Intranasal vasopressin affects pair bonding and peripheral gene expression in male *Callicebus cupreus*

M. R. Jarcho†,*, S. P. Mendoza‡, W. A. Mason†, X. Yang†,§ and K. L. Bales†,‡

†Psychology Department, ‡California National Primate Research Center, and §Department of Public Health Sciences, University of California Davis, Davis, CA 95616, USA. *Corresponding author: M. R. Jarcho, Psychology Department, University of California Davis, Davis, CA 95616, USA. E-mail: mjarcho@ucdavis.edu

Arginine vasopressin (AVP) is a neuropeptide hormone that has peripheral functions in water regulation, and central functions in the stress response and social bonding in male rodents. In this study, we investigated the role of AVP in partner preference behavior in a monogamous primate, the coppery titi monkey (*Callicebus cupreus*). Seven titi males each received three intranasal treatments: saline, low AVP (40 IU) and high AVP (80 IU) in random order, 1 week apart. They experienced a series of stimulus exposures to their female partner, a female stranger and an empty cage. Males were more likely to contact the stimulus and do so faster when either female stimulus was present. When pretreated with saline, males contacted the stranger more frequently than their partner; when pretreated with the high dosage of AVP, males contacted their partner more frequently than the stranger. We used microarray to measure peripheral changes in gene expression associated with intranasal AVP and found reduced expression of several genes coding for proinflammatory cytokines. The data presented here suggest that intranasally administered AVP has both central influences on social behavior and peripheral influences on inflammation in a nonhuman primate.

Keywords: Arginine vasopressin, *Callicebus cupreus*, inflammation, intranasal, partner preference

Received 23 August 2010, revised 04 November 2010 and 17 December 2010, accepted for publication 23 December 2010

Arginine vasopressin (AVP) is a neuropeptide hormone with both central and peripheral functions (Baker et al. 2003; Bielsky & Young 2004; Chassin et al. 2007; Chickanze & Grossman 1998; Englemann et al. 1996; Knepper & Star 2008; Lim et al. 2004; Marler et al. 2005; Shalts et al. 1992). Centrally, AVP plays a role in social behaviors such as initiating pair bonding in male prairie voles (*Microtus ochrogaster*). AVP increases the tendency of male prairie voles to form a selective partner preference following cohabitation (Insel & Hulihan 1995; Insel et al. 1997; Lim & Young 2004; Winslow et al. 1993a). Additionally, AVP has been associated with social memory and social recognition (Bielsky et al. 2005; Danzter et al. 1988; Englemann & Landgraf 1994; Englemann et al. 1996), affiliative behaviors (Young 2002), paternal behavior (Barnshad et al. 1994; Wang et al. 2000) and aggression (Bester-Meredith et al. 1999; Marler et al. 2005).

While the prairie vole has provided an excellent model to study the formation of rodent social bonds, we do not know whether the mechanisms for these bonds differ in primates. Coppery titi monkeys (*Callicebus cupreus*) are socially monogamous New World monkeys that show preferences for a specific partner, distress upon separation from that partner and biparental care of offspring (Mason 1966). As such, they provide an ideal model for investigating the affects of AVP on social behavior and peripheral physiological changes in male primates. In order to assess the behavioral effects of centrally administered exogenous AVP, AVP was administered intranasally in order to bypass the blood brain barrier and ensure central uptake noninvasively. Intranasal administration has been shown to result in efficient absorption by the nasal epithelium reaching maximum uptake in approximately 60 min (Born et al. 2002) and has been used previously in nonhuman primate studies with the closely related peptide oxytocin (OT; Parker et al. 2006; Smith et al. 2010). We predicted that exogenous AVP would, in titi monkeys as in rodents, increase the preference a male had for his pair-mate relative to a female stranger.

Central AVP is also involved in the hypothalamic–pituitary–adrenal (HPA) axis stress response. HPA activation in primates begins with perception of a stressor, which results in the release of corticotropin releasing hormone (CRH) from the parvocellular neurons of the paraventricular nucleus (PVN) of the hypothalamus. These same neurons produce and secrete AVP, resulting in simultaneous release of both AVP and CRH. Activation of receptors in the anterior pituitary results in the release of adrenocorticotropic hormone (ACTH) into peripheral circulation. While CRH is capable of stimulating ACTH release independently, it is well established that maximal ACTH release is achieved following coactivation of pituitary receptors by both CRH and AVP (Lolait et al. 2007; Rabadan-Diehl & Aguilera 1998; Tanoue et al. 2004). Increasing doses of exogenous AVP result in correspondingly increasing ACTH responses (Gillies et al. 1982; Turkelson et al. 1982). ACTH activates receptors on the adrenal cortices resulting in the release of cortisol into circulation. A greater ACTH response has the potential for a more pronounced cortisol response. Therefore, central AVP concentrations can affect peripheral cortisol concentrations.

