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Social isolation alters central nervous system monoamine content in prairie voles following acute restraint

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Abstract

Animal models have shown that social isolation and other forms of social stress lead to depressive- and anxiety-relevant behaviors, as well as neuroendocrine and physiological dysfunction. The goal of this study was to investigate the effects of prior social isolation on neurotransmitter content following acute restraint in prairie voles. Animals were either paired with a same-sex sibling or isolated for four weeks. Plasma adrenal hormones and ex vivo tissue concentrations of monoamine neurotransmitters and their metabolites were measured following an acute restraint stressor in all animals. Isolated prairie voles displayed significantly increased circulating adrenocorticotrophic hormone (ACTH) levels, as well as elevated serotonin and dopamine levels in the hypothalamus, and potentially decreased levels of serotonin in the frontal cortex. However, no group differences in monoamine levels were observed in the hippocampus or raphe. The results suggest that social stress may bias monoamine neurotransmission and stress hormone function to subsequent acute stressors, such as restraint. These findings improve our understanding of the neurobiological mechanisms underlying the consequences of social stress.

Keywords

prairie vole; dopamine; serotonin; hypothalamus; social stress

Introduction

Social bonds play an important role in mediating both physical and psychological health. The lack of social bonds negatively affects health and increases susceptibility to later stressors (Heinrichs, Baumgartner, Kirschbaum, & Ehlert, 2003; Kikusui, Winslow, & Mori, 2006; Uchino, 2006; Uchino, Uno, & Holt-Lunstad, 1999). In addition, individuals who are lonely experience high rates of mood disorders and physical health disturbances (Cacioppo, Hawkley, & Berntson, 2003; Cacioppo et al., 2002; Cacioppo, Hawkley, & Thisted, 2010; Kaplan et al., 1988; Shankar, McMunn, Banks, & Steptoe, 2011; Uchino, 2006). Exemplifying this, decreased social engagement is associated with an increased risk of cardiovascular disease mortality in men (Ramsay et al., 2008). Further, perceived social

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isolation is associated with elevated blood pressure, endocrine dysregulation, decreases in physical activity, and reduced cognitive performance and life satisfaction (Adam, Hawkley, Kudielka, & Cacioppo, 2006; Cacioppo & Hawkley, 2009; Gow, Pattie, Whiteman, Whalley, & Deary, 2007; Hawkley, Masi, Berry, & Cacioppo, 2006; Hawkley, Thisted, & Cacioppo, 2009; Wen, Hawkley, & Cacioppo, 2006). Together, these studies indicate that the lack or loss of social relationships is associated with increased morbidity and mortality in humans.

In addition to evidence that social stressors have negative consequences on human health, the disruption of social bonds and social isolation are associated with behavioral and physiological disturbances in animals (Grippe et al., 2011; Grippe, Gerena, et al., 2007; Grippe, Lamb, Carter, & Porges, 2007; Manuck, Clarkson, Lusso, Taub, & Miller, 1983; Peuler, Scotti, Phelps, McNeal, & Grippe, 2012; Shively et al., 2005). For example, adult female cynomolgus monkeys living as socially stressed subordinates display low levels of physical activity, autonomic dysregulation, endocrine disturbances, and increased mortality (Shively et al., 2005; Shively, Laber-Laird, & Anton, 1997; Shively, Grant, Ehrenkauf, Mach, & Nader, 1997; Watson, Shively, Kaplan, & Line, 1998; Shively, 1998; Williams, Shively, & Clarkson, 1994). Rats and mice exposed to social subordination or defeat stress show depression- and anxiety-like behaviors, disturbances in corticosterone and altered stress response feedback to new stressors (Carnevali et al., 2012; Davies et al., 2016; Haller, Halasz, & Makara, 2000; Heinrichs et al. 1994; Lukkes, Mokin, Scholl, & Forster, 2009; Pisu et al., 2016; Ruis et al., 1999; Weiss et al. 2004). These studies demonstrate that social stress negatively affects the behavior and physiological health of animals, thus enabling them to be used as tools for understanding the mechanisms underlying deleterious effects of environmental stress. An example of an animal model that has been used to investigate the neurobiological mechanisms underlying responses to social stress is the prairie vole.

