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Review

Problems with measuring peripheral oxytocin: Can the data on oxytocin and human behavior be trusted?

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ABSTRACT

Research on the neurobiological and behavioral effects of oxytocin (OT), as well as on its possible therapeutic applications, has intensified in the past decade. Accurate determination of peripheral OT levels is essential to reach meaningful conclusions and to motivate, support and inform clinical interventions. Different, but concordant, methods for measuring plasma OT have been developed over the past four decades, but since 2004 several commercially available methods have been favored in research with humans. Evaluation of these methods reveals that they lack reliability when used on unextracted samples of human fluids, and that they tag molecules in addition to OT, yielding estimates that are wildly discrepant with an extensive body of earlier findings that were obtained using methods that are well validated, but more laborious. An accurate, specific, and readily available method for measuring OT that can be adopted as the standard in the field is urgently needed for advances in our understanding of OT's roles in cognition and behavior.

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1. Introduction

The roles of the neurohypophyseal peptide oxytocin (OT) in human cognition and social behavior have become a topic of intense interest, not only for OT's relevance to questions in neuroscience and the behavioral sciences, but also due to the potential clinical utility of OT for treating conditions such as autism and schizophrenia (Beckmann et al., 1985; Donaldson and Young, 2008; Hammock and Young, 2006; Meyer-Lindenberg et al., 2011; Modahl et al., 1998). The role of OT in human social behavior has been investigated mainly using two approaches. In the first, researchers have examined the differences in social cognition or behavior between or within subjects as a result of exogenous OT administration (usually using an intranasal method; Bos et al., 2012; Churchland and Winkielman, 2012; Guastella and MacLoed, 2012; Van IJzendoorn and Bakermans-Kranenburg, 2012; Zink and Meyer-Lindenberg, 2012). The main technical issues dogging the intranasal technique are twofold. The first is that the OT pharmacokinetics are not fully understood and it is not known how much OT reaches target sites in the brain. Neumann et al.

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(2013) did report that nasal and intraperitoneal administration of OT in rodents leads to increased OT levels in brain extracellular fluid, with correspondent increases in OT plasma levels, which is encouraging, but similar evidence is not currently available for human subjects. Second, and related, it remains unknown whether exogenously administered OT interacts with endogenous levels of OT or other hormones (Churchland and Winkielman, 2012; MacDonald et al., 2011; Neumann and Landgraf, 2012).

In the second approach, which is our focus here, researchers measure participants' levels of endogenous OT to determine whether those levels change as a function of experimental manipulation (for example, experimental exposure to a particular type of social interaction, such as massage or an expression of trust) or whether individual differences in either basal levels or task-related reactivity are associated with other individual difference variables (e.g., Bello et al., 2008; Feldman et al., 2010; Strathearn et al., 2009; Tabak et al., 2011; Zak et al., 2005).

Whether peripherally measured OT is indicative of central release patterns and activities within the brain and therefore associated with social and emotional behaviors remains still unknown: Some studies suggest that certain stimuli lead to coordinated release of central and peripheral oxytocin, whereas other studies indicate that certain stimuli change central levels while leaving peripheral levels unchanged (for review see Neumann and Landgraf, 2012). Recent studies addressed this issue by measuring plasma and cerebrospinal fluid levels of OT in human subjects and found no correlation between OT concentrations in the two compartments, suggesting that plasma OT concentrations are uninformative about central concentrations, at least under the basal conditions studied (Jokinen et al., 2012; Kagerbauer et al., 2013). Whether plasma OT can serve as a global biomarker of behaviorally relevant changes in central OT release remains an important research question. However, for the purposes of this review, we hold this issue aside and inquire more deeply into the validity of the methods that are currently in circulation for measuring oxytocin in human plasma and other peripheral fluids.

Pressed for time, many readers of these research reports will skip the methodological details about assay methods and sample extractions—taking for granted that measuring OT in plasma, saliva, or other human bodily fluids is a straightforward matter and that the methods have acceptable levels of sensitivity and specificity. Readers are also likely to expect that the peer review process included reviewers with the expertise to discern whether reliable methods had been used. Regrettably, we have discovered that some of the most widely used techniques for assaying OT in humans have dubious validity. Bluntly put, it is unclear just how much OT the most commonly used assays are measuring. Here, we briefly review the history of OT measurement with the goal of bringing these measurement problems to the attention of researchers in hopes that reliable standards can be set and met.

