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ORIGINAL ARTICLE

Cerebrospinal fluid and plasma oxytocin concentrations are positively correlated and negatively predict anxiety in children

DS Carson[1], SW Berquist[1], TH Trujillo[1], JP Garner[1,2], SL Hannah[3], SA Hyde[1], RD Sumiyoshi[1], LP Jackson[1], JK Moss[1], MC Strehlow[4], SH Cheshier[5], S Partap[6], AY Hardan[1] and KJ Parker[1]

The neuropeptide oxytocin (OXT) exerts anxiolytic and prosocial effects in the central nervous system of rodents. A number of recent studies have attempted to translate these findings by investigating the relationships between peripheral (e.g., blood, urinary and salivary) OXT concentrations and behavioral functioning in humans. Although peripheral samples are easy to obtain in humans, whether peripheral OXT measures are functionally related to central OXT activity remains unclear. To investigate a possible relationship, we quantified OXT concentrations in concomitantly collected cerebrospinal fluid (CSF) and blood samples from child and adult patients undergoing clinically indicated lumbar punctures or other CSF-related procedures. Anxiety scores were obtained in a subset of child participants whose parents completed psychometric assessments. Findings from this study indicate that plasma OXT concentrations significantly and positively predict CSF OXT concentrations ($r = 0.56$, $P = 0.0064$, $N = 27$). Moreover, both plasma ($r = -0.92$, $P = 0.0262$, $N = 10$) and CSF ($r = -0.91$, $P = 0.0335$, $N = 10$) OXT concentrations significantly and negatively predicted trait anxiety scores, consistent with the preclinical literature. Importantly, plasma OXT concentrations significantly and positively ($r = 0.96$, $P = 0.0115$, $N = 10$) predicted CSF OXT concentrations in the subset of child participants who provided behavioral data. This study provides the first empirical support for the use of blood measures of OXT as a surrogate for central OXT activity, validated in the context of behavioral functioning. These preliminary findings also suggest that impaired OXT signaling may be a biomarker of anxiety in humans, and a potential target for therapeutic development in individuals with anxiety disorders.

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INTRODUCTION

Animal models have implicated the neuropeptide oxytocin (OXT) as a potent prosocial, anxiolytic and anti-stress molecule in the central nervous system. It is well established that centrally administered OXT facilitates affiliation, social bond formation and social learning and memory in various rodent species.[1,2] OXT has also been shown to have behavioral and physiologic stress-attenuating and anxiolytic effects in preclinical animal models. Central OXT administration attenuates stress-induced anxiety behavior and corticosterone release in rats and OXT gene deletion mice,[3,4] and the pathological high anxiety of selectively bred Wistar rats.[5]

The behavioral and neuroendocrine effects of exogenously administered OXT in animals translate to humans. Intranasal OXT administration has been shown to attenuate anxiety in social contexts, *via* diminished amygdala activation to fearful stimuli.[6] Intranasal OXT administration also increases positive communication and reduces cortisol concentrations during couples' conflict.[7] OXT treatment likewise improves symptoms in several patient populations. For example, intranasal OXT administration increases eye gaze frequency and decreases salivary cortisol concentrations in patients with Fragile-X Syndrome, who often exhibit extreme social anxiety.[8]

A number of recent studies have attempted to extend these pharmacological findings by investigating relationships between peripheral (e.g., blood, urinary, salivary) OXT concentrations and their potential as a biomarker of behavioral functioning in humans.[9–15] Specifically, several groups have provided evidence for an inverse relationship between peripheral OXT concentrations and anxiety. For example, in patients with fibromyalgia, a disorder characterized by widespread musculoskeletal pain accompanied by fatigue, sleep, memory and mood issues, a negative correlation between plasma OXT concentrations and symptom severity of both anxiety and depression is observed.[16] Similarly, research has outlined a significant negative correlation between plasma OXT concentrations and symptoms of anxiety and depression in patients with major depressive disorder.[17] Recent evidence also suggests that plasma OXT concentrations may act as a buffer against psychosocial stressors and enhance positive affect *via* an interaction with specific OXT receptor single nucleotide polymorphisms.[18,19]