Prairie voles are rodents that form monogamous social bonds similar to humans (Carter, DeVries, & Getz, 1995; Carter & Getz, 1993; Young & Wang, 2004), display depression- and anxiety-relevant behaviors, and several physiological alterations following long-term social isolation (Grippe, Cushing, & Carter, 2007; Grippe, Gerena, et al., 2007; Grippe et al., 2012). Isolated adult prairie voles show physiological disruptions similar to other species including autonomic dysregulation and elevated stress hormones during periods of low activity and when exposed to additional short-term stressors (Grippe, Gerena, et al., 2007; Grippe, Sgoifo, Mastorci, McNeal, & Trahanas, 2010; McNeal et al., 2014). Further, both young and adult prairie voles display elevations in plasma corticosterone and increased immunostaining of corticotropin-releasing hormone cells and fibers in the paraventricular nucleus of the hypothalamus as a function of social isolation (Grippe, Gerena, et al., 2007; Ruscio, Sweeny, Hazelton, Suppatkul, & Carter, 2007). The accumulated previous evidence that social isolation in prairie voles is associated with behavioral and physiological changes also reported in humans who experience isolation as a chronic stressor indicates that this species is a valuable animal model for investigating the complex interactions of social isolation with brain function. Combined, the previous findings using the prairie vole model provide evidence that social stress contributes to poor health outcomes.

The prairie vole model can be used to better understand how chronic social stress may alter the neurobiological mechanisms regulating the response to acute stressors. Exposure to

stress alters central neurotransmitter systems, and these changes are hypothesized to mediate the behavioral and physiological effects observed following stress, including social stress (McKittrick, Blanchard, Hardy, & Blanchard, 2009). Social separation of male rats for 14 days increased serotonin turnover in the hippocampus (dos Santos, de Andrade, & Graeff, 2010), and this has been replicated following chronic variable stress as evidenced by an increase in 5-hydroxyindoleacetic acid (5HIAA)/serotonin ratio (Gamaro, Manoli, Torres, Silveira, & Dalmaz, 2003). Further, subordinate rats living in a complex social system displayed greater serotonin metabolite levels, 5HIAA, in several forebrain regions and in the preoptic area of the hypothalamus compared to dominant rats (Blanchard et al., 1991). Social isolation rearing decreased dopamine and its metabolite, 3, 4-dihydroxyphenylacetic acid (DOPAC), in the frontal cortex in rats (Möller, Du Preez, Viljoen, Berk, & Harvey, 2013), while three episodes of social defeat increased DOPAC in forebrain regions in mice (Puglisi-Allegra & Cabib, 1990). In vivo neurotransmitter monitoring has demonstrated that prior exposure to repeated social stress or isolation potentiates stress-induced increases in dopamine and serotonin release in the prefrontal cortex following an additional stressor (Ago et al., 2013; Miura, Qiao, Kitagami, Ohta, & Ozaki, 2005; Tidey & Miczek, 1996). The increased neurotransmitter response to an acute stressor suggests that prior exposure to chronic social stress affects the neurotransmitter system's ability to respond to a subsequent stressor, which could then influence hypothalamic-pituitary-adrenal (HPA) activity, subsequent behavior, and overall health.

Although the effects of social stress on brain function have been investigated in rats and mice, there has been little study of neurotransmitter changes in the prairie vole following social isolation. Therefore, the current experiment measured neurotransmitter and metabolite levels in key neuronal structures that play a role in regulating responses to stress, including the hippocampus, hypothalamus, frontal cortex, and raphe (Ago et al., 2013; Cryan, Valentino, & Lucki, 2005; Dent & Neill, 2012; Dronjak & Gavrilovic, 2006; Krystal & Neumeister, 2009; Möller et al., 2013). Specifically, this study assessed dopamine, serotonin, and their metabolites, as well as circulating hormones levels, following an acute restraint stressor in prairie voles that experienced prior long-term social isolation or control (paired) housing. We hypothesized that social isolation would negatively influence neurotransmitter levels and neuroendocrine function following acute restraint stress, compared to paired control housing.

Methods

Animals

Fifteen male prairie voles (60–90 days old) were used from the colony at Northern Illinois University. All prairie voles were removed from breeding pairs at 21 days of age, and housed in same-sex sibling pairs until the commencement of experimentation. Animals were allowed *ad libitum* access to food and water, maintained at a room temperature of 20–21°C and a relative humidity of 40–50%, under a standard 14:10 light/dark cycle (lights on at 06:30). All experimental protocols were approved by the Northern Illinois University Institutional Animal Care and Use Committee and followed National Institute of Health

guidelines as stated in the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 2011).

