Central and Peripheral Actions of Arginine Vasotocin on Courtship Behavior in Male White Perch *Morone americana*

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Running Headline: AVT and courtship behavior

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Abstract
Arginine vasotocin (AVT) and its mammalian homologue arginine vasopressin (AVP) have been shown to have widespread behavioral effects in vertebrates. AVT was evaluated for its effectiveness in stimulating an important courtship behavior in male white perch *Morone americana*. Male white perch exhibit a stereotypical courtship behavior termed 'attending' prior to spawning. Attending is defined as extremely close and continuous following of the female while occasionally contacting her abdominal area with the snout. We tested the behavioral effectiveness of AVT in stimulating attending when AVT was administered intraperitoneally (IP) and intracerebroventricularly (ICV). We also tested AVT in combination with the V₁₄ receptor antagonist Manning compound. Both IP and ICV injections of AVT produced significant increases in attending behavior compared to saline-injected controls. The minimum effective ICV dose (0.02 µg/fish) was 1500 times lower than the minimum effective IP dose (30.0 µg/fish). Manning compound did not block AVT induced attending behavior when administered IP, but did reduce attending when co-administered ICV with AVT. The greater effects observed with central administration as opposed to peripheral administration suggests a central site of action for this behavioral effect. The attending stimulated in white perch males injected with AVT is a complex affiliative behavior that shows intriguing similarities to the affiliative behaviors mediated by AVP in mammals. These similarities may point to a conserved behavioral role for AVP and AVT in vertebrates.

**KEYWORDS:** AVT, courtship behavior, intraperitoneal, intracerebroventricular.
Introduction

Pickford (1952) first demonstrated that neurohypophysial extracts induced a 'spawning reflex' in hypophysectomized killfish (*Fundulus heteroclitus*). Subsequently, experiments with pure forms of specific neurohypophysial factors suggested arginine vasotocin (AVT) was responsible for this effect. Isotocin (IST) could also induce this behavior but only at tenfold higher doses (Wilhelmi, Pickford, and Sawyer, 1955; Pickford and Strecker, 1977). In other closely related species of Cyprinodontids, AVT was shown to have similar effects (Macey, Pickford, and Peter, 1974; Peter, 1977; Liley and Stacey, 1983).

Following the initial demonstrations of AVT effects on behavior in Cyprinodontids, behavioral effects of this family of neuropeptide hormones have been demonstrated in representatives of all other vertebrate classes. Examples include effects on clasping behavior in newts (*Taricha*) (Moore and Zoeller, 1979; Moore and Miller, 1983; Moore, 1992), vocal and locomotory behaviors in anuran amphibians (Diakow, 1978; Boyd, 1991; Boyd, 1994; Marler, Chu, and Wilczynski, 1995; Propper and Dixon, 1997; Sensar, Klomberg, and Marler, 1998), singing and sexual behavior in birds (Kihlstrom and Danninge, 1972; Maney Goode, and Wingfield, 1997), and communicative and affiliative behavior in voles, mice, and hamsters (Ferris, Albers, Wesolowski, and Goldman, 1984; Hennessey, Whitman, and Albers, 1992; Winslow Hastings, Harbaugh, and Insel, 1993). The V1a form of the AVP receptor is found in the brain and is the receptor subtype that is important behaviorally in mammals (Young Nilsen, Waymire, MacGregor, and Insel, 1999). The AVT receptor has been partially cloned from several species of fish and an anuran and shows strongest sequence...
homology to the AVP V1a receptor subtype (Mahlmann, Meyerof, Hausmann, Heirhorst, Shonrock, Zwiers, Lederis, and Richter, 1996; Godwin, unpublished data). Behavioral data are consistent with the tissue distribution and inferred relationships of these receptors. Specifically, the AVP V1a receptor antagonist Manning compound ([β-Mercapto-β, β-cyclopentamethylene-propionyl1, O-Me-Tyr2, Arg8]-Vasopressin) interferes with AVT or AVP induced behavioral effects (Moore and Miller, 1983; Winslow et al., 1993; Propper and Dixon, 1997; Goodson and Bass, 2000).

