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Vasopressin needs an audience: Neuropeptide elicited stress responses are contingent upon perceived social evaluative threats

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ABSTRACT

The nonapeptide arginine vasopressin (AVP) plays an important role in hypothalamus–pituitary–adrenal axis regulation and also functions as a social hormone in a wide variety of species, from voles to humans. In the current report we use a variety of stress inducing tasks, including the Trier Social Stress Test (TSST) and intranasal administration of AVP to show that intranasal administration of this neuropeptide leads to a significant increase in salivary cortisol and pulse rate, specifically in conditions where subjects perform tasks in the presence of a social evaluative threat (task performance could be negatively judged by others). In contrast, in conditions without a social evaluative threat (no task condition, modified TSST without audience and bike ergometry), subjects receiving AVP did not differ from subjects receiving placebo. Thus exogenous AVP's influence is contingent upon a circumscribed set of initial conditions that constitute a direct threat to the maintenance of our social selves. Stress evoked by social threat is an integral part of social life and is related to self-esteem and in extreme forms, to poor mental health (e.g., social phobia). Our findings suggest that AVP is a key component in the circuit that interlaces stress and social threat and findings offer inroads to our understanding of individual differences in sociability and in stress response elicited in threatening social situations.

Introduction

Social behavior requires the drive to approach others and the diminution of stress and fear that is naturally elicited by proximity to others. The nonapeptide arginine vasopressin (AVP), which has been related to social behavior and hypothalamus–pituitary–adrenal (HPA) axis regulation, seems to be a prime candidate for modulating stress in social situations (Goodson, 2008).

To begin with, AVP is an important chemical messenger mediating stress since it is secreted by hypothalamic neurons along with corticotrophin releasing hormone (CRH) (Aguilera and Rabadan-Diehl, 2000). Both hormones act synergistically on the pituitary to finally release corticotrophin (adrenocorticotrophic hormone, ACTH) and other peptides. Additionally, AVP is a neuromodulator within the brain and its dual function both peripherally and centrally have deep evolutionary roots (Goodson, 2008). AVP, by modulating regions of the limbic system such as the amygdala, nucleus accumbens and subgenual cingulate (Caldwell et al., 2008; Zink et al., 2010), has an influential role in regulating affiliative and aggressive tendencies (Ferris et al., 1994; Carter et al., 2008; Ferris et al., 2008; Young et al., 2008; Ebstein et al., 2009). Concomitantly with the important role of AVP in stress regulation, chronic over-secretion of this peptide is accompanied by untoward side effects including depression and anxiety (Keck, 2006). Moreover, there is a growing consensus that stress, hostility and social isolation confer vulnerability to some diseases (Miller et al., 2009).

Several studies have shown that neuropeptides bypass the blood–brain barrier after intranasal (IN) administration, providing a useful method for studying central nervous system effects of AVP in humans (Born et al., 2002; Thompson et al., 2006). Evidence suggests that IN-AVP stimulates agonistic facial motor patterns in response to the faces of unfamiliar men in men (Thompson et al., 2006), suggesting that AVP may lead to aggressive behavior in response to threat in men. IN-AVP also substantially increased the electrophysiological response to an event related potential (ERP) (Pietrowsky et al., 1996), showing the effect on central processes in the brain. In a recent fMRI study (Zink et al., 2010) IN-AVP also mediated the activity in limbic regions in the brain during a negative emotional task.

To shed light on the neurobiological substrate of AVP in modulating social behavior in the context of social stress, we employed the Trier Social Stress Test (TSST) (Kirschbaum et al., 1993b), a paradigm that has proven particularly effective in

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evaluating psychosocial stress under controlled laboratory conditions. Gender (Kirschbaum et al., 1995; Uhart et al., 2006), genetics (Kumsta et al., 2007; Shalev et al., 2009), and environmental stressors (Macmillan et al., 2009) among other factors (Kudielka et al., 2009) influence individual's responses during the TSST. In this context, HPA axis reactivity is indexed by measuring salivary cortisol while central nervous system reactivity is measured by monitoring blood pressure and pulse rate. Given AVPs established dual roles in modulating both the HPA axis and social signaling, we hypothesized that IN-AVP prior to the TSST would lead to an interaction with the stress response and heightened cortisol reactivity when compared to placebo. Furthermore, given the long evolutionary history of AVP as a social hormone (Goodson, 2008), we hypothesized that the effects of AVP on the stress response could be specifically attributed to the social evaluative elements of the TSST. We therefore designed a set of studies to investigate the influence of AVP on HPA reactivity under a set of conditions varying in social evaluation and exposure to stress. We began by investigating the effect of IN-AVP on HPA axis reactivity in the full TSST and hypothesized that IN-AVP would enhance salivary cortisol output.

Despite indirect evidence for its contribution to social signaling (Goodson, 2008), little is known regarding the role of AVP in the context of human social stress. For example, it could be the case that IN-AVP, even in the absence of stressful cues, would directly activate the HPA axis, resulting in increased cortisol levels. Furthermore, it may also be the case that stressors in general, even those absent the social evaluative threat produced in the TSST, may interact with AVP to trigger a rise in cortisol. Hence, we tested the hypothesis that the effect of IN-AVP on the salivary cortisol response is contingent upon social contexts. To address this issue, we implemented three additional experimental conditions.

The first experiment was entitled the “no task” group and controlled for direct physiological influences of AVP administration on HPA reactivity under a no stress condition. In this experiment, subjects were simply administered IN-AVP or placebo while sitting by themselves in a controlled environment, absent stressful stimuli. The second experiment was entitled the “no audience,” in which participants engaged in a modified TSST, absent audience and cameras, and consequently absent social evaluative threats yet still retaining enough stressors to trigger a cortisol response. The third experiment employed an exercise bike (“bike ergometry”), also absent audience and cameras, which was designed to evoke physiological stress (cortisol, blood pressure and heart rate) but not a social stress response. The purpose of these three additional experiments was to isolate the social evaluative threat and determine the specificity of AVP effect on this component of the TSST procedure.

If IN-AVP resulted in a direct physiological response then we would expect to observe increases in cortisol levels in the AVP group in all four conditions. Alternatively, if IN-AVP was sensitive to stress responses in general, then we would expect to observe AVP's effects on cortisol reactivity for the full TSST, the “no audience” and the “bike ergometry” conditions. However, if AVP's effects were specific to contexts which contained social evaluative threats, then we would expect to observe an effect of AVP on cortisol response in the full TSST condition only.