General Procedure

Prairie voles were randomly divided into two groups of either paired (control; $n = 8$ isolated ($n = 7$) conditions for four weeks. After this period, all animals were exposed to a 60 minute restraint session (Smith, Lieberwirth, & Wang, 2013). Immediately following the restraint, plasma and brain tissue samples were collected. Serotonin, dopamine, and their metabolites were assayed via high-performance liquid chromatography (HPLC) from tissue dissections of the frontal cortex, hippocampus, hypothalamus, and raphe. Finally, circulating adrenocorticotrophic hormone (ACTH) and corticosterone were measured via enzyme-linked immunosorbent assay (ELISA) from plasma samples. All handling, cage changing, and collection of tissue and plasma (described below) were matched between the two groups.

Housing Conditions

Isolated animals were separated from their siblings and housed individually for four weeks, without visual, olfactory or auditory cues from the sibling. Paired control animals were continually housed with their same-sex siblings. For each group, only one animal from each pair was used in this investigation. The four-week manipulation period was selected based on previous results demonstrating that this time frame is sufficient to induce disruptions of affective behaviors and associated health consequences (Grippe et al., 2010; Grippe, Cushing, et al., 2007; Grippe, Gerena, et al., 2007).

Restraint

All prairie voles were exposed to 60 minutes of restraint during the light period in a custom-designed apparatus (Pournajafi-Nazarloo et al., 2009). Briefly, each animal was placed in a wire mesh screen that wrapped around the animal and was closed with binder clips (Dayas, Buller, Crane, Xu, & Day, 2001).

Sample Collection

Immediately following the 60-minute restraint session, animals were anesthetized for sample collection using procedures described previously (Grippe, Gerena, et al., 2007; McNeal et al., 2014). Briefly, voles were deeply anesthetized with a mixture of ketamine (67 mg/kg, subcutaneous (sc); NLS Animal Health, Owings Mills, MD) and xylazine (13.33mg/kg, sc; NLS Animal Health). Blood was sampled within two minutes of the anesthetic injection, from the periorbital sinus via a heparinized capillary tube, and was collected during a period not exceeding 1.5 minutes. The blood was placed immediately on ice, and then centrifuged at 4°C, at 3500rpm, for 15 minutes to obtain plasma. Plasma aliquots were stored at -80°C until assayed for circulating ACTH and corticosterone via ELISA. Immediately after blood was collected, animals were sacrificed and brains were carefully removed. The hypothalamus, raphe, frontal cortex, and hippocampus were dissected, placed in Eppendorf tubes, frozen on dry ice, and then stored at -80°C until assayed for neurotransmitter and metabolite levels via high performance liquid chromatography (HPLC).

Enzyme-Linked Immunosorbent Assay

Stress hormone levels were determined using commercially available ELISA kits (ACTH, EK-001–21, Phoenix Pharmaceuticals, Burlingame, CA; corticosterone, ADI-900–097, Enzo Life Sciences, Farmingdale, NY). Plasma samples were analyzed according to the kit instructions, and diluted to give results reliably within the linear portion of the standard curve (ACTH, 1:7; corticosterone, 1:500). The sensitivity of the kit for ACTH is 0.08 ng/ml (range 0–25 ng/ml), and for corticosterone is 27.0 pg/ml (range 32–20,000 pg/ml).

High Performance Liquid Chromatography

For the neurotransmitter measures, dissected brain tissue samples were thawed, sonicated in 0.1 M perchloric acid and then centrifuged at $11,000 \times g$ for 6 minutes. The supernatant was assayed for dopamine and dopamine metabolites homovanillic acid (HVA) and DOPAC, as well as, serotonin and the serotonin metabolite 5HIAA, using HPLC procedures described previously (Matuszewich & Yamamoto, 2004). Protein content was determined for each sample using the Quick Start Bradford protein assay (Bio-Rad, Hercules, CA, USA) and read on a Multiscan Plus microplate reader at 620 nm (Thermo Electron Corporation, Waltham, MA, USA).