Glucocorticoids (GCs) have strong anti-inflammatory properties, and the cytokines that initiate the inflammatory...
response are influenced by GCs [Blotta et al. 1997; Dekruyff et al. 1998; Elenkov et al. 1996; reviewed in Sapolsky et al. (2000) and Calcagni & Elenkov (2006)]. Central administration of AVP may be expected to cause an anti-inflammatory state via amplified secretion of cortisol. CRH and GCs also modulate pair bonding in prairie voles. In male prairie voles, treatment with CRH or corticosterone facilitates pair bond formation (DeVries et al. 1996, 2002). In female prairie voles, the opposite pattern has been observed, with lower levels of corticosterone being associated with faster pair bond formation (DeVries et al. 1995). The sexually dimorphic nature of the findings in prairie voles may be linked to the neuropeptides associated with pair bonding in male and female voles: AVP and OT, respectively (Young & Wang 2004). As previously mentioned, AVP generally has a stimulatory effect on HPA activity, and OT has a suppressive effect (Gibbs 1986).

In an attempt to capture peripheral changes associated with changes in central AVP, we also investigated peripheral blood mononuclear cell (PBMC) gene expression profiles with and without AVP administration. We predicted that pharmacologically increasing central AVP would result in increased expression of the AVP receptor genes, and the OT receptor gene, which have been implicated in the social behavior of monogamous males (Winslow et al. 1993b). We further predicted that direct or indirect (e.g. via cortisol or ACTH activation) peripheral actions of AVP would change gene expression of stress-related loci.

Methods

Subjects

Seven captive-born male coppery titi monkeys (C. cupreus) housed at the California National Primate Research Center (CNPRC) were used in this study. All males were sexually mature and had been paired with a sexually mature female for at least 1 year. None of the males were caring for dependent offspring during the study. Males were 5.5 years of age on average (3.3–10.3 years) and were housed with a female pair-mate, identical to the housing described by Mendoza (1998). Twice daily (at 0830 and 1300 h) animals were fed a diet consisting of monkey chow, cottage cheese, marmoset jelly, apples, raisins, bananas, baby carrots and vitamins.

AVP treatment

Each animal received each of three treatments: saline (vehicle control, 300 μl), low dose AVP (40 IU) in 300 μl of saline and high dose AVP (80 IU) in 300 μl of saline. Doses were based on work by Born et al. (2002) indicating what doses would lead to rises in AVP in cerebrospinal fluid. While being manually restrained, treatment was administered intranasally. Treatment solutions were dripped in 50 μl increments into alternate nostrils, and the nostril was then covered immediately to prevent the monkey from expelling the solution. Animals received treatment and were tested once per week for three consecutive weeks, with a single treatment administered on a given test day. Two animals were tested per day, and all seven animals experienced one test session per week. The order of treatments administered was approximately balanced across weeks and subjects.

Behavioral assessment

Following AVP administration, males were returned to their home-cage with their mate for 20 min to allow sufficient uptake prior to beginning observation. During this uptake period animals were video recorded and behaviors involving social interaction with the mate were scored (Table 1).

Approximately 30 min postadministration stimulus presentations began. The partner and female stranger were placed in transport cages and served as the social stimuli, and an empty transport cage was used as a nonsocial control stimulus. The transport cage dimensions were approximately 0.3 m high × 0.3 m wide × 0.6 m long, constructed of wire mesh that allowed visual, olfactory, auditory and physical (e.g. fingers through the mesh) contact. Stimuli were presented into the home-cage consecutively for 5 min each, during which time behavioral scans were made every 15 s. During pilot testing, interobserver reliability was found to be 0.90. Duration of proximity was measured by the amount of time the male spent within arm’s reach of the stimulus cage. Contact was scored as any time the male came into physical contact with the transport cage. All behaviors (Table 1) were scored using Behavior Tracker 1.5 behavioral analysis software. Throughout the 60-min testing period, each of the three stimuli was presented three times, in random order with a given presentation lasting 5 min.