Although peripheral samples are far easier to obtain from humans than more invasive central ones [e.g., cerebrospinal fluid (CSF) samples], their functional significance is unknown. This is because OXT is produced in the hypothalamus and released into the brain *via* distributed neural pathways and into peripheral

[1]Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Stanford, CA, USA; [2]Department of Comparative Medicine, Stanford University School of Medicine, Stanford, CA, USA; [3]Division of Pediatric Hematology, Department of Pediatrics, Oncology, SCT and Cancer Biology, Stanford University School of Medicine, Stanford, CA, USA; [4]Division of Emergency Medicine, Emergency Department, Stanford University School of Medicine, Stanford, CA, USA; [5]Department of Neurosurgery, Stanford University School of Medicine, Stanford, CA, USA and [6]Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, CA, USA. Correspondence: Dr DS Carson or KJ Parker, Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, 1201 Welch Road, MSLS P-124, Mail Code 5485, Stanford, CA 94305-5485, USA.
E-mail: dcarson@stanford.edu or kjparker@stanford.edu
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circulation *via* the posterior pituitary. Once released into circulation, OXT does not cross the blood–brain barrier in significant amounts, given that the epithelial tight junctions that form this barrier are generally considered impermeable to large molecules.[20] Thus, blood measures of OXT are likely only meaningful if they are related to measures of brain OXT activity. However, the relationship between blood and CSF OXT concentrations is not well understood. It has been argued that the central and peripheral OXT systems are functionally independent,[21,22] but experimental evidence from rodent studies has shown that OXT can be synchronously released into both central and peripheral compartments.[23,24] It therefore would be extremely valuable to determine whether blood OXT concentrations are a valid surrogate of CSF OXT concentrations in humans, particularly in the context of behavioral measurement.

The present study was designed to address this important gap in scientific knowledge. It did so by capitalizing on an extremely rare opportunity to concomitantly collect blood and CSF samples from pediatric and adult patients undergoing clinically indicated lumbar punctures and other CSF-related procedures. The experimental aims were two-fold: (1) test whether plasma OXT concentrations positively predict CSF OXT concentrations within individuals; and (2) test whether OXT concentrations in both compartments negatively predict anxiety, in keeping with findings from the preclinical literature and several human investigations.

METHODS

Participant recruitment

The Stanford University Institutional Review Board approved this research study. Twenty-seven pediatric and adult patients (11 males, 16 females) undergoing either clinically indicated lumbar punctures or other CSF-related procedures were recruited to this study. Participants were between four and 64 years of age ($M = 16.88$, s.e.m. = 2.4). Detailed participant demographic and medical characteristics are provided in Table 1.

Participants were recruited from Stanford Hospital & Clinics and the Lucile Packard Children's Hospital. Clinical indications for CSF collection included rule-out diagnoses (i.e., clinical assessment to eliminate from consideration the possible presence of a condition or disease including pseudotumor cerebri, meningitis, and subarachnoid hemorrhage), headaches, ventriculoperitoneal shunt taps, craniectomies and blood/tissue diseases such as leukemia that required CSF access in diagnosis or treatment. Patients scheduled for these clinical procedures were identified by health care providers as potential study participants and consented prior to sample collection. CSF aliquots for this study were either provided as an additional amount to the volume acquired for clinical purposes or reserved at the time of clinical procedure in lieu of disposal.

Inclusion criteria for participants consisted of clinically indicated reason for CSF collection and participant willingness to undergo blood collection. Exclusion criteria consisted of pregnancy at the time of biological sample collection.

Behavioral assessment of trait anxiety

The parent version of the Spence Children's Anxiety Scale (hereafter Spence) was administered to parents of a subset ($N = 10$) of child participants aged 6–18 years old ($M = 14.02$, s.d. = 3.57). The Spence assesses the severity of trait anxiety symptoms broadly in line with the dimensions of anxiety disorder categories proposed by the DSM-IV. It measures six domains of anxiety including generalized anxiety, panic/agoraphobia, social phobia, separation anxiety, obsessive-compulsive disorder and physical injury fears. A higher total score on the Spence is associated with higher levels of overall trait anxiety.[25,26]