Tissue samples were analyzed for dopamine, serotonin, and metabolites with HPLC electrochemical detection. A Rheodyne injector (Cotati, CA, USA) with a 20 μ l loop delivered the sample onto a reverse phase Synergi 4 C18 column 150 mm $\times$ 2 mm (Phenomenex, Torrance, CA). A Shimadzu 10 ADVP pump continuously pumped mobile phase (32 mM citric acid, 54.3 mM sodium acetate, 0.074 mM ethylenediaminetetraacetic acid, 0.32 mM octyl sodium sulfate and 3% methanol) at a flow rate of 0.20 ml/minute. Compounds were detected with an LC-4B amperometric detector (Bioanalytical Systems, West Lafayette, IN, USA), with a 3 mm glassy carbon working electrode maintained at a potential of +0.5V relative to an Ag/AgCl reference electrode. Data were collected using ChromPerfect Spirit Software (Justice Innovations, Inc., Denville, NJ, USA).

Statistical Analyses

Statistical analyses of intracellular neurotransmitter content in the hippocampus, raphe, hypothalamus, and frontal cortex, and circulating hormones, were compared between prairie voles that were previously isolated or pair housed using independent-samples t-tests for each dependent variable. Effect size was calculated using Cohen's d by taking the mean difference and dividing by the pooled standard deviation. For interpretation, a small effect size was classified as $d=.2$, a medium effect size was classified as $d=.5$ and a large effect size was classified as $d=.8$ and greater (Cohen, 1988). The data presented in the figures represent means $\pm$ standard error of the mean (SEM). All data were analyzed using SPSS 22 (IBM Corporation Somers, NY, USA). A probability value of $p<.05$ was considered statistically significant. Using HPLC, we were unable to assess metabolites in some brain regions because of low metabolite levels, however, the attrition of sample size was similar between both conditions. The range of samples sizes for metabolites varies by brain region (hippocampus $n= 3-5$, raphe $n= 4-6$, frontal cortex $n= 4-7$, hippocampus $n= 5-8$).

Results

Serotonin

Serotonin levels in the hypothalamus were significantly greater in prairie voles previously isolated compared to pair house animals following the restraint stressor ($t(13)=2.985$, $p=.011$; $d=1.49$; see Figure 1). Frontal cortex serotonin was marginally decreased in animals that were previously isolated compared to animals that were pair housed ($t(9)=-2.058$, $p=.070$; $d=1.43$). There were no group differences in serotonin levels in the hippocampus ($t(13)=.648$) or in the raphe ($t(10)=-1.475$) following restraint stress.

Despite differences in serotonin levels in the hypothalamus and frontal cortex, there were no differences between paired and isolated animals in the serotonin metabolite, 5HIAA in any of the brain regions (hypothalamus: $t(4)=1.888$; frontal cortex: $t(9)=.456$; hippocampus: $t(13)=-1.319$; raphe: $t(8)=1.306$; see Table 1). There were also no group differences in the turnover ratio of 5HIAA to serotonin (hypothalamus: $t(4)=-.673$; frontal cortex: $t(9)=1.351$; hippocampus: $t(13)=.738$; raphe: $t(10)=1.560$; see Table 1).

Dopamine

Prairie voles that were previously isolated had significantly greater dopamine in the hypothalamus following restraint compared to those that were previously paired ($t(13)=2.372$, $p=.034$; $d=1.19$; see Figure 2). However, there were no group differences in dopamine levels in the frontal cortex ($t(9)=1.476$), hippocampus ($t(13)=-1.088$), or raphe ($t(10)=-1.636$).

Similar to serotonin metabolite, there were no group differences in the dopamine metabolite, DOPAC, in the brain regions of interest (hypothalamus: $t(6)=1.546$; frontal cortex: $t(9)=-.647$; hippocampus: $t(13)=-1.441$; raphe: $t(10)=-.454$). However, homovanillic acid (HVA) in the hypothalamus was slightly higher in isolated compared to paired animals ($t(6)=2.114$, $p=.079$; $d=1.38$), while there was no group difference in the hippocampus ($t(9)=-.340$). There were also no group differences in the ratio of DOPAC to dopamine (hypothalamus: $t(6)=.672$; frontal cortex: $t(9)=-1.028$; hippocampus: $t(13)=-1.425$; raphe: $t(10)=.836$) or the ratio of HVA to dopamine (hypothalamus: $t(6)=.371$; hippocampus: $t(9)=.512$; see Table 1).