Table 1: (a) AVP uptake period ethogram; (b) stimulus testing behavioral categories

<table>
<thead>
<tr>
<th>Behavior Type</th>
<th>Description</th>
<th>Measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Contact Male</td>
<td>Male in physical contact with female</td>
<td>Duration</td>
</tr>
<tr>
<td>Tail twine Male</td>
<td>Male in contact with female and tails are entwined</td>
<td>Duration</td>
</tr>
<tr>
<td>Male approach Male</td>
<td>Male moves toward female and establishes contact</td>
<td>Frequency</td>
</tr>
<tr>
<td>Male leave Male</td>
<td>Male breaks contact with female</td>
<td>Frequency</td>
</tr>
<tr>
<td>Female approach Female</td>
<td>Female moves toward male and establishes contact</td>
<td>Frequency</td>
</tr>
<tr>
<td>Female leave Female</td>
<td>Female breaks contact with male</td>
<td>Frequency</td>
</tr>
<tr>
<td>Vocalize Male</td>
<td>Male engages in calling behavior, usually with female</td>
<td>Frequency</td>
</tr>
<tr>
<td>Chest rub Male</td>
<td>Male rubs chest or armpits with forearms</td>
<td>Frequency</td>
</tr>
<tr>
<td>Face rub/sneeze Male</td>
<td>Male rubs face with hands or on cage, or sneezes</td>
<td>Frequency</td>
</tr>
<tr>
<td>Groom Male</td>
<td>Male grooms female</td>
<td>Frequency</td>
</tr>
<tr>
<td>Autogroom Male</td>
<td>Male grooms himself</td>
<td>Frequency</td>
</tr>
<tr>
<td>Lipsmack Male</td>
<td>Male smacks lips towards female</td>
<td>Frequency</td>
</tr>
<tr>
<td>Arch Male</td>
<td>Male arches back while walking</td>
<td>Frequency</td>
</tr>
<tr>
<td>Tail lash Male</td>
<td>Male wags tail, usually a sign of agitation</td>
<td>Frequency</td>
</tr>
<tr>
<td>(b) Proximity Male</td>
<td>Male comes within arm’s reach of transport cage</td>
<td>Duration</td>
</tr>
<tr>
<td>Contact frequency Male</td>
<td>Male contacts transport cage</td>
<td>Frequency</td>
</tr>
<tr>
<td>Proximity latency</td>
<td>Time between start of trial and time when male comes into proximity with transport cage</td>
<td>Latency</td>
</tr>
<tr>
<td>Contact latency</td>
<td>Time between start of trial and time when male contacts transport cage</td>
<td>Latency</td>
</tr>
</tbody>
</table>

Genes, Brain and Behavior (2011) 10: 375–383
Intranasal vasopressin affects pair bonding and peripheral gene expression

Blood collection and processing
A 3-ml blood sample was collected from the femoral vein after completion of stimuli presentations and 2 h after treatment. Whole blood was collected into sterile vacuum-sealed tubes, and was immediately centrifuged at 4°C to separate and extract plasma. Plasma was stored at −80°C until assay. Prior to assay, samples were diluted 1:4 in PBS gel buffer. Plasma concentrations of cortisol were estimated in duplicate using commercial radioimmunoassay kits (Siemens Medical Solutions Diagnostics, Los Angeles, CA). Assay procedures were modified with the addition of 0.5 and 2.5 μg/dl concentrations of standards along with the provided range of 1.0-50 μg/dl. Assay sensitivity has been determined to be 0.26069 μg/dl. Intra- and interassay coefficients of variation were 4.8% and 6.11%, respectively. OT and AVP were analyzed by enzyme immunoassay (Assay Designs, Ann Arbor, MI) in assays previously validated for titi monkeys (Bales et al. 2005). Intra-assay CV was 2.10% for OT and 3.23% for AVP.

Buffy layer was extracted to isolate PBMC, which were then lysed and RNA was isolated using the RNeasy kit (Qiagen, Valencia, CA). RNA extraction followed protocols and procedures described in the Qiagen RNeasy Mini Handbook (www.qiagen.com).

