Social Stress Buffering by Friends in Childhood and Adolescence: Effects on HPA and Oxytocin Activity

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Dedication

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Abstract

Previous research has demonstrated that before puberty, parents are able to buffer, and often completely block, cortisol responses to social evaluative stressors (e.g., Trier Social Stress Test; TSST). However, after puberty, parents no longer provide a powerful buffer of the HPA axis from a social-evaluative stressor. The current study investigates whether friends can buffer the HPA axis in both children and adolescents compared to parents and whether similar stress-ameliorating patterns can also be observed in oxytocin activity. A total of 109 participants (54 children ages 9-10 and 55 adolescents ages 15-16; approximately half of each sex) completed the TSST and were randomly assigned to prepare for their speech with their parent or friend for 5 minutes beforehand. Salivary cortisol and urinary oxytocin were measured before and after the TSST. For children, cortisol responses were comparable regardless of who helped the child prepare the speech. For adolescents, however, friends actually amplified the cortisol response compared to parents. In addition, adolescents produced less oxytocin than children, as did males compared to females. Notably, for boys, oxytocin levels decreased across the session if participants prepared with a friend rather than their parent. The mean change was in the same direction but not significant for girls. These results indicate that friends do not take over the social buffering role by age 15-16, which may inform interventions in at-risk children and adolescents.

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Chapter 1: Introduction

Chronic stress in childhood, adolescence, and adulthood has been associated with increased rates of physical health problems and psychological disorders (McEwen & Seeman, 1999; Repetti, Taylor, & Seeman, 2002). Adolescence in particular has been characterized by increased rates of internalizing and externalizing disorders, including depression, anxiety disorders, and substance use disorders (see Costello, Copeland, & Angold, 2011, for review). As psychosocial stressors have been shown to contribute to the onset of these disorders, increased attention has been focused on mechanisms by which adolescents can be buffered from stressors to prevent the onset or recurrence of mental illness. For instance, loneliness or a lack of social support predicts both morbidity and mortality in adults (House, Landis, & Umberson, 1988), while having a network of social support is related to more positive outcomes for the cardiovascular, immune, and neuroendocrine systems (Uchino, Cacioppo, & Kiecolt-Glaser, 1996). This is thought to occur through alterations in cardiovascular activity, sleep, immune function, stress reactivity, and health behaviors (Cacioppo et al., 2002; Hawkley & Cacioppo, 2003). There is vast evidence that loneliness during adolescence has negative consequences for mental health and health behaviors (Heinrich & Gallone, 2006). Although these are long-term examples, there is extensive evidence demonstrating that physiological responses to acute stressors can be dampened through a phenomenon termed social buffering.

Social buffering has been defined as the reduction of physiological stress responses to an acute stressful event due to the presence or assistance of another individual, often a person with a close relationship to the person undergoing the stressor. This reduced physiological response may not be specific to a particular system and may

include systems such as the hypothalamic-pituitary-adrenal (HPA) axis and autonomic nervous system. Social buffering by parents may serve to protect adolescents from the deleterious effects of stress by decreasing activation of stress-mediating systems. However, social buffering does not include all social behaviors that can protect against threat or negative social experiences; it is specifically the presence and behaviors of a close social figure (e.g., parent, romantic partner, or friend) that blocks activation of physiological systems in response to an acute stressor. Social buffering may "get under the skin" and affect the neuroendocrine system through physiological and molecular changes that may also be expressed as new or adaptive behavior (e.g., effective coping strategies). Physiological pathways include, but are not limited to, alterations in neural activity, decreased autonomic reactivity, epigenetic alterations, and changes in oxytocin levels (Hostinar, Sullivan, & Gunnar, 2013). Psychological pathways of social buffering include those that are emotional, instrumental, or informational (Cobb, 1976; House & Kahn, 1985), including making the individual feel loved and cared for, actually assisting in the resolution of the stressor, or by supporting coping mechanisms.

Social buffering may prevent some of the deleterious effects of chronic HPA activation by blocking activation of the HPA axis to stressors and thus avoiding chronic HPA activation even in a stressful environment. This phenomenon may be a primary reason why social support has positive effects on mental and physical health. As a result, understanding potential mechanisms of the social buffering effect may inform treatments that decrease wear and tear on the body following stress. A recent theory about why social support is associated with more positive outcomes, termed Social Baseline Theory, posits that being embedded in a social network is an experience that is expected by the

human brain (Beckes & Coan, 2011). Thus, being in a relatively predictable and supportive social network is the brain's "baseline." As neural circuits associated with emotion regulation (e.g., dorsolateral prefrontal cortex) are *less* active when in the presence of social support than without it, the brain may be less vigilant for threat and rely more on supportive individuals in order to conserve valuable metabolic resources (reviewed in Beckes & Coan, 2011). Being with a social buffer, therefore, may help individuals regulate themselves in the face of threat by reducing activation of neural circuits involved in the detection, interpretation, and response to threat. As a result, downstream mediators such as the release of cortisol from the adrenal cortex may dampened by remaining at the social baseline.

Social buffering has been observed across the lifespan, but the majority of research has been on adults who are buffered by friends or romantic partners or on children who are buffered by parents. Previous research has demonstrated that parents fail to buffer the HPA axis response to social stressors during adolescence at age 15-16 but parents remain effective buffers when children are 9-10 years old (Hostinar, Johnson, & Gunnar, 2015). This disappearance of parental social buffering may contribute to increased rates of psychopathology and physical health problems, especially in the context of chronic stress.

Social Buffering in Animal Models

Research in animal models has demonstrated the profound effect of social buffering on dampening of the stress response in a range of species. More long-term studies demonstrate effects from early postnatal life through adulthood. For example, during the first 2 postnatal weeks of the rat pup, feeding and tactile stimulation provided

by the mother dampens HPA activity at basal levels and in response to stress (Levine, 2000). Specifically, maternal licking and grooming of the rat pup decreases adrenocorticotropic hormone (ACTH) and corticosterone reactivity to stress, heightens glucocorticoid feedback sensitivity, and lowers corticotropin-releasing hormone (CRH) messenger RNA in the hypothalamus (Liu et al., 1997). In adulthood, after pair bonding, both male and female tree shrews show a drastic reduction in glucocorticoids, a decrease in heart rate, and improved immune function (von Holst, 1998). In tree shrew pairs that are harmonious, cortisol reactivity decreases compared to individuals in non-harmonious pairs (von Holst, 1998). There is also evidence that being housed in isolation is associated with greater HPA responses to stress in rats than being housed in groups (Bartolomucci et al., 2003; Dronjak, Gabrilovic, Filipovic, & Radojcic, 2004).

Acute stressor studies demonstrate similar effects of maternal and partner support. For parental buffering, the effectiveness is dependent on the attachment pattern. For example, there is a bidirectional HPA buffering effect between squirrel monkey mothers and infants in response to a disturbance manipulation or a disturbance plus novelty manipulation, which reflects the attachment pattern between mother and infant in squirrel monkeys (Wiener, Johnson, & Levine, 1987). However, in titi monkeys, the infant shows greater buffering in the father's presence than the mother's, which is consistent with the father's greater role in caregiving and attachment (Hoffman, Mendoza, Hennessy, & Mason, 1995; Mendoza & Mason, 1986). In titi monkeys, the mother's presence more effectively buffers HPA responses of the infants than having no parental presence, indicating that the maternal effect may be graded by attachment status rather than nonexistent (Hoffman et al., 1995). In adult male guinea pigs, there is an attenuation of

the cortisol response to a novel environment when with a bonded female compared to a strange or acquaintance female (Hennessy, Hornschuh, Kaiser, & Sachser, 2006; Sachser, Durschlag, & Hirzel, 1998). A similar effect of partner buffering of acute stress is observed for both male and female titi monkeys and Wied's black tufted-ear marmosets (Hennessy, Mendoza, Mason, & Moberg, 1995; Smith, McGreer-Whitworth, & French, 1998).

Research on social buffering from the period between weaning and adulthood (potentially the equivalent to adolescence in humans) in animal models is mixed. In adolescent rhesus monkeys, both mothers and peers can buffer cortisol responses to stress, but in an inconsistent manner (Rilling et al., 2001; Winslow et al., 2003). Likewise, squirrel monkeys that have been peer housed show little to no increase in cortisol secretion in response to novelty (Hennessy, 1984; Hennessy, Mendoza, & Kaplan, 1982). In guinea pigs, adolescents are buffered from stress by maternal care weeks beyond the weaning period (Hennessy, Nigh, Sims, & Long, 1995). Interestingly, other adult females are as effective at reducing cortisol reactivity, whether familiar or not (Graves & Hennessy, 2000; Hennessy, Maken, & Graves, 2000, 2002; Maken & Hennessy, 2009), but adult males and familiar sibling cage-mates fail to buffer the response (Hennessy et al., 1995; Hennessy, O'Leary, Hawke, & Wilson, 2002). Taken together, these studies of post-weaning to pre-adulthood social buffering in animal models suggest significant but inconsistent alterations in the effectiveness of social buffering by parents, peers, and partners that may be moderated by a number of factors.

Social Buffering in Humans

In humans, researchers have reported findings similar to the animal literature.

Already by 12-18 months of age, the presence of a parent reduces or completely blocks the cortisol response to both fearful experiences and physical pain as long as the parent and child share a secure attachment relationship (Gunnar, Brodersen, Nachmias, Buss & Rigatuso, 1996; Nachmias, Gunnar, Mangelsdorf, Parritz & Buss, 1996; Spangler & Schieche, 1998). However, inhibited children with an insecure attachment to the parent experience less effective social buffering, suggesting that relationship quality is an important moderator of the effect (Nachmias et al., 1996; Spangler & Schieche, 1998). The physical presence of the attachment figure may be necessary to elicit the buffering effect in early childhood as children who are securely attached show similar elevations in cortisol to insecurely attached children during the first few days of day care after the parent leaves (Ahnert et al., 2004). Physiological social buffering occurs even if the child is exhibiting behaviors of distress (Nachmias et al., 1996). As a result, the HPA system and behavior may act relatively independently of each other, at least at this early period in life, which suggests that other physiological systems may be involved and that increased cortisol reactivity cannot be implied from behavioral distress. One study of mid-to-late childhood reported that for 7-12 year old girls, maternal support following the Trier Social Stress Test for Children (TSST-C), a social evaluative speaking task, reduces elevations in cortisol in a graded manner. Girls who returned to their mother after the TSST-C showed nearly blocked cortisol elevations, while those who were only allowed to have phone contact showed some cortisol reduction compared to girls with no contact with their mother (Seltzer, Ziegler & Pollak, 2010). Thus, social buffering by the mother at this age is effective, and the mother may not have to be physically present to show at least some effect.

Social buffering has been observed in adulthood as well; however, studies focus on the ability of peers or romantic partners rather than the parent to buffer responses to stress as adults may rely on these individuals more for social support. In addition, social evaluative stressors are often used as the psychosocial challenge in the laboratory as they have been shown to consistently activate the HPA axis. For men, preparing for the Trier Social Stress Test (TSST), an evaluative public speaking task, with their female romantic partner reduced cortisol reactivity to the task, but participating with an unfamiliar woman did not buffer the HPA response, indicating that a certain level of intimacy is needed for the buffering effect (Kirschbaum, Klauer, Filipp, & Hellhammer, 1995). However, the buffering effect appears to be different for women, who tended to have increased cortisol when preparing with their male romantic partner (Kirschbaum et al., 1995). It appears that when women receive a massage from their male partner, they then show attenuated cortisol response to stress suggesting that verbal support alone is not enough to experience social buffering effects of male partners for women (Ditzen et al., 2007).

For women, support from a close female friend significantly reduced cardiovascular responses to a social stress test, suggesting that autonomic activity may also undergo social buffering (Fontana, Diegnan, Villeneuve & Lepore, 1999; Uno, Uchino & Smith, 2002). Not all types of social support reduce physiological reactivity, though, as preparing with a stranger elicits a cortisol response to stress for children, adolescents, and adults (Hostinar et al., 2015; Kirschbaum et al., 1995). Even if judges during the TSST are positive and friendly, cortisol still increases compared to the no-audience condition, indicating that social buffering may not be solely due to general positive affect during social interactions (Taylor et al., 2010). However, even with

strangers, eliciting self-disclosure increases intimacy that may block cortisol responses to stress despite having no prior relationship history, suggesting that emotional intimacy may be a key to social buffering effects (Smith, Loving, Crockett, & Campbell, 2009).

