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Neuropeptidergic Regulation of Pair-bonding and Stress Buffering: Lessons from Voles

Kyle Gobrogge[1,*] and **Zuoxin Wang**[2]

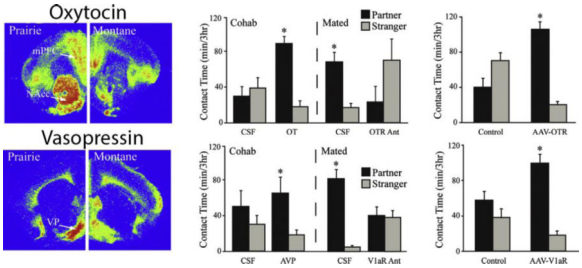
[1]Department of Psychology, Tufts University, Medford, MA 02155, USA

[2]Department of Psychology and Program in Neuroscience, Florida State University, Tallahassee, FL 32306, USA

Abstract

Interpersonal attachment is a critical component of the human experience. Pair-bonding ameliorates the severity of several mental and physical diseases. Thus, a better understanding of how the central nervous system responds to and encodes social-buffering during stress is a valuable research enterprise. The prairie vole (*Microtus ochrogaster*), as a laboratory animal model, provides the gold standard for investigation of the neurobiology underlying attachment. Furthermore, emerging research in voles, additional laboratory rodents, transgenic mice, primates, and humans has provided novel insight into the neurochemical mechanisms underlying the therapeutic effects of social bonds reducing anxiety, depression, and drug abuse liability. In the present review, we highlight the work from this burgeoning field and focus on the role(s) of the neuropeptides oxytocin (OT), vasopressin (AVP), and corticotrophin releasing hormone (CRH) mediating stress buffering. Together, the data suggest that OT underlies social bonding to reduce stress-induced psychological illness while AVP and CRH facilitate arousal to enhance autonomic reactivity, increasing susceptibility to adverse mental and physical health.

Graphical abstract



*Corresponding Author: Kyle Gobrogge, Ph.D., Department of Psychology, Tufts University, Medford, MA 02155, Phone: 617-780-1571, Kyle.Gobrogge@tufts.edu.

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Keywords

vasopressin; corticotrophin-releasing hormone; dopamine; paraventricular nucleus of the anterior hypothalamus; posterior dorsal medial amygdala; nucleus accumbens; ventral tegmental area, social bonding; stress; passive stress-coping, autism; depression, anxiety, & drug abuse

Introduction

Stable social relationships are necessary for maintaining human health. Conversely, breaking bonds can create severe emotional and physical distress including passive-stress coping states involving anxiety, depression, and vulnerability to drugs of abuse. However, we know little regarding how the brain changes and responds to social attachment and partner loss. Thus, in order to better understand the adverse psychological manifestations caused by breaking pair-bonds, the establishment of an animal model that exhibits strong face validity to human attachment is necessary to investigate basic neurobiological mechanisms underlying the effects of social-buffering modulating the stress response. Because male and female prairie voles (*Microtus ochrogaster*) form enduring social pair-bonds after mating and extended cohabitation, these rodents provide an innovative animal model system to investigate the neurobiology of social attachment and partner loss. While a laundry list of neurotransmitters, peptides, molecules, and genes in the central nervous system (CNS) has been implicated in attachment behaviour in voles (Young & Wang, 2004), here we focus primarily on the role of neuropeptides modulating bonding behaviour and stress.

Coping represents a psychological state where an individual consciously establishes a set of specific goals to reduce physiological harm during stressful life experiences (Lazarus & Folkman, 1984, Lazarus, 1999). Due to the extreme frequency of adverse life events people endure during life, several definitions and conceptual frameworks have been proposed. There is a vast literature in which the term “coping”, as it pertains to stress, is classified. Here, we focus on the role of sociality within the context of stress social buffering. Social-buffering is a phenomenon whereby socio-sexual interactions protect an individual from adverse physiological and psychological consequences of stress during challenging situations (Lazarus & Folkman, 1984, Lazarus, 1999). This phenomenon is well known in humans, however - only recently, has it begun to be characterized in laboratory animals.

Because mating induces arousal associated with anxiolytic-like states accompanied by sedation and calmness, it was originally hypothesized that sexual activity reduces anxiety because of central oxytocin (OT) release. In support of this hypothesis, in 1989, Flannery & Wieman found that OT was released in the paraventricular nucleus of the anterior hypothalamus (PVN) of male rats courting a sexually-receptive female. These anxiolytic effects of sexual activity were blocked by central administration of an OT receptor antagonist administered directly after mating but had no effects in non-mated male rats. This work demonstrated a critical role of central OT underlying anxiolytic effects associated with mating and social-buffering of the stress response. Later work revealed exogenous central infusions of OT, but not vasopressin (AVP), reduced stress-induced corticosterone (CORT) release and anxiety-like behaviour in female rats (Windle et al., 1997). These data suggested

that central OT represents a potent anxiolytic, ameliorating both CORT release and anxiety-like states, and thus may play a significant role in buffering physiological responses to stressful life experiences.

In this review, we begin by discussing previous work examining the role of central OT and related neuropeptides (e.g., AVP and CRH) involved in the stress response. We then focus on more recent studies investigating the direct role of OT and its interactions with other peptidergic systems, classical neurotransmitters, and genes underlying social-buffering of stress in voles and expand the review to include information from similar studies using other laboratory rodents, primates, and humans. We conclude by discussing recent work utilizing OT as a potential therapeutic to alleviate stress-induced psychopathology.

Neuropeptides, Attachment, and the Molecular Genetics of Sociality

The functional significance of long-term pair-bonding has been documented cross-culturally in humans. Individuals living in stable paired relationships live longer than their unpaired, single, counterparts - across demographic groups (House et al., 1988, Lillard & Waite, 1995). A significant number of humans grieving the loss of a family member or intimate partner experience long, and sometimes short - but intense - periods of post-traumatic stress, major depression, panic attacks, and generalized anxiety (Byrne & Raphael, 1999, Kristensen et al., 2012, Onrust & Cuijpers, 2006, Zivin & Christakis, 2007). Social-buffering serves as a potent anxiolytic, while social isolation, separation, or loss of a familiar conspecific predicts poor mental and physical health (Cohen & Wills, 1985, Flannery & Wieman, 1989, Heinrichs et al., 2003, Karelina & DeVries, 2011, Maulik et al., 2010, Smith & Wang, 2012, Smith, Lei, & Wang 2013), increasing the risk of developing debilitating psychological diseases (Byrne & Raphael, 1997, Elwert & Christakis, 2008a, Elwert & Christakis, 2008b, Kristensen et al., 2012, Onrust & Cuijpers, 2006, Rozenzweig et al., 1997, Zivin & Christakis, 2007). Previous clinical research has associated these mental health issues with dysregulation in OT, AVP, and CRH (Dinan et al., 1999, Meyer-Lindenberg et al., 2011, Pitman et al., 1993, Purba et al., 1996, Raadsheer et al., 1994). Thus, elucidating neuropeptidergic mechanisms underlying social-buffering of stress should be an important focus in biomedical research.

Neuropeptides and dopamine promote pair-bond formation and maintenance

Pair-bonding behaviour in prairie voles has been extensively studied in the field and laboratory settings. After 24-hours of cohabitation, with successful mating, male prairie voles exhibit partner preference toward their female partner but not a stranger (Figure 1 A–C; Dewsbury, 1975, Dewsbury, 1987, Getz & Carter, 1996, Getz et al., 1981, Getz et al., 1993, Gray & Dewsbury, 1973, Williams et al., 1992) and become highly aggressive toward unfamiliar male and female conspecifics (Aragona et al., 2006, Gobrogge et al., 2007, Gobrogge et al., 2009, Insel et al., 1995, Wang et al., 1997, Wang et al., 1996, Winslow et al., 1993). Bonded males can retain their partner preference even after two weeks of separation from a female partner (Insel et al., 1995, Bosch et al., 2009). Field work demonstrates that over 75% of prairie voles maintain their bond throughout life (Getz & Carter, 1996, Getz et al., 2003) even when a female partner dies or abandons their male partner, 80% of males never bond again (Getz et al., 1993).

In prairie voles, mating increases AVP (Wang et al., 1994) and CRH (Bosch et al., 2009) mRNA expressed in neurons of the bed nucleus of the stria terminalis (BNST) yet decreases AVP processes in the lateral septum (LS) (Bamshad et al., 1994) - brain areas where OT acts to reduce stress. Female prairie voles, on-the-other-hand, exposed to olfactory cues display differences in the density of OT receptors (OTRs) in the accessory olfactory bulb (Witt et al., 1991) and exhibit a significantly higher density of OTRs in the medial prefrontal cortex (mPFC) and nucleus accumbens (NAcc) compared to promiscuous montane voles (Figure 1D). Intra-cerebro-ventricular (ICV) infusion of an AVP or OT receptor antagonist reduces partner preference while AVP or OT receptor activation enhances partner preference without mating, in both male (Cho et al., 1999, Winslow et al., 1993) and female (Cho et al., 1999) prairie voles. Site-specific manipulation of neuropeptides in select brain nuclei supports these correlative anatomical findings. For example, micro-infusion of AVP in the LS of sexually naïve males enhances partner preference in the absence of mating (Lim & Young, 2004, Liu et al., 2001). OT release in the NAcc increases when females mate with male prairie voles (Ross et al., 2009a), and OT micro-infusion into the NAcc increases partner preference in the absence of mating. Partner preference can be blocked with concurrent micro-injection of an OTR antagonist in the NAcc (Figure 1E) and prelimbic cortex (PLC), in the absence of mating (Liu & Wang, 2003, Young et al., 2001). Further, sexually naïve males infused ICV, or intra-NAcc, infused with CRH display partner preference can be blocked by co-infusion with CRH receptor antagonist (DeVries et al., 2002, Lim et al., 2007).