Gene expression
Two blood samples per male were used for microarray analysis on a subset of males (n = 4). From each of the four males two samples were assayed, one following saline treatment, and the second following high dose AVP treatment. Microarray analysis was performed with Rhesus Macaque Genome Array (Affymetrix, Santa Clara, CA). Isolated RNA was prepared and labeled following the protocols and procedures described in the Affymetrix Expression Analysis Technical Manual (www.affymetrix.com). Isolated RNA was used to create cDNA through reverse transcription. The cDNA was then used to synthesize biotin-labelled cRNA by in vitro transcription (IVT) using the GeneChip array IVT Labelling kit (Affymetrix, Santa Clara, CA). Amplified cRNA was fragmented and hybridized to the arrays according to the manufacturer’s procedures (Affymetrix, Santa Clara, CA). The fluorescent signal from each probe on the GeneChip was read by Affymetrix GC3000 Scanner (Affymetrix, Santa Clara, CA). Microarray results were analyzed by robust multiarray analysis (RMA) using ArrayAssist (Iobion Informatics, La Jolla, CA). This software calculates a fold change value, which is a measure of the expression level of the treatment relative to that of the control. In addition, the expression levels of control and treatment were compared by t-test.

Following microarray analysis, several genes were confirmed in their expression levels using quantitative polymerase chain reaction (qPCR). The genes that were investigated with qPCR are shown in Fig. 1. Manufacturer protocols were followed for qPCR technique (Applied Biosystems 7900HT Fast Real-Time PCR System and SDS Enterprise Database User Guide). The TaqMan Gene Expression Assays (Applied Biosystems, Foster City, CA) contain forward and reverse primers, as well as probes, which are marked with a reporter and quencher dye. An increase in fluorescence signal is detected only if the target sequence is complementary to the probe and is amplified during PCR, thus minimizing nonspecific amplification to undetectable levels. Cycle thresholds were determined and treatment samples were compared to the individual control sample to which they corresponded. Each additional cycle in the PCR reaction corresponds to a twofold decrease in starting RNA quantity, so a treatment threshold cycle that is greater than the corresponding control by one cycle represents a twofold down-regulation of the target gene. Microarray and qPCR expression profiles were significantly correlated across the five genes that were investigated with qPCR (rA = 0.921, P = 0.026; Fig. 1).

Statistical analysis
Male behavior during the AVP uptake period was analyzed with mixed models (PROC MIXED) using SAS v. 9.2 (SAS Institute, Cary, NC). The mixed effects model was chosen because the data set contains repeated measures for each animal which violates the default assumption of identical and independent distribution inherent to traditional regression models (i.e. fixed-effect models).

By using mixed effects models, we can describe the relationship between an outcome measure and treatment condition (i.e. fixed-effects) and variation between individuals (i.e. random effects), which is used to adjust the degrees of freedom. For example, we can determine what differences in tail twining are results of the treatments that animals received as opposed to differences that are a result of variation between individual titi males. The effect of AVP treatment during stimulus presentations was analyzed for two behavioral categories: proximity (latency and duration) and contact (latency, duration, frequency and likelihood (see below for description)).

Statistical analyses of stimulus testing were complicated by the fact that in addition to the treatment parameters and individual differences accounted for in the AVP uptake period models, these models had to incorporate variability due to the different stimuli, each of which were presented three times in random order. Therefore, for these analyses, the model incorporates seven animals, three treatment conditions, and three stimuli (presented three times each), resulting in 189 data points, or 189 total degrees of freedom. After subtracting the 11 degrees of freedom used on estimating fixed parameters, there are 178 degrees of freedom in the F-test. Post hoc comparisons were made using t-tests and we used the Bonferroni correction to account for multiple comparisons.

To compare latency variables between treatment/stimulus conditions, we used proportional hazards mixed models for which we report a χ² and a hazard ratio (Xu 2004). The ‘coxme’ procedure of the Kinship library within R (R Foundation for Statistical Computing, Vienna, Austria) was used to fit the models. As latency is defined as the amount of time from the beginning of a given trial to the occurrence of a behavior for our purposes, it is very similar to a survival time measuring the time to ‘death’. Because there were observations censored by the 5-min trial time (i.e. behavior of interest was not observed before the end of the trial), standard linear regression modeling is difficult to apply. Therefore, a proportional hazard model for ‘survival times’ was applied. As multiple latency measures were collected on each animal, we added a random effect reflecting the variation between animals, and the proportional hazard model with mixed effects was used.

