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Short Communication

Reliability of basal plasma vasopressin concentrations in healthy male adults

Quintana DS, Westlye LT, Smerud KT, Mahmoud RA, Djupesland PG, Andreassen OA. Reliability of basal plasma vasopressin concentrations in healthy male adults.

Objective: The neuropeptides oxytocin (OT) and arginine vasopressin (AVP) play important and interrelated roles in modulating mammalian social behaviour. While the OT system has received considerable research attention for its potential to treat psychiatric symptoms, comparatively little is known about the role of the AVP system in human social behaviour. To better understand the intraindividual stability of basal AVP, the present study assessed the reproducibility of basal plasma AVP concentrations.
Methods: Basal plasma AVP was assessed at four sampling points separated by 8 days, on average, in 16 healthy adult males.
Results: Only one out of six comparisons revealed strong evidence for reproducibility of basal AVP concentrations (visit 2 vs. visit 4: $r = 0.8$, $p < 0.001$; all other comparisons $p > 0.1$). The concordance correlation coefficient [0.15, 95% CI (–0.55, 0.73)] also revealed poor overall reproducibility.
Conclusion: Poor reliability of basal AVP concentrations suggests future work covarying AVP with trait markers should proceed with careful consideration of intraindividual fluctuations.

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Significant outcomes

- Basal arginine vasopressin (AVP) concentrations may have poor week-to-week reproducibility.
- Other variables, not necessarily related to behaviour, may influence basal AVP concentrations.

Limitations

- The relatively small sample only included healthy male adults, which limits the generalisability to other populations.

Introduction

The neuropeptides oxytocin (OT) and arginine vasopressin (AVP) play important and interrelated roles in modulating mammalian behaviour and cognition (1). As OT and AVP likely evolved from the same ancestral peptide (arginine vasotocin), these neuropeptides are structurally similar as they differ by only two

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amino acids (2). While the OT system has received considerable research attention for its potential to treat psychiatric symptoms, comparatively little is known about the role of the AVP system in human social behaviour, though its vasoactive and water balance effects have been extensively studied. Intriguingly, animal studies have shown more specific social-behavioural effects with AVP compared with OT administration (3), particularly in males.

Intranasal administration may offer a direct pathway to the central nervous system for large molecules, such as AVP, that have difficulty crossing the blood–brain barrier after peripheral administration (4,5). Despite the promise of pre-clinical studies, studies examining the impact of intranasal AVP administration on human social behaviour and cognition have produced mixed outcomes. For instance, intranasal administration of AVP has been shown to improve social cognition (6) whereas subsequent work has demonstrated opposite results (7). There have also been varied results from case–control studies comparing AVP levels in plasma and cerebrospinal fluid (CSF) between clinical and healthy populations. For example, AVP concentrations have been reported to be increased in autism spectrum disorders (ASD) (8), whereas others have reported no significant differences between an ASD and control group (9). Conversely, others have reported lower AVP in schizophrenia and first-degree relatives of patients with schizophrenia (10,11).

Another line of work has examined the relationship between peripheral AVP and social behaviour in an effort to identify biomarkers of social dysfunction. Research has reported a positive association between AVP and relationship functioning in heterosexual couples (12), along with a relationship between AVP and ASD symptoms in children (13,14). These associations may also be sexually dimorphic, as research has additionally demonstrated a positive correlation between severity of psychosis and repetitive behaviour symptoms in female (but not male) first-episode schizophrenia (15) and ASD patients (9), respectively. This research approach assumes (either explicitly or implicitly) that AVP concentrations are stable on an intraindividual level.

To the best of our knowledge, only two prior investigations have examined the stability of peripheral AVP concentrations in humans. Stachfeld et al. (16) assessed basal plasma AVP levels in nine women during the early follicular and midluteal phases over the course of two menstrual cycles, reporting poor reproducibility within each phase. Conversely, other work examining the stability of plasma AVP measured twice, 6 months apart, in males and females revealed highly correlated measures ($r = 0.8$, $n = 45$) (17). While calculating correlations to determine reliability can be an informative measure of the precision of a linear relationship, there are cases where correlation coefficients can fail to detect non-reproducibility as it is not a sensitive measure of accuracy (18). To illustrate, plotting one measure against another perfectly reproduced measure would reveal that all measurements would fall on a 45° line through the origin (LTO). However, it is possible to have a highly correlated measure that is not reproducible as the correlation coefficient cannot measure a departure from the 45° LTO. For example, the line of best fit location can be shifted above or below the 45° LTO and still generate the same correlation coefficient (see Fig. S1, Supplementary material). To address this weakness of the correlation coefficient, Lin (18) developed the concordance correlation coefficient (CCC), which calculates both precision (how far data deviate from the line of best fit) and accuracy (how far the line of best fit deviates from the 45° LTO). In a repeated-measures context, low precision from repeated measures may occur due to increasing familiarity with the laboratory environment.