Materials and methods

Subjects

Participants were primarily college students at Israeli institutions of tertiary education, recruited by word of mouth and advertisements on campus notice boards for a study on the neurobiological substrates of personality. Selection criteria stipulated that subjects were <35 years old, had no history of psychiatric or endocrine illness (by self-report and standardized questionnaires), were non-smokers, and were not using medication on a regular basis. Individuals with any medical condition were excluded from further study, as were individuals taking any prescription medications that might interact with AVP or with the HPA axis.

Prior to administering AVP or saline control, participants were debriefed about the physiological effects of AVP and informed consent was obtained from each subject. Altogether, 152 male subjects (mean age = 25.11, SD = 2.80) participated in the experiment; 62 participated in the full TSST ($n = 31$ AVP; $n = 31$ placebo), 30 in the “no task” condition ($n = 15$ AVP; $n = 15$ placebo), 30 in the modified TSST “no audience” ($n = 15$ AVP; $n = 15$ placebo), and additional 30 in the “bike ergometry” condition ($n = 15$ AVP; $n = 15$ placebo). The project was approved by the S. Herzog Hospital IRB committee and the Israeli Ministry of Health. All subjects were reimbursed for participating in the experiment. Demographics of the samples are presented in Table 1.

General procedure

Testing was carried out in a laboratory at the Hebrew University Department of Psychology. Subjects were scheduled for testing within a fixed time-window (between 15:00 and 18:00 h) to counter effects of circadian changes in cortisol. To limit variance, subjects were given explicit instructions to refrain from excessive physical activity for 2 h prior to the experiment and from brushing their teeth, eating, and drinking (besides water) for the 90 min prior to the testing session. The full TSST session was carried out as described in previous studies (Kirschbaum et al., 1993b; Shalev et al., 2009). Briefly, the TSST consists of a free speech and a mental arithmetic task of 10 min duration performed in front of a panel of two women with a camera and microphone situated between the interviewers. The modified TSST, “no audience” control protocol, was designed to follow as closely as possible the full TSST, replicating the instructions and time points, however absent the social evaluative component. After IN administration subjects were told that they would play the role of an interviewee for a job and had 5 min to make an argument for their candidacy. Subjects entered the interview room 15 min after IN administration and rather than standing up and speaking in front of a committee for a 5-min interview, were told instead to sit behind a table and write on a piece of paper their suitability for a particular job. The experiment room was absent of committee members, however a timer counting backwards from 5 min was placed on the table and subjects were instructed to sit and write for 4 min. When the timer counted down to 1 min, subjects then had to stand in an upright posture and speak out loud to a virtual committee to create similar motor and physiological response as in the full TSST since an orthostatic response may influence sympathetic nervous system parameters (Januszewicz et al., 1982; Goldstein, 1987; Carnethon et al., 2002).

After 5 min, the second task emphasizing the cognitive load of the TSST commenced. Subjects were instructed to do the second phase for which no details were provided on a computerized program similar to the mental arithmetic task as in the full TSST counting backwards from 1687 in jumps of 13.

The “no audience” task was performed in the same room as the full TSST but all social context elements of the TSST (committee, video camera and microphone) were removed prior to the start of the session. Further to this, to ensure the absence of social evaluative threats, subjects were notified beforehand that they would be alone in the room, and that they would not be observed. In order to ascertain the compliance of the subjects to the task the experimenter (I.S.) watched all subjects behind a one-sided mirror. All subjects complied with the task. After the mental arithmetic task, subjects returned to the waiting room for further sampling, questionnaires and debriefing.

In the “no task” control group subjects were instructed to sit and read National Geographic magazines for 80 min following IN

Table 1
Demographics of the subjects.

	Complete group ($n=152$)	Full TSST ($n=62$)		No audience ($n=30$)		No task ($n=30$)		Bike Ergometry ($n=30$)	
Drug	AVP ($n=61$); Placebo ($n=61$)	AVP ($n=31$)	Placebo ($n=31$)	AVP ($n=15$)	Placebo ($n=15$)	AVP ($n=15$)	Placebo ($n=15$)	AVP ($n=15$)	Placebo ($n=15$)
Age (SD)	25.11 (2.80)	24.53 (2.20)	24.88 (2.93)	24.15 (1.97)	24.15 (1.97)	26.10 (3.60)	26.10 (3.60)	25.57 (4.00)	26.37 (2.69)
BAI (SD)	5.35 (5.28)	5.42 (4.45)	6.48 (6.95)	5.78 (5.02)	5.26 (4.15)	3.93 (5.32)	4.43 (4.03)	4.47 (4.53)	6.47 (6.20)
BDI (SD)	6.42 (5.88)	7.32 (5.82)	6.43 (5.08)	5.93 (4.75)	5.80 (4.24)	6.80 (10.1)	4.43 (4.89)	7.67 (4.98)	6.67 (6.53)
LESS (SD)	177.54 (134.14)	183.03 (114.99)	165.06 (96.51)	223.28 (198.58)	188.47 (138.59)	154.60 (172.75)	139.64 (121.33)	193.60 (140.29)	180.60 (138.85)
Mean AUCi (SD)	1.23 (1.11)	2.38 (0.59)	1.58 (1.10).	0.61 (0.91)	0.92 (0.95)	0.32 (0.67)	0.18 (0.54)	1.31 (0.80)	0.90 (0.98)

*BAI—Beck anxiety inventory (Beck et al., 1988); a 21-question multiple-choice self-report inventory that is used for measuring the severity of an individual's anxiety. The BAI has a maximum score of 63. The standard cut-offs are as follows: 0–7 minimal level of anxiety, 8–15 mild anxiety, 16–25 moderate anxiety and 26–63 severe anxiety (Univariate: BAI × Group: $F=0.585$, $p=0.626$; BAI × Group × Treatment: $F=0.810$, $p=0.490$).
BDI—Beck depression inventory (Beck et al., 1996); a 21-question multiple-choice self-report inventory, one of the most widely used instruments for measuring the severity of depression. The standard cut-offs are as follows: 0–9 indicates minimal depression, 10–18 indicates mild depression, 19–29 indicates moderate depression and 30–63 indicates severe depression (Univariate: BDI × Group: $F=1.000$, $p=0.395$; BDI × Group × Treatment: $F=0.250$, $p=0.861$).
LESS—life-event stress scale (Holmes and Rahe, 1967); a list of forty-two stressful life events. A score of 150 for events occurring within the last year gives a fifty–fifty chance of developing an illness. A score of 300+ gives a 90% chance (Univariate: LESS × Group: $F=0.091$, $p=0.965$; LESS × Group × Treatment: $F=0.443$, $p=0.723$).

administration and to refrain from any other activity. Subjects were left alone in the room and the experimenter only entered the room for cortisol and blood-pressure sampling and for validation of the subject's compliance to the task.