Proximity duration showed a normal distribution, and was therefore analyzed using a linear mixed model (PROC MIXED in SAS v. 9.2; SAS Institute, Cary, NC) to handle within-individual correlations (Littell et al. 1996).
Contact frequency had a zero-inflated distribution (Rodrigues 2006). That is, several of our trials ended without the male contacting the stimulus cage within the 5-min time period. The possibility exists that had the trial been longer, fewer trials would have ended without contact. Therefore, these data were analyzed using a two-step model created and analyzed in R (R Foundation for Statistical Computing, Vienna, Austria). The first step determined whether the male contacted the stimulus. This binary variable was termed ‘contact likelihood’ and is reported as the odds ratio ±95% confidence interval. When the confidence interval does not include 1.0, it suggests that the estimate is significantly different from what would be expected by chance. A confidence interval less than 1.0 represents a reduction in likelihood and is reported as the odds ratio ±95% confidence interval.

Results

AVP uptake observations

The only behavior that showed a significant response to AVP treatment during the uptake period (first 20 min post-treatment) was rubbing the face (F2,12 = 4.19, P = 0.04), which increased when males received AVP (Mean ± SEM Saline: 2.86 ± 1.12 s; Low AVP: 8.14 ± 2.63 s; High AVP: 6.86 ± 1.04 s). Males administered the high dose of AVP showed a nonsignificant trend toward more time with their tails twined with their pair-mate (F2,12 = 3.04, P = 0.09; Mean ± SEM Saline: 108.29 ± 54.65 s; Low AVP: 112.14 ± 111.88 s; High AVP: 259.57 ± 133.08 s). Contact duration with partner, male and female approach and leave during the uptake period were not significantly affected by AVP treatment (all P > 0.25).

Stimulus testing – latency of proximity to stimulus cage

Female behavior in the stimulus cages was taken into account by recording the number of times she grabbed at the male when he approached. Females showed no trend for grabbing, either more or less, with respect to the treatment the males received or to the stimulus type they represented (partner or stranger).

The latency to approach the stimulus cage was predicted by stimulus, but not by treatment (Fig. 2; stimulus $\chi^2 = 41.18$, df = 2, $P < 0.0001$; treatment $\chi^2 = 3.00$, df = 2, $P = 0.22$), and the interaction was not significant. Post hoc analysis of fixed effects of stimulus revealed that males were faster to establish proximity with the stimulus cage when either the female partner or the stranger female was present than they were when the stimulus cage was empty (partner $\chi^2 = 9.10$, df = 1, $P = 0.0026$; stranger $\chi^2 = 38.96$, df = 1, $P < 0.0001$).

Stimulus testing – duration of proximity

Test of fixed effects showed both treatment and stimulus to be significant predictors of the duration of proximity to the stimulus cage (Fig. 3; treatment $F_{2,178} = 6.74$, $P = 0.0015$; stimulus $F_{2,178} = 7.74$, $P = 0.0006$). Post hoc analysis of fixed effects of treatment showed that proximity duration was not significantly different when animals were given high dose AVP as compared to saline ($t_{178} = −0.59$, $P > 0.5$); however, proximity duration was significantly lower when males were administered low dose AVP (Fig. 3a; $t_{178} = −3.43$, $P = 0.0007$). Post hoc analysis of fixed effects of stimulus showed that proximity duration was significantly lower when the empty cage was presented than when either the stranger or partner female was presented (Fig. 3b; $t_{178} = −3.24$, $P = 0.0014$). There was no difference in proximity duration between the partner and stranger female presentations ($t_{178} = 0.31$, $P > 0.5$).

Stimulus testing – latency of contact with the stimulus

Contact latency was predicted by stimulus, but not by treatment (Fig. 4; stimulus $\chi^2 = 40.52$, df = 2, $P < 0.0001$;
Intranasal vasopressin affects pair bonding and peripheral gene expression

Figure 4: Effect of stimulus on latency to contact the stimulus cage. Males contacted the stimulus cage faster when either their partner or the stranger female was presented as compared to an empty stimulus cage. Bars represent mean ± SEM. *P < 0.05.

Stimulus testing – likelihood to contact the stimulus cage
Likelihood to contact the stimulus cage was greatest when the stranger female (OR = 4.90, 95%CI: [1.57, 19.41]) was presented, as compared to an empty cage. There was no difference in contact likelihood between the partner and the empty cage (OR = 1.10, 95%CI: [0.40, 3.09]). Treatment did not significantly affect contact likelihood (Fig. 5b).