We have recently reported the results of a double-blind randomised crossover trial of intranasal OT in healthy volunteers using a novel Breath Powered device (19,20). Within this trial, basal AVP measures were taken at the beginning of four separate laboratory visits before treatment administration, providing the opportunity to assess the reliability of AVP measurements from multiple daytime samples from a series of individuals during the course of ~1 month. Therefore, the goal of this analysis was to assess the intraindividual stability of plasma AVP concentration in healthy individuals using repeated sampling across four different days.

Materials and methods

Participants

Participants were recruited through advertisements at the University of Oslo, and were eligible to participate if male, aged 18–35 (inclusive), and in good physical and mental health. Exclusion criteria included use of any medications within the last 14 days, history of physical or psychiatric disease, and IQ < 75. A screening visit occurred between 3 and 21 days before the first blood sample for AVP analysis at Oslo University Hospital. The Wechsler Abbreviated Scale of Intelligence (21) and the Mini-International Neuropsychiatric Interview (22) were administered to index IQ and confirm the absence of psychiatric illness, respectively. A physical examination was performed by study physicians and nurses, which included 12-lead electrocardiogram and the collection of routine blood

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samples, to confirm the absence of physical illness. This trial was approved by the Regional Committee for Medical and Health Research Ethics (REC South East) and participants provided written informed consent before they participated (19). The study is registered at http://clinicaltrials.gov (NCT01983514).

Study design

Plasma sampling occurred on 4 different days. The average time between consecutive laboratory visits was 8 days (range: 6–20 days, SD = 3.5 days). The average time of day difference between blood collections between the four sampling points was 148 min (range: 22–304 min, SD = 105), with four participant visits during the morning, four during the midday, and eight during the afternoon. Blood samples were collected via intravenous catheter to assess basal levels of peripheral AVP during four laboratory visits on separate days. Blood samples were centrifuged at 4°C within 5 min of blood draw, after which plasma was frozen at −80°C until competitive radioimmunoassay using commercially available kits (BÜHLMANN Laboratories AG, Schönenbuch, Switzerland). The Oslo University Hospital hormone laboratory performed the analyses, following manufacturer specifications (including sample extraction). The intra-assay coefficient of variability (CV) was 7.6% and the inter-assay CV was 11.7%. The sample limits for this assay were 0.63–80 pg/ml. Other routine blood parameters were also tested at each visit, including hematocrit and serum sodium as measures of blood volume and electrolyte levels, respectively. Participants completed the State-Trait Anxiety Inventory (STAI) (23) before blood sampling during each visit to index levels of state anxiety. Shortly after blood sampling, one of four treatments was administered in double-blind fashion using one of four randomised sequences (treatments were 8 international units (IU) intranasal OT, 24 IU intranasal OT, 1 IU intravenous OT, or placebo) (19).

Statistical analysis

Statistical analysis was conducted using IBM SPSS Statistics version 22 (IBM, Armonk, NY, USA) to examine the main effect of time on AVP concentrations and JASP (24) to calculate correlations. While it was not expected that a single dose of OT treatment would influence measures of basal AVP *before* treatment during subsequent visits, the main effect of treatment sequence was also assessed to ensure there were no carryover effects of treatment. As per previous work (19), a linear mixed-model (LMM) approach was adopted for the analysis of AVP, with all models fitted using an unstructured matrix. Experimental treatment was both a fixed and repeated effect in the LMM testing the impact of time and treatment sequence on AVP level. Five AVP concentration assessments from the first visit were not completed due to technical issues related to blood sample collection (e.g. insufficient blood volume, assay failure). One sample above the expected physiological range (20 pg/ml; (25)) was removed from the analysis. A repeated-measures analysis of variance was performed to assess changes in state anxiety between visits, as measured by the STAI (23). The relationship between basal AVP concentrations at the four sampling points, along with AVP concentrations and dehydration markers of hematocrit (red blood cell volume) and serum sodium (electrolyte levels), were calculated using Pearson's correlation coefficient. As null hypothesis significance testing cannot provide evidence for the null hypothesis, Bayes factors (B) were also calculated. A B value <0.33 provides substantial evidence for the null hypothesis, whereas a B value >3 provides substantial evidence for the alternative hypothesis. Values between 0.33 and 3 only provide anecdotal evidence. The CCC was also calculated for comparisons of basal AVP concentrations (18) using the 'agRee' R package (http://CRAN.R-project.org/package=agRee). A CCC of 0 represents perfect disagreement whereas 1 represents perfect agreement, with a CCC < 0.9 considered to represent poor strength of agreement.