Behavioural genetics of partner preference formation and social memory

Neurogenetic research, employing viral-vector-mediated gene transfer methods, has substantiated the role of OT in the NAcc and AVP in the ventral pallidum (VP) in the facilitation of partner preference in both sexes. OTR over-expression in the NAcc of sexually inexperienced female prairie voles enhanced partner preference formation (Figure 1F; Ross et al., 2009b) but had no effect in female meadow voles. Thus, NAcc OTR density correlates positively with affiliation and partner preference in monogamous but not polygamous voles (Ross et al., 2009b). Viral vector expression of AVP receptors (V1aRs) in the VP enhanced partner preference in the absence of mating in male prairie voles (Pitkow et al., 2001). Remarkably, V1aR over-expression in the VP of promiscuous male meadow voles recapitulated a socially monogamous life strategy (Lim et al., 2004).

Neurobiobehavioural Consequences of Stress-Induced Bereavement

Behavioural manifestations and neurochemistry accompanied by partner loss

Because partner loss in humans activates an entire suite of mental and physical health problems (Shear & Shair, 2005), the socially monogamous prairie vole has been utilized to investigate mechanisms associated with bereavement (i.e., bond loss). Several laboratories have demonstrated depressive-like symptomology, by using the prairie vole to model human partner loss-induced depression and anxiety (Bosch et al., 2009, McNeal et al., 2014). In a recent series of experiments, male prairie voles pair-bonded with their female partner for 24-hours and were randomly divided into two groups: (1) remained with their partner or (2) separated from their partner for four weeks. Males separated from their partner exhibited

significantly higher levels of anxiety in the elevated plus maze (Figure 1 G&H, (Sun et al., 2014)) and light-dark box (LDB; Figure 1 I, (Sun et al., 2014). Lone males displayed higher levels of depressive-like behaviour in the forced swim test (FST) but didn't exhibit pair-bonding-induced selective aggression

Next, the density of several neuro-peptide/-transmitter systems, previously implicated in pair-bonding behaviour (e.g., OT, CRH, AVP, dopamine (DA), & CORT) in the PVN, medial supra-optic nucleus (mSON), ventral tegmental area (VTA), and rostral zona incerta (ZIR) were immunocytochemically screened through brain sections processed from lone or paired males. Partner loss significantly increased the density of OT-immunoreactive (-ir), AVP-ir, and CRH-ir neurons in the PVN, with no changes in tyrosine hydroxlase-ir (TH) (Figure 2 A–L; Sun et al., 2014). Males displayed robust partner preference two weeks after partner separation but not after four weeks. Body weight and plasma CORT levels were significantly elevated after four weeks of partner separation with no significant changes in plasma OT or AVP (Sun et al., 2014). These data suggest that pair-bond status, post partner loss, may not be affected by pair-bond maintenance because both partner preference and selective aggression extinguished four weeks after partner separation. These results demonstrate a time dependent dissolving of partner preference behaviour in male prairie voles and highlight the prairie vole as a robust animal model to characterize the neurochemical mechanisms underlying bond loss and bereavement.

Neuropeptides and bond loss

Several neuro-peptide/-transmitter systems interact to regulate stress, partner separation, and social memory associated with pair-bonding. OT, CRH, and AVP are produced, transported, and released from parvo- and magno-cellular neurons in the hypothalamus and extra-hypothalamic areas during stress. OT has a single receptor that has been characterized (i.e., OTR) and is coupled to G_q receptors, and when stimulated, enhances neuronal activation and gene expression.

Two CRH receptors have been cloned CRH-R1 and CRH-R2. The intra-cellular signaling cascade and g-protein coupled receptors (Gs/i) vary for each CRH receptor subtype in the CNS and peripheral nervous system (PNS). CRH-R2 has two alternatively spliced forms found in rodents (α and β) and display distinctly different mRNA expression profiles relative to CRH-R1 (Chalmers et al., 1995; Lovenberg et al., 1995b). CRH-R2α is expressed primarily in the CNS (Chalmers et al., 1995; Lovenberg et al., 1995a) while CRH-R2β is found predominantly in the PNS (Chalmers et al., 1995, Lovenberg et al., 1995a). In the pituitary, CRH-R2 is highly expressed in the posterior pituitary lobe with low mRNA levels in the anterior lobe (Kageyama et al., 2003, Van Pett et al., 2000). Both CRH-type receptors bind CRH and urocortins in the CNS, PNS, and pituitary to modulate stress.

AVP has three receptors that have been discovered: V1/2. V1 is further classified into two receptor subtypes V1a & V1b (V_3). Similar to CRH receptors, V1a/b vary in g-protein coupling - either to stimulatory (s) or inhibitory (i) g-protein coupled receptors in the CNS. V_2 receptors are coupled to G(s) proteins and when activated, cyclic adenosine monophosphate (cAMP) increases – enhancing gene expression and neuronal activity.

Treating pair-bonded males with CRH antagonists has no effect on partner preference formation - even if separated from their female partner (Bosch et al., 2009), while same-sex separation in males doesn't change PVN-CRH-ir or –mRNA levels (Bosch et al., 2009, Grippo et al., 2007b). In other work, however, CRH pharmacology influences bond formation but not maintenance (Sun et al., 2014). Enhanced CRH-ir neuronal expression in the PVN of males is associated with female partner loss and not social isolation (Figure 2I; Sun et al., 2014). Thus, PVN CRH-ir may be responding to bond loss and not social isolation. Additionally, social separation from a same-sex conspecific doesn't affect PVN-OT-/AVP-ir neuronal density in male prairie voles (Grippo et al., 2007b, Ruscio et al., 2007). However, partner loss increases PVN-OT-/AVP-ir neuronal density without changing DA-ergic cell expression in the VTA or ZIR in males (Sun et al., 2014, Figure 2 A–L). Thus, a higher density of PVN-OT-/AVP-ir neurons may indicate an overall change in neuropeptide release and/or limited receptor activity associated with a break in pair-bonds.

Central manipulation of V1a-type receptors has been the focus of several studies examining the neurobiology of pair-bonding behaviour in voles (Young et al., 2011a). However, there is strong evidence for a role of AVP V1b-type receptors implicated in social behaviour in a number of elegant studies conducted in the laboratory of Dr. Scott Young at NIMH. In a recent study manipulated V1b receptors in pyramidal neurons of the CA2 region of the hippocampus in AVPR1B knock-out mice - which exhibit a marked reduction in aggressive behaviour. Lentiviral delivery of AVPR1B into the dorsal CA2 region restored the probability of socially motivated attack behaviour in complete AVPR1B knockout mice, without changing patterns of anxiety-like behaviour. Thus, hippocampal AVP V1b-type receptors in the CA2 region are necessary and sufficient to regulate aggression in mice (Pagani et al., 2015), a brain region where stress has a significant impact on neuronal structure and plasticity (McEwen, Nasca, & Gray, 2015).

OT also robustly modulates social behaviour (Churchland & Winkielman, 2012, Feldman, 2012, Gordon et al., 2011) and produces anxiolytic-like effects in rodents (Neumann & Landgraf 2012, Smith & Wang 2012). Blocking OTR in the PVN exacerbates behavioural stress responses (Figure 3 H&I; Smith & Wang 2014b) whereas activation of OTR in the hypothalamic-pituitary-adrenal (HPA) axis (Bosch et al., 2004) and central nucleus of the amygdala (CeA) (Ebner et al., 2005) ameliorates stress reactivity. Further, the HPA axis utilizes CRH and AVP in the PVN to engage arousal in order to increase the production of CORT from the adrenal gland in rodents and cortisol in humans producing anxiogenic responses to environmental stimuli (Armario, 2006, Lightman, 2008). ICV infusion of OT reduces CRH/AVP synthesis and release in the PVN and dampens HPA functioning during stress (Windle et al., 2004, Windle & Shanks, 1997). However, the precise mechanism of how OT acts on the HPA system to reduce CRH/AVP activity and facilitate anti-anxiety-/-depressive-like behaviour is unclear.

Originally, one theory postulated that OT acted directly on CRH cells in the PVN because OT receptors co-localize on CRH perikarya and suppress action potentials (Bilezikjian & Vale, 1987, Dabrowska et al., 2011, Ludwig & Leng, 2006, Vale et al., 1981). However, the primary effects observed were excitatory (Inenaga & Yamashita, 1986). Additionally, gamma-aminobutyric acid (GABA) signaling provided clues into the inhibitory nature of OT

reducing CRH activity in the HPA axis (Cullinan, 2000, Decavel & Van den Pol, 1990, Herman et al., 2002) because PVN-projecting GABA-ergic neurons inhibit stress-induced CRH neuronal firing via $GABA_A$ receptor activation (Bali et al., 2005, Bali & Kovacs, 2003, Bartanusz et al., 2004). In support of these working hypotheses, suppression of stress-induced PVN CRH release, via ICV injections of OT, was blocked by a $GABA_A$ receptor antagonist (Bulbul et al., 2011). Thus, OT may exert anxiolytic effects indirectly by $GABA_A$ inhibition of HPA CRH release. Prior work suggests correlative anxiolytic effects via PVN-OT release or OT micro-injection studies (Smith & Wang, 2012). Furthermore, PVN-OT attenuates stress-induced CRH neuronal activity, reduces plasma CORT, decreases anxiety, and increases GABA-ergic excitability (Smith et al., 2014a). More specifically, concurrent infusion with a $GABA_A$ recetor antagonist, bicuculline, blocked stress-inhibition produced by exogenous OT-PVN infusion (Smith et al., 2014a). Thus, PVN-OT may represent a viable therapeutic target to reduce activation of the HPA axis to decrease stress-responsivity.