Despite these clear demonstrations of behavioral actions of AVT in other vertebrates, questions remain regarding AVT effects in fishes. The spawning reflexes observed in response to injection with purified pituitary extracts were described by Pickford (1952) as "violent S-shaped spasms" during which "the fish showed no interest in each other but behaved independently." The nature of this response and the large dosages necessary to elicit it in Fundulus and related species suggested that AVT could be acting peripherally through stimulation of smooth muscle (Peter, 1977; Liley and Stacey, 1983). Supporting this interpretation, both Peter (1977) and Pickford, Knight, and Knight, (1980) found that AVT was no more effective when administered intracerebroventricularly than intraperitoneally. In contrast, Macey and coworkers (1974) found that AVT induced spawning reflexes in fish were essentially abolished with lesions of the preoptic nuclei, but not with lesions of comparable size in other brain regions, suggesting AVT may act in this brain region. Recently, Goodson and Bass (2000) showed clear effects of AVT and IST on neurons associated with vocalization behavior when these neuropeptides were administered directly into the preoptic area.
Currently, there are no published reports clearly demonstrating central effects of AVT in intact, freely-behaving fishes.

In the white perch (\textit{Morone americana}), the primary courtship behavior leading to the spawning act is termed “attending”. Attending is close (within 30 cm) and continuous following of a female while occasionally contacting her abdominal area with the snout. In contrast to the spawning reflex of Cyprinodontid fishes, attending is a clear and complex affiliative behavior. Indeed, attending shows strong similarities to the affiliative behaviors measured in studies of AVP effects in mammals (Winslow \textit{et al.}, 1993; Young \textit{et al.}, 1999). We examined AVT effects on attending behavior in this study and found that exogenous AVT can induce attending behavior in white perch and is more effective when administered directly to the brain than when given intraperitoneally. This effect is antagonized by a putative AVT receptor blocker (Manning compound).

**Methods**

**Experimental animals**

We used white perch broodstock maintained at the North Carolina State University Pamlico Aquaculture Field Laboratory. They were transported to the Aquatic Research Facility on the North Carolina State University campus and maintained as described previously until testing (King, Berlinski, and Sullivan, 1995). We conducted these experiments during the breeding season for white perch in March, April, and May of 1998 and 1999. The white perch males used were $207.3 \pm 5.8$ g and $237.9 \pm 2.3$ mm (mean $\pm$ SEM, $N = 94$) in total length. All males were spermiating at the start of studies.

**Injections**
Perch received intraperitoneal (IP) or intracerebroventricular (ICV) injections of peptides under anesthesia (200 mg/l tricaine methane sulfonate in freshwater) (MS-222) Argent, Redmond, WA). Arginine vasotocin and Manning compound were obtained from Sigma Chemical Corporation (St Louis, MO). Intraperitoneal injections of peptides were given with a 1cc Luer LOK® syringe fitted with a 25 Ga needle (Becton Dickinson, Franklin Lakes, NJ) given in 100-300 µl of 0.9% NaCl. Intracerebroventricular injections of peptides were made freehand into the third ventricle of the brain in 2 µl of 0.9% NaCl containing 10% Sheaffer ink (v/v) (Ft Madison, IA). Control animals were given 0.9% NaCl and ink only. Peptides were mixed fresh at the beginning of each study and kept on ice and syringes were filled just prior to injection. A small hole was made in the skull with a 20 Ga needle, then a 10 µl Hamilton syringe (Reno, NV) fitted with a 33 Ga needle was inserted into the hole to a depth of 8 mm. Lining up the syringe with the caudal side of the orbit and angling the syringe perpendicular to the head surface placed the needle in the middle of the brain approximately in the hypothalamus. The depth of the needle was controlled by placing a 200 µl pipette tip over the 33 Ga needle and trimming it so only 8 mm of the needle projected from the tip. We waited 10 seconds after inserting the needle, injected 2 µl over a period of 10 s, then waited 10 more s before removing the syringe. The hole was filled with Triple X antibiotic ointment (Food Lion, Salisbury, NC). Accuracy of injection was confirmed in all studies by opening the skull of each fish and visually inspecting the brain. Samples with visible ink outside the brain were excluded from analyses. Preliminary studies in which we sectioned each brain showed that ink was delivered to the ventricular system if no ink was found on the surface of the brain or in the braincase.
Behavior tests

We compared the display of attending behavior to assess AVT effects. Attending is defined as close (within 30 cm) and continuous investigation of the female's abdominal area which may include physical contact. Behavior tests were executed by introducing an experimental male into the tank of an ovulated female for a period of one hour. During the test period, the courtship behavior attending was scored by one observer unaware of the experimental treatment given to the focal male.