Little research has been done on social buffering in adolescence, so it is unclear at this point which people can buffer stress for adolescents (e.g., parents and/or friends), under what conditions social buffering occurs, and what changes occur across adolescence. A study of adolescents ages 12-16 revealed that recovering from a stressor with a *low negative quality* best friend was related to better HPA axis recovery and that even in a *low positive quality* friendship, higher levels of responsiveness after the stressor aided recovery (Calhoun et al., 2014). However, there was an interaction between positive friendship quality and responsiveness such that *high positive quality* friendship and high levels of responsiveness by the adolescent's best friend was associated with poorer recovery from the stressor, possibly due to co-rumination about the stressor (Calhoun et al., 2014). Thus, both relationship history and quality of support may predict recovery post-stressor. A more ecologically valid study of social buffering, although not experimental, reported that children aged 10-11 years demonstrated lower cortisol levels after a negative event when they reported being with their best friend during that event (Adams, Santo, & Bukowski, 2011).

As noted earlier, previous work from our group has reported that parental support buffers cortisol responses for children ages 9-10 years but not for children ages 15-16 (Hostinar, Johnson, & Gunnar, 2015). Receiving support from a stranger did not buffer cortisol reactivity in either age group, suggesting that the buffering effect is specific to familiar individuals (Hostinar et al., 2015). This decreased efficacy of parental buffering

for older adolescents may increase the likelihood for psychopathology and stress-related disorders if other supportive individuals cannot take over the buffering role. The precise neurobiological mechanisms behind the social buffering effect are not fully understood. In addition, the mechanisms behind the loss of sensitivity to parental support in later adolescence are unclear. It appears that the loss of parental buffering of cortisol reactivity may be more due to the pubertal transition than to age-related changes, suggesting a mechanism associated with hormonal changes during puberty may be responsible for the shift in parental buffering effectiveness (Doom, Hostinar, VanZomeren-Dohm, & Gunnar, 2015). In addition, there were no effects of age or puberty on post-stressor alpha-amylase, an enzyme secreted by the salivary glands that is a marker of autonomic arousal (Doom et al., 2015), indicating that the autonomic nervous systems of adolescents are still responding but that there may be a mechanism blocking the adrenal release of cortisol or an upstream HPA mediator. There is evidence of developmental changes in neural activity in response to threat and social buffering, as with adolescence, there is no difference in right amygdala reactivity when viewing photos of one's mother versus a stranger, even though images of the mother buffered amygdala reactivity compared to images of a stranger during childhood (Gee et al., 2014). Another possible reason for alterations in parental buffering include increased reliance on friends and romantic partners across puberty. Finally, it could be that parents do not fully lose effectiveness in social buffering but the process may be stressor-specific, such that potent stressors (e.g., social evaluative) may be too powerful to buffer. In order to better understand social buffering of the HPA axis, HPA physiology will be reviewed next with an emphasis on

what activates the axis, what blocks activation, and what promotes recovery from challenge.

HPA Axis Physiology

The primary target of HPA measurement in human studies is the glucocorticoid cortisol, which is the end product of the HPA axis, produced by the cortex of the adrenal glands. The signaling cascade that initiates the production of cortisol begins in the paraventricular nucleus (PVN) of the hypothalamus (reviewed by Gunnar & Quevedo, 2007). Upon stimulation, the PVN produces the neuropeptides corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP), which then signal the pituitary gland to produce adrenocorticotropic hormone (ACTH). ACTH is then released into circulation, reaching receptors in the adrenal cortex that stimulate the production of cortisol. Cortisol is also released into the bloodstream, acting throughout the brain and body. Most organs and tissues of the body contain cortisol receptors, and a large number of genes contain glucocorticoid responsive elements, which are responsible for cortisol's widespread effects in the body. In response to stressors, the genomic actions of cortisol increase glucose that is available to cells, inhibit processes not necessary for survival, and reduce inflammation (reviewed in Gunnar, Doom, & Esposito, 2015). The non-genomic actions of cortisol during stressors permissively facilitate the fight-or-flight response.

Mineralocorticoid and glucocorticoid receptors (MRs and GRs) bind cortisol in order to mediate basal and stress-related functions. MRs have higher affinity for cortisol, so cortisol will preferentially bind to MRs unless the enzyme barrier 11B-HSD2 is present to render cortisol inert (Wyrwoll, Homes, & Seckl, 2011). GRs will bind to cortisol after most MRs are bound or if they are not present. In the brain, GR activity is

highest at the peak of the circadian cycle or when the individual is challenged by a stressor. Overall, MRs mediate basal activity of cortisol while GRs are more responsible for stress responses (for review, see Gunnar & Vazquez, 2006). Cortisol binding globulin binds to 80-90% of cortisol in circulation, which prevents interactions with MRs and GRs. Cortisol that is not bound to receptors can enter freely into all cells, where it can interact with its receptors in the cytoplasm. After forming, the hormone-receptor complex interacts with glucocorticoid responsive elements in the nucleus to regulate gene transcription.

Following the onset of a stressor, it generally takes 20-25 minutes for cortisol levels to peak in plasma and another 2 minutes for peak saliva levels (Gunnar et al., 2015). However, there are significant individual differences in stress reactivity and recovery as the same stressor may not activate the axis in all individuals. There is considerable variability in cortisol reactivity as well as the ability to recover to baseline after stressor onset and termination. A meta-analysis of specific types of stressors that activate the HPA axis revealed that several factors were related to variability in cortisol reactivity and recovery (Dickerson & Kemeny, 2004). Both physical and psychogenic stressors reliably activate the HPA axis, especially if they are interpreted as uncontrollable (Dickerson & Kemeny, 2004). It is possible that one reason social support can effectively buffer the HPA axis or return the system to baseline faster is that stressors are interpreted as more controllable when encountering them with a close individual. Other predictors of HPA regulation are the emotions elicited by the stressor. Self-evaluative emotions such as shame may be particularly tied to HPA reactivity and recovery as shame may heighten and/or prolong the stress response (Dickerson &

Kemeny, 2004). Further, ruminating about negative events and emotions with others may prevent the HPA system from returning to baseline post-stressor (Dickerson & Kemeny, 2004). Finally, psychiatric sequelae may predict differences in HPA reactivity and recovery (Dickerson & Kemeny, 2004). From a social buffering standpoint, a lack of social support is a risk factor for a number of psychiatric disorders. Low support and psychiatric symptoms may both be contributing to exaggerated HPA responses and difficulty returning to baseline after the threat. Thus, individual differences such as genetics and history of stress may lead to vastly different HPA responses to the same stressor.

In addition to basal effects on physiological regulation, the HPA axis performs significant actions in response to stressors, which are specific to the type of stressor experienced (Jöels & Baram, 2009). These stressors may be classified as systemic (e.g., infection, heat or cold stress, physical injury) or as psychogenic. Stressors are classified as psychogenic if they require processing and elaboration of the forebrain in order to activate the HPA axis. In order to respond to psychogenic stressors, the central nucleus of the amygdala must be stimulated, triggering a pathway through the bed nucleus of the stria terminalis to the PVN, which releases CRH (Ulrich-Lai & Herman, 2009). For humans, threats to the social self, especially when perceived as unpredictable and/or uncontrollable, are one of the most potent triggers of HPA activation (Dickerson & Kemeny, 2004). As a result, one of the most reliable laboratory stress tasks in the field is the TSST, which consists of a public speaking and mental arithmetic task that is taped and evaluated by live judges (Kirschbaum, Pirke, & Hellhammer, 1993). There is a version for children and adolescents, but for younger children, there may be buffering of

the HPA response depending on the child's relationship with the parent accompanying them (Gunnar, Talge, & Herrera, 2009).

Finally, cortisol can control its own release through negative feedback mechanisms in the hypothalamus, pituitary, hippocampus, and medial prefrontal cortex (mPFC; Tasker & Herman, 2011). Tonic inhibition acts on the HPA axis through GABA-producing cells that surround the PVN. Down-regulation of GABAergic input to the PVN, often due to chronic stress, reduces tonic inhibition of the system. Different levels of the HPA axis also demonstrate negative feedback, including CRH and ACTH regulating their own production and the synthesis of molecules at other levels. Negative feedback of cortisol appears to operate on different time scales as well (Jöels & Baram, 2009). This feedback may be fast through the endocannabinoid system in the PVN or slow through GR-mediated genomic mechanisms in the hippocampus and mPFC (Jöels & Baram, 2009). Negative feedback mechanisms may also be regulated following chronic stress by reduced GR expression in the hippocampus and mPFC, which may protect the brain from the effects of chronic HPA activation (Jöels & Baram, 2009). It is possible that in addition to blocking HPA activation in response to stress, social buffering may also operate to return the system to baseline faster through more sensitive or efficient negative feedback mechanisms.

Pubertal & age-related changes. In general, the transition from childhood to adolescence is accompanied by increased basal cortisol levels (see Gunnar & Vazquez, 2006, for review), and stressors that involve performance and peer evaluation may be particularly susceptible to increased reactivity across adolescence due to both changes in hormones and increased salience of social evaluation (Gunnar, Wewerka, Frenn, Long &

Griggs, 2009; Sumter, Bokhorst, Miers, Van Pelt & Westenberg, 2010; van den Bos, de Rooij, Miers, Bokhorst & Westenberg, 2014). Increased gonadal steroids during puberty facilitate the sexually dimorphic development of the adolescent brain (Neufang et al., 2009), which coincides with the more sexually differentiated HPA system across adolescence. Just after puberty at age 16, girls tend to have higher morning cortisol levels while boys have higher morning ACTH levels (Reynolds et al., 2013). In addition, the cortisol awakening response (CAR) becomes more differentiated with puberty, with males typically demonstrating peak cortisol levels 30 minutes after awakening and females peaking around 45 minutes (Schlotz, Hellhammer, Schulz, & Stone, 2004). Adult men typically respond more strongly to agentic stressor tasks, such as the TSST and matriculation exams, than women due to hormonal differences (Kudielka & Kirschbaum, 2005), and this is only true during certain phases of the menstrual cycle (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999). However, sex differences are unreliable in childhood and tend to emerge during puberty (Gunnar, Wewerka, Frenn, Long, & Griggs, 2009). This time of sexual differentiation and heightened HPA reactivity is thought to contribute to increased rates of mental illnesses like depression, particularly for girls (Dahl & Gunnar, 2009). Thus, adolescence may be an important time to mitigate the effects of chronic HPA hyperreactivity in order to lessen the burden of mental illness. Interestingly, the pubertal transition has been described as a second sensitive period of HPA axis vulnerability which may be modified based on the sex of individual (Eiland & Romeo, 2012).

A number of social changes happening during adolescence parallel those of physical development during puberty. Adolescents report becoming more emotionally

intimate with peers while becoming more distant from parents (Cauce, 1986; Harris, 1995; Hartup, 1996; Hunter & Youniss, 1982). Parental relationships do not typically wane completely, as parents remain nurturers and provide advice to their offspring through young adulthood (Collins & Laursen, 2004; Furman & Buhrmester, 1992; Hunter & Youniss, 1982). The roles of parents do change with increasing time spent with peers and greater emotional closeness with peers (Laursen & Collins, 2009). As a result, there are both social and biological shifts that are preparing adolescents for adulthood, which may affect who acts as stress buffers during this time of transition.

Of course, one's overall support network and the quality of the relationship and supportive behaviors of the social buffer have been shown to be important moderators of stress reactivity. Recent research has attempted to tease apart the concepts of perceived versus received support in order to understand their unique impacts on stress reactivity (Uchino, Carlisle, Birmingham, & Vaughn, 2011). Perceived support is one's perception of their access to supportive individuals, while received support is the actual receipt of social support during a specific time frame (Dunkel-Schetter & Bennett, 1990). One study demonstrated differential susceptibility to a laboratory stressor based on whether individuals had high or low perceived social network support. When told the experimenter would provide no support during the social evaluative stressor, those with high network support showed lower heart rate reactivity to the stressor than individuals with low network support (O'Donovan & Hughes, 2008). However, when offered support from the experimenter, individuals with low perceived social support showed lower heart rate reactivity, suggesting that perceiving support outside of the stressful situation may be a powerful buffer of stress responses but that individuals with low perceived support may

particularly benefit from received support (O'Donovan & Hughes, 2008). Individuals who perceive more support may also have other psychological characteristics such as high self-esteem that may affect stress reactivity (Uchino, 2009). Another study examined the impact of the quality of support behaviors (received support) on cardiovascular reactivity. Participants who received support from an ambivalent friend demonstrated higher systolic blood pressure reactivity than those who received support from a supportive friend, although there were no differences between diastolic blood pressure or heart rate (Reblin, Uchino, & Smith, 2010). Both perceived support and quality of received support will be examined in relation to cortisol reactivity and recovery in the current study to ensure that any differences observed are not solely due to perceived or received support.