Neurobiology of Social-buffering and Mammalian Stress Coping

Stress can manifest in several forms. Social separation-induced stress is one specific subtype of stressor which is highly understudied. Relational life events including partner loss, divorce, or infidelity can cause significant mental health problems in humans (Brown, 1989, Dalgard et al., 2006). Social support through friends, family, or formation of new bonds can alleviate some of these deleterious psychological manifestations (Cohen & Wills, 1985, Smith & Wang, 2012, Smith, Lei, & Wang 2013). Tactile contact between mother and her offspring - via suckling - reduces anxiety (Carter & Altemus, 1997) while monogamous romantic long-term relationships decrease the development of stress-related disorders including frequency and magnitude of panic attacks and psychological distress (Cohen & Wills 1985, Smith & Wang, 2012). However, this is a dearth of basic research elucidating the neurochemical mechanisms programming social-buffering of stress through the formation and maintenance of pair-bonds.

Anxiolytic effects of OT are found when social-buffering is used to cope with stress. OT releases in the PVN during social interaction between pair-bonded prairie vole partners representing a natural anxiolytic (Figure 3 F&G; Smith & Wang, 2014b). In other work, PVN-OT is released during mating in male rats (Waldherr & Neumann, 2007) and in lactating female rats nursing (Neumann et al., 1993). Together, data from these studies supports the notion that social interactions induce OT-PVN release and is associated with reduced anxiety and enhanced calmness. It is also important to note that PVN-OT is released during anxiogenic states and stress (e.g., maternal defeat (Bosch et al., 2004), elevated platform (Blume et al., 2008), immobilization (Babygirija et al., 2012, Smith & Wang 2014b), FST (Wigger & Neumann, 2002, Wotjak et al., 1998), and shaker stress (Nishioka et al., 1998)). This dual function of PVN-OT release may serve as a neurochemical thermostat operating as an inhibitory or negative feedback mechanism for stress-induced HPA axis function. Studies using direct pharmacological manipulation provide evidence to support this theory.

Peripheral, or site-specific, treatment with OT decrease stress responding in many animals (e.g., intra-peritoneal (i.p.; Detillion et al., 2004), subcutaneous (Grippo et al., 2009,

Petersson et al., 1999), intra-nasal (Ditzen et al., 2009, Heinrichs et al., 2003, Linnen et al., 2012, Parker et al., 2005, Quirin et al., 2011), ICV (Bulbul et al., 2011, Lukas et al., 2011, Norman et al., 2010, Windle et al., 2004, Windle et al., 1997, Zheng et al., 2010), and intra-PVN (Blume et al., 2008, Smith & Wang, 2014b)). The data reported in a recent study demonstrate an anxiolytic effect of PVN-OT infusion attenuating stress-induced activation of PVN-CRH neurons, an increase in circulating CORT, and enhanced anxiety-like behaviour in female prairie voles. These effects were only observed when PVN-OT infusion preceded stress but not after (Smith & Wang, 2014b). These data suggest that PVN-OT serves as an inhibitory rather than a negative feedback mechanism attenuating stress.

Chronic partner loss in prairie voles increases circulating CORT, central CRH mRNA, and depressive-like behaviour after stress challenge (Bosch et al., 2009). It has also been shown in a recent study (Smith & Wang, 2014b) that physical interactions between pair-bonded male and female prairie voles alleviate immobilization stress-induced changes at neuronal, physiological, and behavioural levels. Specifically, stressed female prairie voles (recovered “alone” or with their “partner”) exhibited significantly lower levels of OTRs in the PVN (Figure 3A) and V1aRs (Figure 3B) relative to handled (HAN) and social control (SC) females. Recovering from immobilization stress with a familiar partner significantly reduced the level of OT in the PVN (Figure 3C), while no significant tonic or phasic neuronal release adaptations in AVP or CRH were found between groups (Figure 3 D&E). Because OT release in the PVN was increased during stress recovery with a partner this most likely indicates that decreased PVN-OT is attributed to enhanced OT release.

Prior work shows that during stressful life events, social groups and partners cooperate (Zivin & Christakis, 2007) to reduce stress (Karelina & DeVries, 2011) and mammals are more likely to affiliate, rather than fight, during times of stress. Rats will rescue familiar conspecifics from being restrained - resembling empathic responding (Ben-Ami Bartal et al., 2011), and chimpanzees exhibit unique stress coping via consoling one another following conflict (Fraser et al., 2008). Human and primate females receiving more physical attention from their partner exhibit decreases in stress reactivity (Dunbar, 2010). Empathic responding and physical contact between human partners is positively correlated with OT and inversely related to decreased blood pressure and hypertension (Dunbar, 2010). Mating in male rats causes PVN-OT release which is associated with reduced anxiety (Waldherr & Neumann, 2007) while socio-sexual experience in female prairie voles enhances OT release in the NAcc (Ross et al., 2009a). Suckling rat pups display increases in OT release (Kojima et al., 2012), as do female rats having just given birth (Bosch & Neumann, 2012), and inter-generationally in rat mothers ICV injected with OT whom then lick, groom, and nurse their pups more, which - in turn - increases OT release in these offspring (Pedersen & Boccia, 2002). While the precise location of extra-hypothalamic OT neuronal projections originate is contentiously debated, tract-tracing evidence in prairie voles suggests the PVN as one source (Ross et al., 2009a). ICV OTR blockade attenuates social-buffering of stress-induced depression and anxiety in mice and rats (Norman et al., 2010, Waldherr & Neumann, 2007) while site-specific manipulation in the PVN (Bosch et al., 2004) and CeA (Ebner et al., 2005) show similar results.

Neuropeptide interactions underlying social-buffering of stress

CORT and OT represent two of the first neurotransmitter interactions underlying social memory in prairie voles. Sexually naïve female prairie voles exposed to an unfamiliar male conspecific significantly decreased circulating CORT (DeVries et al., 1995) and central OT release (Ross et al., 2009a). These findings support work illustrating ICV OT infusion significantly decreased circulating CORT, indicating potential OT interactions with the HPA axis underlying attachment and social memory (DeVries et al., 1997a). Similar findings have also been observed in studies examining other forms of social behaviour (Amico et al., 1994, Carter & Altemus, 1999, Chiodera et al., 1991, Kikusui et al., 2006). OT interacts with a structurally and chemically related peptide (only differing by two amino acids), AVP, and both receptor subtypes (Barberis & Tribollet, 1996) regulate social memory in prairie voles. For example, male and female prairie voles infused ICV with OT/AVP induces partner preference after only one hour of social cohabitation without mating. ICV-OT-induced partner preference can be blocked by co-administration with a V1aR antagonist and vice versa (Cho et al., 1999). This work is supported by site-specific infusion of AVP into the LS inducing partner preference in male prairie voles which was blocked by co-administration with an OTR antagonist (Liu et al., 2001).

Neuropeptide/Dopamine interactions programming stress social-buffering

Pair-bonding and the presence of a partner are also regulated by the central DA-ergic reward system, thereby reinforcing mental health and wellness (Aragona & Wang 2004, Neumann, 2009). DA in the rostral NAcc shell facilitates pair-bond formation and maintenance (Aragona et al., 2006), while partner loss disrupts the HPA axis causing dysregulated emotive behaviour similar to that observed in human clinical populations of depressed and anxious-prone patients (Kristensen et al., 2012, Onrust & Cuijpers, 2006). Not surprisingly, OT and AVP work in concert with the mesocorticolimbic DA-ergic system to regulate robust social memory and attachment in prairie voles (Young & Wang, 2004). Activation of NAcc DA-type 2 receptors (D2R) attenuate partner preference formation which can be blocked by simultaneous administration of an OTR antagonist (Liu & Wang, 2003). Together, these findings indicate that both D2R and OTR activation is required in female prairie voles to form robust partner preference and social memory toward a male partner. Promiscuous male meadow voles micro-injected with the prairie vole V1aR promoter in the VP exhibited enhanced V1aR expression which facilitated mating-induced partner preference after 24-hours of successful mating with a female.

These effects were abolished by co-infusion with a D2R antagonist before mating and cohabitation (Lim et al., 2004). Anatomically, NAcc-D2R medium spiny neurons project to the VP (Gerfen, 1992), supporting the hypothesis that AVP and DA interact at the level of the VP to encode robust social memory - even in socially promiscuous voles. Work under review by Smith and colleagues report significant changes in the central DA-ergic systems underlying social-buffering alleviating negative effects of social defeat in female prairie voles. Housing with a male partner for five days after a single social defeat experience (i.e., being an intruder in a resident-intruder test) limits the defeat-induced social avoidance response observed in female prairie voles (Smith et al., 2014a). D2R protein levels in the

medial amygdala were also elevated in females post-defeat but only if they recovered with a male partner (Smith et al., 2014a).