Sample collection and processing procedures

CSF was obtained for research purposes using standard sterile procedures by clinical staff following administration of either local or general anesthetic. CSF was collected from the lumbar region in the majority ($N = 24$) of patients, while a small number of patients had CSF collected from rostral regions including the left ventricle ($N = 1$) and the cisterna magna ($N = 2$). CSF samples were immediately aliquoted into siliconized polypropylene tubes and flash-frozen on dry ice. Whole blood was collected (at the same time as CSF) into chilled EDTA-treated vacutainer tubes from a central or arterial line and placed on wet ice. Whole blood samples were promptly centrifuged (1600 *g* at 4 °C for 15 min), the plasma fraction transferred and aliquotted into siliconized polypropylene tubes, and flash-frozen on dry ice. All samples were stored at −80 °C until quantification for OXT concentrations.

Sample preparation and oxytocin quantification

CSF and plasma OXT concentrations were quantified using a commercially available enzyme immunoassay kit (Enzo Life Sciences, Farmingdale, NY, USA). This kit has been validated for use in humans and is highly specific and selectively recognizes OXT and not related peptides. Per the technological division of Enzo Life Sciences, the cross-reactivity with vasopressin is 0.6% and the limit of detection at the time these samples were assayed was 11.7 pg/ml. A trained technician blinded to experimental conditions performed sample preparation and OXT quantification following established procedures.[9,12,23,27]

Briefly, CSF samples (800 µl per participant) were thawed in an ice bath and mixed with an equal volume of ice-cold acetone, vortexed and centrifuged (4000 *g* at 1 °C for 15 min). CSF samples were then evaporated at room temperature using compressed nitrogen. Plasma samples (1000 µl/participant) were thawed in an ice bath, acidified with 0.1% trifluoroacetic acid, and centrifuged (17 000 *g* at 4 °C for 15 min). Phenomenex Strata-X columns (Phenomenex, Torrance, CA, USA) were activated with 4 ml of high performance liquid chromatography-grade methanol followed by 4 ml of molecular biology grade water. Sample supernatants were applied and drawn through columns by vacuum following column activation, and eluted by sequentially applying 4 ml of wash buffer (89:10:1 water: acetonitrile:trifluoroacetic acid) and 4 ml of elution buffer (20:80 water: acetonitrile). Plasma samples were then evaporated at room temperature using compressed nitrogen. It is important to note that there has been debate over the best methodology for processing and measuring plasma OXT concentrations. Specifically, this debate has focused on whether to extract or dilute plasma samples prior to quantification. However, recent empirical evidence (as well as publically available information from the technical division of the assay company, Enzo Life Sciences) unequivocally supports the use of the extraction method over the dilution method in order to eliminate interference from large proteins to accurately measure plasma OXT concentrations.[28,29] We therefore extracted plasma samples prior to quantification in the present study.

Each evaporated CSF and plasma sample was reconstituted in 250 µl of assay buffer prior to OXT quantification to provide sufficient sample volume to run each participant's samples in duplicate wells (100 µl per well). Given the sensitivity limitations of the commercial assay this ensured that the plated samples contained high enough quantities of OXT to be read above the limit of detection. The program used to calculate pg/mL concentrations of OXT allows for extrapolation based on the sample concentration factor. That is, the program extrapolates the final concentrations by dividing the results by the fold-difference in original sample volume. This method, which has been validated in our and other laboratories and is used widely in this research field,[12,28,30–32] increases the concentration of OXT in each well, and ensures that each sample falls within the linear portion of the standard curve, above the assay's limit of detection, when it is initially read. All samples were assayed in duplicate with a tunable microplate reader for 96-well format at 405 nm with correction at 570 nm, according to manufacturer's instructions. All standards were run in triplicate and provided intra- and inter-assay coefficients of variation below 10%.