Stimulus testing – frequency of contact with the stimulus
Males showed increased contact frequency toward either the partner (median difference = 2.27, 95%CI: [1.39, 3.66]) or the female stranger (median difference = 1.82, 95%CI: [1.15, 2.86]) as compared to an empty cage when all treatment conditions were combined. There was no overall effect of treatment (Fig. 5d). However, when saline was administered, males showed higher contact frequency toward the stranger as compared to the partner (OR = 0.221, 95%CI: [0.044, 0.889]), while with low dose AVP there was no difference in the frequency of contact between the partner or stranger (OR = 0.331, 95%CI: [0.080, 0.889]), and with high dose AVP, increased contact frequency was directed toward the partner over the female stranger (OR = 15.625, 95%CI: [1.887, 468.604]).

Hormone analysis
Cortisol did not show a differential response to treatment. Plasma cortisol concentrations when given saline, low AVP and high AVP were 25.9 ± 3.7, 25.3 ± 2.1 and 30.8 ± 8.0 μg/dl, respectively (mean ± SE). Neither plasma OT nor AVP showed a response to AVP treatment at the time of blood collection. Plasma OT concentrations were 82.6 ± 42.4, 100.9 ± 59.2 and 94.6 ± 54.6 pg/ml when given saline, low AVP and high AVP, respectively. Plasma AVP concentrations were 200.2 ± 54.7, 134.2 ± 23.7 and 162.9 ± 22.3 pg/ml when given saline, low AVP and high AVP, respectively.

Gene expression analysis
Gene expression was analyzed first by pooling the results from all four individuals and analyzing by treatment (Table 2).
Table 2: (a) Genes showing significant changes in expression associated with AVP treatment; (b) genes showing consistent and significant changes in expression associated with AVP treatment in at least three of the four males

<table>
<thead>
<tr>
<th>Gene</th>
<th>Fold change</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CASP1</td>
<td>−3.40</td>
<td>Cleave and activate IL1</td>
</tr>
<tr>
<td>CST7</td>
<td>−2.50</td>
<td>Immune response</td>
</tr>
<tr>
<td>LILRB1</td>
<td>−2.25</td>
<td>Immune response</td>
</tr>
<tr>
<td>SPA17</td>
<td>−1.68</td>
<td>Immune cell migration and metastasis</td>
</tr>
<tr>
<td>LTB</td>
<td>1.56</td>
<td>Immune response</td>
</tr>
<tr>
<td>IL13</td>
<td>−1.43</td>
<td>Interleukin 13</td>
</tr>
<tr>
<td>IL7</td>
<td>−1.42</td>
<td>Interleukin 7</td>
</tr>
<tr>
<td>CEACAM8</td>
<td>−1.37</td>
<td>Immune response</td>
</tr>
<tr>
<td>DEFA5</td>
<td>−1.36</td>
<td>Antimicrobial humoral response</td>
</tr>
<tr>
<td>CXCL16</td>
<td>−1.36</td>
<td>Chemotaxis, immune response</td>
</tr>
<tr>
<td>IL10B</td>
<td>−1.31</td>
<td>Interleukin 10 beta</td>
</tr>
<tr>
<td>MH2CTA</td>
<td>−1.30</td>
<td>Parasite perception, immune response</td>
</tr>
<tr>
<td>(b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGJ</td>
<td>−2.77</td>
<td>Immunoglobulin J chain, activation of B lymphocytes</td>
</tr>
<tr>
<td>IGK</td>
<td>−2.66</td>
<td>Immunoglobulin K chain</td>
</tr>
<tr>
<td>IGHCA1</td>
<td>−2.51</td>
<td>Immunoglobulin heavy constant alpha 1</td>
</tr>
<tr>
<td>IL1B</td>
<td>−2.40</td>
<td>Interleukin 1 beta</td>
</tr>
<tr>
<td>IL16</td>
<td>−2.20</td>
<td>Interleukin 16, chemoattractant factor</td>
</tr>
<tr>
<td>NFIL3</td>
<td>−1.90</td>
<td>Nuclear factor, interleukin 3</td>
</tr>
<tr>
<td>CRHR1</td>
<td>−1.86</td>
<td>Corticotropin releasing hormone receptor 1</td>
</tr>
<tr>
<td>CD94</td>
<td>−1.86</td>
<td>Antigen preferentially expressed on natural killer cells</td>
</tr>
<tr>
<td>TNFα-P6</td>
<td>−1.64</td>
<td>Tumor necrosis factor alpha-induced protein 6</td>
</tr>
<tr>
<td>IL10RA</td>
<td>−1.64</td>
<td>Interleukin 10 receptor activity</td>
</tr>
<tr>
<td>TNFSF13B</td>
<td>−1.62</td>
<td>Tumor necrosis factor receptor binding, B cell activation and differentiation</td>
</tr>
<tr>
<td>IL18</td>
<td>−1.60</td>
<td>Interleukin 6 signal transducer</td>
</tr>
<tr>
<td>IL15</td>
<td>−1.46</td>
<td>Interleukin 15, T and natural killer cell activation</td>
</tr>
<tr>
<td>A2M</td>
<td>−1.44</td>
<td>Cytokine transporter</td>
</tr>
</tbody>
</table>