Neuropeptide/Classic Neurotransmitter interactions and social stress buffering

Recent work investigated the anxiolytic nature of interactions between PVN-OT and GABA underlying stress-induced HPA activity and anxiety (Smith et al., 2014a). Elevated platform stress (EPS) experience increased CORT while intra-PVN-OT injections 15-minutes prior to, but not immediately following, attenuated EPS-induced-CORT, blocked c-Fos co-localization in PVN-CRH-ir neurons, and activated GABA-ir neurons instead (Smith et al., 2014a). Behaviourally, OT infusion in the PVN prior to EPS significantly reduced anxiety-like behaviour in the elevated plus maze (EPM) while these effects were reversed with co-injection of a $GABA_A$ receptor antagonist (Smith et al., 2014a). PVN-OT pretreatment blocked stress activation of the HPA axis via GABA (Smith et al., 2014a). As found previously, stress-induced OT release in the PVN of female prairie voles buffered subsequent stress responding (Figure 3 F&G; Smith & Wang 2014b). These results indicate that OT release in the PVN controls GABA neuronal activity via $GABA_A$ receptor-mediated inhibition of the stress response. Similar to most peptides in the CNS, OT can passively diffuse (Ludwig & Leng, 2006, Moos et al., 1984) and/or be coupled to an action potential in cell bodies and dendrites via synaptic release (Van den Pol, 2012). OT is manufactured and synthesized in the PVN and mSON (Ludwig, 1998, Ludwig et al., 1994, Moos et al., 1989, Neumann et al., 1993, Pow & Morris, 1989, Russell et al., 1992) and exogenous OT central infusion excites GABA-ergic cells in select brain regions including the CeA, which has been found to reduce fear and anxiety in rats (Huber et al., 2005, Terenzi & Ingram, 2005). OTRs are co-localized on CRH-ir cells in the PVN (Dabrowska et al., 2011). PVN-CRH neurons receive numerous inputs: 50% representing GABA-ergic synapses confirmed via electrophysiological preparations (Wuarin & Dudek, 1991), electron microscopy (Decavel & Van den Pol, 1990, Decavel & Van den Pol, 1992, Herman et al., 2003, Miklos & Kovacs, 2002), and immunohistochemical studies that reveal an abundance of PVN-CRH neurons co-expressing $GABA_A$ receptors (Cullinan, 2000). Direct pharmacological research in rats indicates that activation of these receptors inhibits stress-induced CRH output by blocking CRH mRNA (Bulbul et al., 2011) and release (Hillhouse & Milton, 1989, Plotsky et al., 1987) in the PVN. Smith et al., 2014a provide direct evidence demonstrating that PVN-GABA-ergic neurons respond to OT infusion because intra-PVN-OT injections enhanced GABA-ergic neural responding that was associated with a significant decrease in basal and stress-induced PVN-CRH neuronal activity.

Activation of PVN-CRH neurons enhanced CORT release from the adrenal cortex via ACTH secretion in the pituitary gland (Bilezikjian & Vale, 1987, Ontjes et al., 1977, Vale et al., 1981). Importantly, female prairie voles infused with OT in the PVN exhibited decreased PVN-CRH activity resulting in significantly lower plasma CORT. These anxiolytic effects of OT were attenuated by site-specific infusion of a $GABA_A$ receptor antagonist in the PVN (Smith et al., 2014a). PVN-OT infusion blocks basal and stimulated HPA stress-response and anxiety-like behaviour via $GABA_A$ receptor-mediated inhibitory neurotransmission in prairie voles. In summary, data generated from our group and others support the notion that social-buffering is mediated by OT neurons localized within the PVN.

Neuronal circuitry programming social-buffering of stress

Previous neuroanatomical data illustrate reciprocal connections in PVN-OT/-CRH neurons that regulate HPA axis functioning. OT and CRH processes form synaptic connections where OT can diffuse or release in the PVN (Ludwig & Leng, 2006) to alter neuronal firing rate properties post-exogenous intra-PVN-OT micro-infusion. In order to precisely examine stress buffering circuitry, until a transgenic prairie vole is generated and made publicly available, future vole research would benefit from performing *in-vivo* electrophysiological experiments examining either intact or brain slices from prairie voles experiencing long-term bond loss. For example, prior work from Nishijo's group has previously demonstrated that the presence of a conspecific rat buffered several stress responses to an aversive conditioned stimulus (CS), including freezing and c-Fos activity in the lateral amygdala (LA) of male rats. In a more recent study by the same group, they analyzed freezing behaviour and local field potentials in the LA of fear-conditioned rats in response to the CS and/or in the presence or absence of a familiar conspecific for two consecutive days. The presence of a conspecific animal significantly decreased peak amplitudes of auditory evoked field potentials, gamma oscillations (25–75 Hz), and high frequency oscillations (100–300 Hz) in the LA (Fuzzo et al., 2015). These neuronal responses were positively correlated with freezing duration in fear-conditioned rats (Fuzzo et al., 2015). These data demonstrate the capacity of social-buffering decreasing CS-induced activation of LA neurons to reduce conditioned fear responding.

Neuropeptides and Pair-Bonding as Viable Therapeutics to Alleviate Stress-Induced Bereavement: Clinical Implications and Future Directions

The prairie vole has recently emerged as a valuable model system to examine interactions between natural (e.g., mating & pair-bonding) and artificial (e.g., drugs of abuse) reward uncovering protective or deleterious effects of addiction, withdrawal, and dependence - which all serve as potent stressors. To date, this body of work demonstrates that psychostimulants dysregulate the mesocorticolimbic DA-ergic system underlying pair-bonding behaviours (Liu et al., 2010, Young et al., 2011a, Young et al., 2011b) which can be ameliorated via OT (Young et al., 2014). Thus, the prairie vole represents an innovative animal model to investigate the neurochemical mechanisms underlying interactions between socio-sexual behaviour, social memory, and stress-related psychopathologies. For example, social separation disrupts HPA axis functioning which enhances isolation-induced stress-related dysfunctional social and emotional behaviour in prairie voles (Grippo et al., 2008, Lieberwirth et al., 2012). Smith & Wang, 2014b, also provide strong evidence demonstrating the long-lasting nature of forming a pair-bond in female prairie voles serves as a potent social buffer ameliorating stress responses in the presence of a familiar partner, similar to what has previously been observed in primates and humans (Dunbar, 2010, Fraser et al., 2008). These vole data demonstrate the effects of social support on engaging the PVN-OT system and its requirement to reduce social separation-induced stress relief in female prairie voles and extends prior work in humans showing peripheral OT release during physical interaction between long-term committed couples (Gordon et al., 2011).

Oxytocin, mammalian social buffering, and stress-induced neuronal plasticity

Prairie voles are not the only animal model where OT is being investigated as a potential neurotherapeutic target for the treatment of social-separation stress-related disorders. Similar to familial/social support, OT works as an anxiolytic agent in many mammals. For example, isolated animals exhibit enhanced HPA axis functioning leading to clinical levels of anxiety and depression in socially monogamous laboratory rodents like prairie voles and California mice as well as more generally gregarious animals like rats and/or inbred strains of mice (Detillion et al., 2004, Grippo et al., 2008, Lieberwirth et al., 2012, Norman et al., 2010, Waldherr & Neumann, 2007). Furthermore, central OT micro-infusion ameliorates the negative psychological consequences of social isolation in many laboratory animals including mice, rats, and voles (Detillion et al., 2004, Grippo et al., 2008, Lieberwirth et al., 2012, Norman et al., 2010). Counterintuitively, in other social contexts, socially isolated female prairie voles exhibit depression after psychological stress which engenders anhedonia (i.e., reduced sucrose preference) while contradictory evidence has shown that OT administration during isolation can enhance depressive symptoms (Grippo et al., 2008). Thus, social stress can be alleviated via OT - independent of the social context. Although OT appears to represent a putative therapy for alleviating stress-related disorders and bereavement, the precise neurochemical mechanism of how OT works in the CNS is still unfolding. For example, social and physical stress have both been found to be associated with increased PVN-OT extracellular release (Bosch et al., 2004, Engelmann et al., 2004) from dendrites (Ludwig & Leng, 2006).

Group housing and PVN-OT infusions both decrease stress-induced PVN-CRH neuronal activation in rats (Blume et al., 2008, Windle et al., 2004) whereas social stress caused by isolation can be ameliorated with intra-PVN-OT micro-injection (Blume et al., 2008, Windle et al., 2004). Data from Smith & Wang, 2014b, show that even in the presence of a familiar partner, social-buffering of the stress response doesn't occur if PVN-OT is blocked via intra-PVN micro-infusion with a selective OTR antagonist (Figure 3 H&I). Thus, these data support the notion that social-buffering reverses adverse effects of social separation stress on dysfunctional psychophysiological behaviour and HPA axis pathology via PVN-OT activation.

Humans experiencing symptoms of major depression exhibit enhanced levels of CORT (Stetler & Miller, 2011, Thase, 2009) and increased expression of PVN-CRH-ir neurons (Raadsheer et al., 1994) facilitated by interactions with the central AVP system (Aguilera & Rabadan-Diehl, 2000, Dinan et al., 1999, Purba et al., 1996, Raadsheer et al., 1994). Thus, increased CORT and PVN-CRH-/AVP-ir may underlie neuroplasticity after repeated stress and enhanced HPA axis priming in male prairie voles experiencing partner loss. Because of the significant alterations in socio-emotional dysregulated behaviour and impaired structural neuronal plasticity caused by partner loss, it may be more beneficial to a species to break a bad bond rather than maintain one (Field, 2006, Stroebe et al., 2010). For example, similar to abstaining from drugs of abuse - maintaining an unhealthy pair-bond (e.g., individuals in co-dependent relationships) can cause severe mental illness by perpetuating an unhealthy partnership and/or "relapsing" with the same or similar partner (Byrne & Raphael, 1997, Byrne & Raphael, 1999, Kristensen et al., 2012, Onrust & Cuijpers, 2006, Zivin &

Christakis, 2007). However, the underlying neurochemical mechanisms programming adaptive versus maladaptive bereavement are, at the present time, unknown.

