a wide range of neural, behavioral, hormonal, and cognitive deficits in the offspring (Hellemans, Sliwowska, Verma, & Weinberg, 2010; Riley, Infante, & Warren, 2011). Neonatal handling is able to rescue

deficits in growth and to abolish hypothermic responses to alcohol challenge induced by prenatal alcohol exposure (Weinberg, Kim, & Yu, 1995). However, handling is ineffective at attenuating HPA hyperresponsiveness to restraint stress (Gabriel, Yu, Ellis, & Weinberg, 2000; Ogilvie & Rivier, 1997) or improving performance on a conditioned taste aversion task (Gabriel & Weinberg, 2001). Moreover, prenatal alcohol-induced deficits in the T-maze reversal task are completely eliminated in handled rats (Lee & Rabe, 1999). However, handling does not ameliorate spatial learning deficits on the Morris water maze (Gabriel, Johnston, & Weinberg, 2002), but rather, handling reveals or enhances differences in spatial navigation following prenatal alcohol exposure. Thus, while neonatal handling can ameliorate some deficits resulting from prenatal alcohol exposure, the effects of handling are limited and vary by task parameters (Hannigan, O'Leary-Moore, & Berman, 2007), and may be negative rather than positive in some instances.

How do we explain such paradoxical results of neonatal handling? One possibility is that the effects of handling may be dependent on numerous variables including pup ultrasonic vocalizations and level of maternal care (Bell, Nitschke, Gorry, & Zachma, 1971; Liu et al., 1997). Thus, neonatal handling might exert differential effects on pups whose responsiveness or behavior has already been altered by prenatal alcohol exposure. For example, because prenatal alcohol exposure decreases pup ultrasonic vocalizations (Kehoe & Shoemaker, 1991), alcohol-exposed pups may fail to elicit the increased licking and grooming by the dam associated with neonatal handling, and which may, at least in part, mediate handling effects. It is also possible that maternal behaviors may be affected by exposure to ethanol during pregnancy, although it appears that altered mother-infant interactions are due primarily to deficits in pups rather than deficits in maternal behavior (Chen, Driscoll, & Riley, 1982).

Prenatal Stress

Unlike the effects of neonatal handling on pups prenatally exposed to alcohol, handling has been shown to reverse the behavioral deficits induced by prenatal stress, including reduced activity in the open field and decreased time in the open arm of the elevated plus maze (Bogoch, Biala, Linial, & Weinstock, 2007; Wakshlak & Weinstock, 1990; Weinstock, 2008), as well as the deleterious effects of prenatal stress on hippocampal neurogenesis (Lemaire, Lamarque, Le Moal, Piazza, & Abrous, 2006). Moreover, ~30% of the hippocampal genes involved in synaptic function and suppressed in prenatally stressed rats are restored by neonatal handling (Bogoch et al., 2007).

Perinatal Malnutrition

Only a few studies have investigated the influence of neonatal handling on anxiety in rats raised under malnourished conditions. Two studies showed that handling was effective in rescuing the decreased open field exploratory activity of malnourished rats to levels observed in controls (Cines & Winick, 1979; Franková, 1968). Surprisingly, non-handled malnourished animals also showed more open field activity than their well-nourished counterparts, and neonatal handling abolished this difference by reducing malnourished animals' activity and increasing the activity of the well-nourished animals (Levitsky & Barnes, 1972). Despite these somewhat contradictory effects of perinatal malnutrition on

open field behavior, neonatal handling was able to eliminate behavioral differences between malnourished and well-nourished animals. However, neonatal handling did not change the increased reactivity to footshock (Rocha & Vendite, 1990) or the accelerated development and increased responsiveness of the stress system (Wiener & Levine, 1978) observed in malnourished rats. Conversely, non-handled malnourished pups exhibited normal basal corticosterone levels, whereas handled malnourished pups exhibited elevated basal corticosterone levels. Thus, neonatal handling appears to interact with perinatal malnutrition to alter the development of the HPA axis, and, similar to its effects on rats prenatally exposed to alcohol, neonatal handling ameliorated some but not all deficits resulting from perinatal malnutrition.

NEONATAL HANDLING AND SOCIAL BEHAVIOR

The quality of environmental experiences during infancy can modulate the development of social behavior and its neurocircuitry (Veenema, 2012). In rats, neonatal handling negatively affects the neurocircuitry that supports social behavior, leading to pervasive deficits in social behavior throughout the lifespan. These negative effects can be observed as early as PN7, with handled females, but not males, showing reduced preference for the maternal odor, indicating a possible deficit in the mother–pup relationship (Rainecki et al., 2009; Rainecki, Lutz, Sebben, Ribeiro, & Lucion, 2013). Peripubertal rats handled during infancy also show a reduction in play behavior with littermates in the home cage (Karkow & Lucion, 2013), but exhibit more play behavior than non-handled controls when tested following a period of social isolation (Aguilar, Caramés, & Espinet, 2009; Sivy & Harrison, 2008). Furthermore, handled rats show reduced play behavior and increased risk assessment behavior in the presence of a predator odor. However, when returned to the same environment where the predator odor had been experienced, handled rats are less likely to exhibit a conditioned suppression of play (Sivy & Harrison, 2008).

