**Supplemental Materials**

**Integrative Pathways Linking Close Family Ties to Health: A Neurochemical Perspective**

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**http://dx.doi.org/10.1037/amp0000049**

In this supplementary material we focus in greater detail on two areas referred to briefly in the text. The first is the unique anatomy and signaling features of the oxytocin system. The second is methodological concerns about the measurements used in the oxytocin literature and the conduct of the studies.

Overview of Oxytocin System

The oxytocin system has several unique features that don’t conform to the traditional model of neurotransmission most psychologists are familiar with and are therefore outlined here. Oxytocin neurons reside in the hypothalamus (paraventricular and supraoptic nuclei). As established over 50 years ago (Bargmann & Scharrer, 1951), these neurons have axonal projections that release oxytocin into the bloodstream via the pituitary. Within the last several years, work has also identified oxytocin projections from the hypothalamus to many forebrain regions including the ventral striatum and amygdala as well as the cingulate, insular, and association cortices (Knobloch et al., 2012). The degree to which there is coordinated release between these forebrain and pituitary projections is unclear even in animal models where both can be measured. Although there is evidence of coincident release into the brain and bloodstream in response to some stressors, there is also evidence of a lack of correspondence, suggesting that the coordination is dependent on the nature of the stimulus (Neumann, 2007). It should therefore be remembered when interpreting measures of peripheral oxytocin that these may or may not correlate with intracerebral signaling. Adding to this complexity, is the ability of oxytocin neurons to release oxytocin from their dendrites and cell-bodies (Ludwig & Leng, 2006), not just their axons, like most other neurons. This can prime oxytocin neurons for as long as 90 minutes and can lead to perpetuated oxytocinergic neuron activity. This appears to be occurring during stress (Neumann, 2007) and the degree to which it occurs during social interactions is still being determined but could be a mechanism by which interacting with others could have protracted effects on physiology. In contrast to the high degree of complexity in the regulation of oxytocin release, there is only one oxytocin receptor (Gimpl & Fahrenholz, 2001) that is located in relatively good correspondence to the regions receiving oxytocinergic projections (Boccia, Petrusz, Suzuki, Marson, & Pedersen, 2013; Freeman, Inoue, Smith, Goodman, & Young, 2014; Grinevich, Knobloch-Bollmann, Eliava, Busnelli, & Chini, 2016).

The specific neural pathways by which social signals communicate with oxytocin neurons has received less attention. But, each of the principal monoamines – dopamine, serotonin, and norepinephrine – can activate oxytocin neurons, while endogenous opioids have an inhibitory influence (Brown, Bains, Ludwig, & Stern, 2013). There are no direct inputs from the cortex to oxytocin neurons in the hypothalamus (Sawchenko & Swanson, 1983), however prefrontal areas involved in visceral regulation (e.g. ventromedial prefrontal cortex) do project to adjacent hypothalamic areas that project to oxytocin neurons (Ongür, An, & Price, 1998). Other potential relays for forebrain input to the oxytocin system are via the bed nucleus of the stria teriminalis (Sawchenko & Swanson, 1983), lateral septum (Oldfield, Hou-Yu, & Silverman, 1985), or medial amygdala (Wang, He, Zhao, & Li, 2013), which are the sole limbic inputs to oxytocin neurons. Thus, there is a degree of integration of input before it reaches the oxytocin system (Herman, Tasker, Ziegler, & Cullinan, 2002).

Methodological Issues in Oxytocin Research

Because of the unusual property of the oxytocin system having axons that release into the blood, it is possible to measure this peripherally released oxytocin non-invasively with a blood draw. However, the optimal methodology for measuring the concentration of oxytocin is controversial. The prevailing view is that blood samples should be run through an extraction procedure before measurement in order to remove factors unassociated with oxytocin within the blood that can give a false signal (Nave, Camerer, & McCullough, 2015). In contrast, there is a counter-argument that this extraction procedure eliminates factors that sequester oxytocin and therefore under-measures the available oxytocin (Carter, 2014). This debate is still ongoing and awaits future work in the area to reach a resolution. This issue is less likely to be a problem when oxytocin is sampled from the cerebrospinal fluid or urine.

The manipulation of oxytocin levels through intranasal administration is also not without controversy, because the precise process by which oxytocin delivered by this route influences behavior has not yet been elucidated. There are two key concerns. The first is that the dose given in most intranasal studies (24 International Units) exceeds the concentration of oxytocin in the entire brain (Leng & Ludwig, 2016). Thus, much of this dose can leech into the bloodstream and these supraphysiologic levels of oxytocin in the blood could potentially act on peripheral oxytocin (or vasopressin) receptors to induce central effects. Controls that block these peripheral effects with an antagonist or that administer high doses of oxytocin into the periphery would help alleviate this concern and have not generally been performed. When peripheral and intranasal oxytocin administration have been compared, the results suggest that intranasal effects of oxytocin are not solely due to peripheral effects (Quintana et al., 2016; Quintana, Alvares, Hickie, & Guastella, 2015; Quintana et al., 2015).

The second concern is the extent to which oxytocin that is delivered intranasally reaches behaviorally relevant brain areas. It is a protracted distance from the olfactory epithelium to the hypothalamus and because oxytocin does not diffuse across cellular membranes (Ermisch, Rühle, Landgraf, & Hess, 1985), the path is torturous. In addition to these physical impediments, there are aminopeptidases that break down oxytocin while it is traveling through the brain. Cerebrospinal fluid studies in humans (Striepens et al., 2013) and non-human primates (Dal Monte, Noble, Turchi, Cummins, & Averbeck, 2014; Freeman et al., 2016; Modi, Connor-Stroud, Landgraf, Young, & Parr, 2014) suggest that there is a slight increase in oxytocin after intranasal administration, but the time course of this effect and the degree to which the increase is due to diffusion of the oxctocin delivered intranasally remains to be clarified. Because of the dose-dependence of different signaling pathways activated by the binding of oxytocin to its receptor can lead to qualitatively different effects (Grinevich et al., 2016), improved understanding and methodologies for measuring the concentrations of oxytocin in the brain following intranasal administration are needed.

More generally, the data linking oxytocin and psychosocial factors should be interpreted in light of the rising methodological concerns in the larger fields of medicine (Ioannidis, 2005), human neuroscience (Button et al., 2013), animal neuroscience (Freedman, Cockburn, & Simcoe, 2015), and psychology (Open Science Collaboration, 2015). Recent reviews have raised concerns that most animal and human studies of oxytocin are underpowered and likely to reflect inflated Type I errors due to undisclosed analytical flexibility (Nave et al., 2015; Walum, Waldman, & Young, 2016). We believe increased methodological rigor will benefit the field and confirm that oxytocin is increased by particular social interactions and can indeed facilitate neural processes that facilitate social engagement in a contextually dependent manner.