Intra-nasal infusion of OT enhances prosocial communication styles between partners (i.e., increased agreeableness, curiosity, emotional support, and sustained eye-to-eye contact (Ditzen et al., 2009)) and facilitates trust between individuals (Kosfeld et al., 2005). One particular social context, in which prosocial forms of communication are affected - in clinical populations - represents the class of stress-related psychopathologies centered on autism spectrum conditions (Heinrichs et al., 2009, Hollander et al., 2007, McDougle et al., 2005, Yirmiya et al., 2006). Furthermore, germane to the current review, the prairie vole has been established as a translational animal model for human disorders, like autism, characterized by deficits in social communication. For example, prairie voles form unique socio-sexual relationships which aren't typically observed in humans with autistic spectrum disorders. Thus, because the prairie vole establishes pair-bonds and long-term social memories - all hallmark deficits of individuals on the autistic spectrum - these social behaviours in voles can be neurobiologicaly manipulated in the lab to hopefully reveal neurochemical mechanisms underlying the many behavioural facets of autism. The prairie vole model is also well suited to investigate the neurobiology of anxiety-/depression-like behaviour associated with social loss in adulthood (Bosch et al., 2009, Grippo et al., 2007a).

Oxytocin/GABA interactions, benzodiazepines, anxiety, and stress social-buffering

Several forms of clinical anxiety disorders are primarily treated pharmacologically with GABA positive allosteric modulators (i.e., benzodiazepines) including lorazepam, diazepam, and xanax. Diazepam, for example, binds allosterically to $GABA_A$ receptors to dampen neuronal excitability (Hadingham et al., 1993, Luddens & Korpi, 1995, Pritchett & Seeburg, 1990). Anxiety levels are also attenuated by OT and diazepam in several behavioural paradigms: (1) EPM (OT – (Griebel et al., 2000, Smith & Wang 2014b)); (2) LDB (OT - (Waldherr & Neumann, 2007); diazepam - (Griebel, Belzung, 2000)); (3) novel object test (OT - (Veenema et al., 2007); diazepam - (Depino et al., 2008); (4) open field test (OT - (Veenema et al., 2007); diazepam - (Veenema et al., 2007)). OT and diazepam enhance GABA neuronal transmisssion in the PVN, presynaptically via OT release (Smith & Wang 2014b) as well as post-synaptically (Zahner et al., 2007). OT with benzodiazepine treatment may work in concert, with one another, to enhance the efficacy of anti-anxiety treatment. The precise mechanism of OT-$GABA_A$-receptor PVN mediated interactions within the HPA axis to reduce stress responsivity is unclear. Future research would benefit from investigating the underlying connections between these systems for anti-anxiety therapeutic application. Preliminary work demonstrates that both OT and GABA work at the level of the PVN to block stress-induced CORT plasma levels to ameliorate anxiety behaviour: (1) OT, (Smith & Wang 2014b) and (2) GABA, (Marques de Souza & Franci, 2008). Forthcoming work by Smith et al., 2014a, demonstrates that $GABA_A$ receptor activity modulates anxiolytic effects of OT in the PVN - providing mechanistic insight regarding neuropeptidergic-classical neurotransmitter interactions modulating social-buffering of stress in monogamous prairie voles.

Neurochemistry underlying interactions between natural/artificial rewards related to stress and social buffering

An emerging field exploring neurobiological interactions between drug abuse, social behaviour, and stress is making headway in the scientific literature (Young et al., 2011b). Addiction can manifest in several forms ranging from drugs of abuse (e.g., cocaine/*dextro*-amphetamine (*d*-AMPH), alcohol, nicotine, marijuana, lysergic acid diethylamide (LSD), molly, ecstasy, etc.) to sex, gambling, excessive exercise, overeating, body modification, and codependency in unhealthy and abusive intimate partnerships that can lead to serious mental and/or physical ailments. For example, addiction impairs social behaviour (Strathearn & Mayes, 2010, Young et al., 2011b) – preventing the formation and maintenance of mother-child attachment, and monogamously committed partnerships leading to child neglect/abuse (Wasserman & Leventhal, 1993), decline in marriage rates, infidelity, and increased separation/divorce (Collins et al., 2007, Kaestner, 1995). Because forming pair-bonds engenders social-buffering of stress leading to both physical and psychological health (House et al., 1988, Kiecolt-Glaser & Newton, 2001) - understanding brain areas vulnerable to stress (e.g., amygdala, mPFC, and hippocampus; McEwen, Nasca, & Gray, 2015) and drug abuse usurping natural reward (Young et al., 2011b) is of critical importance in health and disease-related clinical research.

Prior work from our lab has pinpointed the mesocorticolimbic DA-ergic system as a prime neural circuit involved in drugs of abuse hijacking natural rewards like social pair-bonding (Aragona et al., 2006, Liu et al., 2010, Young et al., 2011a, Young et al., 2011b Young et al., 2014). The VTA-NAcc-mPFC DA-ergic projection programs pair-bonding and encodes social memory (Aragona et al., 2006, Gingrich et al., 2000) as well as undergoes significant neuroadapatations involving changes in DA release kinetics and DA receptor (DAR) signaling with drug experience (Henry et al., 1989, Nestler, 2005), which can permanently rewire parts of the CNS controlling natural desire, as observed in dampening motivation for engaging in socio-sexual behaviour (Clemens et al., 2004, Febo & Ferris, 2007, Liu et al., 2010, Ludwig, 1998). However, the roles of prosocial neuropeptides - like OT - have been understudied in the context of drug abuse and their interactions with the DA-ergic mesocorticolimbic system (McGregor et al., 2008).

Oxytocin reinstates pair-bonding when drugs of abuse hijack mesocorticolimbic dopaminergic circuitry

OT has been well known to play a significant role in regulating prosocial behaviour (Melis & Argiolas, 2011) and when it acts in the mesocorticolimbic DA-ergic pathway it mediates the formation and maintenance of pair-bonds (Bosch & Neumann, 2012, Liu & Wang, 2003, Ross et al., 2009b, Young et al., 2001). Drug exposure has been found to alter OT functioning in this reward circuit (Butovsky et al., 2006, Johns et al., 1997a, Johns et al., 1997b, Sarnyai et al., 1992), which varies by drug type, dose, and social context. Together, these data have paved the way for future research examining the role of central OT as a potential treatment for reversing social deficits caused by addiction. OT and DA have begun to be explored as prime candidates underlying natural (i.e., mating, pair-bonding, food, parenting, social play, etc.) and artificial (i.e., psychostimulants, alcohol, opiates, marijuana, nicotine, etc.) reward. A recent paper by Young and colleagues, 2014, provided evidence

demonstrating a role for OT in the mPFC reversing psychostimulant-induced deficits in pair-bonding in female prairie voles.

Specifically, we found that repeated i.p. treatment with AMPH blocked mating-induced partner preference in female prairie voles (Figure 4C). Treatment with AMPH also induced significant changes in OT and DA neurochemical signaling in brain regions programming pair-bonds. Repeated AMPH significantly decreased OTR-ir in the mPFC, but not the NAcc or VTA - Figure 4A and D2R-ir in the NAcc, but not the mPFC or VTA - Figure 4B and enhanced NAcc DA levels. PLC infusion of OT, in repeatedly AMPH treated female prairie voles, restored partner preference formation (Figure 4C), which significantly altered DA content in the NAcc (Figure 4D). Importantly, all of these effects were dependent on OTR activation (Figure 4C). Repeated psychostimulant experience impairs pair-bonding behaviour via an OT-mediated neurochemical interaction with the mesocorticolimbic DA-ergic system.

OT and DA significantly contribute to the formation and maintenance of pair-bonds (Young et al., 2011a) and are altered by exposure to psychostimulants which impairs natural pair-bonding behaviour (Young et al., 2011b). As described above, the data reported by Young and colleagues, 2014, support the notion that interactions between DA and OT in the mesocorticolimbic circuit can restore partner preference formation in drug-exposed animals. Prior work from our laboratory and others firmly established that 24-hours of mating and social cohabitation induce partner preference formation in female prairie voles (Insel & Hulihan, 1995, Williams et al., 1992). Young et al., 2014, demonstrate that pre-exposure to AMPH blocks the formation of mating-induced partner preference. These effects were specific to AMPH because social memory tests were conducted 48-hours after the last AMPH injection when AMPH was completely metabolized. Psychostimulant withdrawal did not have an effect because mating frequency and affiliative behaviour between drug treated and naïve controls was not statistically different. Importantly, these findings in female prairie voles match those found in male prairie voles (Liu et al., 2010) as well as effects of psychostimulant experience affecting soscio-sexual (Clemens et al., 2007), maternal (Febo & Ferris 2007, Johns et al., 1997b, Slamberova et al., 2005), and social play behaviours (Wood et al., 1994).

Repeated AMPH treatment impaired partner preference formation, significantly decreased OTR-ir density in the mPFC, but not the NAcc or VTA (Figure 4A) - replicating prior work from other labs (Johns et al., 2010, Sarnyai et al., 1992). OTR-ir density was decreased in the mPFC after 24-hours of the final AMPH injection when females were paired with a male conspecific and provided ad libitum mating and extended cohabitation prior to partner preference testing. Previous behavioural pharmacological work indicated that OTR activation in the mPFC, PLC subregion, during mating and cohabitation was necessary for pair-bond formation in female prairie voles (Young et al., 2001). Thus, decreased OTRs in the PLC may represent a neurochemical locus underlying AMPH impairment of mating-induced pair-bonding behaviour.

