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SHORT COMMUNICATION

Neonatal CSF oxytocin levels are associated with parent report of infant soothability and sociability

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Summary Oxytocin (OT) has been linked to social behavior in rodents, non-human primates, and adult humans, but almost nothing is known about brain OT activity in human newborns or its impact on social development. To better understand the role of OT biology in human social functioning, a multi-disciplinary, longitudinal study was conducted. Cerebral spinal fluid (CSF) OT levels from 18 human neonates were evaluated and examined in relationship to social-seeking behavior at term, at 3 months, and at 6 months of age. Higher neonatal CSF OT levels were consistently associated with solicitation of parental soothing and interest in social engagement with others. This is the first study to link CSF OT levels to normative human social functioning. Research is now required to test whether early OT levels serve as a biomarker for subsequent social abnormalities.

Impairments in social functioning—observed in autism or extreme anxiety—can lead to devastating, lifelong consequences for the individual. Understanding the biology of early aberrations in infant social behavior may offer insights into, and early markers for, these developmental disorders. Oxytocin (OT) has emerged as a strong candidate in the search for the biological underpinnings of human social functioning (Meyer-Lindenberg et al., 2011).

Central OT plays a critical role in a variety of mammalian social behaviors including mother—infant attachment, affiliative contact-seeking, and social recognition (Insel, 2010). Young peer-reared rhesus monkeys display aberrant social behaviors; as a group these monkeys had lower cerebrospinal fluid (CSF) OT levels over the course of development when compared to maternally reared controls (Winslow et al., 2003). Strikingly, individual expressions of affiliative

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behaviors also correlated with CSF OT levels. While CSF OT levels are positively correlated with social functioning in this non-human primate model, this relationship is not evident in plasma OT assessments (Winslow et al., 2003), highlighting the complex regulation of central and peripheral OT systems (Amico et al., 1990; Veening and Barendregt, 2010). Finally, central OT blockade, achieved pharmacologically or genetically, results in a variety of social impairments (Carter, 2007; Insel, 2010; Meyer-Lindenberg et al., 2011; Modi and Young, 2012).

There is limited information on the role of central OT biology in adult human social functioning, primarily due to the need for invasive measurement techniques (i.e., lumbar puncture), and nothing is presently known about OT biology in human infants. We therefore capitalized on the unique opportunity to examine CSF from newborns undergoing clinical sepsis evaluation, including lumbar puncture. The vast majority (>95%) of such infants are later found not to be septic, thus allowing measurement of potential biomarkers in normal newborn CSF. Here we describe the results of a pilot study designed to measure newborn CSF OT levels in the immediate postnatal period, followed by periodic behavioral assessments during the first year of life to test for the first time the relationships between human newborn CSF OT levels and later social behavior.

1. Methods

1.1. Participants

The study was approved by the Stanford University Institutional Review Board (Protocol 10663). Eighteen neonates (12 males, 6 females) undergoing clinically indicated sepsis evaluation for standard risk factors (maternal fever, prolonged rupture of membranes, respiratory distress) were included. Exclusion criteria included known chromosomal anomalies and major malformations. With parental consent, 0.1–0.5 ml additional CSF was obtained at the time of lumbar puncture using standard sterile procedures within 72 h of birth. All subjects received 48 h of antibiotics and were found to be sepsis-negative. Gestational age ranged from 27 to 40 weeks (M = 36.14, SD = 3.80). Ethnicity and other demographics are presented in Table 1.

1.2. Fluid storage and processing procedures

CSF was transferred on ice, aliquoted, snap frozen, and stored at −80 °C. Unextracted CSF samples were assayed for OT levels (in duplicate, 100 μl per well) by enzyme immunoassay (Enzo Life Sciences, Farmingdale, NY) as previously described (Parker et al., 2010). Per Enzo Life Sciences literature, the cross-reactivity with vasopressin is 0.6% and the limit of assay sensitivity where the curve is no longer linear is ∼10 pg/ml. All samples were run on a single microplate. The intra-assay coefficient of variation was <10%.

1.3. Soothability at term or term equivalent

Prior to hospital discharge, parents were asked to review a list of soothing techniques and check which ones helped to calm their baby (e.g., talking, touching, holding, providing a pacifier, feeding, not needing to be soothed). Responses were scored categorically. Parents were blind to OT levels.

Table 1 Participant demographics and perinatal characteristics.

	Number of participants
Sex	
Females	6
Males	12
Ethnicity	
Hispanic	8
Non-Hispanic	10
Insurance status	
Public coverage	8
Private coverage	10
Gestation	
Pre-term	9
Term	9
Birth history	
Vaginal	10
C-section	8
Pitocin exposure	
Yes	7
No	11

1.4. Sociability follow-up at 3 months of age

At 3 months term-adjusted age, parents were contacted by phone to complete the Infant Behavior Questionnaire (Rothbart, 1981). Interviews were conducted in English or Spanish, as appropriate. The revised very short form of the instrument consists of 37 items assessing extroversion and positive affect, negative affect, and effortful control.

