Social Buffering by Unfamiliar Adult Males in Preweaning Guinea Pigs (*Cavia porcellus*): The Effects on HPA Activity and Fos Induction in the Medial Prefrontal Cortex

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SOCIAL BUFFERING BY UNFAMILIAR ADULT MALES IN PREWEANING GUINEA PIGS (CAVIA POCELLUS): THE EFFECTS ON HPA ACTIVITY AND FOS INDUCTION IN THE MEDIAL PREFRONTAL CORTEX

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science

By

WITHAYAPON WATANASRIYAKUL
B.S., Wright State University, 2013

2016
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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Withayapon Watanasriyakul ENTITLED Social Buffering By Unfamiliar Adult Males In Preweaning Guinea Pigs: The Effects On HPA Activity And Fos Induction In The Medial Prefrontal Cortex BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science.

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ABSTRACT

Watanasriyakul, Withayapon. M.S. Department of Neuroscience, Cell Biology, and Physiology, Wright State University, 2016. Social Buffering By Unfamiliar Adult Males In Preweaning Guinea Pigs: The Effects On HPA Activity And Fos Induction In The Medial Prefrontal Cortex

Social buffering, a phenomenon in which the presence of a social partner can reduce stress responses, is often most effective between strongly attached partners. Our laboratory previously found a surprising buffering effect of the hypothalamic-pituitary-adrenal (HPA) response in preweaning guinea pigs by unfamiliar adult males. It was hypothesized that this HPA-buffering effect was driven by social interactions between the two partners and may involve an activation of the prelimbic cortex. Therefore, the current study examined these potential associations. To limit social interactions, the adult male was anesthetized in one condition compared to another condition where the adult male remained conscious. Conscious males, but not unconscious males, significantly reduced cortisol levels, suppressed vocalizations, and increased Fos activity in the medial prefrontal cortex (mPFC). In conclusion, unfamiliar adult males can buffer HPA responses in preweaning guinea pigs via social interactions, which may involve an activation of the mPFC to suppress HPA activity.
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I. INTRODUCTION

The hypothalamic-pituitary-adrenal (HPA) axis, the primary neuroendocrine stress-response system, becomes activated when encountering physical and psychological stressors. The cascade begins with the activation of the paraventricular nucleus (PVN) of the hypothalamus, in which the neurons secrete corticotropin-releasing hormone (CRH). CRH then triggers the anterior pituitary gland to release adrenocorticotropic hormone (ACTH) into the general circulation. When ACTH reaches the adrenal cortex, it stimulates the release of cortisol (corticosterone in some species), which is distributed throughout the body. The response is rapid and adaptive since cortisol increases energy to muscles, enhances cardiovascular functions, sharpens cognition, and maximizes cerebral glucose utilization (Sapolsky, Romero, & Munck, 2000). When cortisol travels back to the central nervous system (e.g., cortex, hippocampus, amygdala), it stops the production of CRH and ACTH at the PVN and the pituitary, respectively, as a negative feedback. Therefore, in the absence of continued stress exposure, these effects gradually diminish as the cortisol levels slowly decrease due to negative feedback and metabolic clearance, leading to stress recovery (Cone, Low, Elmquist, & Cameron, 2003). Unfortunately, prolonged exposure to stress and continued high levels of cortisol have been associated with many neuropsychological disorders such as depression, post-traumatic stress disorder, and anxiety (Coplan et al., 1996; Gold, Goodwin, & Chrousos, 1988; Kathol, Jaeckle, Lopez, & Meller, 1989; McEwen, 1998).
There are many methods by which stress and its negative effects can be attenuated, such as pharmacological treatment and cognitive behavioral therapy (Coplan, Gopinath, Abdallah, & Berry, 2014; Fernie, Kollmann, & Brown, 2015; Levinstein & Samuels, 2014; Steenkamp, Litz, Hoge, & Marmar, 2015). Interestingly, social animals appear to utilize their social partners as a coping mechanism to reduce stress responses; this is called social buffering. This phenomenon can be seen across social mammalian species, including humans, non-human primates, and rodents (Hennessy, Kaiser, & Sachser, 2009; Hostinar, Sullivan, & Gunnar, 2013). In addition, there is selectivity in social buffering, as it appears to be most effective between strongly attached individuals such as pair-bonded partners and mothers and infants (Ditzen & Heinrichs, 2014; Gunnar et al., 1992; Hennessy, Hornschuh, Kaiser, & Sachser, 2006; Hennessy, O’Leary, Hawke, & Wilson, 2002; Hoffman, Mendoza, Hennessy, & Mason, 1995; Kirschbaum, Klauer, Filipp, & Hellhammer, 1995; Remage-Healey, Adkins-Regan, & Romero, 2003; Wiener, Johnson, & Levine, 1987).

Therefore, in this introduction section, I will review previous findings on social buffering between pair-bonded partners as well as between mothers and infants across multiple mammalian species particularly humans, non-human primates, and rodents. I will then focus on social buffering and its proposed neural mechanism in guinea pigs. Lastly, I will provide our rationale and hypotheses for the current study.

**Social Buffering Between Pair-Bonded Partners**

*Humans*
In the Trier Social Stress Test (TSST), a commonly used tool to elicit stress responses in humans, the subjects are asked to perform a speech in front of a panel of strangers (i.e., mock job interview). In men, verbal support from their female romantic partners prior to TSST significantly reduced saliva cortisol levels compared to support from strangers or no support (Kirschbaum et al., 1995). In women, receiving neck and shoulder massages from their male romantic partners prior to TSST significantly reduced saliva cortisol levels as well as lowered heart rate compared to no support (Ditzen et al., 2007). Additionally, couples who demonstrated positive social interactions (i.e., displaying empathy, hugging, kissing) after TSST showed faster cortisol recovery time than couples with negative social interactions (i.e., being insensitive, hostile, or distant; Meuwly et al., 2012). These studies illustrate selective social buffering between human couples, as support from strangers was unable to reduce the adrenocortical response along with cardiovascular reactivity triggered by TSST. Furthermore, the type of social interactions (i.e., positive) between couples may mediate HPA reactivity as well.