Results

A total of 57 male volunteers were assessed for study eligibility, with 18 participants aged 20–30 years (M = 23.81, SD = 3.33) included (19). Two participants withdrew after study enrolment, thus basal AVP concentration data from 16 participants were included in the analysis (Fig. 1a). Medical histories and routine blood tests taken during each sampling time point confirmed the absence of illnesses known to influence basal AVP levels (e.g. diabetes insipidus) and of serum evidence for dehydration at the time of assessment (i.e. hematocrit, sodium), which can influence AVP concentrations. Calculating the range of within-subject variance revealed an average 8.8% within-subject fluctuation of basal AVP concentrations across all participants. There was no significant relationship between the time of day range (minutes) and the standard deviation of AVP concentrations across samples [r = 0.35, 95% CI (−0.17, 0.72), n = 16, p = 0.18]. There was also no significant main effect of time on state anxiety, $F(3,45) = 2.25$, $p = 0.1$, $\eta^2 = 0.13$.

While there was some evidence of very modest increases in mean AVP levels across visits [visit

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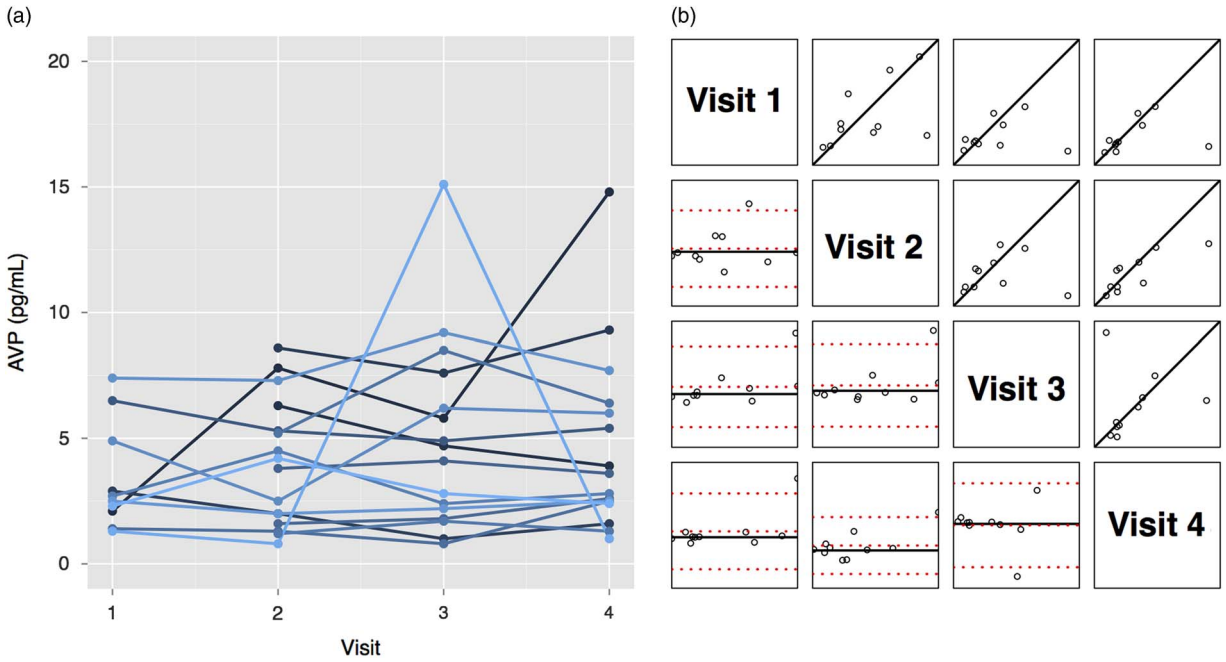

Fig. 1. (a) Arginine vasopressin (AVP) plasma concentrations at each sampling point. (b) A matrix of plots visualising the agreement of AVP plasma concentrations between four sampling points. The upper panel consists of scatterplots with identity line (45° line though the origin). The lower panel consists of Bland–Altman plots with confidence bounds and bias (dotted red line) and the horizontal black line passing through the origin. The confidence bounds show the mean of the difference between two sampling points plus or minus twice of the standard deviation of the difference. The widening spread from left to right of the x-axes suggests poorer replicability with higher AVP basal concentrations.