1.5. Sociability follow-up at 6 months of age

At 6 months term-adjusted age, parents were asked to return for evaluation and to complete two sets of questionnaires: (1) the IBQ and (2) the Relating to Others subscale from the Socialization section of the Vineland Adaptive Behavior Scales, 2nd ed. (Sparrow et al., 2005).

1.6. Statistical analyses

Study data were managed using REDCap and analyzed in SPSS v.19. Univariate statistical assessments were used to test relationships between CSF OT levels, perinatal factors, demographic factors, and behaviors; t-tests were used when the independent variable was dichotomous. Pearson correlations were used when the independent variable was continuous.

2. Results

2.1. CSF OT concentrations and study covariate analyses

CSF OT concentrations in newborn samples ranged from 10.25 to 34.06 pg/ml (M = 20.55, SD = 7.53). OT concentrations

were not related ($p > .10$) to: sex, ethnicity, insurance status, gestational age, birth weight, mode of delivery (vaginal vs. c-section), Apgar scores at 1 min or 5 min, maternal gravida or para status. There was no difference in OT levels in babies whose mothers were exposed vs. not exposed to Pitocin ($t(16) = .17$, ns). For those who were exposed, there was no significant relationship between OT and amount of Pitocin given ($r = -.20$, ns). Therefore, data were pooled across these variables. Neither demographic factors (Table 1) nor parity were statistically associated with parent responses regarding need for soothing at term, 3 or 6 months.

2.2. Newborn CSF OT concentrations positively correlate with need for interaction to soothe at term

Parents were given a checklist of soothing methods (holding, pacifier, feeding, does not require soothing) just prior to hospital discharge when their infant reached term adjusted age. Their responses (needed help soothing using any method vs. no need for soothing) suggested that higher OT levels were associated with increased need for soothing assistance (M for babies who needed assistance being soothed = 21.37, $SD = 8.32$ vs. M for babies who did not need assistance being soothed = 16.18, $SD = .58$, $t(13) = 2.18$, $p < .05$). In particular, higher OT levels were associated with needing feeding to be soothed (M for babies who needed to be fed = 22.69, $SD = 7.45$ vs. M for babies who did not need to be fed = 12.63, $SD = 3.45$, $t(13) = 2.32$, $p < .05$).

2.3. Newborn CSF OT concentrations positively correlate with crying for attention at 3 months of age

Eleven parent phone interviews using the IBQ were completed (3 female and 8 male infants). There was no significant difference in neonatal CSF OT levels in the retained subjects vs. those lost to follow-up ($t(13) = .32$). We created a separate Crying for Contact Index consisting of the mean average of two items assessing infants' need for soothing: "After sleeping, how often did the baby cry if someone doesn't come within a few minutes?" and "When you were busy with another activity, and your baby was not able to get your attention, how often did s/he cry?" Higher neonatal OT levels were significantly associated with greater crying when parents were not immediately responsive (Fig. 1A; $r = .65$, $p < .05$). Notably, IBQ measures of negative affect were not associated with higher neonatal OT levels.

2.4. Newborn CSF OT concentrations positively correlate with crying for attention and social behaviors at 6 months of age

Seven families returned to the hospital clinic for 6 month follow-up (3 female and 4 male infants). Again, despite declining subject retention, there was no significant difference in neonatal CSF OT levels in the retained subjects at 6 months vs. those lost to follow up ($t(13) = .88$). The consistency between the 3-month Crying for Contact Index scores and the 6-month scores was highly significant ($r = .97$, $p < .01$). As at 3 months, higher levels of OT were associated with a greater Contact for Crying at 6 months (Fig. 1A; $r = .72$, $p < .10$), while negative affect was not related. On the Vineland instrument, CSF OT levels were positively related to the Relating to Others subscale (Fig. 1B; $r = .84$, $p < .05$), which includes items about watching other people, smiling, showing affection, and taking interest in other children.