*Non-Human Primates*

In animal research, isolation in novel surroundings is a commonly used procedure to elicit an HPA response. Titi monkeys (*Callicebus cupreus*) are New World primates; they pair bond, are territorial, and typically live with their immature offspring (Mason, 1966; Mason, 1971). When adults living in family groups were isolated in a novel environment for 49 hr, their plasma cortisol levels were significantly elevated; however, the presence of a pair mate in the novel environment attenuated this response (Hennessy,
Mendoza, Mason, & Moberg, 1995). Similarly, black tufted-ear marmosets (Callithrix kuhli), another New World primate, also show a strong, long-term, sociosexual pair bond between male and female (Evans, 1983). Isolation of adult males and females in a novel environment for 48 hr significantly increased urinary cortisol levels whereas the presence of the opposite-sex partner reduced the HPA response during novelty exposure (Smith, McGreer-Whitworth, & French, 1998). In contrast, squirrel monkeys (Saimiri sciureus) are also New World primates, but they are polygamous (Baldwin, 1985; Boinski, 1987). Plasma cortisol concentrations of adults also significantly increased at 1, 25, and 49 hr during novelty exposure while alone. However, the presence of an opposite sex cage mate did not buffer HPA activity (Hennessy et al., 1995a). These studies in New World primates exemplify the importance of strongly attached bonds in social buffering. Titi monkeys and black tufted-ear marmosets form a strong attachment with their partners, and the partner’s presence has been shown to reduce the cortisol response during a stressful situation. Conversely, squirrel monkeys do not form a strong attachment to their mates, and the mate’s presence has not been shown to buffer HPA activity.

**Rodents**

In rodents, attachment between mates has been studied extensively in prairie voles (Microtus ochrogaster), a socially monogamous species in which long-term pair bonds are formed between partners (Wang & Aragona, 2004). McNeal et al. (2014) examined adrenocortical buffering following a 5-day separation period in pair-bonded prairie voles. The animals underwent a Forced Swim Test (FST) after the separation to examine
depressive-like behavior (i.e., floating, immobility). The experimenters observed that isolated voles had significantly longer immobility time, and their heart rate was also higher during FST compared to non-separated voles. In addition, separated animals had significantly elevated plasma corticosterone and ACTH concentrations after FST in comparison to non-separated animals. The presence of a pair-bonded partner may affect the HPA recovery period as well. Smith and Wang (2013) restrained female prairie voles in a plastic tube for 1 hr. The animals were allowed to recover for 30 min after the test either alone or with a male partner before trunk blood was collected for corticosterone analysis. The investigators found that female prairie voles that recovered alone had significantly higher levels of corticosterone as compared to animals that were not restrained. In contrast, female voles that were allowed to recover with their partners showed a buffering of HPA activity, and corticosterone levels were not significantly different than those of the controls. These studies provide further evidence demonstrating the ability of a strongly attached partner to reduce the adrenocortical response.

**Social Buffering between Mothers-Infants**

For mammals, the mother provides not only milk but also other specific survival needs for her offspring including warmth and protection. Furthermore, the mother-infant bond is critical for the infant’s physiological as well as social development (Meyer, Novak, Bowman, & Harlow, 1975). Because the mother-infant relationship is established early on and is fundamental to the infant’s survival, one may predict the mother would also buffer HPA activity of her young.
Humans

Studies in children have examined the effects of the presence or absence of the mother in a novel environment. When mothers left their 9-month-old infants with a female experimenter for 30 min, the babies’ salivary cortisol levels significantly elevated compared to infants who were not separated from their mothers (Larson, Gunnar, and Hertsgaard, 1991). In a similar study, the effects of transitioning to day care were investigated in 15-month-old children. Compared to when tested at home, salivary cortisol concentrations significantly increased on the first day of day care, and this rise of adrenocortical reactivity lasted up to 9 days (Ahnert, Gunnar, Lamb, & Barthel, 2004). Following up on their previous studies, Gunnar et al. (1992) investigated the effects of play behavior during maternal separation in 9-month-old babies. The subjects were assigned to 4 conditions: maternal separation-passive, no maternal separation-passive, maternal separation-play, and no maternal separation-play. In the passive conditions, the infants were allowed to play by themselves while a female experimenter or the mother read a book nearby for 30 min. In the play conditions, the experimenter or the mother engaged the infants to play with toys for the same length of time. Saliva samples were collected before and after the experiment for cortisol analysis. The experimenters found that babies who were separated from their mother and were allowed to play passively by themselves had a significant increase in cortisol levels compared to children in the other conditions. These studies illustrate that maternal separation can activate the adrenocortical response in 9 and 15-month-old human infants. There is also selectivity in
maternal buffering since the presence of a female experimenter or day care staff did not buffer the adrenocortical response; however, play behavior may be able to mediate HPA activity during the separation.

*Non-Human Primates*

HPA buffering in mothers and infants has been studied in many non-human primate species. For example, in squirrel monkeys, mother and infants exhibit evidence of strong emotional bonds (Baldwin, 1969; Baldwin, 1985). Isolation of 3-month-old infants in a novel cage for 30 min significantly elevated their plasma cortisol levels compared to non-separated infants. The mothers’ plasma cortisol levels also increased during the time of separation, suggesting that the separation affects the mother’s HPA axis as well (Mendoza et al., 1978). Stanton and Levine (1984) demonstrated that even a brief separation of 5 min significantly increased the adrenocortical response in both the mother and the infant squirrel monkeys. Infant squirrel monkeys raised on surrogate mothers also show signs of attachment to their surrogate mothers; the infants recognize and prefer their own artificial surrogate mothers to other surrogates (Kaplan, Cubicciotti, & Redican, 1979). The presence of an artificial mother has been shown to buffer the cortisol response in preweaning squirrel monkeys. For instance, isolation of 5-month-old squirrel monkeys in a novel cage for 4 hr resulted in a significant increase of cortisol levels whereas the presence of the artificial mother (a plastic cylinder covered with soft fabric) reduced this HPA response (Hennessy & Kaplan, 1982).
For titi monkeys, the father plays a more active role in infant care-taking; in turn, the infant is more attached to the father (Mendoza & Mason, 1986b). Following disturbances such as handling and capture, isolated preweaning titi monkeys exhibited a significant elevation of plasma cortisol whereas the presence of the father and the mother buffered this HPA reactivity. The presence of only the mother reduced the infant’s cortisol levels elicited by the disturbance, but not to the same level as when both parents were in the cage. Interestingly, the presence of only the father reduced the infant’s adrenocortical response to the same level as when both parents were present (Hoffman et al., 1995). Therefore, it appears that the ability to socially buffer HPA activity in non-human primate infants may be species-specific and depend on the strength of the relationship between the care-giver and the infant.