1 = 3.4 pg/ml (SD = 2.1); visit 2 = 4 pg/ml (SD = 2.5); visit 3 = 4.9 pg/ml (SD = 3.8); visit 4 = 4.4 pg/ml (SD = 3.6)], LMM revealed no significant main effect of visit sequence [$F(3,10.2) = 1.18$, $p = 0.37$] or treatment sequence [$F(3,8.8) = 1.24$, $p = 0.35$] on AVP plasma concentrations. There was also no significant interaction of time and treatment sequence [$F(9,10.2) = 1.62$, $p = 0.22$]. These analyses suggest no carryover effect of treatment condition on AVP levels. There was a significant correlation between age and average AVP concentration [$r = 0.55$, 95% CI (0.08, 0.82), $n = 16$, $p = 0.03$]. Each time point was compared with each other time point within individuals. In these comparisons, a significant correlation was only observed between AVP concentrations at visit 2 and visit 4 ($r = 0.8$, 95% CI (0.51, 0.93), $n = 16$, $p < 0.001$; $B = 172.8$; Fig. 1b). Comparisons of the other five sampling point comparisons revealed no other significant relationships or B values <0.33 or >3 (Table 1; Fig. 1b). There was only one significant relationship out of eight between AVP concentrations and blood markers of dehydration (i.e. hematocrit and sodium) at any time point [AVP and serum sodium at the first visit; $r = -0.69$, 95% CI (–0.91, –0.01), $n = 15$, $p = 0.03$; Table S1, Supplementary material].

The CCC point estimate was 0.15 [$n = 10$, 95% CI (–0.55, 0.73)], which suggests poor reproducibility between sampling points (Fig. 1b). CCC calculation omitting the first sampling point (thus enabling the inclusion of all participants) revealed a similar point estimate of 0.27 [$n = 16$, 95% CI (–0.61, 0.85)]. Creating a measure combining the average of the first two and last two

Table 1. The relationship between basal arginine vasopressin across different sampling points

	Visit 2	Visit 3	Visit 4
Visit 1			
Pearson's *r*	0.517	0.156	0.284
p-value	0.126	0.666	0.427
Upper 95% CI	0.865	0.716	0.775
Lower 95% CI	–0.166	–0.525	–0.421
Bayes factor	1.1	0.42	0.51
Visit 2			
Pearson's *r*	–	0.266	0.801
p-value	–	0.319	<0.001
Upper 95% CI	–	0.673	0.928
Lower 95% CI	–	–0.264	0.505
Bayes factor	–	0.49	172.81
Visit 3			
Pearson's *r*	–	–	0.289
p-value	–	–	0.277
Upper 95% CI	–	–	0.686
Lower 95% CI	–	–	–0.241
Bayes factor	–	–	0.53

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visits generates a large correlation coefficient [$r = 0.62$, 95% CI (0.17, 0.85), $n = 16$, $p = 0.01$] and a Bayes factor ($B = 6$) consistent with a strong relationship. However, the CCC point estimate of 0.52 [$n = 16$, 95% CI (–0.22, 0.88)] is still poor, which is indicative of poor repeated-measure accuracy.

Discussion

Despite showing no significant group differences in AVP over four sampling points, we provide evidence that intraindividual basal AVP measures are weakly related in adults as only one out of six correlations of basal plasma AVP concentrations indicated strong evidence for a relationship according to the calculated Pearson's correlation coefficient and Bayes factor. The CCC point estimate, which indexes both the precision and accuracy of repeated measures, also revealed poor overall reproducibility. Calculating an average of two basal AVP measures appears to only modestly improve the precision of repeated AVP measures. Together, the data indicate that basal AVP concentrations fluctuate from week-to-week – at least in healthy young males. These results have implications for research that covaries basal AVP concentrations with behavioural trait markers, as this approach assumes that AVP levels are stable markers. A failure to find a reliable relationship between repeated AVP measures suggests that a number of other variables, not necessarily related to behaviour, may influence basal AVP concentrations. As dehydration blood marker values fell between typical ranges, and only one out of eight comparisons between AVP concentrations and blood markers were statistically significant, it is unlikely that AVP concentrations were markedly influenced by red blood cell volume and electrolyte level fluctuations.