Out of the 40,000 genes, 372 genes were identified as showing significantly different expression levels after the AVP treatment relative to the controls. Criteria for being significantly different due to treatment included a fold change of at least 1.5 (50% change in expression level), and fulfilling the 0.05 alpha level. Out of the 372 genes showing a change in expression levels, 267 were down-regulated and 105 were up-regulated. We did not see significant changes in any of the genes that we predicted to be affected by AVP administration; neither the AVP receptor genes nor the OT receptor gene showed changes in expression levels. Among the genes that showed significant changes in expression were 24 inflammatory response relevant genes (Table 2). Inflammatory genes were significantly more likely to be down-regulated than up-regulated ($\chi^2 = 13.5, df = 23, P < 0.001$).

Gene expression was then analyzed for consistency across individuals. Genes that showed consistent significant changes in expression in at least three of the four males are shown in Table 2. Eight inflammatory response relevant genes showed consistent differences in regulation across individuals and were all down-regulated when AVP was administered ($\chi^2 = 8.00, df = 7, P < 0.005$).

**Discussion**

Male titi monkeys showed an ability to distinguish between social and nonsocial stimuli in all of the behavioral measures we recorded: they contacted the transport cage more frequently, spent more time in proximity to the cage, and took less time to approach and contact the cage when social stimuli were present. Furthermore, they showed significantly different behavioral responses to the two social stimuli. When given saline, males contacted the stranger female more frequently than the partner, but when given high dose AVP they contacted their partner more frequently than the stranger, thus supporting our prediction that AVP would increase affiliative behavior directed to the partner. It is very common for monogamous male mammals (Smith et al. 2010; Williams et al. 1992), as it is for male mammals in general, to investigate an unfamiliar female for a longer period or prior to a familiar female (Bermant et al. 1968; Crawley 2007); so this result was not surprising. While we believe that central AVP is directly responsible for the observed behavioral changes, there remains the possibility that AVP augmented the peripheral stress response (although we did not observe a change at the time-point we chose). This physiological response may have then altered brain functioning and behavior. Previous work in prairie voles has shown that acute stress, including administration of peripheral corticosterone, results in altered social bonding (DeVries et al. 1996). Future work will investigate the mechanisms driving the behavioral changes observed in this study.

An unexpected finding is that low dose (but not high dose) AVP resulted in decreased proximity to all stimuli, both social and nonsocial. While this contradicts our predictions, previous work has suggested that at some dosages AVP may be anxiogenic (Bielsky et al. 2004; Griebel et al. 2002; Landgraf et al. 1995). Some peptides exert their behavioral effects in an inverted u-shaped dose response fashion, and it is possible that AVP could result in increased anxiety and decreased willingness to approach the stimulus at one dose and not another.

The hormone analyses showed no difference in response to treatment. It is possible the stress response was activated by the handling and administration of AVP but returned to baseline by the time the blood samples were taken (2 h postadministration). The lack of significant differences in OT and AVP 2 h following administration is not surprising considering the short half-life of these peptide hormones (Janáky et al. 1982; Rydén & Sjöholm 1969; Share 1962). While cortisol has a longer half-life than OT and AVP (Kerrigan et al. 1993), it too would be expected to return to basal levels within 2 h. The gene expression data provide evidence of a possible change in peripheral hormone concentrations that occurred before the blood samples were collected. The down-regulation of proinflammatory cytokines suggests
a change in the inflammatory state. Cortisol is a known mediator of cytokine expression; however, the mechanism for the observed changes in gene expression in this study remains unclear.