2. Issues in the measurement of peripheral oxytocin in humans

2.1. Foundational measurement of oxytocin in plasma: RIA on extracted samples

The measurement of peripheral OT was facilitated, as early as 1970, by the advent of radioimmunoassay (RIA) methods (Boyd et al., 1970; Chard et al., 1970). Extraction was shown to be necessary to eliminate interfering substances present in the samples and to concentrate the low plasma levels of OT in order to accurately quantify the peptide in the measurable range of the assay. Using such methods (see Table 1), researchers have determined that in healthy women who are not pregnant or breastfeeding, and in healthy men, normal OT concentrations in plasma are almost always <10 pg/ml, are in the range of ∼1–3 pg/ml for most people, and for many people are too low to detect via RIA (Amico et al., 1981; Chicharro et al., 2001; Cyranowski et al., 2008; Domes et al., 2010; Grewen et al., 2005; Jokinen et al., 2012; Lee et al., 2003; Salonia et al., 2005; Tabak et al., 2011). According to RIA, basal OT levels for men and women rarely differ (Amico et al., 1981; Grewen et al., 2005; Jokinen et al., 2012). Many studies on the role of OT in human social behavior have focused on its roles in pregnancy, parturition and lactation (Blanks and Thornton, 2003; Chard, 1989; Dawood et al., 1978), and using RIA methods, researchers estimated that pregnant women have values in the range of ∼1–10 pg/ml (Amico et al., 1986; Dawood et al., 1978; Sellers et al., 1981), that they have plasma OT levels ∼7–45 pg/ml during early-stage labor (Dawood et al., 1978; Fuchs et al., 1982; Lindow et al., 1998; Rahm et al., 2002; Sellers et al., 1981), and that those levels might peak ∼9–114 pg/ml during advanced stages of labor (Dawood et al., 1978; Fuchs et al., 1982; Sellers et al., 1981; Thornton et al., 1988). Estimates obtained using RIA methods with extracted plasma likewise indicate that nursing mothers have plasma OT levels ∼2–13 pg/ml prior to breastfeeding sessions (Lucas et al., 1980; Nissen et al., 1996; Yokoyama et al., 1994), and that these values can rise to ∼11–24 pg/ml during breastfeeding (Lucas et al., 1980; Nissen et al., 1996; Yokoyama et al., 1994).

Subsequently, some researchers explored RIA methods that were argued not to require sample extraction (Burd et al., 1985; Robinson, 1980), reporting that such assays yielded plasma levels consistent with those previously established with RIA on extracted samples. Unfortunately, these methods have not been rigorously examined in subsequent work with human plasma.

It is worth noting that the RIA methods enumerated above were all developed using independently derived antisera and independently conducted validations. In most cases, validation included evaluation of cross-reactivity with known peptides and proteins (e.g., vasopressin and neurophysin), demonstration of the need to extract samples to eliminate interference and to concentrate the low levels of OT present in plasma, evaluation of the extraction method employed (e.g., recovery of radiolabel and/or recovery after spiking) and in several instances, confirmation of the RIA results by comparison to bioassay (Dawood et al., 1978; Chard et al., 1970) or with chromatographic separation (Robinson, 1975; Stock et al., 1989). In spite of this methodological diversity, these independently developed and validated RIAs have produced consistent results, yielding plasma OT values typically <10 pg/ml in healthy adult subjects.

2.2. The EIA revolution in plasma OT measurement

To avoid the labor-intensive extraction process and the use of radioactive materials, in 1998 researchers began developing enzyme immunoassay (EIA) methods for measure plasma OT without extraction (Prakash et al., 1998). In a sample of four dairy cows, Prakash et al. (1998) found high correlations ($r = .97$) between values using standard RIA methods and their new EIA method, as well has high absolute agreement in the estimated values. These results suggest that a high-quality EIA method might be feasible given proper validation, and the method was subsequently applied to plasma OT measurement in yaks (Sarkar and Prakash, 2006) and mithuns (Mondal et al., 2006). Another research group developed an EIA method that avoided extraction by using a novel filtration method to remove interfering plasma proteins (Péqueux et al., 2001). Use of this method yielded OT estimates in human plasma of ∼7 pg/ml, with standard errors that put those values in the ranges that previously had been established for OT in human plasma using RIA with sample extraction (Amico et al., 1981). This method subsequently has been used in research

Table 1
Typical plasma oxytocin estimates from previously published research for seven different reference groups as a function of assay method (RIA on extracted samples vs. EIA on unextracted samples).