Adult rats handled during the neonatal period show reduced social investigation during the learning phase of a social learning test, although they are still able to differentiate between a novel and familiar social stimulus (Todeschin et al., 2009). Handling also decreases affiliative social behavior (e.g., allogrooming, sniffing) and increases non-affiliative behavior (e.g., aggression) during a social interaction test (Todeschin et al., 2009). Moreover, lactating rats that were handled in infancy show increases in aggressive behaviors against a male intruder (Giovenardi et al., 2005; Padoin et al., 2001), although not all studies reveal these changes (Boccia & Pedersen, 2001). Overall, these findings highlight the importance of the early-life environment for the development of behavioral systems that can modulate the way animals cope with social demands and opportunities.

The neuropeptides oxytocin and AVP have been widely implicated in the regulation of several features of social behavior, including social motivation (Lim & Young, 2006), social recognition (Engelmann, Ludwig, & Landgraft, 1994), maternal nurturing behavior (Ross & Young, 2009), and aggression (Gobrogge, Liu, Young, & Wang, 2009). Importantly, the development of the oxytocin and AVP systems can be altered by the early-life environment, including neonatal handling (Veenema, 2012). Neonatal handling appears to reduce the number of oxytocin-positive neurons in the PVN (Todeschin et al., 2009; Winkelmann-

Duarte et al., 2007) and amygdala (Oreland, Gustafsson-Ericson, & Nylander, 2010), but increases AVP-positive neurons in the PVN (Todeschin et al., 2009). Decreases in oxytocin neurons in the PVN and amygdala may explain, at least in part, some of the deficits in social behavior induced by neonatal handling, while increases in AVP neurons in the PVN may be associated with the increased aggression observed in neonatal handled rats. However, more studies are needed to determine whether causal relationships exist between these peptide hormone systems and deficits in social behavior associated with neonatal handling and to uncover mechanisms that support pathological forms of social behavior.

NEONATAL HANDLING AND REPRODUCTION

Environmental factors that influence early development can exert downstream effects on reproductive strategies and success, as well as on the growth and subsequent reproductive success of offspring (Bateson et al., 2004). Indeed, individuals exposed to adverse environmental experiences early in life may develop mechanisms to overcome the immediate threat; however, the cost of these alternative strategies can be later reproductive success. Even mild environmental experiences during infancy can negatively affect multiple aspects of the male and female reproductive systems in rats.

In females, the first sign of reproductive dysfunction following neonatal handling is delayed pubertal onset, as measured by vaginal opening (Sieck & Ramaley, 1975). In adulthood, neonatal handled rats show a reduction in the number of oocytes, despite exhibiting regular estrous cycles (Gomes, Frantz, Sanvitto, Anselmo-Franci, & Lucion, 1999; Raineke et al., 2008). Neuroendocrine regulation of ovulation requires a dynamic coordination of all elements of the hypothalamic–pituitary–gonadal (HPG) axis (Freeman, 2006). Specifically, the key neuroendocrine events necessary for ovulation involve the sequential surge of gonadotropin-releasing hormone (GnRH) from the hypothalamus, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) from the pituitary, and estradiol and progesterone from the gonads in the preovulatory period (Freeman, 2006). Neonatal handling disrupts many of these neuroendocrine processes: handled females show increased content of GnRH in the medial preoptic area on the afternoon of proestrus, followed by a reduction in plasma concentrations of LH, FSH, and estradiol (Gomes et al., 2005). Additionally, neonatal handling results in reductions in the size and number of neurons in the medial preoptic area (Cammozzato et al., 2009). Neonatal handling also reduces sexual behavior in female rats (Gomes et al., 2006; Raineke et al., 2008). Moreover, the progesterone surge following sexual behavior, which is necessary for blastocyst implantation and successful pregnancy (Adler, Resko, & Goy, 1970), is reduced in neonatal handled females (Gomes et al., 2006), presumably due to reduced copulatory behavior. Overall, these neuroendocrine changes may partially explain changes in sexual behavior and ovulation observed in neonatal handled female rats.

In males, neonatal handling also reduces sexual behavior, including a significant decrease in the frequency of mounts with intromission (Padoin et al., 2001), as well as deficits in the motivational aspects of sexual behavior, such as reduced time spent investigating the sexual partner (Raineke et al., 2013). Neonatal handling also induces morphophysiological changes in the male reproductive system, such as reduced testicular weight, smaller seminiferous

tubule diameter and decreased germinal epithelium thickness, all of which contribute to reductions in daily sperm production (Mazaro & Lamano-Carvalho, 2006).

NEONATAL HANDLING AND FOOD CONSUMPTION

Clinical and preclinical studies have demonstrated that early-life adversity can increase vulnerability to eating disorders and dysfunctional feeding behavior (Groleau et al., 2012). Despite being a mild early-life experience with many positive effects on behavioral and physiological function, neonatal handling can significantly alter feeding behavior in rats (McIntosh, Anisman, & Merali, 1999; Silveira et al., 2008). Animals handled early in life show increased consumption of palatable food, such as sweet and savory snacks (McIntosh et al., 1999; Silveira et al., 2008; Weinberg et al., 1978b), without alteration in standard lab chow ingestion (Silveira et al., 2004). Importantly, this handling-induced increase in consumption of sweet foods emerges only after puberty and is independent of satiety (Silveira et al., 2004, 2008). This ontogenetic pattern corroborates the clinical literature, which suggests that eating disorders like anorexia nervosa and bulimia emerge after puberty (Fairburn & Harrison, 2003). Furthermore, neonatal handling seems to affect only a few metabolic parameters: plasma levels of ghrelin and triglycerides are decreased, but there is no change in insulin, leptin, glucose, or cholesterol levels (Benetti et al., 2007; Silveira et al., 2006). These studies indicate that the neonatal handling model provides an approach for understanding mechanisms underlying the enduring consequences of early experience on the individual's responses to food.