Oxytocin System Physiology

While the study of social stress buffering has focused on the reduction or prevention of heightened reactivity in stress-mediating systems, part of the positive effects of close relationships may also come from the stimulation of hormones and other neurochemicals that have restorative or anti-stress effects. One system that exhibits inhibitory effects on HPA activity is the oxytocin system, which may be a mechanism by which social support blocks activation of the HPA axis following an acute stressor. Studies of prairie voles have demonstrated that oxytocin mediates the impact of partner presence on decreased corticosterone release following an immobilization stressor (Smith & Wang, 2013). Administration of intranasal oxytocin in humans results in decreased anxiety and cortisol reactivity to stress (Ditzen et al., 2009; Heinrichs et al., 2003), and there is correlational evidence that maternal support is related to increased oxytocin and

decreased cortisol in response to stress in children (Seltzer et al., 2010). For these reasons, and due to further evidence discussed in detail below, oxytocin has been targeted as a potential mediator of HPA activity and social buffering in humans. The physiology of the oxytocin system will now be reviewed followed by more specific evidence for the effects of oxytocin on social behavior and the HPA axis.

Physiology and anatomy of the oxytocin system. The hormone oxytocin is a 9 amino acid peptide that forms both temporary and long-lasting bonds with other chemicals to exert diverse physiological actions (Martin & Carter, 2013). Unlike a neurotransmitter, oxytocin acts broadly as a neuromodulator after being released from the soma, axons, and dendrites of neurons, transporting itself through a volume transmission process to eventually effect widespread alterations throughout the body (Neumann & Landgraf, 2012; Stoop, 2012). Hypothalamic midline neurons contain the highest concentration of oxytocin-synthesizing cells, including the paraventricular nucleus (PVN) and the supraoptic nuclei of the hypothalamus (Gainer, 2012). Neuronal projections from the hypothalamus extend to the posterior pituitary, which stores and releases oxytocin into neurohypophyseal capillaries and then into circulation (Brownstein, Russell, & Gainer, 1980). Projections between the PVN and the amygdala allow oxytocin to quickly travel and modulate affective processes (Stoop, 2012), potentially promoting more positive emotions and approach behaviors (Carter, 1998).

The synthesis of oxytocin in the PVN is crucial to its widespread neuromodulatory actions as the PVN integrates input from various systems, including the HPA axis and autonomic nervous system (Herman et al., 2012). Of particular importance, a major regulator of the HPA axis, CRH, is produced in the same subset of PVN neurons

as oxytocin, which allows for potential co-release during both positive and negative challenge (Aguilera, Subburaju, Young, & Chen, 2008; Carter et al., 2008; Neumann & Landgraf, 2012). The PVN not only releases oxytocin but also responds to the molecule, which may impact feedback mechanisms and influence other systems supported by PVN function. In addition, oxytocin is released tonically in the brain and travels throughout circulation after release from the posterior pituitary (Neumann & Landgraf, 2012). Pulsatile release of oxytocin occurs when muscle contractions are warranted, such as in the uterus or mammary glands (Carter, 2014). Plasticity of hypothalamic cells may explain pulsatile oxytocin release, as glial processes separating oxytocin-containing neurons are withdrawn to allow electrical coupling and pulsatile oxytocin release (Carter, 2014). Similar to the HPA system, feedback mechanisms exist in the oxytocin system. Cells that synthesize oxytocin may feed forward, producing additional oxytocin and may also induce endogenous oxytocin production in the central nervous system (Grippo et al., 2012). Oxytocin is highly prevalent in both the brain and serum, but since oxytocin is often bound to other molecules in the blood, certain assays may underestimate the true amount of oxytocin present (Martin & Carter, 2013). In human females, oxytocin receptors have been visualized in the anterior and ventromedial hypothalamus, central and basolateral amygdala, ventrolateral septum, olfactory nucleus, hypoglossal and solitary nuclei, medial preoptic area, and the anterior cingulate (Boccia, Petrusz, Suzuki, Marson, & Pedersen, 2013). Interestingly, oxytocin receptors have not been observed in the hippocampus, raphe nucleus, nucleus ambiguus, parietal cortex, or pons (Boccia et al., 2013). Species differences in brain and blood oxytocin levels are present, and

individual differences in oxytocin levels have been linked to individual variations in social behavior (Gouin et al., 2010; Kramer et al., 2004).

Oxytocin's actions are closely coordinated with vasopressin, a structurally similar neuropeptide that is capable of binding with oxytocin receptors, and vice versa. Unlike oxytocin, which has been associated with anxiolytic and affiliative behaviors (Carter, 2014), vasopressin is linked to anxiety and defense behaviors. In fact, a number of actions of these molecules appear to be directly opposing. For example, oxytocin facilitates maternal nurturance and nursing behaviors (Pedersen, 1997), while vasopressin is associated with both maternal aggression (Bosch & Neumann, 2012) and paternal defense of offspring (Kenkel et al., 2012, 2013). However, vasopressin may also facilitate selective social bonding, including pair bonding, which allows the individual to protect themselves and other members of their social network (Carter, 1998; Winslow et al. 1993). Important sex differences in the actions of vasopressin may be responsible for gender differences in stress management strategies (e.g., fight-or-flight for males and tend-and-befriend for females; Carter, 1998; Taylor et al., 2000). It is thought that the interplay between vasopressin and oxytocin in concert with other molecules such as dopamine and endogenous opioids underlies a number of social behaviors (Carter, 2014).

Like many neuromodulators, the effects of oxytocin cannot be determined solely on the amount of the neuropeptide in circulation but on the activity of oxytocin receptors throughout the brain and body. How these receptors are expressed is influenced by genetic and epigenetic modifications to receptor systems in oxytocin pathways (Ebstein et al., 2012; Gregory et al., 2009), and it is through these modifications that individual differences in behavior and responses to stress may arise. One oxytocin receptor in

particular, and the only one that has been described, is coded for by the gene *OXTR* and is present in both neural and peripheral tissue, including the uterus and the breast (Gimpl & Fahrenholz, 2001; Carter, 2014). For vasopressin, two of the three identified receptor subtypes are related to behavior. V1a is related to cardiovascular function, regulation in response to stress, and social behavior, particularly for males (Carter, 2014). V1b is associated with both physiological and behavioral responses to stress, including aggression (Stevenson & Caldwell, 2012). Species differences in both oxytocin and vasopressin receptor expression have been observed, which may contribute to affective and behavioral differences across species (Carter, 2014). These oxytocin and vasopressin receptors are found in regions of the central and peripheral nervous system supporting social and affective functioning and behavior. These areas include amygdala, which is responsible for affective processing, the autonomic nervous system, and the HPA axis. Certain brain stem structures have particularly high oxytocin receptor concentration, suggesting that oxytocin activity may have special importance for these regions. In addition, there are oxytocin receptors in nearly all of the visceral organs, and there is evidence that oxytocin may be locally synthesized in these regions, which highlights the critical importance of oxytocin as a modulator of numerous physiological systems (Gimpl & Fahrenholz 2001; Welch et al. 2009).

Both positive and negative experiences have been linked to oxytocin release, and oxytocin release appears to be an important component of managing responses to arousing situations by supporting activation of the sympathetic nervous system, vagal system, and the HPA axis (Carter, 1992; Dai et al., 2012; Feldman, 2012; Kenkel et al., 2013). A large increase in oxytocin may lead to occupation of vasopressin receptors, thus

producing defensive behaviors typically associated with vasopressin release (Carter, 2014). Higher intensity stressors are particularly effective at releasing oxytocin in males and females (Neumann & Landgraf, 2012; Pournajafi-Nazarloo et al., 2013), but early social experiences and context are related to individual differences in how much oxytocin is released and what stress-mediating actions are supported (Bartz et al., 2011).

Sex differences in the oxytocin system. Research has confirmed that oxytocin operates in both males and females, although it was once thought of as a female reproductive hormone (Lee et al., 2009). Vasopressin is also important to social behavior in both sexes but may be more salient for behavior in males (Carter, 2014). Although both neuropeptides operate in men and women, some of the effects differ between the sexes (Carter, 2007; De Vries & Panciza, 2006; Taylor et al., 2010), and it is thought these variations may be particularly important early in life for observed sex differences in behavior. Future research must target early sex differences in social behavior to understand the contributions of the oxytocin and vasopressin systems to sexual differentiation in social behavior.

Development of the oxytocin system. Developmental trajectories of oxytocin and vasopressin-containing nuclei have been recorded in the pig hypothalamus, which may indicate similar alterations in humans if the oxytocin system is similar across mammals (van Eerdenburg, Poot, Molenaar, van Leeuwen, & Swaab, 1990). The day after birth, oxytocin and vasopressin-containing neurons in the hypothalamus decrease by approximately half at 16 weeks postnatal (right before puberty; van Eerdenburg et al., 1990). However, the number of oxytocin and vasopressin-containing neurons nearly triples across puberty (between 16 and 30 weeks) (van Eerdenburg et al., 1990). This

finding could indicate greater involvement of oxytocin and vasopressin in stress regulation and social behavior starting in adolescence if replicated in humans. In rats, neuronal oxytocin mRNA has been shown to be upregulated during puberty, and this upregulation is dependent on gonadal steroids as gonadectomized rats did not experience this upregulation for the most part (Chibbar, Toma, Mitchell, & Miller, 1990). Gonadectomized rats that were given estradiol or testosterone did show oxytocin mRNA upregulation, indicating that the increase in gonadal steroids during puberty is needed for oxytocin upregulation (Chibbar et al., 1990). However, gonadal steroids are not the only necessary component, as giving these steroids to prepubertal rats did not increase oxytocin upregulation, indicating that some neural maturation is also needed in concert with gonadal steroids to initiate oxytocin upregulation (Chibbar et al., 1990).

Social Experience and Oxytocin System Interactions

Although often thought of as the "hormone of love", oxytocin has been implicated in a wide range of social behaviors, and it has been proposed that the large cortex, sophisticated social cognition, and complex network of social ties in humans are at least partially attributable to the effects of oxytocin across evolution (Carter et al., 2014). Oxytocin has a role in a range of emotions and social behaviors including empathy (Carter et al., 2009; Hurlemann et al. 2010), cooperation (Rilling et al. 2012), and trust (Kosfeld et al., 2005). Overall, oxytocin supports the ability to be sensitive to others and the formation of selective social behaviors and bonds (Carter et al., 2014). A number of studies conducted in prairie voles first shed light on the capacity for oxytocin to facilitate both social behaviors and selective bonds for males and females (Cho et al., 1999; Williams et al., 1994). It appears that mating initiates pair bonding in prairie voles and

that this process is oxytocin-dependent (Williams et al., 1992, 1994). Further, selective bonding requires the presence of both oxytocin and vasopressin receptors as either receptor type alone only leads to non-selective social behaviors (Cho et al., 1999; Young et al., 2011), but it is unknown whether both of these receptors are needed for bond formation in humans. Interestingly, injecting oxytocin antagonists into dopamine-rich regions, including the nucleus accumbens (NAcc) or prefrontal cortex (PFC) of female prairie voles prevented formation of partner preference, while voles injected with the antagonist in the caudate putamen or vehicle into any brain area still formed a partner preference (Young, Lim, Gingrich, & Insel, 2001). As a result, at least in prairie voles, there is evidence that dopaminergic circuits may be involved in the development of social bonds following oxytocin receptor activation. Further experiments in prairie voles suggests that blocking D2 dopamine receptors in the nucleus NAcc prevents partner formation, and D2 agonists promote partner formation (Gingrich, Liu, Cascio, Wang, & Insel, 2000). At the level of the epigenome, neural activation of brain areas associated with social behavior is also correlated with DNA methylation of OXTR in humans (Jack, Connelly, & Morris, 2012).

Intranasal oxytocin administration has been shown to increase trust and prosocial affiliation while playing games of monetary risk in humans (Kosfeld et al., 2005). Further examination demonstrated that this alteration in behavior was not due to a general increase in risk-taking, but was specific to social games (Kosfeld et al., 2005). Oxytocin has been administered in studies of neural activation in response to social (fearful or angry faces) versus non-social images (frightening situations). Individuals who received oxytocin showed greater overall reduction in amygdala responses but that the effect was

larger for social images (Domes et al., 2007; Kirsch et al., 2005), indicating that oxytocin may have both general effects on emotion processing and more specific effects that modify emotional responses to social information. In a conflict discussion task within couples, oxytocin administration has resulted in more positive communication and reduced salivary cortisol levels compared to individuals who receive a placebo (Ditzen et al., 2009).

In response to chronic stress, oxytocin appears to facilitate more social or passive coping mechanisms accompanied by a sense of safety, termed "immobility without fear" (Porges, 1998) rather than the more mobilized and active coping behaviors initiated by vasopressin (Carter et al., 2007; Taylor et al., 2000, 2010). While undergoing periods of isolation, the oxytocin system may act to protect individuals from the negative effects of loneliness through both physiological and behavioral mechanisms. Although oxytocin receptor expression appears to be down-regulated during periods of isolation (Pournajafi-Nazarloo et al., 2013), elevated oxytocin has been reported in individuals experiencing chronic isolation (Carter et al., 2014). Particularly in postmenopausal women, gaps in social relationships are related to elevated oxytocin levels (Taylor et al., 2006), which may be a physiological mechanism that encourages social interaction and may contribute to regulatory processes in the face of isolation (Taylor et al., 2010). Oxytocin may especially effective in curbing the negative effects of loneliness in women compared to men. Long-term injection of oxytocin into female prairie voles demonstrated positive effects on cardiac function and depression-related behaviors following a period of isolation, which has been known to disrupt cardiac function and vagal tone (Grippo et al., 2009).