In the "bike ergometry" condition subjects were required to ride a stationary bicycle (ergometry), 15 min after IN administration, at a minimum speed of 30 km/h (~19 mph) for a period of 10 min. Subjects were left alone in the room to avoid any social context during the physical task. A timer counting backwards from 10 min was placed on the table and subjects were instructed to continue until the timer stopped.

Approximately 45 min after the sessions ended (full TSST, "no audience" and "bike ergometry"), and 45 min after IN administration in the "no task" condition, all subjects were administered a set of questionnaires (i.e., life-event stress scale (LESS) (Holmes and Rahe, 1967), Beck anxiety inventory (BAI) (Beck et al., 1988) and Beck depression inventory (BDI)) (Beck et al., 1996) to validate whether environmental and self report measures were different between treatment and between groups (Table 1).

Sampling and biochemical analysis

Salivary cortisol was sampled eight times during the 90 min full TSST and the three control sessions at the following time points: 10 min prior to testing (5 min after IN administration), 1 min prior to testing, immediately after testing (10 min after last sample in the "no task" condition), and 10, 20, 30, 45, and 60 min post-test. Blood pressure and heart rate were measured at seven time points: 20 min prior to testing (5 min before IN administration), 10 and 1 min prior to testing (10 min after last sample in the "no task" condition), immediately after testing, and 10, 20, and 30 min post-test. Salivette plugs (Sarstedt, Germany) were used to collect saliva, and the samples were analyzed using the electrochemiluminescence immunoassay as described previously (Shalev et al., 2009). The lower detection limit of the assay was 0.5 nmol/l. Subjects' blood pressure and heart rate were measured, while seated, using a digital automatic blood pressure wrist monitor (Omron R7).

Drug administration

In a between-subject design, under double-blind conditions, a 250 µl solution containing arginine-vasopressin (20 IU in 0.9% NaCl, Sigma, Germany) or sterile saline (0.9% NaCl) was self-administered in the presence of the experimenter by means of intranasal drops applied with a medicine dropper 15 min before the onset of the full TSST, "no audience" and "bike ergometry" as previously described (Pietrowsky et al., 1996). This dose leads to a small elevations in AVP plasma levels (equivalent to 0.025 U intravenous), which does not produce central effects in the brain (Pietrowsky et al., 1996). This method was verified in previous studies (Perras et al., 1996; Pietrowsky et al., 1996; Perras et al., 1997; Born et al., 1998; Born et al., 2002; Thompson et al., 2004; Thompson et al., 2006) which demonstrated the central action of IN-AVP. For application of the nasal drops, participants, while seated, were asked to maximally tilt their head backwards whereby the liquid was applied with a standard medicine dropper into both nostrils. There were no reports of undesired side effects or increased water retention during the test.

Statistical analysis

All statistical tests to analyze cortisol and hear rate changes during the sessions were carried out using SPSS version 15 (Windows) as previously described by us (Shalev et al., 2009) and others (Lackschewitz et al., 2008; Wingenfeld et al., 2008). Statistical analyses of cortisol data used cortisol values at eight time points as repeated measures, as well as area under the curve with respect to increase (AUCi). This latter measure reflect the degree of changes over time with respect to basal levels of cortisol and is well established as reliable measure for cortisol over time (Pruessner et al., 2003; Federenko et al., 2004; Wust et al., 2005; Buske-Kirschbaum et al., 2007). All values were log-transformed to correct skewed distributions. We note that using raw cortisol values does not alter the findings. The measure of area under the curve was calculated by using the trapezoid formula described by Pruessner et al.(2003).

Statistical analysis of sampled cortisol (log-transformed) was subjected to repeated measures general linear models (GLMs), with time as the repeated measure and two-way interactions between Condition (full TSST, modified TSST "no audience,""no task" and "bike ergometry") and Treatment effect (AVP and placebo). In addition to these analyses, univariate tests were applied to supplementary measures (AUCi for cortisol and AUCi for heart rate (Pruessner et al., 2003)) to ascertain reliability of findings. The AUCi for salivary cortisol and heart rate measurement was computed by applying a *z*-score transformation. Huynh–Feldt corrections were applied if sphericity (equality of variances) was violated and only adjusted results are reported.

Results

The demographics of the full TSST, "no task,""no audience" and "bike ergometry" groups are shown in Table 1. There were no statistical differences between groups and treatment including

environmental and psychological inventories (i.e., life-event stress scale (LESS) (Holmes and Rahe, 1967), Beck anxiety inventory (BAI) (Beck et al., 1988) and Beck depression inventory (BDI) (Beck et al., 1996)) (Table 1).

Full TSST

We examined each treatment group in separate GLM repeated measures tests (Fig. 1A, B, C and D) and by univariate analysis comparing AUCi (Fig. 2). IN-AVP augments the response to social stress solely in the experimental condition of full TSST (tests of within subjects measures: Time $F=47.19$, $p<0.0001$; Time×Treatment $F=4.961$, $p=0.004$ Huynh–Feldt corrected) (Fig. 1A). No AVP related augmentation was seen in the three control conditions (Fig. 1B, C and D). Similarly, when comparing AUCi (Fig. 2), there was a highly significant effect of AVP on AUCi in the full TSST condition (Univariate: $F=12.61$, $p=0.001$) but not in the other control conditions.