These data are further supported by direct OT micro-infusion into the PLC before pairing which restored partner preference formation in drug treated females. These effects were

dependent on PLC-OTR activation (Figure 4C; Young et al., 2014) - extending prior work from other laboratories examining social behaviour (Francis et al., 2000, Olazabal & Young, 2006) and pair-bonding (Ophir et al., 2012, Ross et al., 2009b). PLC-OT micro-infusion increased NAcc-DA concentration relative to artificial cerebrospinal fluid (aCSF) infused controls (Figure 4D), and these results are consistent with previous research illustrating reciprocal and functional connectivity between the PLC and NAcc - both targets of DA-ergic cells bodies in the VTA mesocorticolimbic pathway (Carr & Sesack, 1999). Both electrical and chemical activation of mPFC neurons increases the firing rate of VTA-DA-ergic projection neurons while inactivation inhibits DA burst firing events (Tzschentke, 2001). In summary, PLC-OT infusion increased NAcc DA concentration and restored AMPH-induced deficits in social bonding.

The Future of Social-buffering Stress Research in Prairie Voles

At present, there are no commercially available compounds that physicians and/or Psychiatrists can prescribe patients experiencing debilitating psychological illness resulting from adverse social impairments or bereavement-stress-induced adverse psychological conditions. Because OT administration enhances prosocial behaviour, this particular hormone has recently become a viable target for therapeutic intervention aimed at disorders characterized by marked impairment in social communication and/or bond-loss stress-related psychopathology with several ongoing clinical trials (Miller, 2013). OT underlies socio-sexual behaviour (Carter et al., 2008) and therefore, it has been speculated for decades that it may reduce pathological HPA axis dysfunction.

For example, intra-nasal OT delivery decreases social conflict enhanced by cortisol and increases positive prosocial communication (Ditzen et al., 2009) between human couples during inter-personal conflict, while also facilitating social support reduction during periods of stress-induced CORT release, promoting decreased anxiety and enhanced calmness (Heinrichs et al., 2003, Quirin et al., 2011). Central OT levels are also positively correlated with an increase in positive prosocial communication between intimate partners (Gouin et al., 2010, Holt-Lunstad et al., 2008). Future preclinical work in voles would significantly benefit by expanding the research “tool-kit” by implementing more cellular/molecular approaches currently used in the behavioural neuroscience field (e.g., electrophysiological preparations, optogenetics, DREADDS, AAV-viral-based tract-tracing with CLARITY in order to reveal more concrete neurochemical/-circuit mechanisms altered during bond loss.

As an example, in other work, recent neurogenetic viral-vector-based tract-tracing, optogenetic, and DREADD research in transgenic mice from the laboratories of Dr. Klaus Miczek at Tufts University and Dr. Jamie Maguire at the Tufts School of Medicine have identified a novel CRH micro-circuit associated with social defeat stress (Gobrogge et al., 2015). Specifically, using viral-vector-based tract-tracing by stereotaxically infusing an AAV-Flex-ChR2 virus bilaterally in the ventral-posterior-medial (VPM) region of the posterior VTA (pVTA) in CRH-Cre (Cre recombinase) mice, CRH neurons in the lateral hypothalamus (LH) and dorsal raphe nucleus (DRN) made reciprocal projections with the paranigral (PN) and parainterfasicular (PIF) subnuclei of the VPM. Furthermore, DRN-CRH neuronal dendrites formed a significant number of putative synapses onto DA-ergic cell

bodies that co-expressed CRH-type 1 receptors but not CRH-type 2a/b receptors. A significant number of DA-ergic cells in the PN/PIF pVTA co-localized with a post-synaptic density 95 (PSD95) marker, vesicular glutamate transporter 1 (VGLUT1, pre-synaptic label), and DRN CRH/AAV-mCherry (red) dendrites but LH-CRH dendrites did not. Functional activation of the PN/PIF using either optogenetics or Gq(s)-DREADDs in CRH-Cre mice was sufficient to mimic chronic social defeat stress-induced drug-taking behaviour. Together, these data suggest that CRH neurons in the DRN are activated by social defeat stress, releasing CRH in the PN/PIF, which modulates dopamine-dependent behavioural sensitization to psychomotor stimulants. This particular line of work would significantly advance the prairie vole field as well as behavioural neuroscience, in general.

To our knowledge, at the present time, there are two labs attempting to generate transgenic prairie voles: one under the direction of Dr. Larry Young's group at Emory University in Atlanta (Donaldson et al., 2009) and the second principal investigator Nirao Shah's group at the University of California at San Francisco (Manoli et al., 2012). When a Cre/LOX (or similar transgenic prairie vole line is generated) - similar studies, as mentioned above, can commence. For example, with a transgenic prairie vole expressing Cre recombinase driven by a specific promoter sequence encoding either OT, AVP, or CRH neuropeptides, one could examine the consequences of modulating molecularly defined regions of only OT, AVP, or CRH neurons and processes in the vole CNS and examine the behavioural consequences of manipulating these systems using optogenetics and/or DREADDs with regard to pair-bonding behaviour and stress social buffering.

In short, OT is a powerful neuropeptide exerting direct regulation of social behaviour and memory (Ross & Young, 2009), while perturbing the OT system has deleterious effects underlying social deficits characterized in several psychiatric conditions including drug addiction, autism spectrum disorders, post-traumatic-stress-disorder and many other forms of stress-induced psychopathology - like anxiety and depression. Therefore, the central OT system represents a clinically viable neurochemical target treatment of social communication deficits (such as those observed in individuals with autism (Hollander et al., 2007)), addiction (McGregor & Bowen, 2012, Strathearn & Mayes, 2010), and social separation-induced anxiety and major depression. Because the central OT system is highly conserved across species (Burkett & Young, 2012, Meyer-Lindenberg et al., 2011, Ross & Young, 2009) and is altered in human addicts (Light et al., 2004), the prairie vole represents a valuable animal model to investigate the effects of pair-bonding and OT on breaking bad-bonds associated with drugs of abuse, conditioned anxiety/panic attacks, and episodic major depression. Finally, future research would benefit from examining the role of pair-bonding and endogenous OT as a viable treatment for drug-induced impairments in devaluing natural reward to restore healthy decision-making and reduce partner loss-induced drug relapse and bereavement in humans (Dawson et al., 2005, Kosten et al., 1987).

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Highlights

Pair-Bonding Buffers Stress via Extra-Hypothalamic Neuropeptide Systems

Neuropeptidergic Interactions Modulate Stress-Mediated Pathology

Attachment and Neuropeptides Serve as Viable Biomedical Therapies for Stress

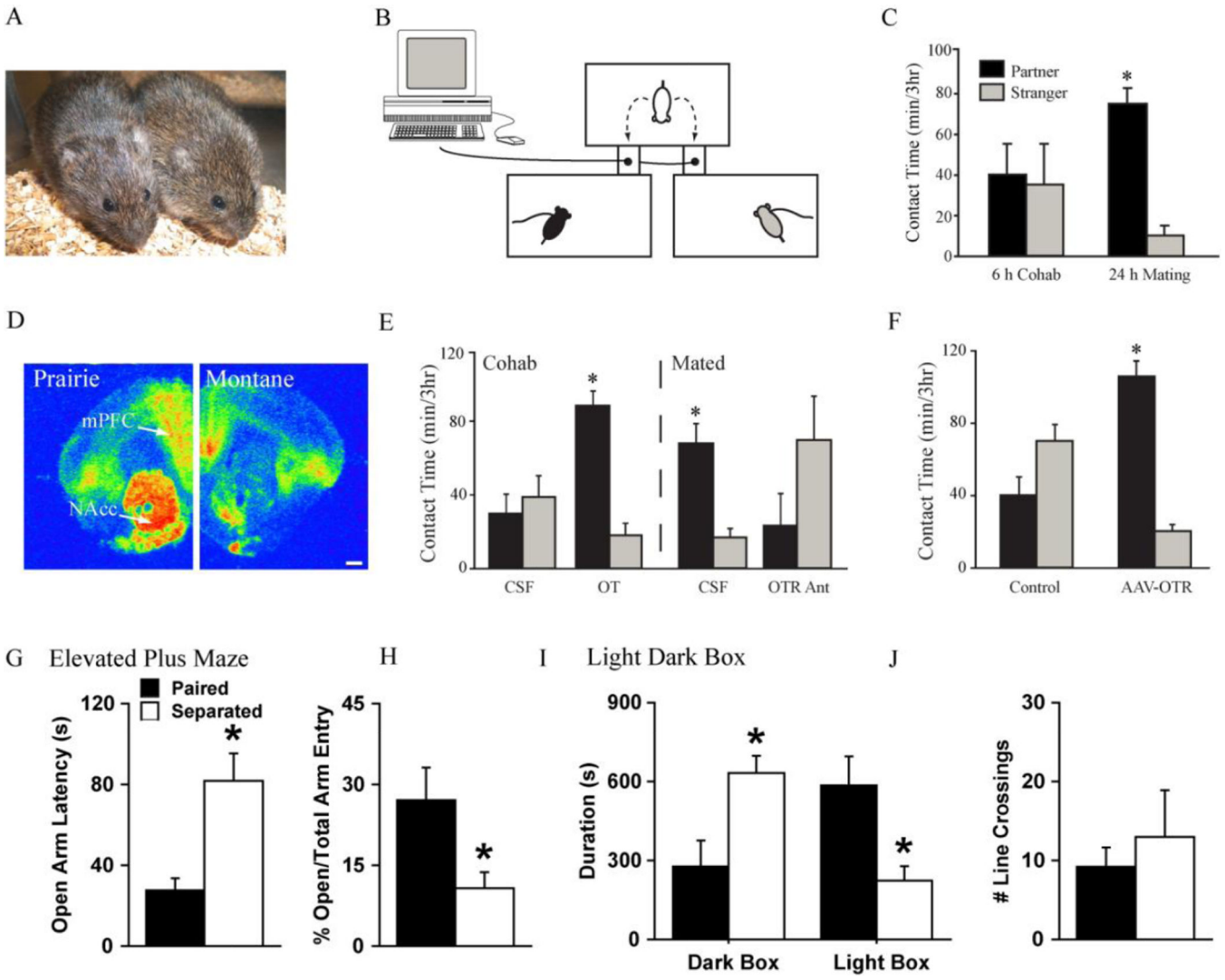

Figure 1.