There appears to be synchrony in parent-child oxytocin levels during interaction with greater parent oxytocin levels in plasma and saliva associated with more positive communication, social engagement, and coordination of affect (Feldman, Gordon, & Zagoory-Sharon, 2011). In both mothers and fathers, plasma and salivary oxytocin levels were associated with attachment relationships across the lifespan, including with their own parents, partner, and child (Feldman, Gordon, & Zagoory-Sharon, 2011). These findings suggest that oxytocin is related to current affect and behavior as well as cumulative relationship history. Levels of oxytocin in the brain and the body early in life have been shown to impact nervous system development throughout the lifespan (Carter et al., 2009), which may contribute to the lasting effects of early social experiences on physical and mental health. Social or medical interventions that potentially alter activity of the oxytocin systems, especially in early life, must be implemented with attention to possible lifelong alterations in social behavior and physiological regulation (Harris & Carter, 2013). With the cumulative evidence on the powerful impacts of relationship history and the oxytocin system on later social development, understanding the potential for oxytocin to be involved in adolescent social buffering is of utmost importance.

Role of Oxytocin in Social Buffering

Several studies have implicated oxytocin as a mediator of the social buffering effect, particularly studies in animal models. It is known that oxytocin administration reduces HPA activity in many animal species, including those that are characterized by close social bonds (DeVries, Cho, Cardillo, & Carter, 1997; Heinrichs et al., 2003; Windle, Kershaw, Shanks, Wood, & Lightman, 2004). Moreover, administration of an oxytocin receptor antagonist directly into the ventricles of the brain increases HPA

activity, which suggests that the interaction between the oxytocin and HPA systems likely occurs—at least to a certain extent—in the brain (Neumann, 2002).

A variety of social acts also increase oxytocin activity (Carter, 1998; Uvnas-Moberg, 1998). One method involves physical touch, especially if done in a soothing way (Uvnas-Moberg, 1998). For mothers, the somatosensory experience of breastfeeding raises oxytocin levels (Uvnas-Moberg, 1998). Massaging the abdomen of rats also results in increased oxytocin levels (Agren et al., 1995). More generally, touch, warmth, and vibration all increase plasma and cerebrospinal fluid oxytocin levels (Stock & Uvnas-Moberg, 1988; Uvnas-Moberg et al., 1993). Thus, social contact that involves physical touch appears to activate somatosensory neurons, increasing the release of oxytocin and buffering HPA responses to stress. Interestingly, in adult women, touch may be a vital component of social buffering, as studies demonstrate that women did not experience social buffering of the HPA axis from male partners unless a component of physical contact was included (Ditzen et al., 2007; Kirschbaum et al., 1995). However, women who experienced physical contact with their partners did not differ in plasma oxytocin levels from women who experienced no contact or only social support (Ditzen et al., 2007).

Beyond physical touch, oxytocin has been shown to promote social interactions and bond formation. In rats, oxytocin has been shown to stimulate social contact and grooming (Argiolas & Gessa, 1991; Witt et al., 1992), and it may enhance the reinforcement of social bonds (Liu & Wang, 2003; Nelson & Panksepp, 1996; Young, Lim, Gringrich, & Insel, 2001). Indeed, social interactions and oxytocin activity appear to operate through positive feedback with more intense interactions linked to higher

oxytocin release and likely dampened HPA activity (Hennessy et al., 2009). As oxytocin is not only released by social contact, situations that involve stressors, which also trigger the release of oxytocin, may result in the highest levels of oxytocin in the presence of a social partner (Hennessy et al., 2009; Nishioka, Anselmo-Franci, Li, Callahan, & Morris, 1998). Thus, it appears that although oxytocin is normally a part of HPA regulation, social interaction further moderates HPA activity. This could occur through assessment of social cues by the PFC, which sends signals to limbic areas of the brain such as the amygdala, NAcc, and bed nucleus of the stria terminalis (BNST), which then communicate with the PVN to coordinate the release of CRH and oxytocin (Hennessy et al., 2009). Classical conditioning may enhance oxytocin activity due to social interaction by pairing an unconditioned stimulus that releases oxytocin (e.g., touch) with other accompanied forms of interaction (e.g., voice or sight), such that future interactions may activate oxytocin neurons and elevate oxytocin levels through conditioned stimuli.

Some of the strongest evidence of the social buffering effect has been conducted with prairie voles, which are studied as they demonstrate long-term pair bonding and the mother and father frequently co-parent offspring (Aragona & Wang, 2004). Recent evidence in female prairie voles suggests that oxytocin in the PVN mediates social buffering effects during a 1-hour immobilization stressor, which may point to a similar mechanism operating in humans (Smith & Wang, 2013). In the study, voles that recovered alone after immobilization showed more anxiety-like behaviors and increased corticosterone levels, which were not observed in voles that recovered with their male partner (Smith & Wang, 2013). Voles who recovered with their partner, and were therefore biologically and behaviorally buffered from the stressor, demonstrated a rise in

oxytocin in the PVN. Furthermore, injections of oxytocin in the PVN reduced behavioral and corticosterone responses to stress, while administration of an oxytocin receptor antagonist blocked social buffering effects provided by partner support (Smith & Wang, 2013). This careful series of experiments suggests that in prairie voles, oxytocin in the PVN plays a causal role in biological and behavioral social buffering, and further mechanistic research is needed to understand whether oxytocin in the PVN also mediates the social buffering effect in humans. In adult humans, nasal oxytocin administration lowers the cortisol response to stress after the TSST with an effect size similar to the buffering effect of friend support (Heinrichs et al., 2003). In this study, oxytocin administration significantly decreased anxiety levels as a result of the TSST, demonstrating oxytocin's anxiolytic effect (Heinrichs et al., 2003). Further, social support plus oxytocin administration was associated with the lowest cortisol reactivity and anxiety levels following the TSST (Heinrichs et al., 2003).

As noted earlier, a study of 7-12 year old girls who were provided with physical, verbal and non-verbal maternal support following the TSST-C showed reductions in salivary cortisol elevations and the quickest return to baseline cortisol levels, accompanied by the highest increase in urinary oxytocin. Girls who were only allowed to have phone contact showed some cortisol reduction compared to girls with no contact with their mother (Seltzer, Ziegler & Pollak, 2010). Interestingly, the phone contact-only group showed increases in oxytocin approaching the full maternal contact group, suggesting that the maternal voice may be an important releasing stimulus of oxytocin through which it at least partially blocks the HPA response (Seltzer et al., 2010). There was no increase in oxytocin and the highest cortisol reactivity for girls who did not

receive maternal support after the TSST. Thus, there appears to be graded influences of social support for children and their HPA and oxytocin responses to stress and maternal support. Overall, there is correlational evidence for the involvement of oxytocin in social buffering in humans and experimental evidence that exogenous administration of oxytocin is related to dampening of the HPA response. Although there is experimental evidence in animal models for the involvement of endogenous oxytocin in the social buffering effect, there is not yet experimental evidence in humans that endogenous oxytocin plays a causal role in social buffering. Although there are studies that demonstrate the link between central or peripheral oxytocin levels and reduced HPA activity, oxytocin may be especially predictive of psychological outcomes following stressful experiences (Pierrehumbert et al., 2010). For example, social support reduces the risk of PTSD development following traumatic events (Ozer, Best, Lipsey, & Weiss, 2003), and oxytocin levels could decrease the incidence of PTSD risk following a major stressor (Olff, Langeland, Witteveen, & Denys, 2010). Thus, oxytocin may not only operate by blocking the HPA axis but by promoting adaptive changes across multiple systems to reduce the risk of disorder.

Oxytocin and HPA System Interactions

Acute stressors lead to elevations in oxytocin concurrent with other stress mediators such as epinephrine and cortisol. However, the effects of oxytocin may either be arousing or calming depending on the activity of other systems and the individual's history (Carter, 2014). The production of both oxytocin and CRF in neurons of the PVN suggests that this location may be prime for interactions between the HPA and oxytocin systems. One study in which oxytocin was administered in the PVN resulted in reduced

HPA responses in rats (Nishioka et al., 1998). C-fos expression, a marker of neuronal activity, is upregulated in several areas of the CNS in response to stress, but is inhibited in the PVN by intra-cerebroventricular administration of oxytocin (Windle et al., 1997). This infusion of oxytocin into the ventricles eliminated c-fos expression in the dorsal hippocampus and ventrolateral septum, two areas that have oxytocin receptors and where c-fos shows increased expression in response to stress (Windle et al., 1997). However, there are some inconsistencies in the literature. Neumann (2002) reported that administration of an oxytocin antagonist in the PVN resulted in blunted ACTH responses in rats. In addition, administering an oxytocin receptor antagonist in the septum increased ACTH responsiveness to stress (Neumann, 2002). Overall, it appears that the presence or delivery of oxytocin to the PVN or other upstream sites may be key to dampening HPA activity.

Under conditions of stress, oxytocin modulates the secretion of ACTH from the anterior pituitary (Gibbs, 1986). The pulsatile administration of oxytocin into isolated rat anterior pituitary cells leads to ACTH release (Link, Dayanithi, Föhr, & Gratzl, 1992). Oxytocin appears to affect ACTH secretion in a synergistic manner when CRH is also added and in an additive manner when vasopressin is applied (Link et al., 1992). This ACTH secretion is then suppressed by negative feedback of glucocorticoids (Link, Dayanithi, & Gratzl, 1993). There is evidence that oxytocin may enhance ACTH release following a stressor after several days but not immediately following the stressor (Nakashima et al., 2002). Chronic oxytocin administration may alter behavioral and emotional responses over time. For example, chronic administration of oxytocin in the ventricles of the brain decreases anxiety levels in rats bred for excessive anxiety (Slattery

& Neumann, 2010). In female oxytocin knockout mice, the corticosterone response to psychological stress is increased compared to wild-type mice, indicating that oxytocin may contribute to the HPA attenuation post-stressor (Mantella, Vollmer, Rinaman, Li, & Amico, 2004). This effect appears to be moderated by oestradiol levels, which may contribute to sex differences observed in social buffering (Ochedalski, Subburaju, Wynn, & Aguilera, 2007). Due to extensive evidence of interactions between the HPA and oxytocin systems and evidence of developmental changes in both systems, the HPA and oxytocin systems' responses to stress will be examined in children and adolescents in the current study.

Chapter 2: The Current Study

There are currently no experimental studies assessing the effectiveness of friends to serve as social stress buffers among either children or adolescents. A study by Adams, Santo, and Bukowski (2011) involved 10-11 year-olds collecting their own saliva at several points throughout the day and recording in a diary what they were doing, who they were with, and how they were feeling in the 20 minutes before the sample. Results indicated that, across participants, children demonstrated lower cortisol levels after a negative event when they reported being with their best friend relative to negative events without their best friend present (Adams, Santo, & Bukowski, 2011). There is also evidence that when allowed to debrief with a friend, 12- to 16-year-olds who had good quality relationships with the friend who debriefed them returned to baseline faster following a social evaluative stressor than did same-aged youth who had poor relationships with the friend who debriefed them (Calhoun et al., 2014). Of course, in both of these cases it could be that being the type of individual who can develop and maintain high quality friendships might be associated with characteristics that make one more stress-resilient. The present study provided an experimental test of whether parents versus same-sex friends provide equivalent or different social stress buffering effects on cortisol levels in children (aged 9 and 10 years) as compared to adolescents (aged 15 and 16 years).

Oxytocin before and after the TSST was assessed in this study to test the hypothesis that changes in oxytocin mediate developmental changes in social buffering of the HPA axis. We measured oxytocin in urine, which reflects oxytocin in the periphery as well as in the brain. As most of the oxytocin in the body is produced in the hypothalamus

(Carter, 2005), the bladder is a reservoir for oxytocin that is non-invasive and thus ideal for use in children. Although more research is needed to understand the association between central and peripheral oxytocin in humans, animal models suggest that central and peripheral oxytocin release is coordinated under certain conditions (Kendrick, Keverne, Baldwin, & Sharman; Landgraf & Neumann; 2004; Wotjak et al., 1998). We asked two questions about oxytocin production in this study: 1) would the pattern of oxytocin production mirror the pattern of social stress buffering observed for parents versus friends at the two ages in our assessment? 2) If so, when entered as a covariate, would it reduce the association between condition and age and the cortisol response to social evaluative stress? Because we are measuring urinary oxytocin, we cannot assume oxytocin is functioning the same way in the brain. Nonetheless, we can conservatively conclude that its levels provide at least one pathway through which social buffering may help protect the individual from deleterious impacts of social evaluative stress. We predicted that if friends become a potent source of stress buffering in adolescence then we would also expect to see them increasing the production of oxytocin by their presence. The specific aims and hypotheses are explicated below.