Plasma oxytocin estimates for. . .	Method	
	RIA on extracted samples	EIA on unextracted samples
Healthy, non-pregnant, non-lactating women (basal levels)	1–5 pg/ml (Amico et al., 1981; Cyranowski et al., 2008; Domes et al., 2010; Grewen et al., 2005; Jokinen et al., 2012; Salonia et al., 2005; Tabak et al., 2011)	200–359 pg/ml (Feldman et al., 2012; Gordon et al., 2008; Schneiderman et al., 2012; Taylor et al., 2010; Weisman et al., 2013)
Healthy men (basal levels)	0–5 pg/ml (Amico et al., 1981; Chicharro et al., 2001; Grewen et al., 2005; Jokinen et al., 2012; Lee et al., 2003)	240–405 pg/ml (Bello et al., 2008; Feldman et al., 2010, 2012; Gordon et al., 2008; Schneiderman et al., 2012; Taylor et al., 2010; Weisman et al., 2013)
Healthy, pregnant women	1–10 pg/ml (Amico et al., 1986; Dawood et al., 1978; Sellers et al., 1981)	264–329 pg/ml (Feldman et al., 2007; Levine et al., 2007)
Healthy women in normal early-stage labor	7–45 pg/ml (Dawood et al., 1978; Fuchs et al., 1982; Lindow et al., 1998; Rahm et al., 2002; Sellers et al., 1981)	–
Healthy women in normal later-stage labor	9–114 pg/ml (Dawood et al., 1978; Fuchs et al., 1982; Sellers et al., 1981; Thornton et al., 1988)	–
Lactating mothers (prior to breastfeeding session)	2–13 pg/ml (Lucas et al., 1980; Nissen et al., 1996; Yokoyama et al., 1994)	–
Lactating mothers (peak during breastfeeding session)	11–24 pg/ml (Lucas et al., 1980; Nissen et al., 1996; Yokoyama et al., 1994)	166 pg/ml (Jonas et al., 2009)

with human cancer cells (Péqueux et al., 2002) and rat plasma (Deblon et al., 2011). Although this latter approach has not, as far as we are aware, been directly compared to RIA methods, these initial encouraging results suggests a promising alternative to RIA with solid-phase or solvent-based extraction.

In 2004, two papers were successively published on a commercially available EIA for OT that allegedly did not require extraction (Kramer et al., 2004; Zak et al., 2004). Kramer et al. (2004) claimed validation in rat and prairie vole plasma for a commercial EIA based on (a) parallelism of diluted plasma compared with a known standard curve, (b) recovery in plasma after spiking with a known quantity of standard OT, and (c) the ability to measure increased plasma OT after a bolus injection of OT into prairie voles. The sample dilution and spiking experiments can be interpreted to indicate that the plasma matrix has little effect on the measured reactivity, thus ruling out non-specific interference. Although promising, these data alone lack the rigor required for bioanalytical method validation (Food and Drug Administration, 2001; Kelley and DeSilva, 2007; Savoie et al., 2010; Shah et al., 2000). Fundamental issues that must be addressed include suitability of sample matrix (e.g., plasma, saliva, etc.), and measurement accuracy, precision, selectivity, sensitivity, reproducibility, and stability. One assumption inherent in the use of immunoassay methods is that the antibody utilized is uniquely specific for binding *only* the analyte of interest. Data to support this latter assumption for currently available OT immunoassays are lacking, as we discuss below.