No task condition

In the "no task" condition there was a significant decline in salivary cortisol (Time: $F=21.09$, $p<0.0001$). In addition, we did not observe an enhancement by IN-AVP of HPA axis response (Time×Treatment: $F=0.634$, $p=0.593$) (Fig. 1B). Glossing over a pile of magazines, a passive task, appears to be a non-stressful, indeed calming condition (c.f. decline in salivary cortisol levels) which is unaffected by IN-AVP. Again, no effect of AVP is observed (Fig. 2) when AUCi is compared between hormone and placebo ($F=0.414$, $p=0.525$).

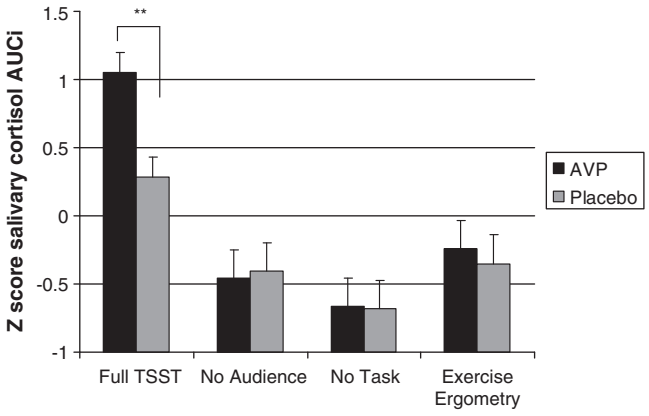

Fig. 2. Area under the curve with respect to increase of salivary cortisol (AUCi, Z-score) (+SEM) grouped by AVP and placebo in all conditions (full TSST, "no audience," "no task" and "bike ergometry"). $^{**}p<0.005$.

No audience condition

In the "no audience" control condition we expected that the task would lead to a modest response due to the physiological and cognitive demands (Dickerson and Kemeny, 2004). Indeed, a significant rise in cortisol was observed (Time: $F=10.13$, $p<0.0001$ Huynh–Feldt corrected) suggesting that the subjects were experiencing some stress due to preparing and delivering a speech and executing an unexpected mental arithmetic computerized task,

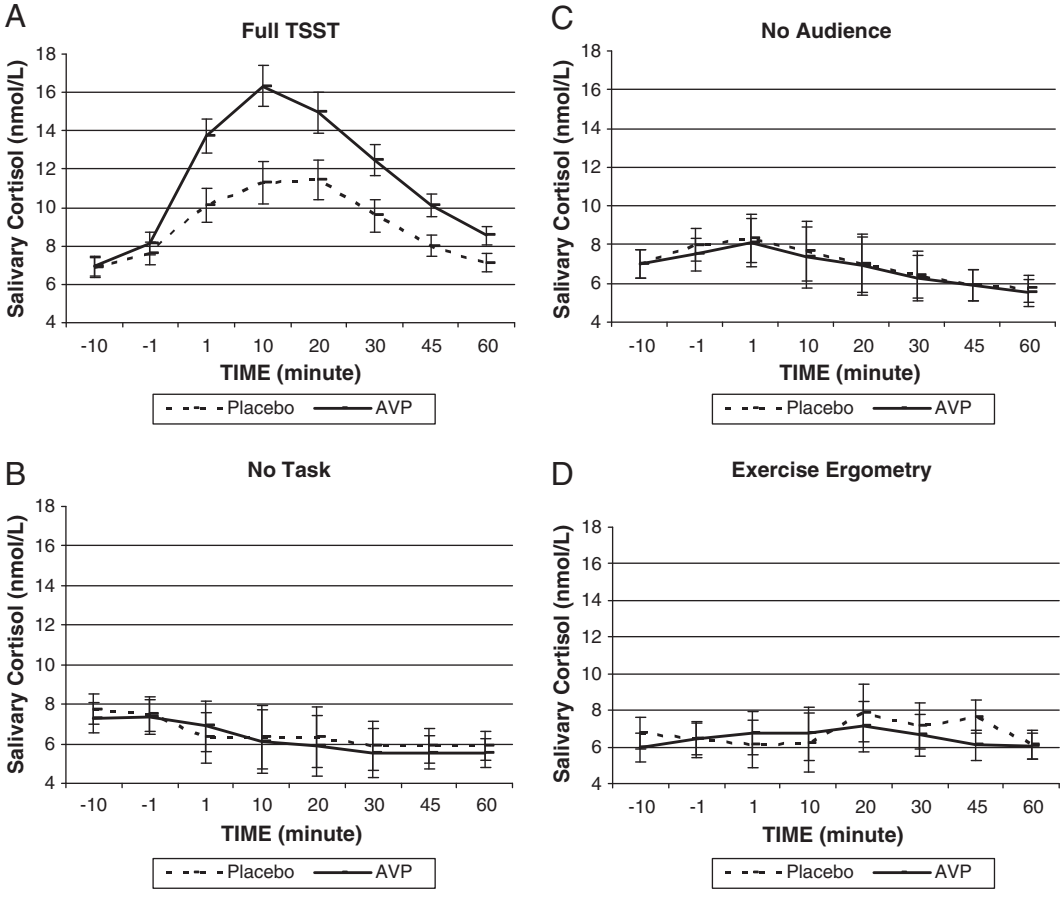

Fig. 1. Modulation of salivary cortisol (±SEM) by AVP and placebo during (A) the full TSST ($n=31$ AVP; $n=31$ placebo), (B) the "no task" condition ($n=15$ AVP; $n=15$ placebo), (C) the modified TSST ("no audience") ($n=15$ AVP; $n=15$ placebo) and (D) the "bike ergometry" ($n=15$ AVP; $n=15$ placebo). GLM repeated measures analysis (Time×Treatment: full TSST, $F=4.961$, $p=0.004$; "no task," $F=0.634$, $p=0.593$; "no audience," $F=0.921$, $p=0.441$; "bike ergometry," $F=0.724$, $p=0.613$).

despite the absence of a social evaluative threat (audience and cameras). As noted above, no enhancement by IN-AVP was observed in this condition (Time×Treatment: $F=0.921$, $p=0.441$) suggesting that the presence of an audience is a necessary condition for the effects of AVP on the cortisol response (Fig. 1C). When AUCi is examined for the "no audience" condition, there is again no effect of AVP ($F=0.843$, $p=0.367$) (Fig. 2).