Each male fish to be tested was captured from the holding tank with a hand net, given an injection (IP or ICV, see below), and placed into a holding tank for 15 minutes so to recover from anesthesia. After this period, we placed the male into the test tank with the ovulated female. The duration of male courtship behavior attending was recorded by one observer for two 10 min intervals; the first interval began immediately after the male was introduced into the tank and the second began 50 min after the introduction. Following the second 10 min interval, the male was removed from the tank and sampled for blood (60 min post introduction). Since males were given a 15 min recovery period after injection, observations began 15 and 65 minutes after injection.

We performed behavior tests in 684-liter rectangular tanks (1.9 m X 0.6 m X 0.6 m), each illuminated with five 75 watt red floodlights (Sylvania). A VHS video camera (Panasonic ag-187u, Seacaucus, NJ) equipped with a standard zoom lens (Panasonic Power Zoom x 12) mated with a wide-angle lens (Panasonic Super Wide Pro 5050-0.5x) and polarizing filter (Toshiba PL 49.0(s), Japan) was installed over each tank on a ball mount (Bogon, Italy) for recording behavior. Direct observations of courtship behavior
were made through blinds located at both ends of the tanks. Most testing occurred between 0700 - 1600 (65 animals) and some occurred between 1600 -2200 (29 males).

We used an ovulated female as a stimulus for male courtship behavior. To induce ovulation in females, each female perch was injected with human chorionic gonadotropin (hCG) (Schein Pharmaceuticals, Florham Park, NJ) at a dose of 330 I.U./kg bodyweight i.m. 24-48-hrs prior to behavioral testing. We assessed maturation of oocytes by anesthetizing females in quinaldine sulfate (50 mg/l) and giving them ovarian biopsies. Only females yielding ovulated eggs were used. Since males and females were similar in size, females were tagged with a silver streamer made of Mylar™ tape (8 cm) inserted through the dorsal fin to mark them for identification during observations.

To assess the precision of our behavioral observations, attending was rescored from videotapes and compared to first person observations in correlation analyses. In our first experiment, all male scores were compared in this manner. In subsequent experiments, only four males scores per experiment were compared in this manner.

**Blood sampling and Hormone assays**

Immediately after behavior testing, fish were removed from the tanks, anesthetized in MS-222 (200 mg/ml), rinsed in fresh water, weighed, and bled by caudal puncture using a 3.0 cc syringe fitted with a 21-gauge heparinized needle. Approximately 1.5 ml blood was collected from each fish. Blood samples were stored on ice until being centrifuged (10 min @ 10,000xG). The plasma was drawn off and stored at -80 °C until assay. Concentrations of KT and T were measured in duplicate 20 µl aliquots of blood plasma by highly specific RIAs. Briefly, samples were triple extracted in 3 ml of diethyl ether, and the ether phase was dried under a stream of nitrogen gas at
37°C before resuspending in 200 µl assay buffer, for RIA as described by Woods and Sullivan (1992).

Statistics

Data were analyzed using JMP statistical software (SAS, Cary, NC). Comparisons of behavioral scores were made by either Student’s t–test or one way analysis of variance (ANOVA) if there were more than two treatments. Individual means were compared using JMP’s compare all pairs command to execute a Tukey-Kramer comparison. Associations between attending scores from videotapes and direct observations were assessed by simple linear correlation (Pearson's R). Potential outliers were tested using Dixon statistics (Barnett and Lewis, 1978).

Results

Behavioral actions of AVT: effects of IP administration

In all experiments, AVT or Manning compound was mixed in 0.9% NaCl whereas control injections were 0.9% NaCl (saline). We evaluated the effects of IP injections in three separate experiments. In experiment 1, males were given an injection with saline or 0.150 µg/g bodyweight AVT. AVT produced almost a doubling in attending behavior 65 min after injection but not 15 min after injection (p < 0.001) (Table 7). In experiment 2, males were given an injection of saline, 0.150 µg/g bodyweight AVT, or 1.0 µg/g bodyweight AVT. In contrast to experiment 1, AVT did not significantly increase attending at either 0.150 µg/g or 1.0 µg/g dose. In experiment 3, males were injected with either saline or Manning compound (1.0 µg/g bodyweight) followed by a second injection of either saline or AVT (1.0 µg/g bodyweight) 15 min later (four treatments:
saline/saline, saline/AVT, Manning/saline, Manning/AVT). There were no significant
differences among treatments. However, the attending scores of males in this experiment
were at the high end of the range seen for males in experiments 1 and 2 (Table 7).