Using the OT method without sample extraction, the researchers went on to use their EIA to determine that the plasma of male and female Sprague-Dawley rats contained OT levels that were 3–25 times higher than those previously published for both male and female rats when RIA methods were used on extracted samples (Bagdy and Kalogeras, 1993; Carter and Lightman, 1986; Melis et al., 1989, 1990; Uvnäs-Moberg et al., 1992, 1996). In the second study, whose data were reported in two different publications (Zak et al., 2004, 2005), research with men and non-pregnant/non-lactating women using commercial EIA kits on unextracted plasma yielded concentrations ∼200–300 pg/ml—*two orders of magnitude* higher than those obtained in previous work in analogous samples using RIA methods with extraction (Amico et al., 1981; Szeto et al., 2011).

These discrepancies notwithstanding, other investigators then began to use the same commercial method for measuring OT in human plasma, often citing Kramer et al.'s (2004) results as support for the assay's validity. Subsequent work using the same commercially available EIA methods, for example, yielded mean plasma OT concentrations ∼200–405 pg/ml for healthy men and healthy non-pregnant/non-lactating women (Bello et al., 2008; Feldman et al., 2010; Gordon et al., 2008; Taylor et al., 2010), with some participants' values exceeding 1000 pg/ml (Gordon et al., 2008). In a sample of women followed throughout their pregnancies, unextracted plasma OT levels using EIA have been estimated ∼300 pg/ml, with estimates for some women exceeding 3000 pg/ml (Feldman et al., 2007; Levine et al., 2007). Thus, the commercially available EIA methods with unextracted plasma can be counted on to produce plasma OT values that are two orders of magnitude higher than those found using RIA on extracted plasma (see Table 1).

Despite its questionable validity, this commercial EIA method (without sample extraction) has been used with increasing frequency since 2004. Consequently, some of the things we thought we knew about OT levels in human plasma have been contradicted in recent research. Based on EIA results, for instance, researchers have concluded that infant suckling raises new mothers' plasma OT levels to approximately 166 pg/ml (Jonas et al., 2009), which exceeds at least sixfold the values previously established with RIA methods (Lucas et al., 1980; Nissen et al., 1996; Yokoyama et al., 1994); that therapeutic massage and reading National Geographic both increase men's plasma OT levels from ∼200 to ∼300 pg/ml (Bello et al., 2008); that watching a two-minute emotional video raises people's plasma OT levels from ∼400 to nearly 600 pg/ml (Barrazza and Zak, 2009), and that sharing a secret with another person raises plasma OT from ∼230 to ∼300 pg/ml (unless they have schizophrenia; Kéri and Kiss, 2011; Kéri et al., 2009; Kiss et al., 2011). Although our discussion is focused on values measured in human samples, similar discrepancies exist when OT is measured in animal plasma (e.g., Table 1 in Szeto et al., 2011).

Researchers' apparent lack of concern about the profound discrepancies between the values obtained using traditional RIA with extracted plasma versus those obtained via EIA on unextracted samples is not reassuring. For instance, Zhong et al. (2012) justified the use of EIA on unextracted samples in their study of the relationship between trust and plasma oxytocin among 1158 Chinese undergraduates (in which they obtained mean oxytocin levels of 214 pg/ml and some values exceeding 900 pg/ml) by arguing that "the oxytocin data from non-extracted samples makes biological sense as compared to those from extracted samples, which often gave rise to non-detectable levels of oxytocin" and, further, that "many other researchers have used the same kit to measure

oxytocin in unextracted samples and found a myriad of associations with relevant physiological outcomes" (p. 3). The application of logic such as this, which we fear might be somewhat prevalent among researchers in this area, is no substitute for unbiased, evidence-based bioanalytical validation as the basis of one's decisions on how to measure oxytocin.

2.3. Commercial RIAs and EIAs both have problems

Two of us (AJM and MEM) were recently involved in an effort to evaluate two commercially available immunoassays (one RIA and one EIA) for measuring OT. We also evaluated whether or not sample extraction was optional or compulsory to obtain values consistent with the pre-EIA literature (Szeto et al., 2011). The commercial RIA method tested lacked sensitivity to measure plasma OT even after extraction in the majority of human samples tested (most samples' values fell below the assay's detection limit). In the EIA, there were divergent results in unextracted compared with extracted samples: Unextracted plasma yielded values 100-fold greater than the same sample after extraction and without correlation.