Bike ergometry condition

Likewise, in the "bike ergometry" condition a significant rise in cortisol was observed (Time: $F=3.70$, $p=0.003$ Huynh–Feldt corrected), reflecting the physical load of the task. Again, no enhancement by IN-AVP was observed in this condition (Time×Treatment: $F=0.724$, $p=0.613$) (Fig. 1D). There was also no significant effect of AVP in the exercise bike condition when comparing AUCi ($F=1.561$, $p=0.222$) (Fig. 2).

Heart rate measure

It has been previously reported that the sympathetic–adrenal–medullary (SAM) system is more sensitive to exercise than the HPA axis (Deuster et al., 1989). Indeed, physical exercise often fails to evoke increases in cortisol response (Yanovski et al., 2000; Cadore et al., 2009) or only a small increase (Kirschbaum et al., 1993a). Moreover, it has been previously shown (Thompson et al., 2006) that AVP also affects autonomic responsiveness to a threatening social stimuli measured via skin conductance. Hence, we further examined the effect of exercise by including the AUCi for heart rate measurements (Fig. 3). AVP significantly increased the AUCi compared to placebo in the full TSST condition ($F=4.692$, $p=0.034$), whereas there was no significant effect of AVP in any of the other three conditions ("no task,"$F=0.465$, $p=0.501$; "no audience,"$F=0.530$, $p=0.473$; "bike ergometry,"$F=0.359$, $p=0.554$) (Fig. 3).

Supplemental analyses

To ascertain the potentially confounding effects of a larger group size in the full TSST, we analyzed a random sample of 30 subjects ($n=15$ AVP; $n=15$ placebo). Again, we observed a significant effect of AVP in the GLM repeated measures test (Time×Treatment: $F=5.644$, $p=0.001$) and when comparing AUCi ($F=9.335$, $p=0.005$ $\eta^2=0.250$), suggesting that the significant difference between AVP and placebo in the full TSST condition is not a function of the increased sample size.

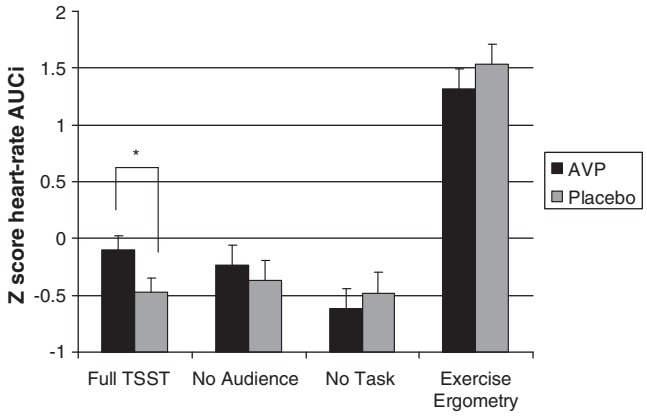

Fig. 3. Area under the curve with respect to increase of heart rate measurement (AUCi, *Z*-score) (+SEM) grouped by AVP and placebo in all conditions (full TSST, "no audience," "no task" and "bike ergometry"). *$p<0.05$.

In a supplemental analysis, we examined whether AVP effects may have been masked by a change in reactivity as a function of high cortisol secretion (Fig. 4A and B). To test this hypothesis we divided the full TSST, "no audience" and "bike ergometry" groups into high and low HPA axis responders based on a median split. We then compared full TSST *low* responders ($n=13$ AVP; $n=17$ placebo) with *high* responders in both the "no audience" and "bike ergometry" conditions ($n=10$ AVP; $n=10$ placebo) for differences in salivary cortisol. Importantly, this latter group of high responders in the "no audience" and "bike ergometry" condition had higher levels of stress response compared to the low responders in the full TSST on mean AUCi ($F=4.00$, $p=0.051$). When examining the effect of AVP between these two sub-groups, we observed a significant effect in the lower responders for the full TSST only (GLM repeated measures: Time $F=10.46$, $p<0.0001$; Time×Treatment $F=3.98$, $p=0.004$ Huynh–Feldt corrected; and AUCi: $F=11.81$, $p=0.002$, $\eta^2=0.297$) (Fig. 4A) and no AVP related augmentation in cortisol for the higher responders in both the "no audience" and "bike ergometry" conditions (Time×Treatment $F=1.21$, $p=0.315$; and AUCi: $F=0.001$, $p=0.980$, $\eta^2<0.001$) (Fig. 4B), suggesting that the presence of an audience is sufficient to evoke an AVP effect in cases where a social element is present despite lower cortisol reactivity. This rules out the hypothesis that the effect of AVP on cortisol reactivity only occur in conditions of higher stress.

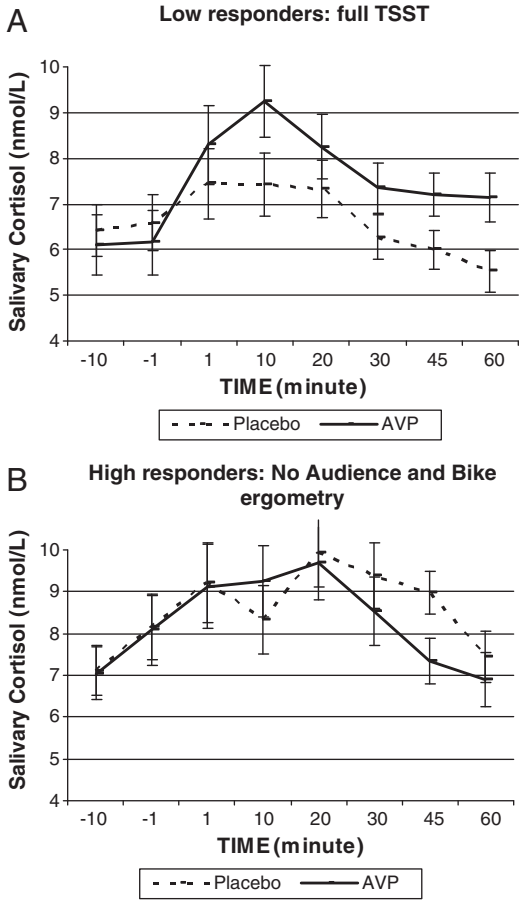

Fig. 4. (A) Significant effect of AVP versus placebo on salivary cortisol (±SEM) in the lower responders cohort of the full TSST ($n=13$ AVP; $n=17$ placebo) (Time×Treatment: $F=3.98$, $p=0.004$). (B) No effect of AVP in the upper responders cohort of the modified TSST ("no audience") and "bike ergometry" conditions ($n=10$ AVP; $n=10$ placebo) (Time×Treatment: $F=1.21$, $p=0.315$).