**Rodents**

In rats (*Rattus norvegicus*), pups recognize their mother and are attracted to her by maternal odor, which is determined by the mother’s diet (Leon, 1992; Raineki et al., 2010). Thus, pups appear unable to distinguish their biological mother from another lactating female fed the same diet as the mother. Therefore, it is not uncommon for maternal buffering studies in rats to use other lactating females as a substitute for the biological mother. For instance, when preweaning rats underwent tail shocks, the presence of an unrelated, anesthetized, lactating female significantly lowered plasma corticosterone levels compared to pups that received shocks while alone (Shionoya, Moriceau, Bradstock, & Sullivan, 2007). In contrast, the presence of non-lactating
females has not been shown to reduce HPA reactivity in preweaning rats. Hennessy and Weinberg (1990) found that the presence of the biological mother significantly lowered the pup’s adrenocortical response to novelty exposure whereas the presence of an unfamiliar non-lactating female did not. In summary, the biological mother and lactating females have been observed to reduce cortisol response in rat pups, suggesting that maternal odor from lactation is critical for HPA buffering.

Social Buffering in Guinea Pigs

Although social buffering has been observed in rodent species such as prairie voles and rats, the topic has been studied most extensively in guinea pigs (Cavia porcellus). Similar to non-human primates, guinea pigs exhibit neuroendocrine and behavioral stress responses during mate/maternal separation (Hennessy et al., 2006a; Hennessy & Moorman, 1989; Hennessy, Tamborski, Schiml, & Lucot, 1989; Hennessy, Zate, & Maken, 2008; Maken & Hennessy, 2009). Selective social buffering is also observed in guinea pigs. For adult guinea pigs, closely attached partners have been shown to buffer the HPA response. For instance, adult male guinea pigs showed a blunted elevation of cortisol when put into a novel environment with their favored female social partner while the presence of an unfamiliar female did not buffer the cortisol response (Hennessy et al., 2006a). Similarly for adult female guinea pigs, the presence of a male partner reduced HPA activity during novelty exposure, whereas the presence of an unfamiliar male did not (Hennessy et al., 2008).
A strong mother-infant attachment is also observed in guinea pigs, as pups recognize and favor the mother over other social stimuli (Hennessy et al., 2006a; Jäckel & Trillmich, 2003). Following a brief maternal separation, the pups exhibit behavioral responses indicative of stress including increased vocalization and locomotion as well as an elevation of plasma cortisol (Hennessy & Moorman, 1989; Hennessy & Ritchey, 1987). Similar to other social mammalian species, the presence of the mother has been shown to attenuate her pup’s HPA as well as behavioral responses to novelty exposure (Hennessy, 2014). Notably, this HPA-buffering effect was still observed when the mother was anesthetized (Hennessy & Ritchey, 1987). Therefore, it appears that it is just the presence of the mother, as a strong attachment figure, that buffer HPA activity of her pups. Moreover, the mother has been shown to buffer these adrenocortical responses from the preweaning age up to adulthood (Hennessy et al., 2006a; Hennessy et al. 2002b). In contrast, the presence of a littermate that had been raised in the same home cage could not moderate its sibling’s HPA activity either during the preweaning or postweaning ages (Hennessy, Miller, & Shair, 2006; Hennessy, Nigh, Sims, & Long, 1995; Hennessy et al., 2015). Studies examining the ability of unfamiliar adult females to buffer HPA activity in preweaning guinea pigs have yielded inconsistent results. For example, Hennessy et al. (2002b) observed a reduction of the cortisol response of preweaning pups to novelty exposure by unfamiliar adult females whereas Hennessy et al. (2006a) did not observe the same effect. Interestingly, in periadolescent guinea pigs (40-50 days old), the presence of an unfamiliar adult female has consistently been shown
to reduce adrenocortical reactivity (Hennessy et al., 2002a; Hennessy et al., 2006a; Hennessy, Maken, & Graves, 2000; Hennessy, Maken, & Graves, 2002; Maken & Hennessy, 2009); however, the same HPA buffering effect was not observed in the presence of an unfamiliar adult male (Hennessy et al., 2002b). In summary, social buffering seems to be more selective in preweaning guinea pigs because only the mother has consistently and potently been shown to reduce cortisol reactivity to novelty exposure. In contrast, both mother and unfamiliar adult females have been shown to reduce the adrenocortical response in postweaning guinea pigs. Taken together, these studies in guinea pigs are in agreement with studies in other species, in which social buffering varies with the strength of the relationship between partners.

**Hypothesized Neural Mechanisms of Social Buffering**

**HPA Activation**

Because HPA activation is initiated by the hypothalamus, inputs to the PVN are critical for the HPA signaling cascade. Research suggests that HPA activation may be stress-dependent because physical and psychological stressors have been shown to activate the HPA axis differently (Herman, Ostrader, Meuller, & Figueiredo, 2005; Hostinar et al., 2013). Physical stressors (e.g., shock) have been shown to trigger A1-A2 noradrenergic nuclei in the ponto-medullary regions to release norepinephrine, resulting in the activation of the PVN (Cunningham & Sawchenko, 1988; Figueiredo et al, 2003; Herman et al., 2003). In contrast, psychological stressors (e.g., novelty exposure, maternal separation, restraint) may engage a pathway from the medial amygdala (MeA)
to the bed nucleus of stria terminalis (BNST) to the PVN. More specifically, the MeA appears to activate the HPA axis indirectly through the inhibition of BNST, which disinhibits the PVN, resulting in the release of CRH (Beaulieu, Di Paolo, & Barden, 1986; Roozendaal, Koolhaas, & Bohus, 1991). Findings from our laboratory also support this MeA-BNST-PVN pathway. Using c-Fos immunoreactivity as a marker for neuronal function, Maken, Weinberg, Cool, and Hennessy (2010) observed a significant increase in Fos induction in the MeA and a significant decrease in Fos induction in the BNST when guinea pig pups were exposed to a novel environment, regardless of whether or not the mother was present. Isolation in a novel environment resulted in significant elevations of ACTH and cortisol concentrations, and the absence of the mother also resulted in an increased Fos activity of the PVN. However, the presence of the mother attenuated these adrenocortical responses. Furthermore, decreased c-Fos expression in the PVN was observed in the presence of the mother. Taken together, the findings suggest that exposure to a novel environment activates the HPA axis by the MeA-BNST-PVN pathway and that the mother buffers HPA activity at the level of the PVN.