(A) Picture represents a pair-bonded male and female prairie vole exhibiting side-by-side affiliative contact. (B) Automated three-chamber partner preference paradigm. Shoebox size cages are connected by hollow Plexiglas tubes with infrared light sensors to beam breaks enabling automated movement quantification. Socio-sexual behaviours between experimental animal (i.e., white cartoon vole), familiar partner (i.e., black cartoon vole), and unfamiliar stranger conspecific (i.e., gray cartoon vole) are videotaped, recorded by an experimentally-blind observer, and subsequently scored. Affiliation is computed in minutes of side-by-side physical body contact. (C) Male and female prairie voles' cohabitating without mating for six hours isn't sufficient to induce a partner preference, as the time spent with a partner and/or stranger is equivalent. Conversely, 24 hours of socio-sexual cohabitation is required to induce partner preference, as mated prairie voles spend significantly more time in side-by-side contact with their familiar partner than with an unfamiliar stranger during a three-hour partner preference test. (D) Vole species differences in oxytocin receptor (OTR) distribution in the medial prefrontal cortex (mPFC) and nucleus accumbens (NAcc) of monogamous prairie (left) and promiscuous montane voles (right).

(E) Six hours of non-sexual cohabitation (i.e., Cohab) with intra-NAcc artificial cerebrospinal fluid (aCSF - control) has no effect on partner preference formation. However, voles micro-injected with OT in the NAcc prior to six hours of nonsexual cohabitation is sufficient to induce a significant partner preference. A full day of socio-sexual cohabitation with mating (Mated) induces a significant partner preference which can be blocked by pretreatment with an intra-NAcc OTR antagonist. (F) Prairie voles infused with an adeno-associated virus (AAV) over-expressing OTRs in the NAcc facilitates partner preference formation in the absence of socio-sexual experience compared to Lac-Z virus controls. (G) Partner separation increased male prairie vole latency to enter the open arm of the elevated plus maze and (H) decreased the percentage of open arm entries versus total arm entries, while partner loss (I) increased time spent in the dark box and decreased light box time in the light dark box test. (J) Partner separation had no effect on locomotor behaviour, as the number of line crossings was equivalent across both separated and paired groups. Bars indicate means ± standard error of the mean. *: $p < 0.05$, OT = oxytocin, scale bar = 50μm. Adapted from Aragona & Wang 2009, Liu & Wang 2003, Ross et al., 2009b, Sun et al., 2014, Young et al., 2011a.

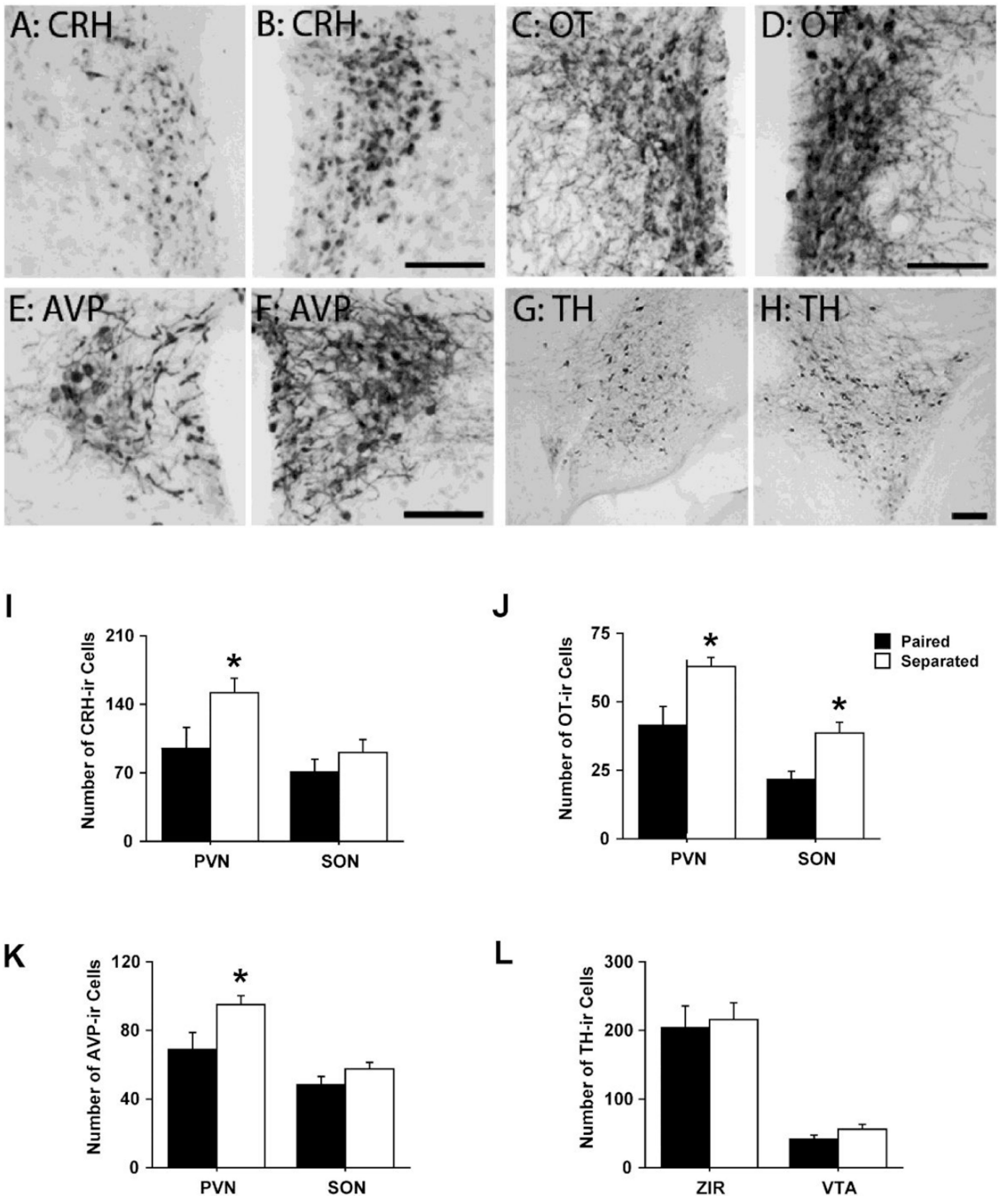

Figure 2.

(A & B) Photomicrographs depicting corticotrophin releasing hormone immunoreactivity (CRH-ir), (C & D) oxytocin (OT-ir), and (E & F) vasopressin (AVP-ir) neurons and processes in the paraventricular nucleus of the anterior hypothalamus (PVN) and (G & H) tyrosine hydroxylase (TH-ir – rate limiting enzyme in dopamine biosynthesis) neurons and processes in the ventral tegmental area (VTA) from male prairie voles that were either paired (A, C, E & G) with or separated (B, D, F and H) from their familiar female partner. (I) Partner separation significantly increased the number of CRH-ir cells in the PVN but not

medial supra-optic nucleus (mSON), (J) increased the number of OT-ir neurons in the PVN and mSON, (K) increased AVP-ir neuronal density in the PVN but not the mSON, and (L) had no effect on TH-ir neuronal density in the rostral zona incerta (ZIR) or VTA. Bars indicate means ± standard error of the mean. *: $p < 0.05$. Scale bars = 50μm & 100μm. Adapted from Sun et al., 2014.