Fractionation studies of plasma revealed at least two macromolecular species present in plasma that showed OT-like immunoreactivity that were removed by extraction. In other words, unextracted samples contained not only OT, but also other molecules that the assay erroneously tagged as OT. Estimates from EIA using extracted samples had similar values to those obtained in earlier reports using RIA with extracted samples (see Table 2).

Further characterization of the extracted samples demonstrated additional molecular species distinct from OT, but which nevertheless demonstrated OT-immunoreactivity, thus leading to the overestimation of authentic OT in the samples by perhaps as much as 200%. In the few samples we tested, the non-OT immunoreactive products account for most of the measured immunoreactivity in both the EIA and the RIA. Although the nature of these molecules remains to be determined, we postulated that these may represent OT-degradation products and thus may reflect the circulating levels of the peptide, but further studies are needed to evaluate this possibility (Szeto et al., 2011).

These results suggest that continued efforts to measure OT in unextracted plasma samples should be discontinued until a rigorously validated method has been established. Although it is currently unknown whether extraction prevents cross-reactivity, or whether it eliminates the effects of binding proteins or other substances that interfere with the assay, oxytocin-free plasma (for instance, obtained from hypophysectomized animals or knockout mice lacking the OT gene) could be used as a negative control to evaluate the issue further. OT-free serum could also be used as the diluent for preparing standards and compared to standards in buffer to directly identify non-specific immunoreactivity or assay interference due to the sample composition. At least one commercial manufacturer of an EIA currently recommends extraction of serum and plasma prior to analysis. When those recommendations are followed, values obtained are on the same order of magnitude as those published earlier with RIA in extracted plasma (Grewen and Light, 2010; Holt-Lunstad et al., 2008). This fact suggests that some of the discrepancies between the "old" RIA results and the "new" EIA results can be alleviated simply by following manufacturers' instructions. On the other hand, even with the commercially available RIA method we evaluated on extracted samples (Szeto et al., 2011), much of what is being tagged as OT is not, in fact, OT.

A recent review of the role of OT and behavior acknowledges the discrepancies in the various methods used to measure OT (Ebstein et al., 2012). Oddly, however, the authors concluded that because the data Kramer et al. (2004) obtained by the use of the EIA method without extraction reflected "biologically relevant results consistent with what is known regarding OT in modulating human behavior," the continued use of the commercially available assays is justifiable. Because much of what is believed about OT modulating human behavior may be based on unreliable methods for measuring OT, however, the defense of this method's validity by referring to "what is known" might be viewed as a form of circular argument. A second assumption operating here may be that whatever immunoreactive macromolecules happen to be included in the OT measures are behaviorally inert, and consequently, that their contaminating effects do not threaten the validity of the conclusions one wishes to draw about the factors that cause OT secretion. Current scientific knowledge does not indicate that this assumption is defensible.

2.4. Assessing oxytocin in saliva, urine, and other fluids

For a variety of practical and theoretical reasons, researchers have attempted to assay OT from other bodily fluids such as saliva (Carter et al., 2007; Feldman et al., 2011; Weisman et al., 2012; White-Traut et al., 2009), urine (Amico et al., 1987; Boyd et al., 1970; Fujiwara et al., 2012; Polito et al., 2006), cerebrospinal fluid (Heim et al., 2009; Jokinen et al., 2012; Kagerbauer et al., 2013), amniotic fluid (Dawood et al., 1978; Kuwabara et al., 1987), and milk (Mishra et al., 2012; Prakash et al., 2009). Additionally, microdialysis has been used for over three decades to directly measure neurotransmitters and neuropeptides, including oxytocin, by sensitive RIA methods in the extracellular fluid of distinct brain areas in animal models, which facilitates the monitoring of neuropeptides at the sites of their release. Cells and macromolecules are excluded by the dialysis probe, so centrifugation and protein precipitation are generally not required prior to analysis (reviewed in Lee et al., 2008; Nandi and Lunte, 2009; Wotjak et al., 2008).