In addition to the limbic structures, the prefrontal cortex (PFC) may be involved in HPA activation as well. For instance, when 2-year-old rhesus monkeys were isolated in a novel environment, positron emission tomography showed a significant activation of the right dorsolateral PFC compared to when the mother was with the infant. The absence of the mother also resulted in a significant elevation of plasma cortisol levels (Rilling et al., 2001). Similarly, Horii-Hayashi et al. (2013) recently observed significant elevations
of plasma corticosterone levels in isolated preweaning rats, and they also observed a significant increase in c-Fos expression in multiple brain regions including the hypothalamic nuclei, PFC, BNST, and amygdaloid nuclei. While this study observed an elevation of Fos activity in the BNST, a study by Maken et al. (2010) observed a decrease in Fos induction during isolation. This discrepancy may be due to the different regions of the BNST (rostral vs. caudal) sampled in both studies as well as differences between species (rats vs. guinea pigs). In any event, current findings suggest that the neural mechanisms of HPA activation may involve the PFC, amygdala, BNST, and PVN.

*The Role of mPFC in Social Buffering*

In looking for a specific brain region responsible for HPA buffering, many researchers have turned to the medial PFC (mPFC) because there are projections from mPFC to many related HPA regions (e.g., BNST, amygdala, hypothalamus, PVN; Hurley, Herbert, Moga, & Saper, 1991; Sesack, Deutch, Roth, & Bunney, 1989), and many studies suggest that the functions of mPFC may be region-specific. Moreover, the prelimbic cortex (dorsal mPFC) appears to be involved in HPA inhibition whereas the infralimbic cortex (ventral mPFC) appears to be involved in HPA excitation (Herman et al., 2003; Jones, Myers, & Herman, 2011; Sullivan & Gratton, 1999; Tavares, Corrêa, & Resstel, 2009). For example, Radley, Arias, and Sawchenko (2006) lesioned either the prelimbic or the infralimbic cortices of rats before inducing stress by brief restraint. Following restraint, rats with a damaged prelimbic region had a significant increase of CRH mRNA expression in the PVN as well as a significant elevation of plasma ACTH
and corticosterone levels compared to animals without mPFC damage. The findings show stress-enhancing effects due to prelimbic ablation, suggesting that the prelimbic cortex normally functions as an HPA inhibitor. In contrast, quite opposite effects were observed when the infralimbic cortex was lesioned. After restraint, animals with infralimbic lesions showed significantly lower levels of CRH mRNA expression in the PVN compared to animals without mPFC damage. Furthermore, ACTH and corticosterone levels of infralimbic-damaged animals were significantly lower than those of intact rats following restraint. The findings show stress-attenuating effects due to infralimbic lesions, suggesting that the infralimbic cortex normally facilitates HPA activation.

However, recent findings by McKlveen et al. (2013) suggest another possible stress-specific role for the prelimbic and infralimbic cortices. More specifically, while the two regions of the mPFC may have oppositional roles (i.e., inhibition vs. excitation), glucocorticoid receptors (GR’s) located in those regions may function according to the type of stressors (i.e., acute vs. chronic). Using a virally mediated knockdown strategy, the investigators injected lentivirus in either the prelimbic or the infralimbic cortices of male rats; the virus was designed to target specific GR genes, resulting in an attenuation of GR signaling. Half of the animals were exposed to chronic variable stress (e.g., cold swim, warm swim, cold room temperature, hypoxia) for 14 days, and all subjects received an acute restraint on day 15 before blood was collected for corticosterone analysis. Subjects with GR knockdown in the prelimbic region had significantly higher levels of corticosterone when exposed to acute but not chronic stress when compared to
GR-intact animals. In contrast, rats with GR knockdown in the infralimbic region had significantly higher levels of corticosterone when exposed to both acute and chronic stress when compared to GR-intact animals. The findings suggest that GR’s in the prelimbic cortex may modulate HPA responses to acute stressors whereas GR’s the infralimbic cortex may modulate HPA responses to both acute and chronic stressors. Furthermore, GR’s in the prelimbic cortex and perhaps the infralimbic cortex may play an inhibitory role in HPA activation; however, the prelimbic cortex as a whole may have an HPA-inhibitory effect, whereas the infralimbic cortex as a whole may have an HPA-excitative effect.

Rationale for Current Study

Selective social buffering is observed in the preweaning guinea pig. More specifically, only the mother has consistently and effectively been shown to buffer HPA activity of her pups during threatening situations. As the guinea pigs grew and were weaned, not only the mother but also unfamiliar adult females reduced the cortisol response to novelty exposure. The presence of an unfamiliar adult male did not buffer this adrenocortical response in periadolescence guinea pigs; their cortisol levels were as high as those animals exposed to a novel cage alone (Hennessy et al., 2002b). Therefore, it was surprising that a recent finding from our laboratory showed stress buffering effects by unfamiliar adult males in preweaning pups (Hennessy et al., 2015). Preweaning 16-day-old guinea pigs were exposed to a novel environment for 2 hr either alone or with a social partner (i.e., mother, littermate, or unfamiliar adult male). Behavioral stress
responses as well as social interactions between the pup and the stimulus animal were scored during the novelty exposure, and blood was collected for plasma cortisol analysis. Because previous studies suggest a potential HPA inhibiting effect by the prefrontal cortex (Herman et al., 2005; Hostinar et al., 2013), brain tissue was also collected for Fos immunoreactivity analysis in the prefrontal cortex (using the infralimbic cortex as a comparison). We found that pups exposed to a novel environment alone had a significant elevation of cortisol levels compared to undisturbed control pups. The presence of the mother attenuated this adrenocortical response whereas the presence of a littermate did not. Contrary to expectations the presence of an unfamiliar adult male buffered HPA activity as effectively as did the mother. Examining neuronal activity in the mPFC, we found that only the presence of the adult male resulted in a significant increase of c-Fos expression in the prefrontal cortex. The presence of other social partners did not have the same influence on Fos levels in the mPFC.