Thus, to summarize, the aims of the current study are to test the effectiveness of parents versus friends in buffering the HPA response to a social evaluative stressor in childhood and adolescence. In addition, the oxytocin system will be tested as a potential correlate of developmental changes in social buffering.

Specific Aims

Aim 1: *To test the hypothesis that post-pubertal adolescents will transition from using parents to their friends as buffers from stress, as measured by decreased cortisol*

reactivity to acute stress.

It is hypothesized that for children ages 9-10, preparing with a parent for a social evaluative stressor (TSST) will be associated with lower cortisol reactivity and faster recovery than preparing with a friend. Conversely, 15-16 year olds are expected to have lower cortisol reactivity and faster recovery when preparing with a friend than with a parent due to adolescents reporting becoming more emotionally close to friends than parents. However, because part of the stress stimulus in the TSST is the expectation that a group of peers will be evaluating their recorded speech, it is possible that by bringing a friend to the session, it increases sensitivity to the peer-evaluation component of the task, and thus increases the stressfulness of the task. Due to this possibility and research on heightened sensitivity to social evaluation in adolescence, friends might actually increase the response to threat compared to parents.

Aim 2: *To test whether the parent and/or peer are effective in stimulating oxytocin increases and whether that differs with age*.

I hypothesize that there will be a larger increase in oxytocin levels following preparation with a parent than with a friend during childhood but larger oxytocin increases when preparing with a friend versus a parent during adolescence.

Aim 3: *To test whether oxytocin mediates the effectiveness of the parent or peer in reducing the cortisol response to an acute stressor*.

I hypothesize that changes in oxytocin will parallel changes in cortisol levels, suggesting that oxytocin may be involved in developmental changes in social buffering and HPA regulation. To examine whether oxytocin might be a potential mechanism of stress buffering, we will examine its role as a covariate of the responses observed,

recognizing that we are only examining statistical mediation, and not a true causal relationship.

Aim 4: *To test whether relationship quality and the quality of supportive behaviors may account for differences in the effects of parent and friend support on cortisol and oxytocin levels.*

Due to evidence discussed above about the effects of perceived versus received support on social buffering, both perceived (relationship quality) and received (supportive behaviors) support will be examined to test whether any differences by age, condition, or sex are due to variations in relationship quality or supportive behaviors. I hypothesize that children will report better relationship quality with parents than friends on the Network of Relationships Inventory compared to adolescents, which will partially explain lower cortisol reactivity, faster cortisol recovery, and higher oxytocin levels when children are preparing with parents versus friends. Conversely, I hypothesize that adolescents will report better relationship quality with friends than parents compared to children, which will partially explain lower cortisol reactivity, faster cortisol recovery, and higher oxytocin levels when adolescents are preparing with friends versus parents. Likewise, I hypothesize that with children, parents will exhibit more supportive behaviors than friends during speech preparation compared to adolescents, which will partially explain lower cortisol reactivity, faster cortisol recovery, and higher oxytocin levels when children are preparing with parents versus friends. I hypothesize that with adolescents, friends will exhibit more supportive behaviors than parents during speech preparation compared to children, which will partially explain lower cortisol reactivity, faster cortisol recovery, and higher oxytocin levels when adolescents are preparing with friends versus

parents.

Methods

Participants

A total of 54 children ages 9-10 and 55 adolescents ages 15-16 were recruited from a department-maintained participant pool and were enrolled in the study. These age ranges were selected as the children were either pre-pubertal or in the very early stages of puberty, and adolescents at this age were either post-pubertal or in the late stages of puberty (pubertal screening described below). A total of 4 pubertal 9-10 year olds were excluded and all numbers and demographics in this study are reported without these participants. Exclusion criteria included the use of steroid medications, and diagnosis of Autism Spectrum Disorder, Fetal Alcohol Spectrum Disorder, or any other developmental disorder. These 54 typically developing children (*M* age = 9.9 years, *SD* = 0.5, range = 9-10.8 years; 26 females) and 55 adolescents (*M* age = 15.8 years, *SD* = 0.5, range = 15.1-16.9 years; 30 females) were included in all analyses. Participants reported the following racial/ethnic backgrounds: white/Caucasian (87.2%), African American (2.8%), Hispanic (1.8%), Asian (0.9%), other (0.9%), and more than one race (6.4%).

Annual household income ranged from $15,000-25,000 to over $200,000. The percent of families that had incomes greater than $150,000 was 21.1%, 56.8% had incomes from $75,001-150,000, 15.6% had incomes from $35,001-75,000, and 4.6% had incomes less than $35,000. There were 2 individuals who refused to report income. The distribution for parental educational level (of the parent who attended the session) was 7.3% high school or GED graduate, 15.6% 2-year college or associate's degree, 39.4% bachelor's or 4-year college degree, and 36.7% postgraduate degree. One individual did

not report education level. Neither parent education nor family income differed as a function of sex, age group (child/adolescent), or parent/friend condition. The University of Minnesota Institutional Review Board approved all study procedures. Parents were recruited by phone, and those who did not meet exclusion criteria and agreed to have their child participate were scheduled. Parents of the friend who attended the session either filled out a consent form online before the session or the friend brought a consent form signed by their parent to the session.

Procedures

Pubertal status. Once at the laboratory, pubertal status was assessed using adolescent self-report. This was done to ensure that all 9-10 year olds were pre-pubertal and all 15-16 year olds were past the halfway point in puberty. The Pubertal Development Scale (Petersen et al., 1988; Carskadon and Acebo, 1993) assessed the extent of participants' sex-specific bodily changes associated with puberty onset: growth in height, body hair, skin changes, deepening of voice, and facial hair for males; growth in height, body hair, skin changes, breast development, and menstruation for females. Responses were 1 = not yet started, 2 = barely started, 3 = definitely started, and 4 = seems complete (Carskadon & Acebo, 1993). Menstruation was coded as 1 if it had not begun and 4 if it had begun. This measure yields a mean score from 1 (puberty has not begun) to 4 (puberty is complete) in order to exclude pre-pubertal adolescents and pubertal children. Consistent with previous studies of pubertal timing (Doom et al., 2015), any 9-10-year-old who reported above a 2.5 on the scale was excluded from analyses for being pubertal, and any 15-16-year-old below a 2.5 was likewise excluded for being pre-pubertal.

Session timeline. Participants were scheduled for one session in which all data were collected. All participants were accompanied by a parent and arrived to the laboratory between 14:30 and 16:30 in order to account for diurnal variation in cortisol. Parents were told over the phone that the primary caregiver was preferred to accompany the participant to the session. Mothers (88.1%) were the parent in most of the sessions with no difference in sex of parent across age group, $\chi^2(1, N = 109) = 2.08$, $p = 0.15$, sex, $\chi^2(1, N = 109) = 2.51$, $p = 0.11$, or condition, $\chi^2(1, N = 109) = 0.99$, $p = 0.32$. All participants also arrived for testing with a friend. Friends were the same sex as the participant within 2 years of their age and could not be a sibling.

Once at the laboratory, each participant was randomly assigned to prepare for the stress task with their parent or with their friend (see Table 1 for summary of age/sex/condition). Sessions adhered to the following timeline (see Figure 1): (1) Parent, friend, and participant worked independently on questionnaires in the laboratory after the consent process (25 min), (2) urine sample #1 collection (5 min), (3) saliva sample #1 collection, then participant was told who they were going to prepare the speech with (parent or friend), moved to a separate room with that individual, and received TSST-M instructions for the speech preparation period (5 min), (4) speech preparation with parent or friend (5 min), (5) sample #2; participant moved to speech room and completed TSST alone regardless of condition (10 min), (6) samples #3-7; participant relaxed with parent and friend regardless of condition while working on questionnaires (samples collected every 10 min), (7) urine sample #2 was collected within 10 minutes of the final saliva sample, (8) debriefing of participants, parents, and friends was conducted. The 5-minute speech preparation period with the parent or friend was the only part that differed by

condition. All participants were with their parent and friend before speech preparation and after the TSST-M.

Table 1

Group	Male	Female	Total
Younger/Parent Condition	15	11	26
Younger/Friend Condition	13	15	28
Older/Parent Condition	15	12	27
Older/Friend Condition	10	18	28
Total	53	56	109

Note. Total N = 109. Number of male and female participants by each age group and study condition (parent vs. friend).

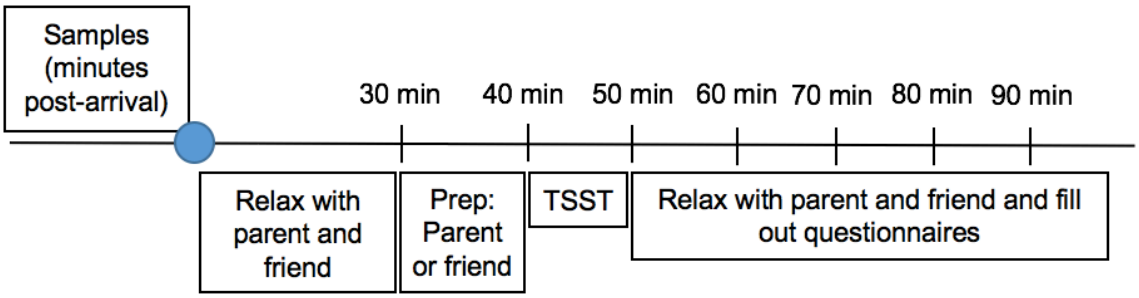

Figure 1. A timeline of the TSST-M protocol by minutes since the beginning of the session (marked by the blue circle). The first saliva sample used to compute reactivity was collected at 30 minutes after the start of the session, and the following 6 samples were collected every 10 minutes after the first. Note that the only difference in parent vs friend condition is during speech preparation.

Stress paradigm. A modified Trier Social Stress Test (TSST-M; Yim, et al., 2010) was used in which participants are asked to imagine they are introducing themselves to a classroom of students and that they should tell the class about their personality and some good and bad characteristics about themselves. The speech was

followed by the standard TSST-C mental arithmetic (Buske-Kirschbaum et al., 1997). Rather than two live judges, the experimenter told the participant that there were two teachers behind a one-way mirror who would judge their speech and math performance and that a classroom of students would later evaluate their recorded speech. The teachers introduced themselves through a pre-recorded audiotape. Participants in the parent condition received support for 5 minutes before the TSST from their parent, who was instructed to assist their child in any way thought useful. In the friend condition, the participant's parent remained in the waiting room while the friend assisted the participant in speech preparation for 5 minutes in any way they deemed useful. In both conditions, parents and friends were given the same instructions about assisting the participant, and the participants were alone in the room during the TSST-M.

Cortisol. Seven saliva samples were collected throughout the session to provide pretest, response, and recovery cortisol levels. Participants used the passive drool method to collect saliva through a straw into 1.5mL Eppendorf (Hamburg, Germany) tubes. Participants were asked not to consume large, protein-filled meals, milk, caffeine, or energy drinks for two hours before the session. After sample collection, saliva was stored in a -80°C laboratory freezer until being shipped to the University of Trier, Germany for assay. A time-resolved fluorescence immunoassay (dissociation-enhanced lanthanide fluorescent immunoassay [DELFIA]) detected cortisol levels. Intra-assay CVs ranged from 4.0% to 6.7%, and inter-assay CVs ranged from 5.1% to 7.2%. All seven samples from each participant were assayed in duplicate and in the same batch to prevent between-batch variation. An average of duplicate samples was used for the final analyses;

these values were log-transformed. A total of 6 cortisol values were considered outliers (> 3 SD from the mean) and were winsorized.

Oxytocin. Two urine samples were collected to measure pretest oxytocin and oxytocin levels in response to the TSST-M. Urine was collected approximately 25 minutes after arrival for pretest levels and again within approximately 10 minutes of the final saliva sample. At least 5 mL of urine was snap frozen on dry ice in 15mL vials immediately after urination, and these samples were stored in a -80°C freezer until shipment on dry ice to the University of Wisconsin-Madison National Primate Research Center for assay of oxytocin and creatinine to adjust for sample volume. Urine samples were subjected to controlled thawing and then to solid-phase extraction with 1 ml SepPak C18 cartridges (Waters, no. WAT023590). Pretreatment of each column was done with 1 mL methanol, 1 mL water, and then 1 mL urine, and this was followed by a 10% acetonitrile (ACN) plus 1% trifluroacetic acid (TFA) wash (1 mL). The elutant was then collected via application of 1 mL of 80/20 percent ACN solution with 1% TFA. Samples were dried in a water bath with air stream and were then reconstituted in the buffer supplied in the 96-well enzyme linked immunosorbent assay (ELISA) kit used (Assay Designs. no. 901-153). Intra- and intercoefficients of variation were determined using oxytocin standards (intra-assay/inter-assay coefficient of variation = 6.0%/10.6%). A Molecular Devices Spectramax 340PC 384 at 405 nm was used to read each plate. Weighted least-squares regression was used for data analysis and log-logit transformation was used for reduction for peptide concentrations. Variation in water content was corrected by dividing the simple creatinine value by peptide concentration (Ziegler et al., 1995). Coefficients of variation for the creatinine assay were 1.7% for intra-assay and

5.2% for inter-assay. The final adjusted value was pg oxytocin/mg creatinine. Both pretest oxytocin and oxytocin at the end of the session were log-transformed for analysis. One sample was considered an outlier and was winsorized.