NEONATAL HANDLING AND RENAL FUNCTION

Intriguingly, neonatal handled animals show decreased kidney function and altered hydroelectrolytic regulatory mechanisms. For example, neonatal handling appears to reduce kidney weight in the absence of morphological changes, reduces urinary volume, water intake, creatinine clearance, aldosterone and angiotensin II plasma concentrations, and increases sodium fraction excretion (Donadio et al., 2009). The renin–angiotensin system plays a critical role in the development of normal renal function (Guron & Friberg, 2000), and neonatal handling appears to increase mRNA expression of renin and angiotensin receptor type 2, and decrease mRNA expression of angiotensin receptor type 1 in the kidney (Rodriguez et al., 2012). Together, these results indicate that even mild manipulations like neonatal handling can have long-lasting, negative consequences for renal function.

SUMMARY AND CONCLUSIONS

The neonatal handling model has been extremely valuable for enhancing our understanding of the effects of early-life environmental experiences on developmental processes. Moreover, the data reviewed here question the general belief that handling during the early postnatal period has *only* beneficial consequences for the animal. While studies measuring emotionality, stress-related hormonal reactivity, and learning and memory suggest that handling enhances the ability to cope with stress, improves adaptation to the environment, and results in a more emotionally stable organism, studies evaluating other behavioral, physiological, and neural systems or using neonatal handling as a potential intervention

indicate that handling may not always be beneficial. Indeed, dysfunctional reproductive and renal function, altered social and feeding behavior, and deficits in aversive memories have been reported in animals handled during the early postnatal period. Even some of the presumably beneficial effects of neonatal handling require more nuanced interpretations. For example, the idea that neonatal handling results in an animal that is better adapted emotionally arise from studies demonstrating that handled rats show increased exploratory behavior in the open field, including more time in the center. However, as rodents are a prey species, an appropriate response to the open field would be to stay out of the center and stick to the periphery of the apparatus. In the words of Martin Daly, “It should be obvious that any small rodent who unhesitatingly enters a brightly lit novel environment is pathologically fearless” (1973). This “pathological fearlessness” becomes even more apparent in light of the observation that neonatal handled rats do not inhibit this increased exploratory behavior in the open field even in the presence of a predator such as a cat (Padoin et al., 2001).

This review highlights the importance of using caution when interpreting results from animal models of early-life experience as beneficial or negative. Indeed, our interpretations of the effects of neonatal handling presented here are by no means meant to be conclusive. Nevertheless, this model has provided us with a unique opportunity to understand how even small variations in the infant’s environment can have significant and enduring effects on neurobiological/-behavioral development.

Acknowledgments

Funding for research in the authors’ laboratories comes from NIH/NIAAA grants R37AA007789 and R01AA022460, and NeuroDevNet (Canadian Networks of Centers of Excellence) 20R64153 to J.W., Canadian Foundation on Fetal Alcohol Research (CFFAR) grant to C.R. and J.W., and CNPq, FAPESP, and CAPES to A.B.L.

REFERENCES

- Adler NT, Resko JA, Goy RW. The effects of copulatory behavior on hormonal change in the female prior to implantation. *Physiology and Behavior*. 1970; 5:1003–1007. [PubMed: 5522514]
- Aguilar R, Caramés JM, Espinet A. Effects of neonatal handling on playfulness by means of reversal of the desire to play in rats (*Rattus norvegicus*). *Journal of Comparative Psychology*. 2009; 123:347–356. [PubMed: 19929103]
- Andersen SL, Teicher MH. Stress, sensitive periods and maturational events in adolescent depression. *Trends in Neuroscience*. 2008; 31:183–191.
- Arbroelius L, Eklund MB. Both long and brief maternal separation precedes persistent changes in tissue levels of brain monoamines in middle-aged female rats. *Neuroscience*. 2007; 145:738–750. [PubMed: 17222517]
- Avishai-Eliner S, Eghabal-Ahmadi M, Tabachnik E, Brunson KL, Baram TZ. Down-regulation of the hypothalamic corticotropin-releasing hormone messenger ribonucleic acid (mRNA) precedes early-life experience induced changes in hippocampal glucocorticoid receptor mRNA. *Endocrinology*. 2001; 142:89–97. [PubMed: 11145570]
- Baldini S, Restani L, Baroncelli L, Coltelli M, Franco R, Cenni MC, Berardi N. Enriched early life experiences reduces adult anxiety-like behavior in rats: A role for insulin-like growth factor 1. *Journal of Neuroscience*. 2013; 33:11715–11723. [PubMed: 23843538]
- Bale TL, Baram TZ, Brown AS, Goldstein JM, Insel TR, McCarthy MM, Nestler EJ. Early life programming of neurodevelopmental disorders. *Biological Psychiatry*. 2010; 68:314–316. [PubMed: 20674602]
- Bateson P, Barker D, Clutton-Brock T, Deb D, D’Udine B, Foley RA, Sultan SE. Developmental plasticity and human health. *Nature*. 2004; 430:419–421. [PubMed: 15269759]