In summary, the only condition where an effect of AVP is observed is in the presence of an audience.

Discussion

Humans are driven to preserve the social self and are vigilant to threats that may jeopardize their social esteem or status. The current study focused on the relationship between AVP and physiological reactions to social stress by testing for a selective AVP rise in the face of social stress challenge (Dickerson and Kemeny, 2004). We employed the robust TSST paradigm that exposes subjects to the social stress of "performing" before two (still faced) "judges" followed by a mental arithmetic task. Within this context of perceived threats to subjects' self-esteem and social status (Dickerson and Kemeny, 2004), exogenous AVP enhanced salivary cortisol rise in the full experimental condition (presence of human "judges"—the audience) but not in the modified TSST "no audience" or the "bike ergometry" conditions where the task was carried out in the absence of an audience and cameras. Similarly, no effect of exogenous AVP on the "no task" condition was observed when subjects browsed through a pile of National Geographic magazines, monitored but alone in isolation. In addition, AVP augmented the response in a random sample of the full TSST ($n=30$), thus ruling out issues of statistical power contributing to the presence of an effect.

Further to this, IN-AVP evoked a significant increase in sympathetic activity for heart rate measure which also was insensitive in the "no audience" and "bike ergometry" conditions. Finally, we observed a significant effect of AVP in a low responder's cohort of subjects in the full TSST condition, underscoring that even with low HPA axis activation, the presence of an audience is sufficient to evoke AVP effect. Conversely, when comparing the high responder's cohort in the "no audience" and "bike ergometry" conditions, both tasks in the absence of an audience, we also failed to observe an effect of IN-AVP, emphasizing that moderate to high activation is not a necessary condition to evoke AVP effect. Only in the presence of an audience does AVP evoke an increase either in salivary cortisol or heart rate measurement.

Despite the intense interest generated by the role of neuropeptides as social hormones that modulate dyadic and group interactions with conspecifics across vertebrates (Ebstein et al., 2009), the current study is only one of a very few investigations (Pietrowsky et al., 1996; Born et al., 2002; Thompson et al., 2006; Zink et al., 2010) to use IN-AVP towards unraveling the mode of AVP action on the human social brain and particularly its role in social stress. AVP enhanced the impact of the audience on the TSST task and appears to increase the subjects' sensitivity to the social milieu and specifically the presence of observers. As argued by Dickerson and Kemeny(2004), status in humans is conferred through hedonic processes that relate to respect, self-esteem, acceptance and positive social attention.

Thompson et al.(2006) showed that IN-AVP affects social perception by decreasing approachability ratings of unfamiliar men displaying happy expressions. Therefore, men with high levels of endogenous AVP may be more vulnerable to generate threatening or negative gestures in social encounters. The TSST plunges subjects into a theater that contains a social evaluative threat by the presence of the judges in the room. AVP mobilizes resources, within this context, to preserve the social self by increasing cortisol release. In turn, cortisol release mobilizes energy and other physiological resources that are likely crucial towards maintaining self-esteem and status and that may generate, in men, threatening gestures in social encounters.

Since IN-AVP was shown to affect central processes in the brain (Pietrowsky et al., 1996; Born et al., 2002; Thompson et al., 2006; Zink et al., 2010) we suggest the notion that specialized social networks in the brain might be involved in the regulation of AVP in the context of social stress. In several animal studies, AVP modulated the activity in the anterior hypothalamus (Ferris et al., 1994) and also in the periaqueductal gray (PAG) (Albers and Cooper, 1995), a region that contains both CRF neurons and receptors and involved in the acquisition and expression of defensive responses to fear conditioning environments in rats (De Oca et al., 1998; Bowers et al., 2003).

In a recent study by Zink et al.(2010) on healthy human subjects using fMRI and IN-AVP in a negative emotional task, AVP abolished the decrease in subgenual cingulate activity and altered the connectivity between subgenual cingulate and supragenual cingulate, a region that is involved in the cognitive control of emotion (Matthews et al., 2008). The subgenual cingulate is an important region that sends direct or indirect inputs to limbic areas including the hypothalamus, PAG, striatum, nucleus accumbens, thalamus, amygdala and hippocampus (Price, 1999). Studies in healthy human subjects suggested that the subgenual cingulate modulates autonomic and emotional responses and also suggested the involvement in processing of negative self-perception information (Kringelbach, 2005; Vogt, 2005; Johnson et al., 2009). All of these brain regions are therefore potential targets for AVP and are likely involved in the mediation of perception of threats to the self and subsequent activation of the HPA axis.

Since several studies in both animals (de Vries, 2008) and humans (Thompson et al., 2006) suggest that some effects of AVP on social behavior are sexually dimorphic (Bielsky and Young, 2004) only healthy males participated in this study in order to avoid potential confounding effects of gender and female hormonal influences. It is clearly of considerable interest to investigate the effects of IN-AVP on social stress in female subjects as well. Additionally, it should be noted that in our experiment we employed only female judges whereas all subjects were male. Opposite sex "tensions" may have made the TSST procedure somewhat more stressful under these conditions. However, female judges were employed in all the experimental procedures and whatever effect was manifested would have been uniform across protocols.

In summary, AVP enhances HPA axis response solely in the presence of an audience. It is now shown that the social context for AVP is apparently crucial to their role in the management of social skills and performance in humans. Understanding the underlying basis of the salience of social stress vis-a-vis HPA axis response is an important avenue of research given the prevalence of disorders such as autism and social phobia that have at their core, dysfunctional social communications and deficits in social skills (Baron-Cohen, 2009). Moreover, such disorders, e.g., autism, are often accompanied by comorbid mood and anxiety disorders (Hofvander et al., 2009) suggesting a further involvement of AVP. Future research would benefit from the convergence of imaging genomics combined with pharmacological paradigms towards enhancing our understanding of the neuroanatomical pathways that regulate social stress in humans and in clinical populations.

Conflict of interest

The authors declare no conflict of interest.