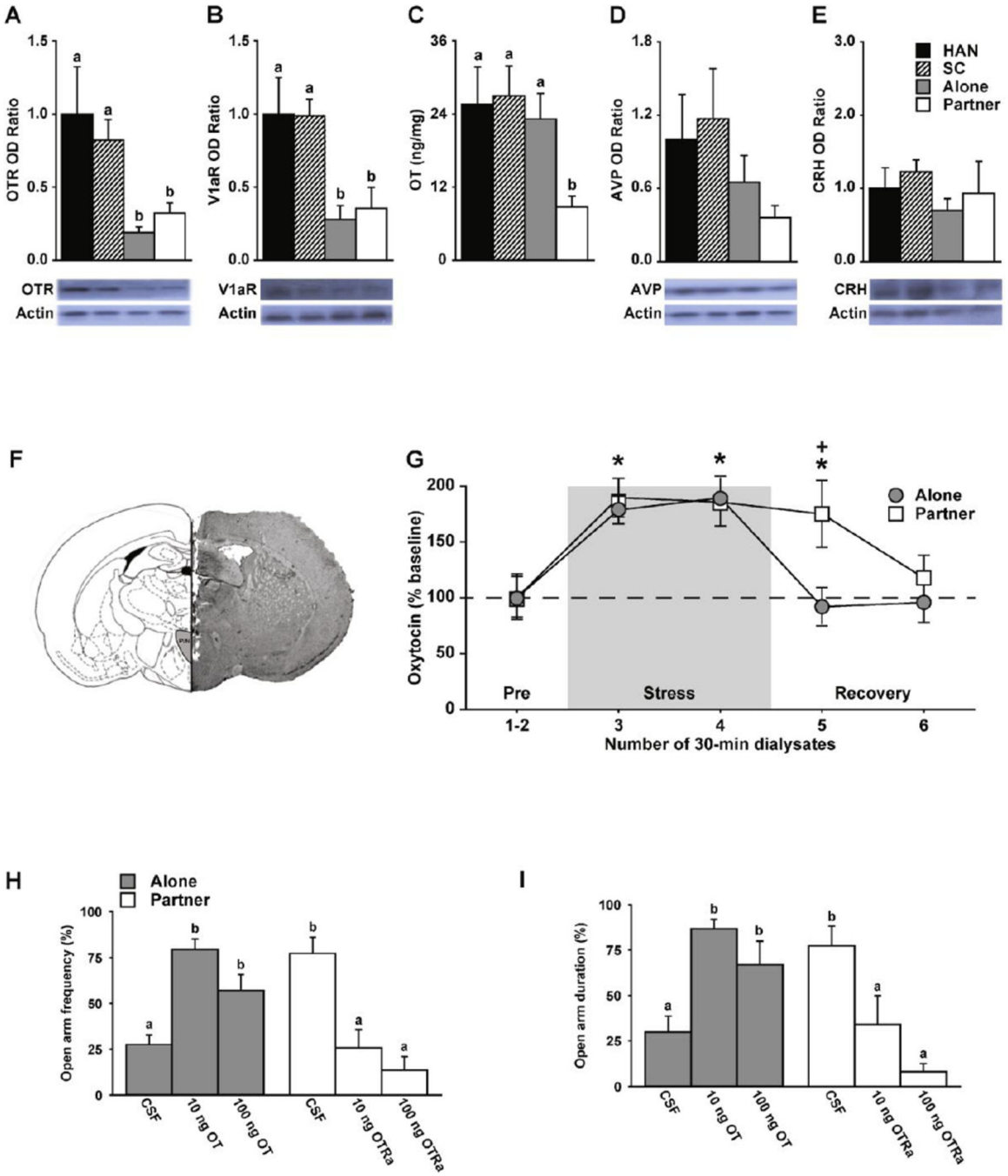

Figure 3.

(A) Immobilization stressed female prairie voles (recovered “alone” or with their “partner”) exhibited significantly lower optical density (OD) in western blot detecting oxytocin receptor (OTR) protein in the PVN and (B) V1a receptor (V1aR) protein relative to handled (HAN) and social control (SC) female controls. (C) Recovering from immobilization stress with a familiar partner significantly reduced the level of oxytocin (OT) content in the PVN; while (D, E) no significant protein levels, detected via western blotting, for vasopressin (AVP) or corticotrophin-releasing hormone (CRH) was found between groups. (F)

Photomicrograph illustrating microdialysis probe placement in the PVN. (G) Immobilization stress significantly increased OT release in the PVN across both groups of females relative to baseline. Recovery with a partner maintained a significantly higher OT tone 30 minutes after immobilization stress. (H & I) Intra-PVN aCSF infused female voles, recovering alone, exhibited enhanced elevated plus maze anxiety-like behaviour (i.e., reduced open arm frequency (H) and duration (I)) unless they received bilateral intra-PVN-OT (e.g., 10ng/100ng) infusion. Conversely, female voles recovering with their male partner exhibited decreased elevated plus maze anxiety-like behaviour (i.e., increased open arm frequency (H) and duration (I)) relative to females recovering alone. Finally, female voles recovering with their partner infused intra-PVN with a selective OTR antagonist attenuated social-buffering effects on anxiety-like behaviour at both low (10ng) and high (100ng) OT doses. Bars labeled with different letters (A–E, H, & I) differ significantly by post hoc Student Newman-Keuls (SNK) tests of significance examining both main effects or interactions with multiple analysis of variance tests and p value set to $< .05$. Bars indicate means ± standard error of the mean. Asterisks (*) indicate differences from baseline and the plus sign (+) indicates group differences at a specific time point, determined by post hoc SNK tests examining significant main effects or interactions with multiple analysis of variance tests and p value set to $< .05$. Adapted from Paxinos & Watson 1998 & Smith & Wang 2014b.

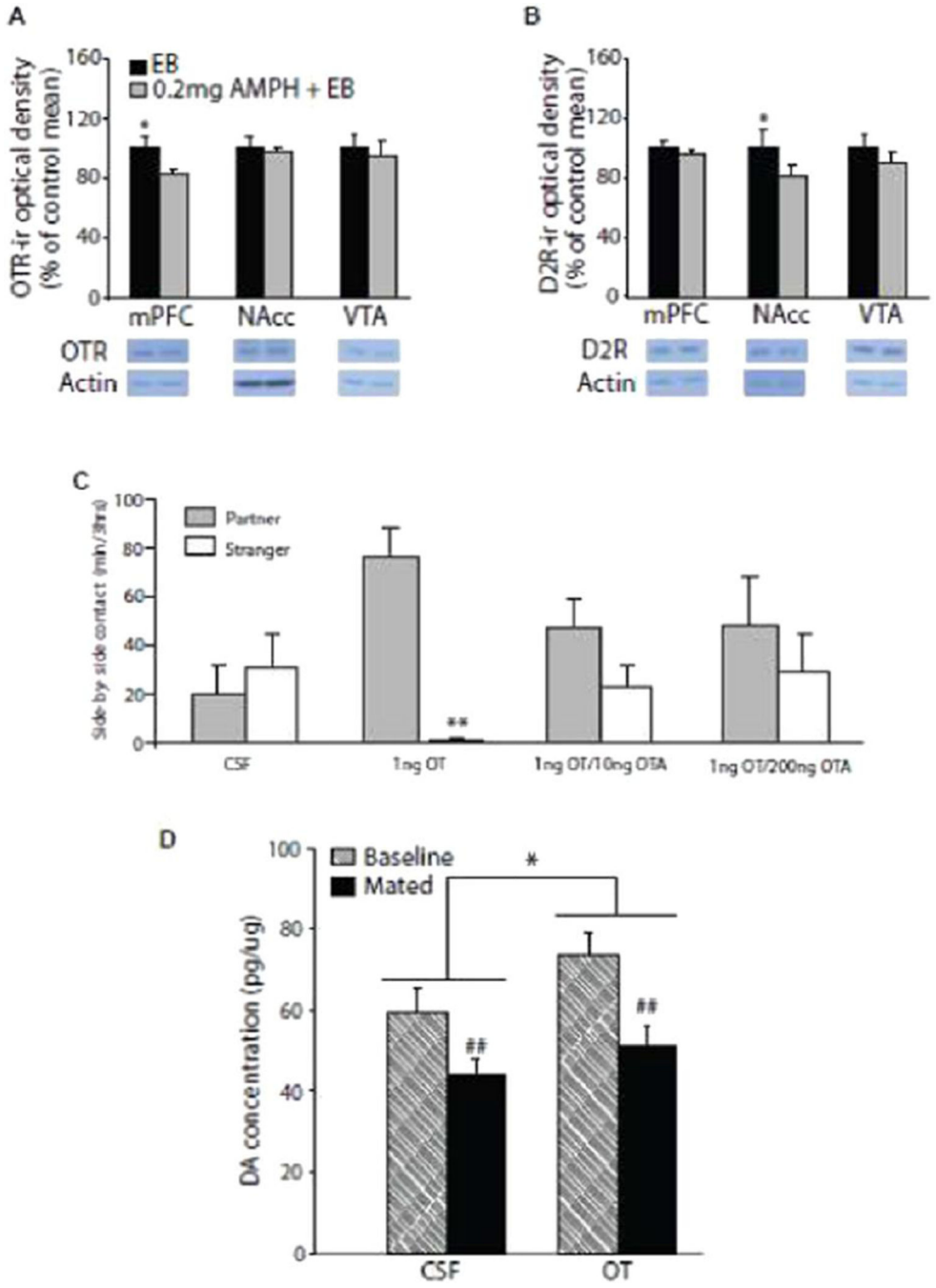

Figure 4.

(A) Western blot data revealed that female prairie voles repeatedly treated with i.p. *d*-AMPH displayed significantly lower OTR-ir in the mPFC, but not NAcc or VTA, relative to estradiol benzoate (EB)-treated control females. (B) NAcc, but not mPFC or VTA, D2R-ir protein levels were significantly lower in female prairie voles repeatedly treated with AMPH compared to EB-treated controls. OT infusion into the prelimbic cortex (PLC) restores mating-induced partner preference formation in AMPH-treated females. (C) Sexually naïve females infused with aCSF into the PLC did not display partner preference while females

injected with 1ng OT intra-PLC, in the absence of mating, displayed robust partner preference and this effect was blocked with concurrent low (10ng OTA) or high (100ng OTA) infusion with the selective OT receptor antagonist. (D) Finally, females infused with OT into the PLC displayed significantly higher levels of dopamine (DA) in the NAcc relative to females treated with intra-PLC aCSF. Furthermore, mated females displayed significantly lower DA levels relative to sexually naïve females (Baseline). Bars indicate means ± standard error of the mean. *$p < .05$, main treatment effects; ##$p < .001$, main sample type effects. Adapted from Young et al., 2014.