- Bell BC, Nitschke W, Gorry TH, Zachma T. Infantile stimulation and ultrasonic vocalization signaling: A possible mediator of early handling phenomena. *Developmental Psychobiology*. 1971; 4:181–191. [PubMed: 5162547]
- Benetti CS, Silveira PP, Portella AK, Diehl LA, Nunes E, Oliveira VS, Goldani MZ. Could preference for palatable foods in neonatally handled rats alter metabolic patterns in adult life? *Pediatric Research*. 2007; 62:405–411. [PubMed: 17667857]
- Boccia ML, Pedersen CA. Brief vs. long maternal separations in infancy: Contrasting relationships with adult maternal behavior and lactation levels of aggression and anxiety. *Psychoneuroendocrinology*. 2001; 26:657–672. [PubMed: 11500248]
- Bogoch Y, Biala YN, Linial M, Weinstock M. Anxiety induced by prenatal stress is associated with suppression of hippocampal genes involved in synaptic function. *Journal of Neurochemistry*. 2007; 101:1018–1030. [PubMed: 17254015]
- Bohn MC. Granule cell genesis in the hippocampus of rats treated neonatally with hydrocortisone. *Neuroscience*. 1980; 5:2003–2012. [PubMed: 7432634]
- Bowlby, J. *Attachment and loss*. Vol. 1. New York: Basic Books; 1969.
- Caldji C, Francis D, Sharma S, Plotsky PM, Meaney MJ. The effects of early rearing environment on the development of GABAA and central benzodiazepine receptor levels and novelty-induced fearfulness in the rat. *Neuropsychopharmacology*. 2000; 22:219–229. [PubMed: 10693149]
- Cammozzato TSC, Winckmann-Duarte EC, Padilha CB, Miguel SPR, Bonzanini L, Anselmo-Franci JA, Lucion AB. Neonatal handling reduces the number of cells in the medial preoptic area of female rats. *Brain Research*. 2009; 1247:92–99. [PubMed: 18977206]
- Chapillon P, Patin V, Roy V, Vincent A, Caston J. Effects of pre- and postnatal stimulation on developmental, emotional, and cognitive aspects in rodents: A review. *Developmental Psychobiology*. 2002; 41:373–387. [PubMed: 12430161]
- Chen J, Driscoll CD, Riley EP. Ontogeny of suckling behavior in rats prenatally exposed to alcohol. *Teratology*. 1982; 26:145–153. [PubMed: 7157191]
- Cines BM, Winick M. Behavioral and physiological effects of early handling and early malnutrition in rats. *Developmental Psychobiology*. 1979; 12:381–389. [PubMed: 456763]
- Cirulli F, Berry A, Alleva E. Early disruption of the mother-infant relationship: Effects on brain plasticity and implications for psychopathology. *Neuroscience and Biobehavioural Reviews*. 2003; 27:73–82.
- Daly M. Early stimulation of rodents: A critical review of present interpretations. *British Journal of Psychology*. 1973; 64:435–460. [PubMed: 4584330]
- Davidson RJ, McEwen BS. Social influences on neuroplasticity: Stress and interventions to promote well-being. *Nature Neuroscience*. 2012; 15:689–695. [PubMed: 22534579]
- de Kloet ER, Vreugdenhil E, Oitzl MS, Joëls M. Brain corticosteroid receptor balance in health and disease. *Endocrine Reviews*. 1998; 19:269–301. [PubMed: 9626555]
- Denenberg VH, Karas GG. Effects of differential handling upon weight gain and mortality in the rat and mouse. *Science*. 1959; 130:629–630. [PubMed: 13815895]
- Donadio MVF, Jacobs S, Corezola KL, Melo DAS, Dias HB, Reichel CL, Sanvitto GL. Neonatal handling reduces renal function in adult rats. *Kidney and Blood Pressure Research*. 2009; 32:286–292. [PubMed: 19797913]
- Engelmann M, Ludwig M, Landgraft R. Simultaneous monitoring of intracerebral release and behavior: Endogenous vasopressin improves social recognition. *Journal of Neuroendocrinology*. 1994; 6:391–395. [PubMed: 7987369]
- Erkine MS, Geller E, Yuwiler A. Effects of neonatal hydrocortisone treatment on pituitary and adrenocortical responses to stress in young rats. *Neuroendocrinology*. 1979; 29:191–199. [PubMed: 226903]
- Escorihuela RM, Tobeña A, Fernández-Teruel A. Environmental enrichment and postnatal handling prevent spatial learning deficits in aged hypoemotional (Roman high-avoidance) and hyperemotional (Roman low-avoidance) rats. *Learning and Memory*. 1995; 2:40–48. [PubMed: 10467565]
- Fairburn CG, Harrison PJ. Eating disorders. *The Lancet*. 2003; 361:407–416.