ACTH and Corticosterone

Prairie voles that were previously isolated displayed higher plasma levels of ACTH following the restraint compared to animals that were pair housed ($t(13)=2.476$, $p=.028$; $d=1.23$; Figure 3). Corticosterone levels did not differ following restraint stress in animals that were previously isolated or pair housed ($t(13)=.326$; Figure 3).

Discussion

The current study investigated the influence that prior social isolation has on neurotransmitter content following acute restraint in prairie voles. Although several studies have focused on the effects of acute or chronic stress on neurotransmission (Ago et al., 2013; Cryan, Valentino, & Lucki, 2005; Dent & Neill, 2012; Dronjak & Gavrilovic, 2006; Krystal

& Neumeister, 2009; Möller et al., 2013), it is unknown whether the same neurotransmitter alterations occur as a function of exposure to a combination of chronic and acute stress in prairie voles. Therefore, the current study used the prairie vole model to assess the influence of chronic social isolation or paired housing on tissue neurotransmitter and metabolite concentrations in brain regions that are relevant to stress and circulating hormone concentrations, following subsequent acute restraint. Isolated prairie voles displayed significantly increased serotonin and dopamine levels in the hypothalamus and marginally decreased serotonin levels in the frontal cortex, as well as increased circulating ACTH following restraint, versus pair housed controls. There were no observed group differences in circulating corticosterone or in neurotransmitter, metabolite or ratio of neurotransmitters to metabolite levels in the hippocampus or raphe. Combined, these alterations in neurotransmission offer unique insight into possible mechanisms by which chronic social stress can adversely influence subsequent responses to acute stress.

The present study demonstrated that social isolation influenced central serotonin function in key brain regions associated with stress, emotion, and physiological functioning. Specifically, socially isolated prairie voles showed significantly increased serotonin in the hypothalamus and marginally significantly decreased frontal cortex serotonin following acute restraint. Alterations in central serotonin are associated with both behavioral and physiological dysregulation (Jacobs & Fornal, 1991). In the hypothalamus, serotonin may coordinate behavioral and endocrine responses to stressors, as well as downstream cardiovascular function (Van de Kar & Blair, 1999). For example, serotonin plays a significant role in behaviors that are disrupted during stress (e.g., mood, sleep), and depression is associated with changes in central serotonin signaling (e.g., impaired serotonin synthesis or release, malfunctions at postsynaptic serotonin receptors, alterations in serotonin transporter density) (Cryan, Valentino, & Lucki, 2005; Grippo et al., 2005; Lucki, 1998; Meltzer et al., 2004; Ressler & Nemeroff, 2000; Way & Taylor, 2010). Additionally, altered central serotonin levels have been found in rodents with myocardial ischemia, and a specific serotonin transporter gene polymorphism has been associated with a higher risk of myocardial infarction in men who survived an initial heart attack (Fumeron et al 2002; Sole, Versteeg, de Kloet, Hussain, & Lixfeld, 1983). The elevated hypothalamic serotonin levels displayed by socially isolated animals following restraint may therefore be one mechanism through which social and environmental stressors lead to dysregulation of nuclei that control biological systems and associated behavioral consequences (Nalivaiko, 2006).

In addition to serotonin, dopamine neurotransmission is also associated with stress responses and emotion dysregulation (Gambardella, Greco, Sticchi, Bellotti, & Renzo, 1994; Kehoe, Shoemaker, Arons, Triano, & Suresh, 1998; Tidey & Miczek, 1996). The present study demonstrated that prairie voles housed in isolation exhibited significantly increased dopamine in the hypothalamus following restraint compared to prairie voles that were pair housed. These findings are similar to those of Dronjak & Gavrilovic (2006), who observed greater dopamine stores in the hypothalamus following 120 minutes of restraint in rats previously exposed to 21 days of social isolation, compared to group housed rats. The increased dopamine in the hypothalamus in isolated prairie voles observed in the present study may be associated with alterations in hedonic behavior. For instance, prairie voles

exposed to long-term social isolation showed a decrease in sucrose intake and preference, suggestive of anhedonia (Grippe, Cushing, & Carter, 2007; Grippe, Gerena, et al., 2007).