While the blood collection time was not ideal for hormone analyses, 2 h following a stressor is sufficient time to observe changes in inflammatory gene expression (Breen et al. 2000; Louis et al. 2007), and we found changes in the expression of several inflammatory genes following AVP administration. The proteins corresponding to the down-regulated genes include interleukin (IL)-1β, IL-7, IL-13, IL-15 and IL-16. IL-1β is well known for its role in inflammatory processes, and is suppressed by glucocorticoids [reviewed by Sapolsky et al. (2000)]. IL-7 is capable of expanding lymphocyte populations, leading to increases in both the B and T cell populations (Hofmeister et al. 1999), leading to humoral and cell-mediated immune response, respectively. IL-13 is secreted primarily by Th2 cells, and is an important mediator of allergic inflammation (Wills-Karp et al. 1998). IL-15 and IL-16 are critical to the induction of inflammatory processes (Center et al. 2000) and IL-15 is elevated during chronic inflammation. Taken together, reduced activity of these genes suggests a suppression of inflammatory processes. Therefore, manipulation of central AVP appears to have affected peripheral inflammatory state. One possible explanation is that intranasally administered exogenous AVP could have co-activated pituitary receptors with CRH and resulted in an increased adrenocortical response. Acutely increased peripheral cortisol concentrations would exert anti-inflammatory effects, resulting in decreased gene expression of genes coding for proinflammatory cytokines in any tissues containing glucocorticoid receptors. PBMC have glucocorticoid receptors (Lippman & Barr 1977), and are responsive to changes in glucocorticoid levels (Adcock et al. 1995).

An alternative explanation for the changes in gene expression is that they were caused by the altered social behavior. Growing evidence supports a role for social condition affecting inflammatory state [reviewed in Uchino et al. (1996), Kiecolt-Glaser (1999), Klein & Nelson (1999), and DeVries et al. (2003)]. This idea predicts that individuals with better social support experience an anti-inflammatory state, while those without social support experience chronic inflammation. Chronic inflammation is currently known to be associated with several chronic health problems (e.g. cardiovascular disease, stroke, viral-mediated cancer). The results of this study, therefore, warrant further investigation of AVP as a possible mechanism mediating chronic inflammation and its downstream consequences.

There are a number of ways in which future studies, with different design parameters, could add to the information gained from this study. For example, in this study, we administered treatments once per week. We chose this timing because we believed that it would allow more than enough time for the subjects to return to pre-treatment conditions. On the basis of the clearance rate of AVP, it is safe to assume that no exogenous AVP remained from a given treatment at the time of the second treatment a week later (Janáky et al. 1982; Rydén & Sjöholm 1969; Share 1962). However, we cannot rule out the possibility that the physiological cascade of effects initiated by exogenous AVP administration remained beyond 1 week. A second choice we made with our behavioral assessment was to present stimuli sequentially rather than simultaneously. This allowed us to assess behavior in the subjects’ home cages rather than in a novel apparatus, which had advantages because these animals are strongly neophobic (Fragaszy & Mason 1978; Hennessy et al. 1995). However, a simultaneous choice would have been closer to the model used in rodent research. A third design choice was that all three stimuli were presented during the same 60-min period. While this was based on designs used in previous titi monkey studies (Fernandez-Duque et al. 1997; Mendoza & Mason 1986), it is possible that this testing method yielded different results than would be seen had each stimulus been presented on separate days.

In summary, intranasal AVP appeared to alter both central and peripheral physiology, resulting in both changed behavior and peripheral gene expression. Male titi social behavior changed in the predicted direction, as males contacted their female pair-mates more frequently when given high dose AVP. Proinflammatory cytokine gene expression was reduced in the periphery 2 h after treatment with high dose AVP. Future work will further investigate the directionality of relationships between AVP, social behavior, and gene expression of proinflammatory cytokines.

References


Intranasal vasopressin affects pair bonding and peripheral gene expression


Acknowledgments

We thank M. George and M. Rolston for assistance with microarray assay and data analysis and K. Abel and J. Lee for assistance with quantitative PCR assay and data analysis. We acknowledge our funding sources from the NIH (Grant nos. HD053555 and RR00169) and the Good Nature Institute.