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References

Aguilera, G., Rabadan-Diehl, C., 2000. Vasopressinergic regulation of the hypothalamic–pituitary–adrenal axis: implications for stress adaptation. Regul. Pept. 96, 23–29.

Albers, H.E., Cooper, T.T., 1995. Effects of testosterone on the behavioral response to arginine vasopressin microinjected into the central gray and septum. Peptides 16, 269–273.
Baron-Cohen, S., 2009. Autism: the empathizing–systemizing (E–S) theory. Ann. N.Y. Acad. Sci. 1156, 68–80.
Beck, A.T., Epstein, N., Brown, G., Steer, R.A., 1988. An inventory for measuring clinical anxiety: psychometric properties. J. Consult. Clin. Psychol. 56, 893–897.
Beck, A.T., Steer, R.A., Ball, R., Ranieri, W., 1996. Comparison of Beck Depression Inventories-IA and -II in psychiatric outpatients. J. Pers. Assess. 67, 588–597.
Bielsky, I.F., Young, L.J., 2004. Oxytocin, vasopressin, and social recognition in mammals. Peptides 25, 1565–1574.
Born, J., Pietrowsky, R., Fehm, H.L., 1998. Neuropsychological effects of vasopressin in healthy humans. Prog. Brain Res. 119, 619–643.
Born, J., Lange, T., Kern, W., McGregor, G.P., Bickel, U., Fehm, H.L., 2002. Sniffing neuropeptides: a transnasal approach to the human brain. Nat. Neurosci. 5, 514–516.
Bowers, L.K., Swisher, C.B., Behbehani, M.M., 2003. Membrane and synaptic effects of corticotropin-releasing factor on periaqueductal gray neurons of the rat. Brain Res. 981, 52–57.
Buske-Kirschbaum, A., Krieger, S., Wilkes, C., Rauh, W., Weiss, S., Hellhammer, D.H., 2007. Hypothalamic–pituitary–adrenal axis function and the cellular immune response in former preterm children. J. Clin. Endocrinol. Metab. 92, 3429–3435.
Cadore, E.L., Lhullier, F.L., Alberton, C.L., Almeida, A.P., Sapata, K.B., Korzenowski, A.L., Kruel, L.F., 2009. Salivary hormonal responses to different water-based exercise protocols in young and elderly men. J. Strength Cond. Res. 23, 2695–2701.
Caldwell, H.K., Lee, H.J., Macbeth, A.H., Young III, W.S., 2008. Vasopressin: behavioral roles of an "original" neuropeptide. Prog. Neurobiol. 84, 1–24.
Carnethon, M.R., Liao, D., Evans, G.W., Cascio, W.E., Chambless, L.E., Heiss, G., 2002. Correlates of the shift in heart rate variability with an active postural change in a healthy population sample: The Atherosclerosis Risk In Communities study. Am. Heart J. 143, 808–813.
Carter, C.S., Grippo, A.J., Pournajafi-Nazarloo, H., Ruscio, M.G., Porges, S.W., 2008. Oxytocin, vasopressin and sociality. Prog. Brain Res. 170, 331–336.
De Oca, B.M., DeCola, J.P., Maren, S., Fanselow, M.S., 1998. Distinct regions of the periaqueductal gray are involved in the acquisition and expression of defensive responses. J. Neurosci. 18, 3426–3432.
de Vries, G.J., 2008. Sex differences in vasopressin and oxytocin innervation of the brain. Prog. Brain Res. 170, 17–27.
Deuster, P.A., Chrousos, G.P., Luger, A., DeBolt, J.E., Bernier, L.L., Trostmann, U.H., Kyle, S. B., Montgomery, L.C., Loriaux, D.L., 1989. Hormonal and metabolic responses of untrained, moderately trained, and highly trained men to three exercise intensities. Metabolism 38, 141–148.
Dickerson, S.S., Kemeny, M.E., 2004. Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. Psychol. Bull. 130, 355–391.
Ebstein, R.P., Israel, S., Lerer, E., Uzefovsky, F., Shalev, I., Gritsenko, I., Riebold, M., Salomon, S., Yirmiya, N., 2009. Arginine vasopressin and oxytocin modulate human social behavior. Ann. N.Y. Acad. Sci. 1167, 87–102.
Federenko, I.S., Nagamine, M., Hellhammer, D.H., Wadhwa, P.D., Wust, S., 2004. The heritability of hypothalamus pituitary adrenal axis responses to psychosocial stress is context dependent. J. Clin. Endocrinol. Metab. 89, 6244–6250.
Ferris, C.F., Delville, Y., Irvin, R.W., Potegal, M., 1994. Septo-hypothalamic organization of a stereotyped behavior controlled by vasopressin in golden hamsters. Physiol. Behav. 55, 755–759.
Ferris, C.F., Stolberg, T., Kulkarni, P., Murugavel, M., Blanchard, R., Blanchard, D.C., Febo, M., Brevard, M., Simon, N.G., 2008. Imaging the neural circuitry and chemical control of aggressive motivation. BMC Neurosci. 9, 111.
Goldstein, D.S., 1987. Stress-induced activation of the sympathetic nervous system. Baillieres Clin. Endocrinol. Metab. 1, 253–278.
Goodson, J.L., 2008. Nonapeptides and the evolutionary patterning of sociality. Prog. Brain Res. 170, 3–15.
Hofvander, B., Delorme, R., Chaste, P., Nyden, A., Wentz, E., Stahlberg, O., Herbrecht, E., Stopin, A., Anckarsater, H., Gillberg, C., Rastam, M., Leboyer, M., 2009. Psychiatric and psychosocial problems in adults with normal-intelligence autism spectrum disorders. BMC Psychiatry 9, 35.
Holmes, T.H., Rahe, R.H., 1967. The Social Readjustment Rating Scale. J. Psychosom. Res. 11, 213–218.
Januszewicz, W., Sznajderman, M., Wocial, B., Chodakowska, J., Feltynowski, T., Zukowska-Grojec, Z., 1982. Sympathetic reactivity to upright posture in borderline and established hypertension. Cor Vasa 24, 429–440.
Johnson, M.K., Nolen-Hoeksema, S., Mitchell, K.J., Levin, Y., 2009. Medial cortex activity, self-reflection and depression. Soc. Cogn. Affect. Neurosci. 4, 313–327.
Keck, M.E., 2006. Corticotropin-releasing factor, vasopressin and receptor systems in depression and anxiety. Amino Acids 31, 241–250.