- Fenoglio KA, Brunson KL, Avishai-Eliner S, Chen Y, Baram TZ. Region-specific onset of handling-induced changes in corticotropin-releasing factor and glucocorticoid receptor expression. *Endocrinology*. 2004; 145:2702–2706. [PubMed: 15044366]
- Fenoglio KA, Brunson KL, Avishai-Eliner S, Stone BA, Kapadia BJ, Baram TZ. Enduring, handling-evoked enhancement of hippocampal memory function and glucocorticoid receptor expression involves activation of the corticotropin-releasing factor type 1 receptor. *Endocrinology*. 2005; 146:4090–4096. [PubMed: 15932935]
- Fox SE, Levitt P, Neslon CA III. How the timing and quality of early experiences influence the development of brain architecture. *Child Development*. 2010; 81:28–40. [PubMed: 20331653]
- Francis DD, Caldji C, Champagne F, Plotsky PM, Meaney MJ. The role of corticotropin-releasing factor-norepinephrine systems in mediating the effects of early experiences on the development of behavioral and endocrine responses to stress. *Biological Psychiatry*. 1999; 46:1153–1166. [PubMed: 10560022]
- Franková, S. Nutritional and psychological factors in the development of spontaneous behavior in the rat. In: Scrimshaw, NS.; Gordon, JE., editors. *Malnutrition. Learning and behavior*. Cambridge: MIT Press; 1968. p. 312-322.
- Freeman, ME. Neuroendocrine control of the ovarian cycle of the rat. In: Neill, JD., editor. *Knobil and Neill's physiology of reproduction*. Philadelphia: Elsevier; 2006. p. 2327-2388.
- Freud, S. *The problem of anxiety*. New York: Norton; 1936.
- Gabriel KI, Johnston S, Weinberg J. Prenatal ethanol exposure and spatial navigation: Effects of postnatal handling and aging. *Developmental Psychobiology*. 2002; 40:345–357. [PubMed: 12115293]
- Gabriel KI, Yu W, Ellis L, Weinberg J. Postnatal handling does not attenuate hypothalamic–pituitary–adrenal hyperresponsiveness after prenatal alcohol exposure. *Alcoholism: Clinical and Experimental Research*. 2000; 24:1566–1574.
- Gabriel KI, Weinberg J. Effects of prenatal ethanol exposure and postnatal handling on conditioned taste aversion. *Neurotoxicology and Teratology*. 2001; 23:167–176. [PubMed: 11348834]
- Garoflos E, Panagiotaropoulos T, Pondiki S, Stamatakis A, Philippidis E, Stylianopoulou F. Cellular mechanisms underlying the effects of an early experience on cognitive abilities and affective states. *Annals of General Psychiatry*. 2005; 4:8. [PubMed: 15876359]
- Giovenardi M, de Azevedo MS, da Silva SP, Hermel EES, Gomes CM, Lucion AB. Neonatal handling increases fear and aggression in lactating rats. *Physiology and Behavior*. 2005; 86:209–217. [PubMed: 16099482]
- Gobrogge KL, Liu Y, Young LJ, Wang Z. Anterior hypothalamic vasopressin regulates pair-bonding and drug-induced aggression in a monogamous rodent. *Proceedings of the National Academy of Sciences of the United States of America*. 2009; 106:19144–19149. [PubMed: 19858480]
- Gold PW, Chrousos GP. Organization of the stress system and its dysregulation in melancholic and atypical depression: High vs. low CRH/NE states. *Molecular Psychiatry*. 2007; 7:254–275. [PubMed: 11920153]
- Gomes CM, Donadio MVF, Anselmo-Franci JA, Franci CR, Lucion AB, Sanvitto GL. Neonatal handling induces alteration in progesterone secretion after sexual behavior but not in angiotensin II receptor density in the medial amygdala: Implication for reproductive success. *Life Science*. 2006; 78:2867–2871.
- Gomes CM, Frantz PJ, Sanvitto GL, Anselmo-Franci JA, Lucion AB. Neonatal handling induces anovulatory estrous cycles in rats. *Brazilian Journal of Medical and Biological Research*. 1999; 32:1239–1242. [PubMed: 10510261]
- Gomes CM, Raineki C, Ramos de Paula P, Severino GS, Helena CVV, Anselmo-Franci JA, Lucion AB. Neonatal handling and reproductive function in female rats. *Journal of Endocrinology*. 2005; 184:435–445. [PubMed: 15684351]
- Groleau P, Steiger H, Bruce K, Israel M, Sycz L, Ouellette AS, Badawi G. Childhood emotional abuse and eating symptoms in bulimic disorders: An examination of possible mediating variables. *International Journal of Eating Disorders*. 2012; 45:326–332. [PubMed: 21656539]