As these participants were taking part in a larger trial with single-dose OT administration it is plausible that manipulation of the closely related OT system with exogenous OT (administered both intranasally and intravenously) may have influenced basal AVP concentrations. However, the likelihood that these treatments had any effects on the present results is small, as the order of treatment administration was randomised and there was no main effect for treatment sequence order. AVP measures were also collected five times after the basal measures (20, 30, 50, 80, 140 mins after treatment administration), which revealed no significant time × treatment interaction (19). It is not well understood if peripheral neuropeptide measures can be used to index central concentrations however, a meta-analysis addressing this research question is underway (26). Research suggests that there is no simple relationship between CSF and plasma concentrations in adults (27) (but for children, see (13)) so peripheral concentrations cannot be used to approximate central levels of AVP with certainty. Regardless, research is yet to examine if central AVP measurements offer less intraindividual variation than peripheral measures – which is more pressing than investigating peripheral stability considering that central activity more likely influences behaviour.

It is not clear why a relationship between plasma AVP concentrations was observed for samples taken during visits 2 and 4. However, performing multiple tests raises the risk of a type I error, which may have occurred here. There are two potential explanations for the poor sample reproducibility. First, radioimmunoassay may be a suboptimal method to measure neuropeptides compared with alternative methods such as liquid chromatography-mass spectrometry due to molecule mislabelling (28). However, sample extraction, which was included in the present analysis, removes most products that can cross-react with target neuropeptide measures. Second, and more likely, real basal AVP concentrations fluctuate from day-to-day due to fluid homeostasis alterations not captured by the administered blood tests, which could influence reproducibility. As the present sample included unmedicated young males free of readily identifiable physical and psychiatric illnesses it is unlikely these intraindividual differences have a pathological origin. As there was no significant relationship between the time of day range and the standard deviation of AVP concentration within participants this indicates that diurnal fluctuation may have only had a limited influence on intraindividual differences. Other variables that were not collected in the present study – such as food intake, physical activity, and sleep quality – may have also influenced these fluctuations. However, research suggests that poor basal AVP reproducibility is still observed when precisely controlling food and fluid intake, at least in females (16). Inter and intra-assay variability may have also partially contributed to poor reproducibility, however, both CV values were within acceptable ranges, according to the manufacturer. While the present research improves on prior work by assessing basal AVP at four sampling points, this investigation was limited by the unavailability of sleep quality and time of last meal data, along with the relatively small sample size and the fact that the present research was part of a larger study. Moreover, the Bayes factors associated with the non-significant associations indicate that the data may be insensitive, rather than supporting the null hypothesis, which could improve with greater participant numbers in future research.

Peripheral AVP concentrations are often used as a biomarker of trait human behaviours. The poor reliability of basal AVP concentrations observed here suggests

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future work covarying AVP with trait markers should proceed with careful consideration of intraindividual fluctuations. Future work will need to investigate intraindividual stability in females, especially in light of AVP's potentially sex-specific influences on social communication (29), and in psychiatric populations.

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Conflicts of Interest

P.G.D. is an employee of OptiNose AS, Oslo, Norway and owns stock and stock options in OptiNose. R.A.M. is an employee of OptiNose US, Yardley, PA, USA and owns stock and stock options in OptiNose. K.T.S. is employed by Smerud Medical Research International AS, a CRO receiving fees for clinical trial services from OptiNose AS. D.S.Q. is supported by a Research Based Innovation Grant from the Research Council of Norway and OptiNose AS, and an Excellence Grant from the Novo Nordisk Foundation. O.A.A. has received speaker's honoraria from GSK, Lundbeck, and Otsuka for work not directly relevant to the manuscript. P.G.D. and R.A.M. are named inventors on relevant patents owned by OptiNose AS not directly relevant to the manuscript. Sigma-Tau Industrie Farmaceutiche Riunite S.p.A. donated the oxytocin used in the study. L.T.W. declares no conflict of interest.

Ethical Standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

Supplementary material

To view supplementary material for this article, please visit https://doi.org/10.1017/neu.2016.67

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