Statistical analyses

Study data were managed using REDCap[33] and analyzed using JMP V.10 (SAS Institute, Cary, NC, USA). CSF and plasma OXT concentrations were log transformed to meet the assumptions of parametric linear models (homogeneity of variance and normality of error). Comparisons of mean OXT concentrations in CSF and plasma was determined using a paired student *t*-test. Relationships between plasma and CSF OXT concentrations, and between OXT concentrations in both compartments and Spence anxiety scores, were assessed using general linear models, which included the following blocking factors: age, sex, ethnicity, sample collection time and type of anesthetic treatment. These blocking factors were selected by the research team as the most likely to contribute extraneous sources of variability without leading to significant loss in the number of degrees of

Table 1. Patient demographics and medical characteristics

Patient number	*CSF OXT (pg/mL)*	*Plasma OXT (pg/mL)*	*Sex*	*Age (years)*	*Ethnicity*	*Type of anesthetic*	*Sample collection time*	*Indications for CSF procedure*	*Diagnosis/pathology report*
1	31.86	5.65	Male	17.26	Caucasian	Local	14:27	Worst headache[a]	No abnormality determined
2	23.39	5.08	Female	13.41	Caucasian	Local	18:30	Rule-out meningitis[a]	No abnormality determined
3	27.71	6.10	Female	44.61	Asian	Local	15:43	Rule-out subarachnoid hemorrhage[a]	Subarachnoid hemorrhage
4	17.10	7.17	Female	11.58	Hispanic	General	12:45	VP shunt tap[b]	Hydrocephalus
5	15.25	7.45	Female	18.51	Caucasian	General	20:15	Pituitary abnormality[a]	Pituitary abnormality
6	50.84	5.37	Male	64.38	Caucasian	Local	22:22	Breathing problem/hypotension[a]	Disseminated malignant neoplasm
7	32.12	5.72	Female	15.48	Caucasian	Local	13:32	Rule-out pseudotumor cerebri[a]	No abnormality determined
8	14.24	5.57	Male	11.22	Caucasian	General	09:15	Maintenance chemotherapy[a]	ALL in remission
9	15.79	6.28	Female	6.04	Asian	General	09:10	Maintenance chemotherapy[a]	ALL in remission
10	25.18	5.07	Male	21.26	Asian	General	12:10	Maintenance chemotherapy[a]	ALL in remission
11	21.13	5.82	Female	14.16	Hispanic	General	09:50	Maintenance chemotherapy[a]	ALL in remission
12	26.48	9.95	Female	15.72	Caucasian	General	03:50	Maintenance chemotherapy[a]	ALL in remission
13	18.27	5.20	Female	5.86	Caucasian	General	10:45	Maintenance chemotherapy[a]	AML in remission
14	16.95	4.77	Female	18.70	Caucasian	General	01:47	Maintenance chemotherapy[a]	ALL in remission
15	15.55	5.13	Female	14.82	Caucasian	General	09:23	Maintenance chemotherapy[a]	ALL in remission
16	18.10	6.04	Female	8.65	Black	General	08:16	Maintenance chemotherapy[a]	ALL in remission
17	27.30	13.14	Male	5.44	Caucasian	General	11:35	Chiari craniotomy[c]	Chiari malformation
18	25.00	4.62	Male	24.09	Asian	General	14:05	Maintenance chemotherapy[a]	ALL in remission
19	15.91	4.55	Female	5.23	Caucasian	General	10:52	Maintenance chemotherapy[a]	ALL in remission
20	17.08	7.86	Female	14.99	Black	General	08:23	Maintenance chemotherapy[a]	ALL in remission
21	28.33	6.93	Male	10.41	Black	General	08:56	Maintenance chemotherapy[a]	ALL in remission
22	28.32	10.28	Male	15.37	Asian	General	09:34	Maintenance chemotherapy[a]	ALL in remission
23	16.92	5.74	Male	18.24	Hispanic	General	10:30	Maintenance chemotherapy[a]	ALL in remission
24	20.15	5.16	Female	4.01	Asian	General	09:30	Maintenance chemotherapy[a]	ALL in remission
25	24.17	6.55	Female	15.81	Asian	General	09:18	Induction chemotherapy[a]	ALL
26	45.07	12.92	Male	14.98	Caucasian	General	13:47	Chiari craniotomy[c]	Chiari malformation
27	28.55	6.41	Male	25.40	Caucasian	Local	21:44	Unexplained change in mental state[a]	No abnormality determined

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloblastic leukemia; CSF, cerebrospinal fluid; OXT, oxytocin; VP, ventriculoperitoneal. [a]Indicates CSF collected from lumbar puncture. [b]Indicates CSF collected from left ventricle. [c]Indicates CSF collected from the cisterna magna.