- Levine S. The ontogeny of the hypothalamic–pituitary–adrenal axis: The influence of maternal care. *Annals of the New York Academy of Sciences*. 1994; 746:275–288. [PubMed: 7825883]
- Levine S, Chevalier JA, Korchin SJ. The effects of early shock and handling in later avoidance learning. *Journal of Personality*. 1956; 24:475–493. [PubMed: 13320272]
- Levine S, Haltmeyer GC, Karas GG, Denenberg VH. Physiological and behavioral effects of infantile stimulation. *Physiology and Behavior*. 1967; 2:55–59.
- Levitsky DA, Barnes RH. Nutritional and environmental interactions in the behavioral development of the rat: Long-term effects. *Science*. 1972; 176:68–71. [PubMed: 5062235]
- Lim MM, Young LJ. Neuropeptidergic regulation of affiliative behavior and social bonding in animals. *Hormones and Behavior*. 2006; 50:506–517. [PubMed: 16890230]
- Liu D, Caldji C, Sharma S, Plostky PM, Meaney MJ. Influence of neonatal rearing conditions on stress-induced adrenocorticotropin responses and norepinephrine release in the hypothalamic paraventricular nucleus. *Journal of Neuroendocrinology*. 2000; 12:5–12. [PubMed: 10692138]
- Liu D, Diorio J, Tannenbaum B, Caldji C, Francis D, Freedman A, Meaney MJ. Maternal care, hippocampal glucocorticoid receptors and hypothalamic–pituitary–adrenal responses to stress. *Science*. 1997; 277:1659–1662. [PubMed: 9287218]
- Lucion AB, Pereira FM, Winkelman EC, Sanvito GL, Anselmo-Franci JA. Neonatal handling reduces the number of cells in the locus coeruleus of rats. *Behavioral Neuroscience*. 2003; 117:894–903. [PubMed: 14570540]
- Macrì S, Würbel H. Developmental plasticity of HPA and fear responses in rats: A critical review of the maternal mediation hypothesis. *Hormones and Behavior*. 2006; 50:667–680. [PubMed: 16890940]
- Madruga C, Xavier LL, Achaval M, Sanvito GL, Lucion AB. Early handling, but not maternal separation, decreases emotional responses in two paradigms of fear without changes in mesolimbic dopamine. *Behavioral Brain Research*. 2006; 166:241–246.
- Mazaro R, Lamano-Carvalho TL. Prolonged deleterious effects of neonatal handling on reproductive parameters of pubertal male rats. *Reproduction, Fertility and Development*. 2006; 18:497–500.
- McEwen BS. Understanding the potency of stressful early life experiences on brain and body function. *Metabolism Clinical and Experimental*. 2008; 57:S11–S15. [PubMed: 18803958]
- McIntosh J, Anisman H, Merali Z. Short- and long-periods of neonatal maternal separation differentially affects anxiety and feeding in adult rats: Gender-dependent effects. *Developmental Brain Research*. 1999; 113:97–106. [PubMed: 10064879]
- Meaney MJ, Aitken DH. The effects of early postnatal handling on hippocampal glucocorticoid receptor concentrations: Temporal parameters. *Developmental Brain Research*. 1985; 22:301–304.
- Meaney MJ, Aitken DH, Bodnoff SR, Iny LJ, Sapolsky RM. The effects of postnatal handling on the development of the glucocorticoid receptor systems and stress recovery in the rat. *Progress in Neuropsychopharmacology and Biological Psychiatry*. 1985; 9:731–734.
- Meaney MJ, Aitken DH, Bodnoff SR, Iny LJ, Tatarewicz JE, Sapolsky RM. Early postnatal handling alters glucocorticoid receptor concentrations in selected brain regions. *Behavioral Neuroscience*. 1985; 99:765–770. [PubMed: 3843740]
- Meaney MJ, Aitken DH, van Berkel C, Bhatnagar S, Sapolsky RM. Effect of neonatal handling on age-related impairments associated with the hippocampus. *Science*. 1988; 239:766–768. [PubMed: 3340858]
- Meaney MJ, Aitken DH, Viau V, Sharma S, Sarrieau A. Neonatal handling alters adrenocortical negative feedback sensitivity and hippocampal type II glucocorticoid receptor binding in the rat. *Neuroendocrinology*. 1989; 50:597–604. [PubMed: 2558328]
- Meaney MJ, Bhatnagar S, Larocque S, McCormick C, Shanks N, Sharma S, Plotsky PM. Individual differences in the hypothalamic–pituitary–adrenal stress response and the hypothalamic CRF system. *Annals of the New York Academy of Sciences*. 1993; 697:70–85. [PubMed: 8257024]
- Meerlo P, Horvath KM, Nagy GM, Bohus B, Koolhaas JM. The influence of postnatal handling on adult neuroendocrine and behavioural stress reactivity. *Journal of Neuroendocrinology*. 1999; 12:925–933. [PubMed: 10583727]
- Myers B, McKlveen JM, Herman JP. Neural regulation of the stress response: The many faces of feedback. *Cellular and Molecular Neurobiology*. 2012; 32:683–694.