We also found that the pups had significantly more social interactions with the adult male than with other social partners. One would not expect these interactions to have HPA-buffering effects because the adult male engaged the pup in physical interactions. More specifically, the adult male often interacted vigorously with the pup by using its snout to push and lift the pup around the cage. These interactions between the pup and the adult male are different from the way the pups interacted with other social partners, as the pup and the mother as well as the pup and the littermate often remained in close contact and huddled without much physical activity.
Play fighting behavior is commonly observed in many rodent species as a part of normal social and physiological development (Vanderschuren, Niesink, & Van Ree, 1997). Previous studies in rats and hamsters showed an activation of both the prelimbic and the infralimbic regions during play fighting behavior (Achterberg et al., 2015; Cheng, Taravosh-Lahn, & Delville, 2008; Gordon, Kollacl-Walker, Akil, & Panksepp, 2002; van Kerkhof et al., 2013). Perhaps physical interactions in guinea pigs between the adult male and the pup may have similar effects on mPFC activity to play fighting behavior in juvenile of other rodent species. Because these social interactions were the one clue for the unexpected HPA buffering by the unfamiliar adult males, perhaps interacting with an adult male resulted in an activation of the prelimbic cortex, and subsequently a buffering of the pup’s HPA activity.

Therefore, the current study examined potential stress-buffering effects of social interactions by unfamiliar adult males. Because social interactions between the pup and the unfamiliar adult male were believed to be a crucial factor in HPA buffering in the previous study, we decided to limit social interactions between these social partners. The adult males were anesthetized in one condition for comparison with another condition, in which the pup and the adult male were allowed to freely interact. We also used undisturbed pups and pups isolated in a novel environment as negative and positive controls, respectively. From previous studies, we anticipated a high level of cortisol in pups isolated in a novel environment in contrast to undisturbed control pups. We predicted a reduction in cortisol response when the pup was exposed to novelty with a
conscious male, but not in the presence of an unconscious male. We also wanted to examine the association between social interactions and the activation of the prelimbic cortex. As in our previous study, we evaluated neuronal activity using Fos immunoreactivity in the prelimbic cortex in each condition, using the infralimbic cortex as a comparison. We predicted an increase of c-Fos expression in the prelimbic cortex in the Conscious Male condition, but not in the Unconscious Male condition.
II. METHODS

Animals and Experimental Conditions

Albino guinea pigs (*Cavia porcellus*) of the Hartley strain were bred in our laboratory. Following birth (Day 0), each mother was housed with her litter in an opaque plastic cage (73.7 cm X 53.4 cm X 25.4 cm) with a wire front and sawdust bedding. Water and guinea pig chow were available ad libitum. Animals were maintained on a 12:12 light/dark cycle with lights on at 07:00 hours. All procedures were approved by the Wright State University Animal Care and Use Committee. Twelve pups (six males, six females) were assigned to each of four experimental conditions: Home Cage, Alone, Conscious Male, and Unconscious Male. A random sample of eight pups from each condition (four animals of each sex) was used for the measurement of Fos immunoreactivity. In the Home Cage condition, pups were left with the mother until they were sacrificed for the collection of blood and brain tissue. In the remaining conditions, pups were tested alone or with a conscious or unconscious male. The adult male in the Unconscious Male condition received an IP injection of ketamine (35 mg/kg) and xylazine (5 mg/kg) cocktail (dosage: 0.5 ml/kg) prior to testing. It should be noted that although stimulus males and test pups had not had direct contact prior to testing, mothers and litters were housed in the general colony room for approximately the first 10 days of life. As such, the pups had been exposed to odors of all breeding males. Four adult males served as a social stimulus once in the Conscious Male and Unconscious Male
conditions, and four adult males served twice in each condition. Testing was conducted on Day 16 (± 1 day). Weaning for guinea pigs is approximately 25 days of age.

Testing Procedure and Behavioral Scoring

In all but the Home Cage condition, the pup was removed from its home cage and taken quietly in a carrying cage to the nearby testing room where it was placed into a clear, empty plastic cage (55 cm X 32 cm X 18 cm) under full room lighting for 2 hr. The floor of the cage was divided by tape into four equal-size segments. A trained observer behind one-way glass scored behavior during Min 0-30 and 90-120. These intervals were chosen to capture behaviors of the active and passive stages, respectively. For active behaviors, the observer tallied the number of whistle vocalizations (Berryman, 1976) and the number of times pups crossed lines into different areas of the cage. For passive behavior, the number of 1-min intervals in which pups exhibited the crouched stance or lying down, eye-closure, and extensive piloerection were scored. Our dependent measure of “full passive” behavior was a composite of the number of 1-min intervals that pups exhibited all three passive behaviors.

Because the adult males in the Unconscious Male condition sometimes began to regain consciousness during the last 30 min of testing, we closely examined the differences in social interactions between the pup and the adult male in the Conscious Male and Unconscious Male conditions. Hence, in these two conditions (as in our previous study), we scored the frequency of several non-agonistic social behaviors that occurred between animals (nose-nose) or were directed at the pup by the male (anogenital
investigation, fur sniff). We also recorded the number of instances that the adult male
directed any of three mild agonistic behaviors (kick, nip, thrust/lunge/lift) toward the pup.
See Table 1 for full definitions of behavioral categories. A microphone transmitted
vocalizations to the headphones of the observer who tallied them with a handheld
counter. Other behaviors were scored on check sheets. Testing cages were cleaned with
detergent after each test.

**Blood Sample Collection and Cortisol Determination**

Immediately following behavioral testing and at the same time of the day in the
Home Cage condition (14:00 – 16:00 hours), the pup was transferred into a nearby
chamber and rendered unconscious using CO$_2$ gas. Blood was then collected directly
from the heart using cardiac puncture; all samples were collected under 4 min from the
conclusion of behavioral testing. Blood was collected on heparin, and plasma was
separated by centrifugation (3000 rpm for 15 min) and frozen until assayed with a
radioimmunoassay kit routinely used in this laboratory (ImmuChem Coated Tubes
Cortisol RIA, MP Biomedicals, Orangeburg, NY). Samples were assayed in one run. The
intra-assay coefficient of variation was measured at 2.1%.