freedom in each analysis. To correct for the blocking factors in the analysis, the regression line is partialed (controlled) for other variables in the analysis, and calculated at the mean value of those variables.[34]

RESULTS

Mean OXT concentrations were 23.95 pg/mL (s.d. = 8.87, $N = 27$) in CSF and 6.73 pg/ml (s.d. = 2.36, $N = 27$) in plasma. CSF OXT concentrations were significantly higher than plasma OXT concentrations ($T_{25} = 18.34$, $P < 0.0001$, $N = 27$). Plasma OXT concentrations significantly and positively predicted CSF OXT concentrations ($F_{1,20} = 9.30$, $r = 0.56$, $P = 0.0064$; Figure 1) in all study participants. This significant and positive relationship was maintained ($F_{1,15} = 15.68$, $r = 0.70$, $P = 0.0013$, $N = 22$) when only child ($\leqslant$ 18 years) participants were included in the analysis. Both plasma ($F_{1,3} = 16.81$, $r = -0.92$, $P = 0.0262$, $N = 10$; Figure 2a) and CSF ($F_{1,3} = 13.91$, $r = -0.91$, $P = 0.0335$, $N = 10$; Figure 2b) OXT concentrations significantly and negatively predicted Spence Total scores in child participants for whom we had behavioral data. Importantly, the previously observed relationship between plasma OXT concentrations and CSF OXT concentrations remained highly significant ($F_{1,3} = 30.91$, $r = 0.96$, $P = 0.0115$, $N = 10$; Figure 2c) in this subset of behaviorally phenotyped participants.

DISCUSSION

There is a large body of literature reporting relationships between peripheral OXT concentrations and a broad range of complex psychological processes and clinical symptoms in healthy and psychiatric populations, respectively.[9–15] These studies typically have drawn strong conclusions about the importance of OXT signaling in the brain-based processes that were under investigation, despite the fact that the functional significance of peripheral OXT measurements was unknown. The present study bridged this important gap in knowledge by providing empirical evidence that plasma OXT concentrations significantly and positively ($r = 0.56$) predict CSF OXT concentrations in humans. This critical discovery supports the assertion that plasma OXT concentrations can be used as a valid proxy for CSF OXT concentrations in subsequent human research studies.

The current study also demonstrated that both plasma ($r = -0.92$) and CSF ($r = -0.91$) OXT concentrations negatively predict trait anxiety in child participants who completed psychometric assessments. Importantly, plasma OXT concentrations significantly and positively ($r = 0.96$) predict CSF OXT concentrations in this subset of child participants. These data are in line with the preclinical literature as well as several prior studies of human patients which showed that plasma OXT concentrations are negatively correlated with trait anxiety in depressed individuals,[16,17] and that adult survivors of childhood abuse have lower CSF OXT concentrations and higher anxiety scores than non-abused individuals.[35] These collective findings suggest the intriguing notion that plasma OXT concentrations may have clinical utility as a biomarker of anxiety.

The identification of novel blood-based biomarkers of anxiety symptoms is urgently needed to inform the streamlined development of more effective and personalized anxiolytic treatments.[36] Intranasal OXT administration is already being tested as a treatment for anxiety disorders, but previous studies have reported robust treatment responders and non-responders.[37–40] Results from the present study suggest that *a priori* stratification of participants in OXT treatment trials on the basis of known plasma OXT signaling deficits may enhance: (1) assessment of the therapeutic potential of OXT to ameliorate anxiety symptoms, and (2) prediction of patients most likely to benefit from OXT treatment. Based on our findings, we hypothesize that patients with lower plasma OXT concentrations and higher levels of anxiety will benefit most from OXT pharmacotherapy.