The observations of metabolite and metabolite/neurotransmitter ratios in the current study may offer a more complex interpretation of central nervous system changes following a combination of social isolation and acute restraint. Differences in the pathways of dopamine metabolites, HVA and DOPAC, may contribute to the disparity in findings between metabolites (Elsworth & Roth, 1997), and between dopamine and metabolites (for review see Meiser, Weindl, & Hiller, 2013). In the current study, HVA in the hypothalamus showed an upward trend in isolated prairie voles, following a similar pattern of dopamine content in the hypothalamus. Conversely, the other dopamine metabolite DOPAC did not show this trend. Dopamine is metabolized into DOPAC through monoamine oxidase both intra- and extracellularly, whereas HVA is metabolized further via catechol-O-methyl transferase which is exclusively localized extracellularly (for review see Meiser, Weindl, & Hiller, 2013). Thus, DOPAC levels can be influenced by many different synaptic processes such as reuptake and recycling of dopamine, availability of monoamine oxidase, and then the rate of further metabolism to HVA (for review see Meiser, Weindl, & Hiller, 2013). The increase in monoamines in the hypothalamus without significant changes in metabolite levels may indicate that neurotransmitter synthesis is higher in isolated prairie voles following restraint stress.

In the current experiment with all groups exposed to restraint stress, the increased neurotransmitter content may reflect an increase in synthesis due to chronic isolation rather than the interaction between acute stress and chronic isolation. Similarly, the current study found an increase in hypothalamic serotonin tissue content and a trending decrease in the frontal cortex in prairie voles that were previously isolated, with no differences in 5HIAA. These findings may be the result of increased synthesis of serotonin, while also having a decrease in metabolism resulting in similar 5HIAA levels. Conversely, there may be different levels of reuptake of serotonin from the synapse, resulting in similar levels of degradation and therefore similar levels of 5HIAA. To better understand the impact of chronic isolation with and without an acute stressor, future research could use an in vivo model of measuring both basal and stimulated monoamine and metabolite levels.

In addition to the central measures in this study, corticosterone and ACTH were measured following restraint, to better understand the influence that chronic isolation had on stress-induced alterations of the HPA axis. Relative to paired prairie voles, isolated animals in the current study displayed increased ACTH levels following restraint; however, corticosterone did not differ between paired and isolated groups. This finding is not entirely consistent with previous studies that have investigated the combination of isolation and an acute stressor using the prairie vole model. For example, social isolation combined with a 5 minute resident-intruder stressor elevated neither ACTH nor corticosterone in male prairie voles (however these hormones were elevated in female prairie voles; Grippe, Gerena, et al., 2007). By contrast, social isolation combined with a 5 minute forced swimming stressor increased both ACTH and corticosterone in male and female prairie voles (McNeal et al., 2014). The inconsistencies in HPA axis reactivity to a combination of social isolation and additional acute stressors may be a function of the specific type of acute stressor employed.

HPA axis reactivity may also differ as a function of the timing or predictability of the acute stressor. For example, female prairie voles exposed to restraint at predictable times displayed a habituation of corticosterone levels; however, those that received restraint sessions at unpredictable times did not habituate (Smith, Lieberwirth, & Wang, 2013). Further research is therefore necessary to determine the specific conditions under which social isolation produces exaggerated HPA axis reactivity to additional acute stressors.

Taken together, the present results demonstrate that social isolation may bias neurotransmission toward a subsequent acute stressor in global brain regions associated with stress, emotion, behavior, and endocrine regulation. The lack of group differences in dopamine and serotonin in the hippocampus, frontal cortex, and raphe in the present study may be a function of the analysis of entire brain regions, therefore preventing conclusions regarding possible subregion changes. For example, the ventral hippocampus has been related to stress whereby the dorsal hippocampus is related to both stress and cognitive functions (Fanselow & Dong, 2010). Social isolation may have produced changes in one subregion of the hippocampus such as the ventral, but not dorsal, region. Future studies will extend the current findings by incorporating discrete subregion analyses and experimental approaches that would allow for better localizations of neurotransmitter alterations.