**Brain Tissue Collection**

Following the blood collection procedure, pups were given an IP injection
overdose of Euthasol. The animals were transcardially perfused with 0.01% heparinized
1X phosphate-buffered saline (PBS) followed by 4% paraformaldehyde. Brains were
post-fixed in 4% paraformaldehyde for 48 hr and submerged in 30% sucrose solution for
48 hr for cryoprotection. The brains were partially blocked towards the caudal end, coated in CRYO-OCT compound (Tissue Tek: Fisher Scientific, Pittsburgh, PA), and flash frozen in isopentane at -30º to -40ºC for approximately 20 sec prior to being stored at -80ºC until sectioned in 16µm coronal slices using a cryostat (Microm HM 550: Thermo Scientific, Waltham, MA). Atlases of the guinea pig forebrain (Luparello, 1967; Tindal, 1965) were used to aid in the identification of the medial prefrontal regions. Frozen sections were thawed-mounted onto gelatin-coated slides (Goal Seal UltraStick micro adhesion slides: VWR International, LLC, West Chester, PA) and stored at -20ºC.

**Fos Immunohistochemistry and Cell Quantification**

All immunohistochemistry procedures were performed at room temperature except for the incubation of the primary antibody, which was performed at 4ºC. Two sections spaced ~16 µm were used for Fos analysis for both regions. The slides containing the tissue sections were removed from the -20ºC freezer and allowed to thaw for 10 min. A PAP pen (Invitrogen, Frederick, MD) was used to encircle the sections on each slide, which were allowed to dry for 2 – 5 min. First, sections were washed three times in PBS/0.2% Triton then incubated in 0.5% peroxide solution for 5 min. Sections were washed twice with PBS/0.2% Triton, incubated in 10% normal goat serum (Sigma-Aldrich Co. LLC, St. Louis, MO) for 1 hr, and then incubated overnight for ~12 hr with a primary antibody for Fos (1:50; rabbit polyclonal IgG Santa Cruz Biotechnology, Inc., catalog number SC52, Santa Cruz, CA) in PBS/0.2% Triton. The sections were washed three times with PBS/0.2% Triton and then incubated for 1 hr with biotinylated secondary
antibody (1:200; anti-rabbit polyclonal IgG BA-1000, Vector Laboratories, Burlingame, CA) in PBS/0.2% Triton. The sections were washed three times with PBS and then incubated in an AB enzyme reagent (Vector Laboratories) for 30 min, after which they were washed three times in PBS and then incubated in diaminobenzidine solution with nickel intensification (Vector Laboratories) for 8 min. The sections were washed in distilled water and coverslipped with Permount mounting medium and were allowed to dry overnight and stored at room temperature.

Fos was measured in equal-sized fields of the prelimbic and the infralimbic cortex as illustrated in Figure 1. Images were analyzed by counting the number of Fos-positive cells using ImageJ 1.48v with the cell counter tool. All quantification was performed blind.

**Data Analysis**

When appropriate, data were analyzed with Analysis of Variance (ANOVA), with factors of Condition X Sex for cortisol and Condition X Sex X Side for Fos. Values from two sections were averaged for each side of the brain for Fos data. Primary post hoc paired comparisons were performed with Newman-Keuls procedures.

Because the distributions lacked normality, non-parametric tests were performed on all behavioral measures. Initially, the examination of sex differences was conducted with Mann-Whitney U tests. When significant, male and female data were analyzed separately; otherwise they were combined for analyses reported below. Overall effects of Condition were assessed with the Kruskal-Wallis procedure. For significant Kruskal-
Wallis effects, post hoc tests were performed with the Dunn-Bonferroni multiple comparison procedure. Except where indicated, a two-tailed value of $p = .05$ was used for determining significant effects. Data analysis was performed using SPSS 22.0.
III. RESULTS

Cortisol

Home cage control pups had low concentrations of cortisol; in contrast, pups separated in a novel environment alone had an elevation of cortisol levels. The presence of a conscious male suppressed HPA activity while the presence of the unconscious male did not. ANOVA yielded significant main effects of Condition, $F(3, 49) = 14.89, p < .001$, and Sex, $F(1, 49) = 9.40, p < .01$ (females > males, as is typical for guinea pigs). For the Condition effect, planned comparisons indicated that cortisol levels in the Alone and Unconscious Male conditions did not differ from each other, but both were greater than those in the Home Cage and Conscious Male conditions, $p$’s < .05 (Fig. 2).

Behavior

As expected, isolated pups vocalized at a high rate, while the presence of the conscious adult male suppressed vocalizations. A Kruskal-Wallis test revealed a significant effect of Condition, $p < .001$. Post hoc tests showed that the presence of a conscious male significantly reduced vocalizing when compared to the Alone and Unconscious Male conditions, $p$’s < .05 (Fig. 3 left panel). Although the median levels of vocalizations were much lower in the pups tested with an unconscious male than pups tested alone, the difference between these conditions was not significant.

For locomotor activity, a Mann-Whitney U test indicated a significant effect of Sex, with male pups being more active than female pups, $p < .05$. Data for each sex were further analyzed separately. However, Kruskal-Wallis tests did not show significant
effects of Condition for either sex, $p’s > .05$ (Table 2). Little passive behavior was observed in any condition. A Kruskal-Wallis test was not significant (Fig. 3 right panel).

Social interactions were frequently observed in pups tested with conscious adult males. Anesthetized adult males sometimes started to regain consciousness during the last 30 min of testing, exhibiting disoriented behaviors such as crawling and stumbling. Four out of twelve pups briefly engaged in social interactions with the adult males in the Unconscious Male condition. Kruskal-Wallis tests revealed that there were significantly more non-agonistic and mild agonistic social interactions observed in the Conscious Male condition relative to the Unconscious Male condition, $p’s < .001$ (Fig. 4).

**Fos**

For the prelimbic cortex, there was no significant effect of Condition, $p > .05$; however, a planned comparison between the Conscious Male and the Unconscious Male conditions revealed that significantly more Fos-positive cells were observed for pups tested with a conscious male than for those tested with an unconscious male, $p < .05$ (1-tailed t-test; Fig. 5 top panel and Fig. 6).

For the infralimbic cortex, ANOVA indicated a significant Condition X Sex interaction, $F(3, 32) = 3.28, p < .05$. Follow up analyses across conditions for each sex showed no significant effect for male pups, $p > .05$, but ANOVA revealed a significant effect for female pups, $p < .05$. Paired comparisons showed that there were significantly more Fos-positive cells observed for female pups tested with a conscious male than for females in all other conditions, $p < .05$ (Fig. 5 bottom panel).
IV. DISCUSSION

The purpose of the current study was to examine the HPA-buffering effect of social interactions between preweaning guinea pigs and an unfamiliar adult male during novelty exposure. To limit social interactions, the adult male was anesthetized in one condition whereas the adult male and the pup were allowed to freely interact in another condition. As expected, the pups interacted with a conscious adult male more than they did with an unconscious adult male. We did observe a low level of social interactions between partners in the Unconscious Male condition, as some adult males started to regain consciousness and attempted to interact with the pup at the end of the test in a disoriented fashion.