Of particular note, with respect to research on humans, are recent efforts to measure OT in saliva (Carter et al., 2007; Feldman et al., 2011; Weisman et al., 2012; White-Traut et al., 2009). Salivary assays typically yield estimates of $\sim$10 pg/ml of saliva, and correlate weakly with EIA-based estimates of OT in unextracted plasma (with r values ranging from .41 to .59; Feldman et al., 2010, 2011; Grewen and Light, 2010). To neuroscientists and behavioral researchers who are unfamiliar with validation standards for new assays, correlations on this order might look like reasonably flattering evidence for the validity of salivary OT assays, but correlations of 0.41 and 0.59 imply coefficients of determination (i.e. percentages of shared variance between the salivary and plasma measures) of 0.17 and 0.35 (i.e., 17% and 35% shared variance), respectively. Coefficients of determination of this low magnitude are somewhat faint praise, especially when one considers that salivary measures of cortisol and testosterone, for example, typically correlate with plasma measures with rs > 0.90—implying >81% shared variance (Arregger et al., 2007; Kaufman and Lamster, 2002; Kirschbaum and Hellhammer, 2000). Moreover, a rigorous evaluation of whether bioavailable OT can be measured in saliva led Horvat-Gordon et al. (2002) to conclude that "measurement of OT in saliva does not yield meaningful indices of individual differences or intraindividual change" (p. 445).

Not only do the existing attempts to measure OT in saliva require further validation, but Horvat-Gordon et al.'s initial reasons for skepticism about the presence of bioactive OT in saliva remain unaddressed ten years on (Gröschl, 2009). We hasten to note that none of Horvat-Gordon et al.'s misgivings are entirely assuaged by recent evidence (Weisman et al., 2012) that intransasal oxytocin administrations cause increases in estimates of salivary OT. This is because (a) the method Weisman et al. (2012) used to assay salivary OT has not, to our knowledge, received full validation using established bioanalytical methods (Kelley and DeSilva, 2007; Shah et al., 2000); (b) the putative OT that the assay did identify could

Table 2
Effects of immunoassay method and extraction on assay validity for two commercial oxytocin immunoassays.

Method	Mean for extracted samples	Mean for unextracted samples	Correlation of values in extracted and unextracted samples	Spike recovery extracted samples	Spike recovery-unextracted samples	Amount of authentic oxytocin in extracted samples (via HPLC)
RIA	22/25 samples < assay detection limit	19/25 samples < assay detection limit	Could not assess because not enough samples had values above assay's detection limit	85%	18.8%	22–43%
EIA	1.8 pg/ml	358 pg/ml	−0.14–0.09 (depending on correlation statistic used and whether data were log-transformed)	97%	364%	7–25%

Szeto et al. (2011).

plausibly have originated from "dripping back into the mouth parts of the sniffs of OT" (Weisman et al., 2012, p. 4) rather than from the salivary glands (other results are vulnerable to the same interpretation; Huffmeijer et al., 2012; van IJzendoorn et al., 2012); and (c) the method returns values for OT in saliva that are (as is typical) conspicuously higher than those obtained from human plasma using RIA on extracted samples following intranasal administrations of equivalent dosages (Landgraf, 1985).

As mentioned above, attempts also have been made to measure OT in urine with RIA (Amico et al., 1987; Boyd et al., 1970; Polito et al., 2006) and EIA (Feldman et al., 2011), suggesting concentrations in the range of 10–50 pg/ml in humans. At least one high-profile research report (Fries et al., 2005) has been criticized (Young and Anderson, 2010) for relying on HPLC-UV approach to measuring OT in urine that gave values that were as much as one million times higher than those previously reported using RIA, and at least one paper using EIA without sample extraction suggests that OT measured in urine does not correlate with levels in blood or saliva (Feldman et al., 2011) although this did not deter the authors from interpreting the urinary values as valid reflections of actual OT in urine. Using a validated RIA with sample extraction, clearance studies of infused OT established that less than 1% of intact plasma oxytocin is cleared by the urine and that randomly collected urine levels are poorly predictive of plasma levels (Amico et al., 1987). The relationship between circulating and urinary excretion levels of OT need to be identified before such measures can be of utility.