The changes in neurotransmitter concentrations and hormone expression observed in the present study provide evidence that long-term social isolation influences the neurobiological responses to an acute stressor. Mechanistically, stress may bias an organism to negative affective behaviors by decreasing serotonergic neurotransmission from the raphe to the prefrontal cortex (via GABAergic inhibition of transmission in the raphe; Cryan et al., 2005). Further, chronic stress has been shown to decrease dendritic spine density, branch, and length within the prefrontal cortex (Holmes & Wellman, 2009; Radley et al., 2006), which may suggest emotional dysregulation and cognitive inflexibility. Stress also influences the hypothalamus which is involved in both the HPA axis and the hypothalamic-pituitary-gonadal axis. Because of the involvement in both axes, alterations within the hypothalamus can affect a variety of hormones, thereby influencing behaviors ranging from reproduction to stress responses (for review see Toufexis, Rivarola, Lara, & Viau, 2014). Together these neurobiological changes may be associated with decreased intracellular serotonin within the frontal cortex as a function of long-term social isolation. Given the significant reliance on the social environment in prairie voles, studies that focus on central neurotransmission may reveal a unique pattern of responses (versus other rodent species whose behavior and physiology are comparatively less dependent on the surrounding social context). The current findings provide a foundation for future studies using the prairie vole model to improve our understanding of social and behavioral influences on central nervous system functioning.

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Figure 1.

Mean ($\pm$ SEM) serotonin concentration observed in the hypothalamus (top left), raphe (top right), frontal cortex (bottom left), and hippocampus (bottom right) following 60 minutes of restraint in prairie voles exposed to prior social isolation or pairing. Social isolation was associated with altered serotonin levels in the hypothalamus and frontal cortex but not in the hippocampus or raphe. * indicates $p < .05$ vs. paired group, + indicates $p < .10$ vs. paired group. Note the scale differences among the 4 panels.

Figure 2.

Mean ($\pm$ SEM) dopamine concentration observed in the hypothalamus (top left), raphe (top right), frontal cortex (bottom left), and hippocampus (bottom right) following 60 minutes of restraint in prairie voles exposed to prior social isolation or pairing. Social isolation was associated with altered dopamine levels in the hypothalamus but not in the other brain regions. * indicates $p < .05$ vs. paired group. Note the scale differences among the 4 panels.

Figure 3.

Mean ($\pm$ SEM) circulating levels of adrenocorticotrophic hormone (ACTH) and corticosterone following 60 minutes of restraint in prairie voles exposed to prior social isolation or pairing. Social isolation was associated with increased circulating ACTH but not corticosterone. * indicates $p < .05$ vs. paired group.

Table 1.

Mean ($\pm$ SEM) metabolite and turnover ratio within the hypothalamus, raphe, frontal cortex, and hippocampus following 60 minutes of restraint in prairie voles exposed to prior social isolation or pairing. There were no statistically significant differences in metabolite or turnover ratios between the two groups. + indicates $p < .10$ vs. paired group; - indicates that the metabolite was not detectable. 5HT=serotonin, DA=dopamine.

	Metabolites			Turnover Ratio		
	5HIAA	DOPAC	HVA	5HIAA/5HT	DOPAC/DA	HVA/DA
Hypothalamus						
Paired	41.55 $\pm$ 0.41	0.56 $\pm$ 0.10	1.85 $\pm$ 0.66	1.32 $\pm$ 0.05	0.07 $\pm$ 0.01	0.29 $\pm$ 0.10
Isolated	74.51 $\pm$ 17.45	1.04 $\pm$ 0.38	3.09 $\pm$ 0.25 #	1.21 $\pm$ 0.15	0.08 $\pm$ 0.03	0.34 $\pm$ 0.09
Raphe						
Paired	2.24 $\pm$ 0.22	4.79 $\pm$ 0.70	-	0.31 $\pm$ 0.02	0.04 $\pm$ 0.01	-
Isolated	3.47 $\pm$ 1.14	4.45 $\pm$ 0.33	-	0.38 $\pm$ 0.04	0.05 $\pm$ 0.01	-
Frontal Cortex						
Paired	1.22 $\pm$ 0.44	0.68 $\pm$ 0.15	0.66 $\pm$ 0.36	0.11 $\pm$ 0.03	2.32 $\pm$ 0.68	1.53 $\pm$ 0.61
Isolated	1.59 $\pm$ 0.76	0.54 $\pm$ 0.08	-	0.24 $\pm$ 0.12	1.31 $\pm$ 0.50	-
Hippocampus						
Paired	19.80 $\pm$ 2.39	0.31 $\pm$ 0.15	0.94 $\pm$ 0.22	5.28 $\pm$ 1.47	1.96 $\pm$ 1.00	4.27 $\pm$ 1.10
Isolated	15.33 $\pm$ 2.38	0.07 $\pm$ 0.01	0.85 $\pm$ 0.15	4.10 $\pm$ 0.23	0.43 $\pm$ 0.05	5.00 $\pm$ 0.84