Isolation of preweaning guinea pigs in a novel environment for 2 hr greatly increased plasma cortisol levels. The presence of a conscious male buffered this HPA response, and the pups’ cortisol levels were not significantly different than those in the home cage control group. In contrast, the presence of an unconscious male did not buffer adrenocortical reactivity, and the pups’ cortisol levels were not significantly different than those of pups isolated in the novel cage. Cortisol results from the current study are in agreement with the results from our previous study, in which the presence of an unfamiliar adult male also reduced plasma cortisol levels of pups exposed to a novel environment. The current study suggests that interactions between social partners can have HPA buffering effects, as the presence of an anesthetized male did not reduce cortisol levels of pups. In comparison, we only observed low levels of social interactions...
between the mother and her pup during maternal buffering (Hennessy et al., 2015). Importantly, however, the adult males buffer HPA activity differently (i.e., via social interactions) than the mother, for which only her presence is enough (Hennessy & Ritchey, 1987).

In an earlier study, periadolescent guinea pigs were exposed to a novel environment in the presence of an unfamiliar adult male for 20 and 60 min (Hennessy et al., 2002b). The researchers did not observe a reduction of plasma cortisol at either time point, and the animals’ cortisol levels were as high as those of pups in a novel cage alone. In comparison, the current study and the study by Hennessy et al. (2015) found an HPA-buffering effect by unfamiliar adult males in preweaning guinea pigs after 2 hr of novelty exposure. We speculated that there might be an age effect, in which the presence of an adult male may be able to reduce the adrenocortical response of younger but not older guinea pigs. There might also be a time effect because the time points in which the cortisol levels were measured were shorter in the previous study. A recent study from our laboratory found that the presence of an adult male was able to reduce plasma cortisol levels of periadolescent guinea pigs after 2 hr in a novel environment (unpublished). Another follow-up study from our laboratory found that the adult male also reduced HPA activity of preweaning guinea pigs after 1 and 2 hr of novelty exposure (unpublished). In summary, results from our studies suggest that the adult male may buffer cortisol response in younger guinea pigs at a faster rate than in older guinea pigs, as the effect took longer than 60 min to occur in periadolescent animals.
Pups isolated in the novel cage also exhibited a high level of high-pitch vocalizations. As for cortisol, the presence of a conscious male significantly reduced the rate of vocalizing whereas the presence of an unconscious male did not. This was an interesting finding because this phenomenon is not observed in other rodent species. For example, Takahashi (1992) observed that while the presence of an adult male rat could reduce vocalizations by rat pups, the pup’s adrenocortical response was elevated. This may be an adaptive behavior in rats, as infanticide by adult males is commonly observed in this species. The presence of an adult male rat may be perceived as a threat by the pup. By being quiet, they are less likely to be noticed.

We found a sex difference in line-crossing behavior, with male pups being more active than female pups. However, further analysis within each sex revealed that there was no significant difference in locomotor activity among conditions. Pups exhibited little full passive behavior (i.e., crouching/lying down, piloerection, eye closure) across conditions, and there was no significant effect among conditions on full passive behavior.

Our previous study (Hennessy et al., 2015) found an elevation of neuronal activity in the prelimbic cortex of pups exposed to a novel environment with an adult male compared to other social partners; this was juxtaposed with a low level of plasma cortisol. Therefore, in the current study, we examined Fos protein levels in the prelimbic cortex of pups across conditions, using the infralimbic cortex as a comparison. For the prelimbic cortex, we observed significantly more Fos-positive cells in pups exposed to a novel environment with a conscious male compared to those exposed to a novel
environment with an unconscious male. The prelimbic cortex is traditionally thought to play an important role in HPA inhibition (Herman et al., 2005). Therefore, we not only replicated the findings from our previous study, but our current results are also in agreement with the literature.

For the infralimbic cortex, we found significantly more Fos-positive cells in female pups exposed to a novel environment with a conscious male compared to other conditions. There was no significant effect in Fos induction among conditions in male pups. The reason for this sex difference is unclear. Nevertheless, the activation in females is of interest because the infralimbic cortex has been traditionally associated with HPA activation mechanisms (Herman et al., 2005; Hostinar et al., 2013); the current study observed an activation of the infralimbic as well as a reduction of cortisol levels.

An increase activity in both the prelimbic and the infralimbic cortices may be a result of social interactions between partners. Studies in other rodent species have found an activation of the mPFC during play fighting behavior. For example, Cheng et al. (2008) examined brain activity of juvenile golden hamsters (Mesocricetus auratus) after a 10-min play fighting session. The researchers observed a significant increase of Fos induction in both prelimbic and infralimbic cortices of hamsters that underwent play fighting compared to control animals that were simply placed in a novel cage. This study illustrates that social interactions may activate both regions of the mPFC. In the current study, it is possible that social interactions with the adult male increased neuronal activity in the mPFC. Even though the activation of the infralimbic cortex may increase the
adrenocortical activity, the prelimbic cortex may act more prominently and suppress overall HPA function. In sum, the current findings suggest an association between high levels of social interactions, a buffering of HPA activity, and an increase in neural activity in the mPFC.

Social interactions with the adult males involved tactile stimulation as well as physical activity. Studies across species including humans, rodents, canines, and zebra fish have shown that forms of tactile stimulation (e.g., massage, petting) may reduce HPA activity (Freitas et al., 2015; Pinar & Afsar, 2015; Schirmer, Jesuthasan, & Mathuru, 2013; Shiverdecker, Schiml, & Hennessy, 2013). In addition, results from various species including humans have shown that various physical activity such as short-term aerobic exercise can reduce glucocorticoid levels, decrease depressive-like behaviors, and improve mood (Basso et al., 2015; Salmon, 2001; Sigwalt et al., 2011). Interestingly, physical activity has been associated with an activation of the prefrontal cortex via noradrenergic systems. For example, Dunn et al. (1996) trained rats to run a treadmill for 8 weeks and found a significantly higher level of 3-methoxy-4-hydroxyphenylglycol, a norepinephrine metabolite, in the prefrontal cortex compared to rats that did not receive training. Therefore, noradrenergic systems may be involved in the activation of the mPFC during social interactions with the adult male.