Daily diary. The parent and participant both completed a diary to report information about the participant relevant to cortisol collection. This information included time of wake, medication usage, physical activity, caffeine consumption, distressing events that day (e.g. arguments), and number of hours of sleep the previous night. Participant reports were the primary source of information. However, for type of medication used by the adolescent, the parent's report on this variable was used when the child's information was incomplete. After excluding participants taking medications with substantial effects on cortisol, a medication count variable was created using the method by Granger and colleagues (2009; $M = .39$, $SD = 0.77$, range: 0-2). Time since wake was calculated by subtracting the participant's reported time of wake from the time of first saliva collection.

Self-reported stress. Participants completed a questionnaire about their stress level at 5 points in the assessment: arrival, speech preparation, speech delivery, math assessment, and the end of the session (e.g., "How stressful was giving the speech?"). Responses included: 1 = calm, 2 = low stress, 3 = medium stress, 4 = somewhat high stress, 5 = very high stress. Participants generally reported large increases in perceived stress levels during the speech and math portions of the TSST-M: arrival ($M = 1.8$, $SD = 1.0$), speech preparation ($M = 2.7$, $SD = 1.2$), speech ($M = 3.8$, $SD = 1.2$), math ($M = 4.2$, $SD = 1.1$), and end of session ($M = 1.5$, $SD = 0.9$).

Quality of parent and friend relationships. As a check on whether there were group differences in the perceived quality of parent or friend relationships, we measured parent and friend relationship quality using the Network of Relationships Inventory: Social Provisions Version (NRI), which has been validated in samples of children and adolescents for assessing the quality of their relationships (Furman & Burhmester, 1985, 2009). For positive relationship qualities, a total of seven scales are measured: Companionship, Intimate Disclosure, Affection, Instrumental Aid, Nurturance, Reliable Alliance, and Reassurance of Worth. These scales were computed using Likert-scale responses to items such as, "How much does this person like or love you?" Scales ranging from 1 (little or none) to 5 (the most) were used. In this study, the participants' ratings of the parent and friend who attended the session were used by creating an average of the seven positive quality scales to yield a positive relationship quality variable for the parent (Cronbach's $\alpha = 0.86$) and for the friend ($\alpha = 0.88$). Parental positive relationship quality ranged from 1.95-4.86 ($M = 3.69$, $SD = 0.68$), and friend positive relationship quality ranged from 1.48-5 ($M = 3.42$, $SD = 0.73$).

Parent and friend behavioral coding. Speech preparation with the parent and the friend was video-taped and coded by trained coders using 5-point Likert scale items. This coding system has been used previously in the literature to code speech preparation (Hostinar et al., 2015). Coders rate the frequency of behaviors during the prep period on a scale of 1 (never) to 5 (the entire time). Factor analysis with principal component analysis yielded a positive support component with loadings all over 0.66, which included the following variables: criticism (reverse-coded), validation, positive affect towards the participant, sensitivity, helpfulness, and intrusiveness (reverse-coded). Each of the scales

had good inter-rater reliability, with the following intraclass correlations for each item: criticism (α = 0.73), validation (α = 0.74), positive affect towards the participant (α = 0.94), sensitivity (α = 0.81), helpfulness (α = 0.78), and intrusiveness (α = 0.85). The mean of these scales was calculated for the composite, and the final supportive behavior composite had good internal reliability (α = 0.84). This data was collected for 91 participants in the current sample due to difficulties with recordings for 12 participants.

Data Analytic Plan

A piecewise latent growth curve model was conducted to analyze cortisol around a theoretically meaningful time point (TSST onset). For a fine-grained analysis, cortisol was divided into reactivity and recovery (Juster et al., 2012). Reactivity and recovery slopes were extracted using Mplus in order to examine reactivity and recovery with linear regression models. Although most individuals' cortisol levels were highest 10 minutes post-TSST-M, some individuals peaked 10-20 minutes later. As a result, landmark registration was used so that each person's peak cortisol response was the point of highest reactivity and the beginning of recovery. Thus, reactivity was the cortisol slope between speech preparation onset and their peak cortisol level. Recovery was measured from the peak to the final sample. Two linear regressions were conducted for cortisol (one for reactivity and one for recovery) with age group (-1 = 9-10 year olds, 1 = 15-16 year olds), sex (-1 = female, 1 = male), and condition (-1 = parent, 1 = friend) as effect-coded independent variables in Step 1 of the regressions. Step 2 had all the 2-way interactions between age, sex, and condition, and Step 3 had the 3-way interaction. Medication count and time-since-wake were included as covariates. For cortisol reactivity, the log-transformed cortisol value after the 30-minute rest period (pretest) was used as a

covariate to control for initial cortisol levels. For cortisol recovery, both the pretest cortisol level and the reactivity slope were included as covariates. To examine predictors of pre- and post-test oxytocin corrected for fluid volume (pg/mg creatinine), a repeated measures ANCOVA was conducted with Time 1 and Time 2 oxytocin as dependent variables to assess within- and between-subjects differences by sex, condition, and age group, and all 2-way and 3-way (e.g., time x sex x condition) interactions. The 4-way interaction was not tested due to power concerns. If the age x condition interaction significantly predicted both cortisol reactivity and change in oxytocin across the session, as hypothesized, change in oxytocin would be examined as a mediator between the age x condition interaction and cortisol reactivity. Regression analyses were then conducted with self-reported stress reactivity (difference between stress level at arrival and during speech/math) and recovery (speech/math to end of session) as dependent variables and age group, sex, and condition (and their interactions) to determine whether stress buffering acts by decreasing subjective feelings of stress.

To examine whether parent/friend relationship quality or support behaviors may be responsible for differences in cortisol or oxytocin levels, 3 univariate ANOVAs were conducted with age group, sex, and condition as fixed factors to test for differences in the following dependent variables: parent positive relationship quality, friend positive relationship quality, and supportive behaviors of the parent/friend during speech prep. If there were significant group differences or interactions that could account for the cortisol and oxytocin results, that variable would then to be added to the respective analysis (cortisol reactivity/recovery or oxytocin) to test whether the results were changed by adding relationship quality or supportive behaviors.

Results

Cortisol

Tables 2 and 3 show the cortisol regression results. Higher pretest cortisol predicted lower reactivity, and more time since morning awakening also predicted lower reactivity. Sex and medication count did not predict reactivity. Likewise, neither age group nor condition yielded main effects. In step 2 of the regression, there was a significant age group by condition effect (see Table 2 and Figure 2). Follow-up analyses indicated that the effect of condition was significant in the 15-16 year olds, such that individuals in the friend condition showed greater reactivity than individuals in the parent condition, $\beta = 0.33$, $t(54) = 2.63$, $p = 0.01$. There was no effect of condition on cortisol reactivity in the 9-10 year olds, $t(53) = -0.65$, $p = 0.52$. The reactivity slope for each age x condition group was significantly greater than zero, ps < 0.01, indicating that each group showed significant cortisol reactivity to the TSST-M.

Table 2

Variable	B	SE(B)	β	t-value	p-value
Step 1					
Pretest Cortisol	-0.10	0.03	-0.37	-3.85	0.00
Med Count	-0.00	0.02	-0.01	-0.14	0.89
Male	0.00	0.02	0.02	0.22	0.83
Time Since Wake	-0.00	0.00	-0.31	-3.26	0.00
Age Group	0.02	0.02	0.11	1.06	0.29

Condition	0.03	0.02	0.15	1.66	0.099
Step 2					
Pretest Cortisol	-0.10	0.03	-0.40	-4.17	0.00
Med Count	0.00	0.02	0.02	0.23	0.82
Male	0.00	0.02	0.03	0.32	0.75
Time Since Wake	-0.00	0.00	-0.33	-3.53	0.00
Age Group	0.02	0.02	0.11	1.12	0.27
Condition	0.02	0.02	0.14	1.64	0.10
Age x Condition	0.03	0.02	0.21	2.33	0.02

Note. N = 109. Hierarchical linear regression results with cortisol reactivity (Mplus-generated) as the dependent variable. Age group was coded as -1 = 9-10 year olds, 1 = 15-16 year olds. Condition was coded as -1 = parent, 1 = friend.

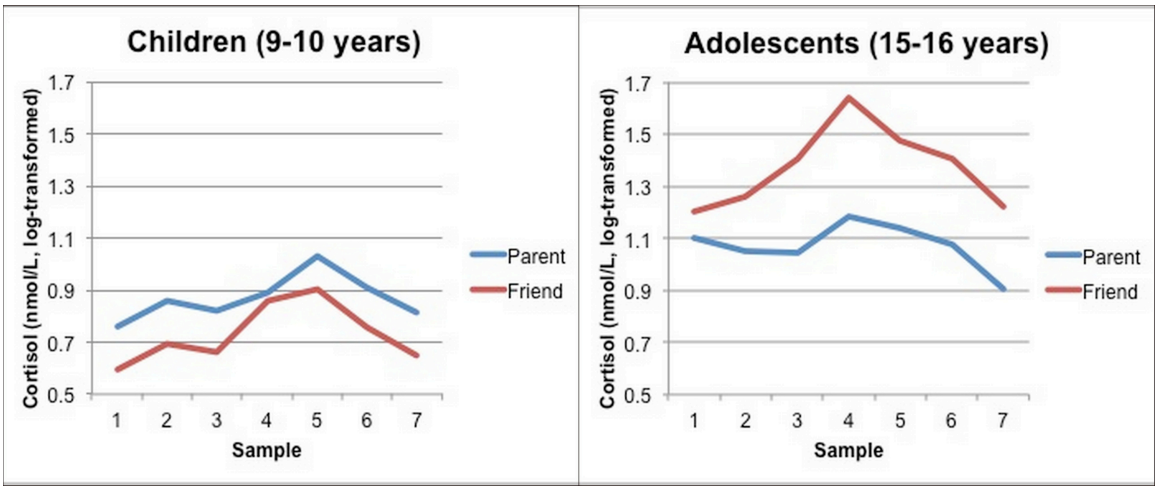

Figure 2. N = 109. Cortisol levels in log-transformed units by age group (9-10 years vs. 15-16 years) and speech preparation condition (parent vs. friend). Sample 1 was collected at the beginning of the speech preparation period, and samples 2-7 were collected every 10 minutes thereafter. Samples 4-6 represent the peak of cortisol production post-TSST that occurred 20-40 minutes after the onset of the TSST. Means for each group were calculated controlling for the effect of sex and time since wake.

In the regression with cortisol recovery as the dependent variable (Table 3), there were no significant effects of medication count, age group, condition, sex, reactivity, pretest cortisol, and time since awakening. In addition, none of the interactions significantly predicted cortisol recovery.

Table 3

Variable	B	SE(B)	β	t-value	p-value
Step 1					
Pretest Cortisol	0.02	0.02	0.12	1.02	0.31
Cortisol Reactivity	0.10	0.06	0.17	1.57	0.12
Time Since Wake	0.00	0.00	-0.11	-1.05	0.30
Med Count	0.02	0.01	0.18	1.81	0.07
Male	-0.01	0.01	-0.15	-1.53	0.13
Age Group	-0.01	0.01	-0.15	-1.41	0.16
Condition	-0.00	0.01	-0.01	-0.11	0.91
Step 2					
Pretest Cortisol	0.02	0.02	0.12	1.07	0.29
Cortisol Reactivity	0.10	0.06	0.18	1.61	0.11
Time Since Wake	-0.00	0.00	-0.11	-0.98	0.33
Med Count	0.02	0.01	0.17	1.72	0.09
Male	-0.01	0.01	-0.15	-1.54	0.13
Age Group	-0.01	0.01	-0.15	-1.41	0.16
Condition	-0.00	0.01	-0.01	-0.12	0.91

Age x Condition	-0.00	0.01	-0.04	-0.36	0.72

Note. N = 109. Hierarchical linear regression results with cortisol recovery (Mplus-generated) as the dependent variable. Age group was coded as -1 = 9-10 year olds, 1 = 15-16 year olds. Condition was coded as -1 = parent, 1 = friend.