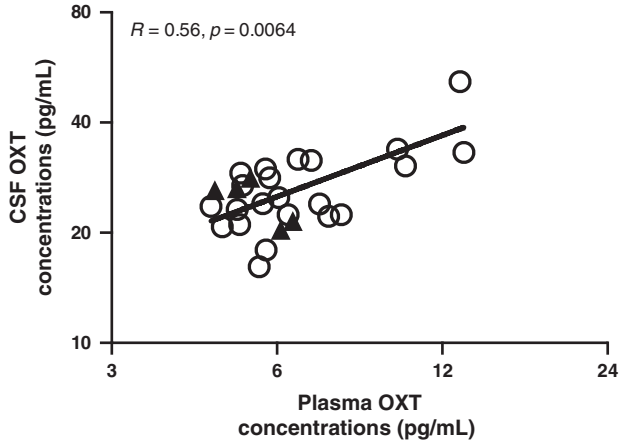

Figure 1. Plasma oxytocin concentrations significantly and positively predict cerebrospinal fluid (CSF) oxytocin (OXT) concentrations in adult and child participants ($N = 27$). Data have been corrected for the following blocking factors: age, sex, ethnicity, sample collection time and type of anesthetic treatment. Open circles denote child participants ($N = 22$); closed black triangles denote adult participants ($N = 5$).

The mechanisms by which OXT acts as an anxiolytic molecule have been investigated in both preclinical animal models (rodent and nonhuman primate) and early human clinical treatment trials. Research in rat species indicates that in response to anxiogenic stimuli, OXT exerts potent anxiolytic effects and modulates neuronal activity related to physiological stress, at the levels of the hypothalamic paraventricular nucleus and amygdala.[41–43] Behavioral stress decreases plasma OXT concentrations in rhesus monkeys and administration of dexamethasone (a synthetic steroid analog of cortisol) leads to a significant increase in plasma OXT concentrations in these animals.[44] In addition, intranasal OXT administration attenuates the adrenocorticotropic hormone stress response in socially isolated squirrel monkeys.[45] Overall, this body of research shows that OXT potently reduces anxiety-like behavior and that the hypothalamic-pituitary-adrenal axis plays a key regulatory role in this process. These findings translate to early clinical trials with a growing number of studies showing intranasal OXT administration decreases anxiety, and decreases basal and stress-induced cortisol concentrations in both healthy participants and patients with clinical anxiety symptoms.[7,8,46–48]

It is important to note that the findings reported here are preliminary and that there are several limitations that require acknowledgement. First, this study was a sample of convenience because of the challenges and ethical considerations involved in obtaining CSF samples from healthy individuals, including children. Many of these patients had serious medical conditions, and although there is some evidence indicating that these conditions do not alter CSF or blood OXT concentrations,[49] it is nevertheless possible that our findings were influenced by the various medications and physical conditions of our study population. Certain medications and disease states are known to lead to alterations in the anatomy of the blood–brain barrier such that certain patient groups may be more likely to show evidence of a relationship between peripheral and central neuropeptide concentrations.[31,50] Further, in a small number of patients in our study CSF was collected from rostral regions including the left ventricle ($N = 1$) and cisterna magna ($N = 2$), while the remainder ($N = 24$) underwent lumbar puncture. There is some evidence from preclinical research that OXT concentrations are highest in the lumbar region, compared to thoracic or cervical spinal regions, in rhesus macaques.[51] Owing to the small sample size and the mixed