Altogether, an increase of Fos induction in both regions of the mPFC as well as a reduction of HPA activity observed in the Conscious Male condition may have resulted from social interactions, which included tactile stimulation and physical activity provided
by the adult males. This may be the most plausible explanation because we only observed an HPA-buffering effect in a condition in which social partners physically interacted. Furthermore, these social interactions seem to be different than social interactions between the pup and the mother or between the pup and the littermate (Hennessy et al., 2015). It is important to note, however, that the current study did not directly examine the ability of the mPFC to reduce plasma cortisol levels in this context. Future studies might investigate this question by pharmacologically inactivating the interested areas using a GABA$_A$ receptor agonist such as muscimol.

In conclusion, the current study provides evidence that social interactions by unfamiliar adult males can buffer HPA activity of preweaning guinea pigs during novelty exposure. Importantly, there seems to be two types of social buffering in guinea pig pups: via attachment and via social interactions. As a significant attachment figure, just the mother’s presence can buffer HPA activity of her pups behaviorally as well as physiologically; this type of social buffering is specific to the mother and her infant. In contrast, unfamiliar adult males can reduce the adrenocortical response of pups through social interactions. In addition, these social interactions may involve an activation of the mPFC (specifically the prelimbic cortex) to suppress HPA activity. We previously observed a different pattern of neuronal activation during maternal buffering (Hennessy et al., 2015; Maken et al., 2010), suggesting the presence of the mother may buffer the HPA activity of pups through other mechanisms, such as activation of oxytocin neurons.
in the PVN (Smith & Wang, 2013) or inhibition of excitatory noradrenergic input to this region (Sarro, Wilson, & Sullivan, 2014).
V. REFERENCES


anxiety-like behaviors and potentiates sertraline action in young rats.


Figure 1. Approximate location of regions selected for analysis of Fos staining in prelimbic and infralimbic cortex. Section displayed in 14.2mm anterior to the intraaural axis. Adapted from Stereotaxic Atlas of the Forebrain of the Guinea Pig (Luparello, 1967).
Figure 2. Mean levels of plasma cortisol of Home Cage control pups and those exposed to a novel cage alone or with either a conscious or unconscious male. Vertical lines indicate standard errors of the means. Horizontal lines indicate significant paired comparisons, $p < .05$. 
Figure 3. Median number of vocalizations (left panel) and 1-min intervals of full passive behavior (right panel) exhibited by pups during the first and last 30 min of a 2-hr exposure to a novel cage while alone or with either a conscious or an unconscious male. SIR indicates the semi-interquartile range. Horizontal lines indicate significant paired comparisons, $p < .05$. 
Figure 4. Median number of non-agonistic (top panel) and agonistic (bottom panel) interactions between the pup and either a conscious or an unconscious adult male during the first and last 30 min of a 2-hr exposure to a novel cage. SIR indicates the semi-interquartile range. Horizontal lines indicate significant paired comparisons $p < .001$. 
Figure 5. Mean number of Fos-positive cells in the prelimbic (top panel) and infralimbic (bottom panel) cortex of pups that were undisturbed or exposed to a novel cage while alone, or with either a conscious or unconscious male. Vertical lines indicate standard error of the means. Horizontal lines indicate significant paired comparisons, $p < .05$. 
Figure 6. Representative Fos-positive cells of the prelimbic cortex in each of the four conditions: A. Home Cage; B. Alone; C. Conscious Male; D. Unconscious Male.
Table 1.

Behavioral definitions

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active</td>
<td></td>
</tr>
<tr>
<td>Vocalization</td>
<td>High pitched “whistles” (F)</td>
</tr>
<tr>
<td>Motor activity</td>
<td>Pup crosses lines (all four paws) dividing long axis of cage into four equal-sized sections (F)</td>
</tr>
<tr>
<td>Passive</td>
<td></td>
</tr>
<tr>
<td>Crouch</td>
<td>Hunched stance with feet tucked beneath body (1-min)</td>
</tr>
<tr>
<td>Lie</td>
<td>Trunk supported by cage floor (1-min)</td>
</tr>
<tr>
<td>Eye-close</td>
<td>Closure of one or both eyes for at least 1 s (1-min)</td>
</tr>
<tr>
<td>Piloerection</td>
<td>Piloerection over at least half of the body surface (1-min)</td>
</tr>
<tr>
<td>Full passive</td>
<td>Occurrence of eye-close, piloerection, and either crouch or lie (1-min)</td>
</tr>
<tr>
<td>Non-agonistic social</td>
<td></td>
</tr>
<tr>
<td>Nose–nose</td>
<td>Physical contact between noses of the two animals (F)</td>
</tr>
<tr>
<td>Anogenital investigation</td>
<td>Partner places nose in contact or within 1 cm of pup’s anogenital region (F)</td>
</tr>
<tr>
<td>Fur sniff</td>
<td>Partner lifts section of pup’s fur with snout (F)</td>
</tr>
<tr>
<td>Agonistic</td>
<td></td>
</tr>
<tr>
<td>Kick</td>
<td>Partner kicks or attempts to kick pup (F)</td>
</tr>
<tr>
<td>Nip</td>
<td>Partner nips or attempts to nip pup (F)</td>
</tr>
<tr>
<td>Thrust/lungo/lift</td>
<td>Partner jabs head or makes short run at pup or uses snout to lift pup (F)</td>
</tr>
</tbody>
</table>

Note. F, frequency of behavior; 1-min, number of 1-min intervals in which behavior occurred.
Table 2

Median number (and semi-interquartile range) of line-crossings of males and females in the Alone, Conscious Male, and Unconscious Male conditions

<table>
<thead>
<tr>
<th></th>
<th>Alone</th>
<th>Conscious Male</th>
<th>Unconscious Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>27 (6.8)*</td>
<td>37 (9.3)*</td>
<td>20.5 (5.1)*</td>
</tr>
<tr>
<td>Females</td>
<td>5.5 (1.4)</td>
<td>5.5 (1.4)</td>
<td>9 (2.3)</td>
</tr>
</tbody>
</table>

Note. * p < .05 vs females.