Oxytocin

A repeated-measures ANCOVA was conducted with oxytocin at pretest (Time 1) and posttest (Time 2) corrected for fluid volume (pg/mg creatinine) to examine within- and between-subjects differences by sex, condition, and age group, and all 2-way and 3-way interactions. Overall, there was a main effect of age with adolescents producing less oxytocin than children (see Table 4, Figure 3). There was also a significant main effect of trials with oxytocin decreasing from pre- to post-test. However, this trials effect was qualified by significant trials by condition and trials by sex by condition effects (Table 4 and Figure 4). As the age x condition interaction did not significantly predict change in oxytocin, it was not examined as a potential mediator between the age x condition interaction and cortisol reactivity.

Table 4

Variable	Mean Square	F-value	p-value
Within-Subjects			
Time	1.98	5.57	0.02
Time x Condition	2.60	7.29	0.01
Time x Male	0.88	2.46	0.12
Time x Age Group	0.08	0.22	0.64

Time x Condition x Male	1.55	4.36	0.04
Time x Condition x Age Group	0.00	0.00	0.98
Time x Male x Age Group	0.01	0.02	0.89
Between-Subjects			
Male	7.95	7.15	0.01
Age Group	11.90	10.71	0.00
Condition	2.83	2.54	0.11
Male x Condition	0.00	0.00	0.98
Male x Age Group	1.36	1.22	0.27
Condition x Age Group	0.22	0.20	0.66

Note. N = 95. Within- and between-subjects results of the repeated measures ANCOVA with log-transformed oxytocin at pre- and posttest as the repeated measures.

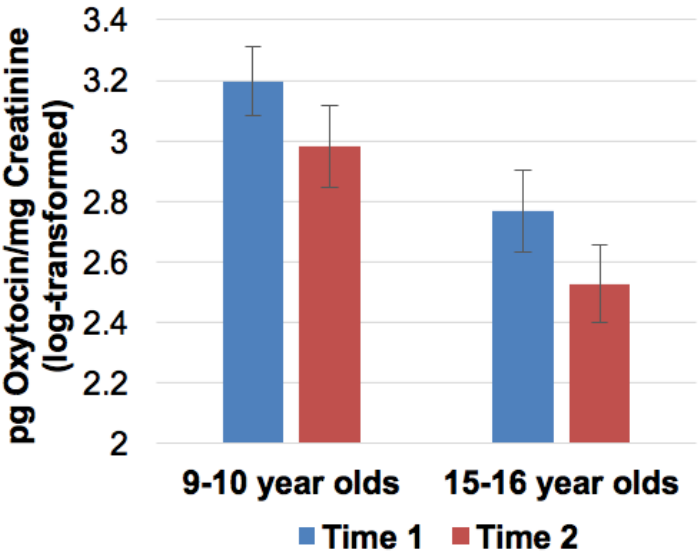

Figure 3. Oxytocin adjusted for urine volume (pg oxytocin/mg creatinine, log-transformed) 30 minutes after arrival (Time 1) and at the end of the session (Time 2) by age group.

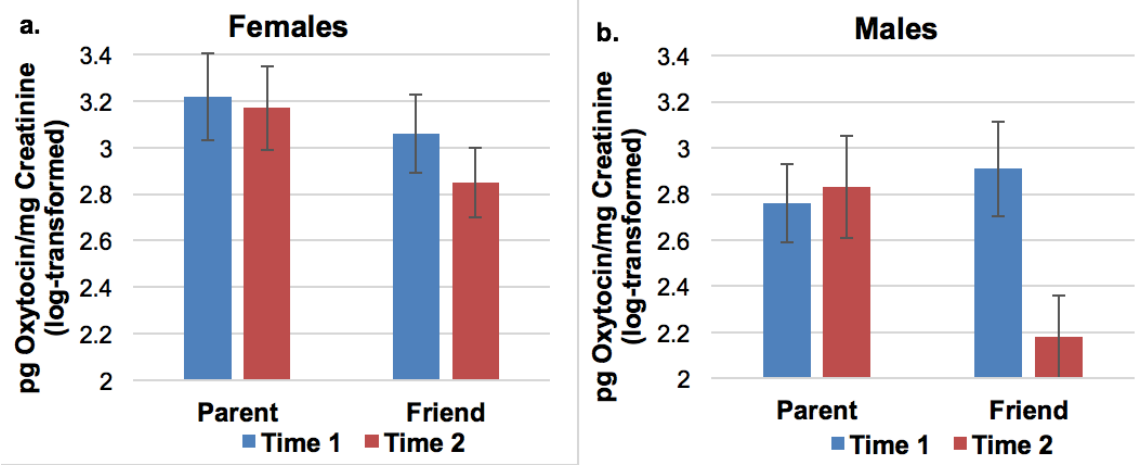

Figure 4. Oxytocin adjusted for urine volume (pg oxytocin/mg creatinine, log-transformed) 30 minutes after arrival (Time 1) and at the end of the session (Time 2) by condition for females (panel a) and males (panel b).

Self-Reported Stress

With regard to self-reported stress reactivity between arrival and speech/math, neither sex, age group, condition, nor their interactions predicted differences in reported stress that might account for the cortisol reactivity findings, *p*s > .10. However, there was a significant increase in self-reported stress overall with participants on average reporting a 2.65-point increase (SD = 1.08) from arrival to speech/math. Similarly, self-reported stress recovery from speech/math to the end of the session was not predicted by sex, age group, condition, or the interactions, *p*s > .10. Only self-reported stress reactivity predicted the amount of self-reported recovery from the stressor, with higher reactivity linked to greater recovery, β = - 0.39, $t(108) = -4.39$, $p < .001$. All other predictors were not significant, *p*s > 0.10.

Relationship Quality

Age group, sex, condition, age group x sex, and condition x sex were not associated with parent positive relationship quality, *p*s > 0.10. The age group x condition

interaction was associated with parent relationship quality at the trend level, $F(1, 101) = 3.74$, $p = 0.056$ (see Table 5 for group means), and thus it was added as a covariate to the cortisol reactivity analyses to examine whether it accounted for the significant age group x condition interaction. Parent relationship quality significantly predicted reactivity, with a better relationship predicting lower cortisol reactivity, $\beta = -0.21$, $t(112) = -2.42$, $p = 0.02$. The age x condition interaction was reduced to significance at the trend level, $\beta = 0.17$, $t(108) = 1.96$, $p = 0.053$, suggesting that some but not all of the effect of the age x condition interaction is due to differences in the reported parent relationship quality. Parental relationship quality predicted cortisol recovery at the trend level, $\beta = 0.18$, $t(108) = 1.81$, $p = 0.07$, with a better relationship associated with slower recovery. Parental relationship quality did not predict within-person change in oxytocin, $p = 0.91$, or between-person oxytocin levels, $p = 0.75$.

Table 5

Group	Mean	SE
Age x Condition		
Child with Parent	3.52	0.14
Child with Friend	3.65	0.13
Adolescent with Parent	3.98	0.13
Adolescent with Friend	3.59	0.13

Note. N = 109. Parental relationship quality means and standard error presented for each age x condition group.

Age group, sex, and condition x age group all significantly predicted positive friendship quality, *p*s < 0.05, and condition predicted friendship quality at the trend level, $p = 0.06$ (see Table 6 for group means). A positive relationship with the friend who attended the session predicted cortisol reactivity at the trend level, $\beta = -0.17$, $t(108) = -1.77$, $p = 0.08$, with a more positive relationship associated with lower cortisol reactivity. The age x condition interaction predicting cortisol reactivity remained significant, $\beta = 0.18$, $t(108) = 2.04$, $p = 0.04$, even after controlling for friendship quality. Friendship quality did not predict cortisol recovery, $p = 0.98$. Likewise, friend relationship quality did not predict within-person change in oxytocin, $p = 0.94$, or between-person oxytocin levels, $p = 0.67$.

Table 6

Group	Mean	SE
Sex		
Male	3.23	0.10
Female	3.60	0.09
Age		
Younger	3.22	0.09
Older	3.61	0.09
Condition		
With Parent	3.29	0.09
With Friend	3.54	0.09
Age x Condition		

Child with Parent	2.96	0.14
Child with Friend	3.48	0.13
Adolescent with Parent	3.62	0.13
Adolescent with Friend	3.60	0.13

Note. N = 109. Friend relationship quality means and standard error presented for each age, sex, condition, and age x condition group.

Supportive Behaviors

Sex, age group x sex, and condition x age group all significantly predicted supportive behaviors by the parent or friend, *p*s < 0.05 (see Table 7 for group means). When positive support behaviors were added to the model predicting cortisol reactivity, the behaviors did not predict cortisol reactivity, $p = 0.74$, and the interaction between behavior and condition was not significant, $p = 0.37$. Positive support behaviors did not predict cortisol recovery, $p = 0.35$, and there was no interaction between behaviors and condition, $p = 0.36$. Supportive behaviors did not predict within-person change in oxytocin, $p = 0.95$, or between-person oxytocin levels, $p = 0.94$.

Table 7

Group	Mean	SE
Sex		
Male	3.03	0.12
Female	3.49	0.12
Age x Condition		

Child with Parent	3.53	0.18
Child with Friend	2.91	0.16
Adolescent with Parent	3.18	0.17
Adolescent with Friend	3.43	0.17
Age x Sex		
Male Child	2.79	0.16
Female Child	3.65	0.18
Male Adolescent	3.27	0.18
Female Adolescent	3.34	0.16

Note. N = 90. Parental relationship quality means and standard error presented for each sex, age x sex, and age x condition group.

Chapter 3: Discussion & Future Directions

The results of the current study demonstrate that parents and friends have similar ability to buffer the HPA axis from a psychosocial stressor at 9-10 years of age, but by 15-16 years of age, the effects of parents and friends diverge. Specifically, having a same-sex friend help adolescents prepare for a social evaluation stressor increased cortisol reactivity to the stressor compared to having a parent help them prepare. In addition, preparing for the speech with a parent resulted in higher levels of urinary oxytocin than preparing with a friend. This was true at both ages, although with age pretest oxytocin levels were lower. These effects were not due to different self-reported experiences of the stressfulness of the task as a function of preparation with a parent versus a friend for children and adolescents. Thus, consistent with much of the social stress buffering literature (Hostinar, Sullivan, & Gunnar, 2013), social partners modify physiological response to the psychological experience of stress.

Although we hypothesized that friends could potentially buffer the HPA axis, particularly for adolescents, we found the opposite. Preparing for this social evaluation task with a friend markedly amplified the HPA response to stress for adolescents and decreased the overall amount of oxytocin produced, regardless of age. There could be a number of possible explanations for these findings. First, researchers have observed a general increase in stress sensitivity in adolescence that is closely related to pubertal development (Sumter, Bokhorst, Miers, Van Pelt, & Westenberg, 2010). Peers may further increase this sensitivity post-puberty as their salience increases across adolescence (Nelson et al., 2005). Thus, planning a self-focused speech with a close friend during adolescence appears to make the social evaluative nature of the stressor more potent and

the stress system more sensitive to this threat, contrary to the hypothesis that buffering from stress may switch from parents to friends across puberty. Interestingly, studies in adults have demonstrated that friends and romantic partners are able to buffer the autonomic nervous system and HPA axis responses to stress (Fontana, et al., 1999; Kirschbaum, et al., 1995; Uno et al., 2002). Thus, adolescence might be a time between childhood and adulthood when friends do not buffer the response to a social evaluative stressor, but actually amplify it. This result is similar to studies of social buffering in adult females in the presence of their male romantic partners, who, unlike men with female romantic partners, show increased cortisol in response to the TSST (Kirschbaum et al., 1995). It may be that similar stress sensitization may be operating in adolescents in the presence of friends, potentially due to increased salience of friends and their friends' opinions.

The increased cognitive ability of adolescents compared to children may allow for greater reflection and rumination about the meaning of a social evaluative task, which may increase anxiety about the task and potentially increase cortisol reactivity (Adam, 2006; Nelson et al., 2005; Westenberg et al., 2004, 2007). This may be amplified in the presence of a salient social figure. In addition to increased cognitive ability, heightened emotional reactivity that has been consistently reported in adolescents may further intensify negative emotions surrounding social evaluation (Dahl & Gunnar, 2009). Increased emotional reactivity, greater cognitive ability to reflect on social evaluation, and an amplified HPA response to social stress in the presence of peers may be related to the increased vulnerability of adolescents to emotional disorders and substance abuse (Paus et al., 2008; Spear, 2009). The dramatic shift in the social context of adolescence

towards independence and greater interaction with peers may place an enhanced meaning on social interactions and increase the amount of time that adolescents spend in these peer environments with high levels of perceived social evaluation (Nelson et al., 2005; Pine et al., 1998; Steinberg & Morris, 2001).