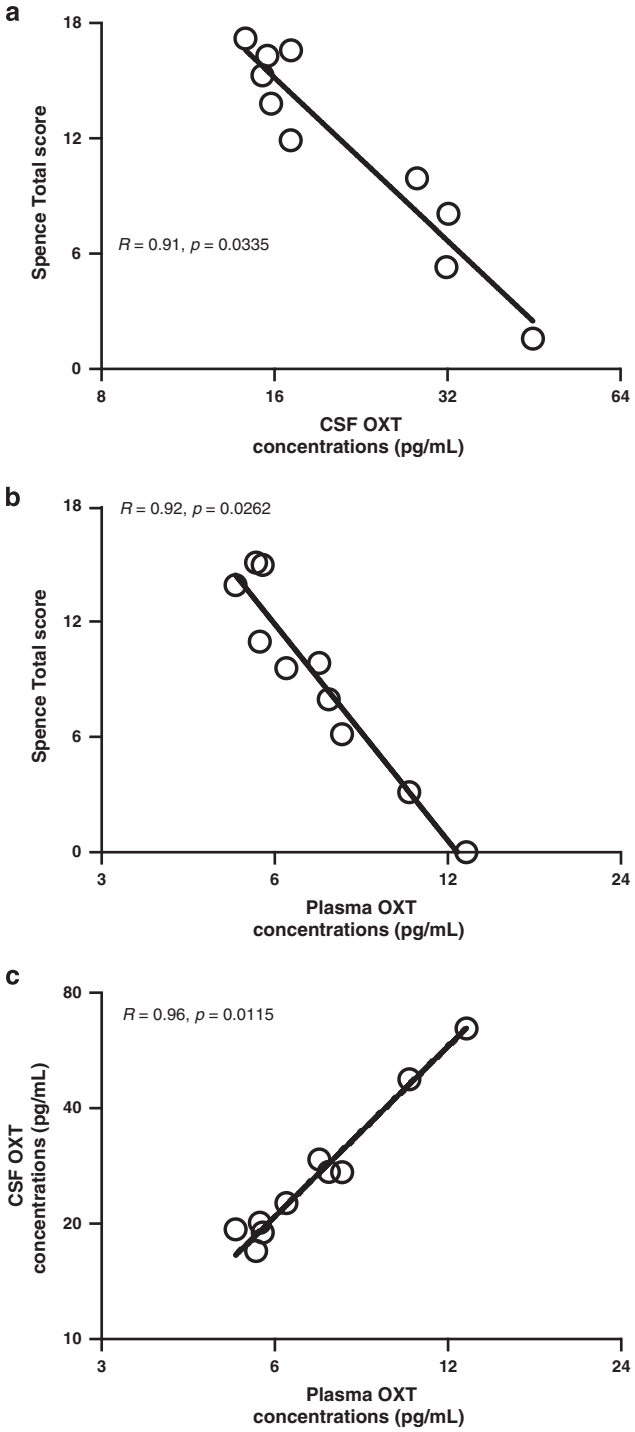

Figure 2. Both plasma (**a**) and cerebrospinal fluid (CSF) (**b**) oxytocin (OXT) concentrations significantly and negatively predict trait anxiety scores in a subset ($N = 10$) of child participants for whom we had behavioral data. The positive relationship between plasma oxytocin concentrations and cerebrospinal fluid oxytocin concentrations remains in this subset of participants (**c**). Data have been corrected for the following blocking factors: age, sex, ethnicity, sample collection time and type of anesthetic treatment.

diagnostic outcomes of the patients in our study it is difficult to perform formal analyses to test how these factors might influence OXT concentrations. However, visualization of the current data set provides no evidence for an impact of the specific medical

conditions or CSF collection sites on OXT concentrations in either compartment (see Table 1).

Although the present study and one other (with adult headache sufferers) found a strong positive relationship between plasma and CSF OXT concentrations, several other studies of adult surgical patients, adult patients with aneurysmal subarachnoid hemorrhage, pregnant women, and adult suicide attempters have found no such relationship.[22,52–55] The majority (81%) of the participants in our study were children ($\leqslant$ 18 years) and the fact that the relationship between OXT concentrations in CSF and plasma was strongest when only children were included in the analysis ($r = 0.70$) suggests that these findings might be more representative of pediatric populations. Due to the small number of adult patients in our study the available data do not help differentiate whether the observed findings are due to developmental differences, medical status, or both. Although extremely difficult to perform, future studies would benefit from determining the relationship between OXT concentrations in medically healthy participants across a range of developmental time-points as well as under a variety of physiological and psychological conditions. An additional limitation of this study is that we obtained behavioral data from only a small number of child participants. Future studies should aim to recruit a larger sample and to conduct a detailed examination of both trait and state anxiety levels in child as well as adult participants.

In conclusion, this study provides the first experimental evidence that plasma OXT concentrations are a valid surrogate for CSF OXT concentrations in a sample inclusive of pediatric and adult participants. This research also showed that both plasma and CSF OXT concentrations significantly and negatively predict trait anxiety in children. These findings provide an exceptional platform for developing personalized diagnostic and outcome measures to improve the efficacy of psychiatric treatments. Replication in patients with clinical diagnoses of certain anxiety disorders (e.g., posttraumatic stress disorder, social anxiety disorder, obsessive-compulsive disorder, panic disorder and generalized anxiety disorder) is now warranted to test whether the present findings generalize to a psychiatric population, and to test the specificity of OXT as a biomarker of anxiety in each of these closely related, but distinctly different, psychiatric disorders.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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