3. Conclusions and recommendations

These observations raise several questions. First, what it is that commercial OT assays are measuring in human plasma? Clearly, the measurements include OT, but evidently they often measure other molecules that are erroneously tagged as OT. Using commercially available EIA assays without extraction, one is guaranteed to obtain values that are two orders of magnitude higher than those obtained using conventional RIA methods with extraction. This discrepancy between results by different methods has rarely been addressed. Why not just divide the values obtained using "new" EIA methods on unextracted plasma by 100 or so? Because the samples will contain multiple immunoreactive products other than OT—and (importantly) in concentrations (relative to actual OT) that appear to vary both across individuals (Amico et al., 1985; Szeto et al., 2011) and physiological states (Mitchell et al., 1998; Mueller-Heubach et al., 1995), thereby rendering such ratios incommensurable.

By following commercial assay manufacturers' recommendations to extract samples prior to conducting the assays, it is possible to obtain levels that are, at least, on the same order of magnitude as those obtained by RIA in extracted plasma (Grewen and Light, 2010; Holt-Lunstad et al., 2008). Even so, these samples also contain multiple immunoreactive products that remain to be identified. Some of these non-OT immunoreactive molecules may be OT degradation products that could reflect the metabolism of bioactive OT, but they might also be non-specific reactants that do not reflect OT turnover. To our knowledge, nobody knows the answer to this question. Questions about the reliability of OT measurement in plasma must also be raised when other biological fluids (e.g., saliva and urine) are used for testing and appropriate validation for all samples types used are needed. In particular, as Horvat-Gordon et al. (2002) explained, there are good reasons to doubt that saliva contains bioavailable OT. Clearly, the field knows less about the measurement of OT in human fluids than many published reports might imply. As a consequence, we also know less about the neurobiological and behavioral significance of peripheral levels of OT than those reports imply.

It is worth noting that RIA and EIA are not the only possible approaches worthy of further exploration. For example, Zhang et al. (2011) described a physio-chemical method for measuring oxytocin in human plasma that used a sample extraction step followed by two-dimensional liquid chromatography separation with tandem mass spectrometry detection. The assay was sensitive to 1.0 pg/ml in human samples and highly specific for intact OT. In a small set of human samples ($n = 8$) plasma levels were below 4 pg/ml, consistent with reports from studies using RIA with sample extraction (see Table 1). Although the instrumentation need for this assay is likely only available in specialized laboratories, its high degree of specificity and sensitivity suggest that this method could serve as an excellent reference for validation of method modalities such immunoassays and—importantly—for confirming the presence of oxytocin in biological samples other than plasma (e.g., saliva and urine).

In addition to the issues related to analytical method, pre-analytical sample processing is one of the most critical elements in obtaining accurate and reliable biochemical assay results. Many errors affecting laboratory test results occur in the preanalytical phase during sample collection (Lippi et al., 2006). Factors that require consideration include whether samples are collected from subjects in a fasted or post-prandial state, sample type (serum or plasma), time of day and temperature during collection and pre-analysis; tube additives (e.g., EDTA and citrate), use of protease inhibitors, and storage conditions. High levels of oxytocinase activity during pregnancy necessitate further precautions. The field requires systematic evaluation, and then standardization, of these pre-analytical sample processing procedures.

Until methods for measuring OT in any human fluid are validated and standardized, interpretation of reported results remains murky at best. Method validation here is an urgent matter, not only because of the importance of understanding the role of OT in physiology and behavior, but also because exploration of the clinical significance of OT is currently under way. Method validation research might lack the romance of seeking substantive scientific discoveries that could find their way into the national newspapers. Nevertheless, if the results of discovery-oriented research are to

be meaningful, the measurement tools upon which they rely must measure what they are claimed to measure.

Finally, the current state of the scientific database on OT in human plasma and other fluids suggests that editorial care should be exercised to insure that manuscript reviewers understand the field standards for bioanalytical method validation (Kelley and DeSilva, 2007; Shah et al., 2000) and the OT measurement problems we have identified herein. Our scientific journals archive forever the progress of scientific knowledge, as well as our avoidable errors and our lapses in due diligence. Thus, editors and reviewers alike bear a heavy responsibility for insuring that scientific findings result from trustworthy methods and yield biologically plausible results (Young and Anderson, 2010).

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