- Núñez JF, Ferré P, García E, Escorihuela RM, Fernández-Teruel A, Tobeña A. Postnatal handling reduces emotionality ratings and accelerates two-way active avoidance in female rats. *Physiology and Behavior*. 1995; 57:831–835. [PubMed: 7610131]
- Ogilvie KM, Rivier C. Prenatal alcohol exposure results in hyperactivity of the hypothalamic–pituitary–adrenal axis of the offspring: Modulation by fostering at birth and postnatal handling. *Alcoholism: Clinical and Experimental Research*. 1997; 21:424–429.
- Oreland S, Gustafsson-Ericson L, Nylander I. Short- and long-term consequences of different early environmental conditions on central immunoreactive oxytocin and arginine vasopressin levels in male rats. *Neuropeptides*. 2010; 44:391–398. [PubMed: 20591479]
- Padoin MJ, Cadore LP, Gomes CM, Barros HMT, Lucion AB. Long-lasting effects of neonatal handling stimulation in the behavior of rats. *Behavioral Neuroscience*. 2001; 115:1332–1340. [PubMed: 11770063]
- Plotsky PM, Meaney MJ. Early, postnatal experience alters hypothalamic corticotropin-releasing factor (CRF) mRNA, median eminence CRF content and stress-induced release in adult rats. *Molecular Brain Research*. 1993; 18:195–200. [PubMed: 8497182]
- Plotsky PM, Thrivikraman KV, Nemeroff CB, Caldji C, Sharma S, Meaney MJ. Long-term consequences of neonatal rearing on central corticotropin-releasing factor systems in adult male rat offspring. *Neuropsychopharmacology*. 2005; 30:2192–2204. [PubMed: 15920504]
- Pryce CR, Bettschen D, Feldon J. Comparison of the effects of early handling and early deprivation on maternal care in the rat. *Developmental Psychobiology*. 2001; 38:239–251. [PubMed: 11319730]
- Pryce CR, Bettschen D, Nanz-Bahr NI, Feldon J. Comparison of the effects of early handling and early deprivation on conditioned stimulus, context, and spatial learning and memory in adult rats. *Behavioral Neuroscience*. 2003; 117:883–893. [PubMed: 14570539]
- Pryce CR, Feldon J. Long-term neurobehavioral impact of the postnatal environment in rats: Manipulations, effects and mediating mechanisms. *Neuroscience and Biobehavioral Reviews*. 2003; 27:57–71. [PubMed: 12732223]
- Rainecki C, Cortés MR, Belnoue L, Sullivan RM. Effects of early-life abuse differ across development: Infant social behavior deficits are followed by adolescent depressive-like behaviors mediated by the amygdala. *Journal of Neuroscience*. 2012; 32:7758–7765. [PubMed: 22649253]
- Rainecki C, Lutz ML, Sebben V, Ribeiro RA, Lucion AB. Neonatal handling induces deficits in infant mother preference and adult partner preference. *Developmental Psychobiology*. 2013; 55:496–507. [PubMed: 22644994]
- Rainecki C, Souza MA, Szawka RE, Lutz ML, Vasconcellos LFT, Sanvitto GL, Lucion AB. Neonatal handling and maternal odor preference in rat pups: Involvement of monoamines and cyclic AMP response element-binding protein pathway in the olfactory bulb. *Neuroscience*. 2009; 159:31–38. [PubMed: 19138731]
- Rainecki C, Szawka RE, Gomes CM, Lucion MK, Barp J, Bello-Klein A, Lucion AB. Effects of neonatal handling on central noradrenergic and nitric oxidergic systems and reproductive parameters in female rats. *Neuroendocrinology*. 2008; 87:151–159. [PubMed: 18057864]
- Riley EP, Infante MA, Warren KR. Fetal alcohol spectrum disorders: An overview. *Neuropsychology Review*. 2011; 21:73–80. [PubMed: 21499711]
- Rocha JBT, Vendite D. Effects of undernutrition and handling during suckling on shuttle avoidance and footshock escape behavior and on plasma glucose levels of young rats. *Developmental Psychobiology*. 1990; 23:157–168. [PubMed: 2114330]
- Rodriguez DL, Mesquita FC, Attolini D, Borba BS, Scherer PS, Almeida PH, Donadio MVF. Evaluation of the brain and kidney renin–angiotensin system and oxidative stress in neonatal handled rats. *Developmental Psychobiology*. 2012; 54:706–713. [PubMed: 22127847]
- Ross HE, Young LJ. Oxytocin and neural mechanisms regulating social cognition and affiliative behavior. *Frontiers in Neuroendocrinology*. 2009; 30:534–547. [PubMed: 19481567]
- Roth TL, Sweatt JD. Annual research review: Epigenetic mechanisms and environmental shaping of the brain during sensitive periods of development. *Journal of Child Psychology and Psychiatry*. 2011; 52:398–408. [PubMed: 20626526]
- Sapolsky RM, Meaney MJ. Maturation of the adrenocortical stress response: Neuroendocrine control mechanisms and the stress hyporesponsive period. *Brain Research Reviews*. 1986; 11:65–76.

- Sapolsky RM, Romero LM, Munck AU. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews*. 2000; 21:55–89. [PubMed: 10696570]
- Severino GS, Fossati IAM, Padoin MJ, Gomes CM, Trevisan L, Sanvitto GL, Lucion AB. Effects of neonatal handling on the behavior and prolactin stress response in male and female rats at various ages and estrous cycle phases of females. *Physiology and Behavior*. 2004; 81:489–498. [PubMed: 15135021]
- Sieck G, Ramaley JA. Effects of early handling upon puberty: Correlations with adrenal stress responsiveness. *Physiology and Behavior*. 1975; 15:487–489. [PubMed: 1221456]
- Silveria PP, Benetti CS, Ayres C, Pederiva FQ, Portella AK, Lucion AB, Dalmaz C. Satiety assessment in neonatally handled rats. *Behavioural Brain Research*. 2006; 173:205–210. [PubMed: 16889839]
- Silveira PP, Portella AK, Clementte Z, Bassani E, Tabajara AS, Gamaro GD, Dalmaz C. Neonatal handling alters feeding behavior of adult rats. *Physiology and Behavior*. 2004; 80:739–745. [PubMed: 14984809]
- Silveira PP, Portella AK, Clemente Z, Gamaro GD, Dalmaz C. The effect of neonatal handling on adult feeding behavior is not an anxiety-like behavior. *International Journal of Developmental Neuroscience*. 2005; 23:93–99. [PubMed: 15730891]
- Silveira PP, Portella AK, Crema L, Correa M, Nieto FB, Diehl L, Dalmaz C. Both infantile stimulation and exposure to sweet food lead to an increased sweet food ingestion in adult life. *Physiology and Behavior*. 2008; 93:877–882. [PubMed: 18191962]
- Siviy SM, Harrison KA. Effects of neonatal handling on play behavior and fear towards a predator odor in juvenile rats (*Rattus norvegicus*). *Journal of Comparative Psychology*. 2008; 122:1–8. [PubMed: 18298275]
- Stamatakis A, Pondiki S, Kitraki E, Diamantopoulou A, Panagiotaropoulos T, Raftogianni A, Stylianopoulou F. Effects of neonatal handling on adult rats spatial learning and memory following acute stress. *Stress*. 2008; 11:148–159. [PubMed: 18311603]
- Stanton ME, Gutierrez YR, Levine S. Maternal deprivation potentiates pituitary–adrenal stress responses in infant rats. *Behavioral Neuroscience*. 1988; 102:692–700. [PubMed: 3196438]
- Stevenson CW, Meredith JP, Spicer CH, Mason R, Marsden CA. Early life programming of innate fear and fear learning in adult female rats. *Behavioral Brain Research*. 2009; 198:51–57.
- Suchecki D, Nelson DY, van Oers HJJ, Levine S. Activation and inhibition of the hypothalamic–pituitary–adrenal axis of the neonatal rat: Effects of maternal deprivation. *Psychoneuroendocrinology*. 1995; 20:169–182. [PubMed: 7899536]
- Suchecki D, Rosenfeld P, Levine S. Maternal regulation of the hypothalamic–pituitary–adrenal axis in the infant rat: The roles of feeding and stroking. *Developmental Brain Research*. 1993; 75:185–192. [PubMed: 8261610]
- Swinny JD, O’Farrel E, Bingham BC, Piel SA, Valentino RJ, Beck SG. Neonatal rearing conditions distinctly shape locus coeruleus neural activity, dendritic arborization, and sensitivity to corticotropin-releasing factor. *International Journal of Neuropsychopharmacology*. 2010; 13:515–525. [PubMed: 19653930]
- Todeschin AS, Winkelmann-Duarte EC, Jacob MHV, Aranda BCC, Jacobs S, Fernandes MC, Lucion AB. Effects of neonatal handling on social memory, social interaction, and number of oxytocin and vasopressin neurons in rats. *Hormones and Behavior*. 2009; 56:93–100. [PubMed: 19324045]
- Vallée M, Maccari S, Dellu F, Simon H, Le Moal M, Mayo W. Long-term effects of prenatal stress and postnatal handling on age-related glucocorticoid secretion and cognitive performance: A longitudinal study in the rat. *European Journal of Neuroscience*. 1999; 11:2906–2916. [PubMed: 10457187]
- Vallée M, Mayo W, Dellu F, Le Moal M, Simon H, Maccari S. Prenatal stress induces high anxiety and postnatal handling induces low anxiety in adult offspring: Correlation with stress-induced corticosterone secretion. *Journal of Neuroscience*. 1997; 17:2626–2636. [PubMed: 9065522]
- Veenema AH. Toward understanding how early-life social experiences alter oxytocin- and vasopressin-regulated social behaviors. *Hormones and Behavior*. 2012; 61:304–312. [PubMed: 22197269]