Kirschbaum, C., Strasburger, C.J., Langkrar, J., 1993a. Attenuated cortisol response to psychological stress but not to CRH or ergometry in young habitual smokers. Pharmacol. Biochem. Behav. 44, 527–531.
Kirschbaum, C., Pirke, K.M., Hellhammer, D.H., 1993b. The 'Trier Social Stress Test'—a tool for investigating psychobiological stress responses in a laboratory setting. Neuropsychobiology 28, 76–81.
Kirschbaum, C., Klauer, T., Filipp, S.H., Hellhammer, D.H., 1995. Sex-specific effects of social support on cortisol and subjective responses to acute psychological stress. Psychosom. Med. 57, 23–31.
Kringelbach, M.L., 2005. The human orbitofrontal cortex: linking reward to hedonic experience. Nat. Rev. Neurosci. 6, 691–702.
Kudielka, B.M., Hellhammer, D.H., Wust, S., 2009. Why do we respond so differently? Reviewing determinants of human salivary cortisol responses to challenge. Psychoneuroendocrinology 34, 2–18.
Kumsta, R., Entringer, S., Koper, J.W., van Rossum, E.F., Hellhammer, D.H., Wust, S., 2007. Sex specific associations between common glucocorticoid receptor gene variants and hypothalamus–pituitary–adrenal axis responses to psychosocial stress. Biol. Psychiatry 62, 863–869.
Lackschewitz, H., Huther, G., Kroner-Herwig, B., 2008. Physiological and psychological stress responses in adults with attention-deficit/hyperactivity disorder (ADHD). Psychoneuroendocrinology 33, 612–624.
Macmillan, H.L., Georgiades, K., Duku, E.K., Shea, A., Steiner, M., Niec, A., Tanaka, M., Gensey, S., Spree, S., Vella, E., Walsh, C.A., Bellis, M.D., Meulen, J.V., Boyle, M.H., Schmidt, L.A., 2009. Cortisol response to stress in female youths exposed to childhood maltreatment: results of the youth mood project. Biol. Psychiatry 66 (1), 62–68.
Matthews, S.C., Strigo, I.A., Simmons, A.N., Yang, T.T., Paulus, M.P., 2008. Decreased functional coupling of the amygdala and supragenual cingulate is related to increased depression in unmedicated individuals with current major depressive disorder. J. Affect. Disord. 111, 13–20.
Miller, G., Chen, E., Cole, S.W., 2009. Health psychology: developing biologically plausible models linking the social world and physical health. Annu. Rev. Psychol. 60, 501–524.
Perras, B., Molle, M., Born, J., Fehm, H.L., 1996. Sleep and signs of attention during 3 months of intranasal vasopressin: a pilot study in two elderly subjects. Peptides 17, 1253–1255.
Perras, B., Droste, C., Born, J., Fehm, H.L., Pietrowsky, R., 1997. Verbal memory after three months of intranasal vasopressin in healthy old humans. Psychoneuroendocrinology 22, 387–396.
Pietrowsky, R., Struben, C., Molle, M., Fehm, H.L., Born, J., 1996. Brain potential changes after intranasal vs. intravenous administration of vasopressin: evidence for a direct nose-brain pathway for peptide effects in humans. Biol. Psychiatry 39, 332–340.
Price, J.L., 1999. Prefrontal cortical networks related to visceral function and mood. Ann. N.Y. Acad. Sci. 877, 383–396.
Pruessner, J.C., Kirschbaum, C., Meinlschmid, G., Hellhammer, D.H., 2003. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology 28, 916–931.
Shalev, I., Lerer, E., Israel, S., Uzefovsky, F., Gritsenko, I., Mankuta, D., Ebstein, R.P., Kaitz, M., 2009. BDNF Val66Met polymorphism is associated with HPA axis reactivity to psychological stress characterized by genotype and gender interactions. Psychoneuroendocrinology 34, 382–388.
Thompson, R., Gupta, S., Miller, K., Mills, S., Orr, S., 2004. The effects of vasopressin on human facial responses related to social communication. Psychoneuroendocrinology 29, 35–48.
Thompson, R.R., George, K., Walton, J.C., Orr, S.P., Benson, J., 2006. Sex-specific influences of vasopressin on human social communication. Proc. Natl. Acad. Sci. U. S. A. 103, 7889–7894.
Uhart, M., Chong, R.Y., Oswald, L., Lin, P.I., Wand, G.S., 2006. Gender differences in hypothalamic–pituitary–adrenal (HPA) axis reactivity. Psychoneuroendocrinology 31, 642–652.
Vogt, B.A., 2005. Pain and emotion interactions in subregions of the cingulate gyrus. Nat. Rev. Neurosci. 6, 533–544.
Wingenfeld, K., Heim, C., Schmidt, I., Wagner, D., Meinlschmidt, G., Hellhammer, D.H., 2008. HPA axis reactivity and lymphocyte glucocorticoid sensitivity in fibromyalgia syndrome and chronic pelvic pain. Psychosom. Med. 70, 65–72.
Wust, S., Federenko, I.S., van Rossum, E.F., Koper, J.W., Hellhammer, D.H., 2005. Habituation of cortisol responses to repeated psychosocial stress-further characterization and impact of genetic factors. Psychoneuroendocrinology 30, 199–211.
Yanovski, J.A., Yanovski, S.Z., Boyle, A.J., Gold, P.W., Sovik, K.N., Sebring, N.G., Drinkard, B., 2000. Hypothalamic-pituitary-adrenal axis activity during exercise in African American and Caucasian women. J. Clin. Endocrinol. Metab. 85, 2660–2663.
Young, K.A., Liu, Y., Wang, Z., 2008. The neurobiology of social attachment: a comparative approach to behavioral, neuroanatomical, and neurochemical studies. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 148, 401–410.
Zink, C.F., Stein, J.L., Kempf, L., Hakimi, S., Meyer-Lindenberg, A., 2010. Vasopressin modulates medial prefrontal cortex–amygdala circuitry during emotion processing in humans. J Neurosci 30, 7017–7022.