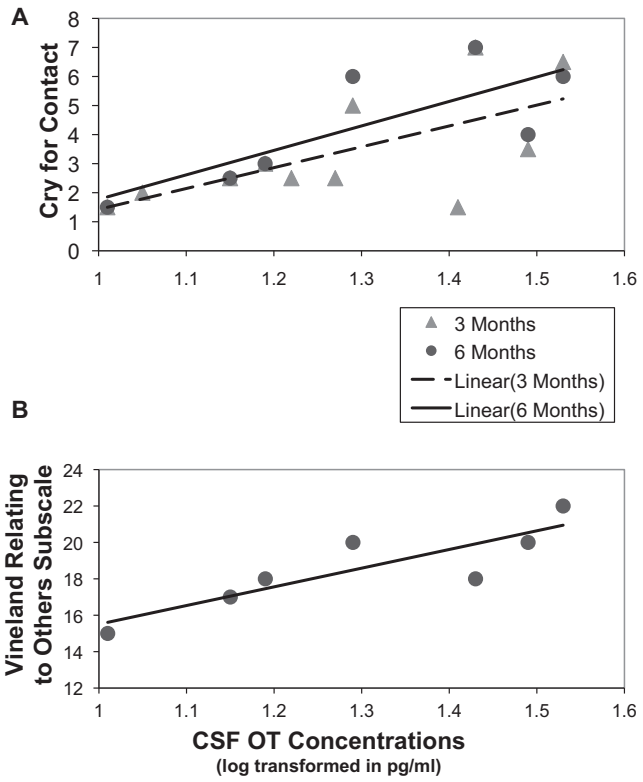

Figure 1 Neonatal CSF OT levels positively correlate with parental reports of infant behavior. (A) Neonatal CSF OT levels correlate with Infant Behavioral Questionnaire scores for "Crying for Contact" at 3 and 6 months (B) Neonatal CSF OT levels positively correlate with parent report of prosocial behaviors at 6 months of age.

3. Discussion

This pilot study provides the first evidence that CSF OT levels are associated with prosocial behavior at term, 3 months, and 6 months of age. These data suggest that infants with higher CSF OT levels appear to actively seek parental social interaction for soothing, and have a greater interest in social interaction as measured at 6 months of age, in agreement with prior studies indicating that OT biology drives affiliative and attachment behaviors (Insel, 2010; Meyer-Lindenberg et al., 2011).

This study is unique in several ways. The majority of studies examining OT concentrations have been conducted in older children and adults, primarily using plasma measurements, with a focus on a limited high-risk population (Heim et al., 2009; Modahl et al., 1998). Our neonatal study population has both biological and behavioral measurements across the normal range of early social function. Moreover, we know of no

prior systematic attempt to test the relationship between specific CSF biomarkers and later human social functioning.

Although few studies have examined CSF OT levels, those that have are in agreement with our findings. Two monkey studies have shown that CSF OT levels are higher in more social bonnet macaques compared to pigtail macaques, and are positively associated with affiliation in rhesus macaques (Rosenblum et al., 2002; Winslow et al., 2003). Moreover, two studies of adult humans have shown that CSF OT levels are diminished in adult survivors of childhood abuse and are negatively correlated with suicide intentionality (Heim et al., 2009; Jokinen et al., 2012). These collective data suggest that CSF OT levels are a promising means by which to assess social functioning in human and non-human primates.

As with any study, there are potential limitations to this work. Given the pilot nature of this study, we were underpowered to detect small effects of gestational age, birth weight, diurnal variation in CSF OT levels, intrapartum Pitocin, and gender. In adults, there is diurnal variation in CSF OT levels (Amico et al., 1983), but the cyclicity of these levels in human newborns has not been established; therefore, a far larger cohort would be needed to assess any association with time of delivery, time of sample collection or sleep/wake cycles of the newborn. Additionally, genetic polymorphisms in the CD38 gene that have been associated with changes in OT release (Higashida et al., 2012) may be relevant and should be measured in larger studies.

Attrition of participants was significant in our study, likely due to the migrant nature of the Hispanic population in our patient catchment area. The lack of differences in OT measurements between those infants who remained in the study and those lost to follow up suggests a lack of sample bias. The positive association between neonatal CSF OT levels and social interactions measured in the full cohort at term is supported by the stability of these correlations at 3 and 6 months of age, despite attrition and use of different questionnaires. Second, we were unable to concomitantly assess plasma OT levels because the plasma volume required for the OT assay exceeded the amount of blood that can safely be obtained from most newborns for research purposes. Third, parent measures were used as a proxy of infant social functioning. No well-validated tests of newborn sociability are available, making our in-hospital questionnaire results the least reliable despite their proximity to the time of OT measurement. Future studies should include more sophisticated laboratory assessments of social functioning (e.g., social initiation; eye gaze to social cues), and more thoroughly assess the roles of the variables outlined above.

In conclusion, these findings support the hypothesis that CSF OT levels may be a biomarker for later social functioning. At present, the relationships between central and peripheral OT levels are not well understood (Veening and Barendregt, 2010), but further investigations and refinement of techniques to measure OT levels should allow us to test whether peripheral measurements can be used in lieu of CSF measurements in future studies. With continued investigation this line of research could provide a critically needed early screening tool, identifying infants at high risk for developing social impairments on the basis of low early OT levels.

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Conflict of interest statement

None declared for any authors.

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