- Viau V, Sharma S, Plotsky PM, Meaney MJ. Increased plasma ACTH response to stress in nonhandled compared to handled rats require basal levels of corticosterone and are associated with increased levels of ACTH secretagogues in the median eminence. *Journal of Neuroscience*. 1993; 13:1097–1105. [PubMed: 8382733]
- Wakshlak A, Weinstock M. Neonatal handling reverses behavioral abnormalities induced in rats by prenatal stress. *Physiology and Behavior*. 1990; 48:289–292. [PubMed: 2255733]
- Walker CD, Perrin M, Vale W, Rivier C. Ontogeny of the stress response in the rat: Role of the pituitary and the hypothalamus. *Endocrinology*. 1986; 118:1445–1451. [PubMed: 3004915]
- Weinberg J, Kim CK, Yu W. Early handling can attenuate adverse effects of fetal ethanol exposure. *Alcohol*. 1995; 12:317–327. [PubMed: 7546327]
- Weinberg J, Krahn EA, Levine S. Differential effects of handling on exploration in male and female rats. *Developmental Psychobiology*. 1978a; 11:251–259. [PubMed: 658605]
- Weinberg J, Levine S. Early handling influences on behavioral and physiological responses during active avoidance. *Developmental Psychobiology*. 1977; 10:161–169. [PubMed: 838160]
- Weinberg J, Smotherman WP, Levine S. Early handling effects on neophobia and conditioned taste aversion. *Physiology and Behavior*. 1978b; 20:589–596. [PubMed: 567356]
- Weinstock M. The long-term behavioural consequences of prenatal stress. *Neuroscience and Biobehavioral Reviews*. 2008; 32:1073–1086. [PubMed: 18423592]
- Wiener SG, Levine S. Perinatal malnutrition and early handling: Interactive effects on the development of the pituitary–adrenal system. *Developmental Psychobiology*. 1978; 11:335–352. [PubMed: 566686]
- Wilber AA, Southwood CJ, Sokoloff G, Steinmetz JE, Wellman CL. Neonatal maternal separation alters adult eyeblink conditioning and glucocorticoid receptor expression in the interpositus nucleus of the cerebellum. *Developmental Neurobiology*. 2007; 67:1751–1764. [PubMed: 17659594]
- Wilber AA, Southwood CJ, Wellman CL. Brief neonatal maternal separation alters extinction of conditioned fear and corticolimbic glucocorticoid and NMDA receptor expression in adult rats. *Developmental Neurobiology*. 2009; 69:73–87. [PubMed: 19025931]
- Winkelmann-Duarte EC, Padilha-Hoffman CB, Martins DF, Shurh AFS, Fernandes MC, Santin R, Lucion AB. Early-life environmental intervention may increase the number of neurons, astrocytes, and cellular proliferation in the hippocampus of rats. *Experimental Brain Research*. 2011; 215:163–172. [PubMed: 21969209]
- Winkelmann-Duarte EC, Todeschin AS, Fernandes MC, Bittencout LC, Pereira GAM, Samios VN, Lucion AB. Plastic changes induced by neonatal handling in the hypothalamus of female rats. *Brain Research*. 2007; 1170:20–30. [PubMed: 17692831]
- Wong R, Judd M. Infantile handling and successive spatial reversal learning in rats. *Behavioral Biology*. 1973; 8:391–397. [PubMed: 4705981]