Interestingly, preparing with friends before the TSST did not stimulate oxytocin production. In fact, oxytocin production decreased across the task when the participants prepared with a friend. When they prepared with the parent oxytocin levels did not decline. Thus, it was not that preparing with the parent increased oxytocin production as much as preparing with the friend inhibited the production of this anti-stress hormone. Our measure of oxytocin in urine does not allow us to make direct comparisons to oxytocin present in the central nervous system, although oxytocin is excreted into the urine between 30 minutes to one hour after release. Indeed, it has been noted that intranasal oxytocin is capable of markedly reducing cortisol increases to the TSST among adults. The oxytocin system has been shown to be protective against acute and chronic stress, often by promoting antioxidant and anti-inflammatory processes (Gutkowska & Jankowski, 2012; Szeto et al., 2008). We found no evidence that urinary levels of oxytocin statistically mediated age, sex or condition effects on cortisol responses in our participants. However, as cortisol and oxytocin have different time courses for response to acute stress, and the development of the HPA and oxytocin systems may proceed at different rates over time, this conclusion should be viewed cautiously.

It is important to note that the paradigm used in the current study is different from the one used in our earlier studies (Doom et al., 2015; Hostinar et al., 2015), as in the earlier paradigm, participants came to the lab with only their primary caregiving parent

and only directly interacted with their parent and the experimenter throughout the session. In this paradigm, children and adolescents came with their friend *and* their parent, and they interacted with their friend and parent both before and after the TSST. This may help explain why our parent findings differed from previous studies. Specifically, unlike previously, we obtained a statistically significant elevation in cortisol among the 9- and 10-year-olds, although the response was fairly small. It may be that having a friend come to the session, even if they did not help you prepare, made the whole session more arousing for the children and adolescents. Indeed, it is critical for interpretation to recognize that in this paradigm, parent and friends are with the participants from arrival until speech preparation and speech/math delivery and then they are present with the participant once the task is over and throughout recovery. The only time that differs is the five minutes of speech preparation when the participant is either with the parent or the friend and the time during the speech and math when the participant is alone with the assessors. Interestingly, social buffering effects have been observed when the supportive figure is present before, during, and after the stressor (e.g., Calhoun et al., 2014; Coan, Schaefer, & Davidson, 2006; Hostinar et al., 2015), but the time course of these effects (reactivity vs. recovery) likely differs depending on the timing of the supportive figure's presence.

In addition, the results of the present study should temper our view of whether parents can provide a stress-buffering role in adolescence. In this study, parents of adolescents were certainly more effective than friends in reducing, or at least not amplifying, the cortisol response. Parents were also associated with higher urinary oxytocin levels than were friends at both ages. Again, this difference in oxytocin was due

to the few minutes of speech preparation when the paradigm differed for the two conditions. Thus, this study may be only the tip of the iceberg regarding differences in oxytocin production during stress when children and adolescents have access to attachment figures. These results strongly suggest that parents continue to be capable of providing stress-protecting effects, at least in the oxytocin system, well into the adolescent period.

The sex differences in oxytocin production in both childhood and adolescence are intriguing considering a vast literature on sex differences in mental and physical health problems across the lifespan. Finding that even in childhood, social challenge is associated with lower oxytocin production in males than females might suggest that oxytocin should be targeted as a potential contributor to sex differences in mental and physical health outcomes in response to stress. Oxytocin has been associated with more social, passive coping strategies in the face of stress, which some theorize is directly related to sex differences in coping strategies between men and women (e.g., tend and befriend vs. fight or flight; Carter, 2007; Taylor et al., 2000). Our finding that females produce more oxytocin than males in response to social evaluation in childhood and adolescence further supports the idea that there are significant sex differences in oxytocin responses to stress at multiple points in development.

These analyses provide support for the argument that perceived social support may be a more powerful buffer of the HPA axis than quality of received support. Greater parental relationship quality predicted lower cortisol reactivity, and friend relationship quality predicted lower reactivity at the trend level, but actual supportive behaviors did not. Thus, having available social resources during a psychosocial stressor may be a more

potent way to dampen the HPA response than to have a parent or friend who demonstrates positive support behaviors. Of course, this may not be solely due to perceived social support, but rather to personality and other factors such as high self-esteem that may influence both perceived support and an individual's HPA response to stress. Interestingly, even though participants were randomly assigned to condition, there were some effects of condition on perceived relationship quality of both parents and friends. A potential explanation for this finding was that participants filled out questionnaires for 30 minutes before speech preparation and then again after the TSST until the final saliva/urine samples were collected. The NRI was typically located in the second half of the questionnaire packet for participants, and thus, many participants may have filled it out after speech prep and that experience with the parent or friend may have affected their responses about relationship quality. Most of the results still remained after accounting for relationship quality, so the age x condition effects are not solely due to differences in perceived relationship quality.

Study Limitations

The study did have limitations that must be considered. First, we had half of participants of each sex within each age by condition group. Due to low power, we should be cautious about the lack of any interaction effects of sex in the cortisol results. Future research must examine whether there are sex differences in peer and parental social buffering before and after puberty. Second, it must also be noted that we asked for the primary caregiver to attend the session with the child. Most of the time, the mother accompanied the participant, but it was the father in 13 cases. Sex of the parent did not differ across sex, age group, or condition, so our results cannot be attributed to having the

mother versus father attend, but it would be interesting to examine how mothers and fathers may differentially impact social buffering. Third, regardless of condition, children and adolescents were with their parent and friend for 30 minutes before speech preparation and again after the TSST-M until the end of the session. This time spent with their parent and friend may have influenced cortisol levels throughout the session. However, it is interesting to note the effects on cortisol and oxytocin observed when the only difference between the parent and friend conditions was the 5-minute speech preparation period. Finally, there are different TSST protocols that have been used in the literature, and these might lead to differences in cortisol reactivity and recovery. In this study, the participants were asked to give a speech about their good and bad qualities (self-referential speech), while in other speech-stems the participant does not talk about themselves. It might be especially threatening to have a friend help you prepare a self-referential speech because doing so, by definition, involves the friend evaluating the participant. In the present version of the TSST there were no judges in the room with the participant, and although each group showed significant cortisol reactivity, cortisol levels could be different in a protocol where judges are in the room. Future research should explore this possibility. Finally, it is possible that these findings are particular to social evaluative stressors. Having a friend or your parent present during other types of stressors, such as facing a painful medical procedure or entering a new situation, might have different social stress buffering effects. This needs to be explored.

Future Directions

Experiments in animal models have yielded valuable information about potential mechanisms of the social buffering effect. Unfortunately, mechanistic studies of oxytocin

mediation in the PVN that have been performed in animals are not possible in humans (e.g., Smith & Wang, 2013). As a result, studies in humans may be limited to intranasal administration or peripheral measurement of oxytocin unless scientists develop safe and effective ways of manipulating different levels of the oxytocin system. In addition, administration of intranasal oxytocin may not be ethical or feasible for studies of children, although recent research has indicated that this method may be safe for young adolescents with autism (Tachibana et al., 2013). As a first step, examining peripheral levels of oxytocin in a correlational manner may be preferred in order to understand basic associations between oxytocin, the HPA axis, and socioemotional processes related to social buffering. Until more is understood about the impacts of oxytocin on development and the development of the oxytocin system in children, experimental studies may remain elusive.

There has been debate in the field about how to measure oxytocin levels in humans as we cannot directly assess levels within the PVN, pituitary, amygdala, etc. A number of peripheral methods have been used with inconsistent results. One study reported that urinary oxytocin was unrelated to serum or saliva levels although plasma and saliva oxytocin were correlated (Feldman et al., 2011). Salivary oxytocin has demonstrated correlations with plasma with a range of r-values from .41 to .59 (Feldman et al., 2010, 2011; Grewen, Davenport, & Light, 2010). Salivary, plasma, and urinary oxytocin appear to be related to different components of social behavior as salivary and plasma levels may be more related to attachment while urinary levels may be more associated with relationship anxiety and stress (Feldman et al., 2011). Oxytocin in urine is more stable than in plasma likely due to acidity, and urine levels are higher and

therefore easier to detect than in plasma (Amico, Ulbrecht, & Robinson, 1987). In addition, as release of oxytocin into the plasma is pulsatile, urinary oxytocin may produce more consistent results as it reflects oxytocin accumulation over time (Aroskar et al., 1964). Newer and more sensitive enzyme immunoassay methods using concentrated samples have demonstrated reproducible increases in salivary oxytocin to lactation and massage (Carter et al., 2007), which are known to increase oxytocin levels.

Alternate Explanations for Loss of Parental Social Buffering. Although the oxytocin system is a powerful regulator of social behavior and appears to be related to social buffering of the stress response, it is probable that the loss of parental effectiveness of social buffering in adolescence is not solely due to changes in the oxytocin system. For example, there are several important developmental changes occurring in the adolescent brain that may contribute to the failure of parents to buffer stress. A recent study of maternal buffering revealed attenuated amygdala activity when viewing images of their mother compared to a female stranger in children, but this attenuation was not observed in adolescents (Gee et al., 2014). Further analysis indicated that children showed less amygdala-prefrontal connectivity when viewing images of the stranger, but connectivity between these regions strengthened when viewing images of their mother (Gee et al., 2014). Children were also better at regulating affect while in their mother's presence. Adolescents showed similar connectivity whether they were presented with maternal or stranger stimuli (Gee et al., 2014). It is possible that children need maternal support to demonstrate more mature affective processing while adolescents have more developed connectivity that allows them to process in a more independent manner. However, adolescents may not have fully developed the capabilities to appropriately deal with

stressors independently, which could result in psychiatric symptoms and disorders without significant support. This hypothesis should be tested in future research as PFC-amygdala connectivity may moderate HPA and oxytocin activity under conditions of psychosocial stress.

Pubertal alterations in neurotransmitter systems and other neuromodulators may be critical to changes in social buffering. For example, recent work in prairie voles demonstrates that the dopamine system, which is responsible for reward processing, is related to processing of emotions and social behaviors and ultimately, social bond formation (Aragona & Wang, 2009). As the dopamine system undergoes significant developmental changes during adolescence (reviewed in Wahlstrom, Collins, White, & Luciana, 2010), it could be that modification of dopamine pathways renders bonds with peers and potential romantic partners more rewarding than those with parents. Similarly, alterations in other neuromodulatory systems across adolescence could contribute to differences in social buffering, including but not limited to serotonin, endogenous opioids, epinephrine and norepinephrine.

Adolescents' conceptualizations of parental support may also shift with age or puberty, which then alters effectiveness of parental buffering. Prepubertal adolescents may conceptualize stressors as being more controllable with a parent present, which is linked to dampened cortisol reactivity (Dickerson & Kemeny, 2004), while pubertal adolescents may not experience feelings of control when supported by a parent. Social stressors may be especially potent during adolescence as demonstrated by increases in neural activation to social evaluation and greater self-conscious emotions (Somerville et al., 2013). It could be that no form of social support is enough to dampen the HPA

response following social stressors from puberty until adulthood. The results of current study support this hypothesis and also suggest that support from friends may actually *heighten* reactivity.

Although there has been a growing body of research on adolescent brain development and potential mechanisms leading to the onset and maintenance of psychopathology, not enough is known about the processes by which stressors contribute to dysregulation in cognition, affect, and behavior. Further, little is known about the mechanisms by which adults and peers can buffer adolescents from acute stressors. There appears to be a shift away from using parents as social buffers during puberty, but the mechanism behind this shift is elusive, as are the psychological consequences of losing parents as buffers of HPA activity.

As higher early adolescent friendship quality has been shown to predict better physical health in adulthood (Allen, Uchino, & Hafen, 2015), the association between adolescent peer relationships and stress reactivity must be examined longitudinally to understand whether stress reactivity may mediate the association between child/adolescent friendship and later physical and mental health. In addition, longitudinal studies across childhood, adolescence, and adulthood should investigate *when* friends become effective social buffers. In adults, there is a great deal of evidence that friends and romantic partners can buffer the HPA response to social evaluation. These findings indicate that at ages 15-16, adolescents have not yet transitioned to using friends as a social buffer, and studies that target at what point friends become effective buffers are greatly needed. Increased HPA reactivity to social evaluative threat, especially in the presence of peers, may contribute to heightened risk for stress-related psychopathology in

adolescence (Costello, Copeland, & Angold, 2011), so understanding when friends can block HPA activation is crucial. It is also unknown what mechanisms may underlie the shift from enhanced to attenuated reactivity in the presence of friends. Although the sex of the child/adolescent and the context appear to affect oxytocin production, the lack of change between childhood and adolescence for oxytocin production in response to stress signals that other neurobiological systems may be associated with the shift in social buffering of the HPA axis over time. There is strong evidence in the animal literature that oxytocin in the PVN mediates the social buffering effect (Smith & Wang, 2013), and this has not been examined in humans, so it could be that peripheral oxytocin does not accurately reflect oxytocin and CRH interactions in the PVN and that there could be a role for oxytocin in developmental shifts in social buffering. Future studies should examine social buffering in contexts other than social evaluation to understand whether parents and friends may be helpful or stress provoking in the face of other challenges. Research on both acute and long-term effects of social relationships on the HPA and oxytocin systems may inform interventions that improve mental and physical health, especially in the face of chronic or severe life stress.

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