**Behavioral actions of AVT: effects of ICV administration**

We evaluated the effects of ICV injections in two separate experiments. In
experiment 4, we injected males with 0.2 µl of 0.9% NaCl with 10% ink containing no
peptide, 0.02 µg AVT, or 0.2 µg AVT. AVT significantly stimulated attending when
administered at 0.02 µg/fish and 0.2 µg/fish (p < 0.05) (Table 7). In experiment 5, males
received a single ICV injection containing either 0.2 µg AVT or both Manning
compound and AVT in a 5:1 (1.0 µg Manning : 0.2 µg AVT) or 1:1 (0.2 µg AVT : 0.2 µg
Manning) ratio. We analyzed the data in three treatments first and then finding no
difference between the 5:1 and 1:1 Manning: AVT, we pooled these two treatments and
tested the one-tailed hypothesis that Manning would depress attending levels relative to
males treated with AVT alone. Indeed, Manning compound significantly depressed
attending compared to AVT injected control males (p = 0.05). In these experiments,
attending scores from selected males were compared to their individual scores from
videotape analysis and were shown to be highly correlated (R = 0.85 p < 0.0001).

**Effects of AVT: Circulating androgen levels**

Regardless of the method of administration (IP or ICV), circulating levels of
testosterone (T) and 11-ketotestosterone (KT) in samples taken approximately 80 minutes
post-injection showed no significant differences across treatments (saline, AVT,
Manning; Table 8).
Discussion

Numerous studies have identified a role for both AVP and AVT in stimulating affiliative and communicative behaviors in vertebrates. Evidence for similar effects in fishes has been controversial because earlier studies examined a relatively simple behavior that could have resulted from hormonal effects on peripheral tissues (Pickford et al., 1980). This study provides clear evidence that AVT acts centrally to stimulate a complex affiliative behavior in a freely-behaving teleost fish. We contend that AVT acts centrally because we observed rapid elevations in attending behavior with ICV injections and these AVT effects could be reduced by a putative AVT receptor antagonist.

We found that IP administration of 0.150 µg/g bodyweight AVT induced significant elevations of attending behavior in male white perch in experiment 1 at the later time point. However, this effect was not reproducible even when AVT was given at a higher dose of 1.0 µg/g in experiment 2. In contrast, administering AVT directly to the brain approximately doubled attending behavior at the early time point. Comparing the effectiveness of delivery, we found that ICV treatment produced the highest overall attending scores observed in these experiments and the effective dose injected into the brain was at least 1500 times lower than the minimum effective dose administered IP (0.02 µg/fish vs. 30 µg/fish). These differences in effectiveness depending on administration method suggest AVT is acting in the brain to mediate this behavioral effect. Where effective, the IP doses of AVT that influenced attending in white perch were comparable to effective doses in other species: Fundulus heteroclitus 0.25-1.25 µg/g bodyweight (Pickford and Strecker, 1977), Thalassoma bifasciatum 1.0 µg/g bodyweight, (Semsar and Godwin, 2000), Rana catesbeianna 0.1 µg (Boyd, 1991).
*cognatus* 1.0 µg/g bodyweight (Propper and Dixon, 1997). The ICV doses found effective in *this study were also comparable to those used in other studies: Zonotrichia leucophrys gambelii 0.1 µg (Maney et al., 1997) and Taricha granulosa 0.1 µg (Moore and Miller, 1983).

The AVP V₁a receptor antagonist (Manning compound) effectively decreased AVT-induced attending behavior in male white perch. Manning compound is an effective blocker of AVT-induced reproductive behavior in a variety of species (Moore and Miller, 1983; Winslow et al., 1993, Propper and Dixon, 1997). Specifically in teleosts, Manning compound blocks AVT effects both on neurons of the preoptic area in the plainfin midshipman (Goodson and Bass, 2000) and in white sucker AVT receptors expressed in *Xenopus* oocytes (Mahlmann et al., 1994). Other investigations have found Manning compound to be effective when administered IP. For example, Propper and Dixon (1997) found that Manning compound did reduce AVT induced calling behavior in *B. cognatus* when administered IP. We did not see a reduction in AVT induced attending when Manning compound was administered IP 15 min prior to AVT. All groups in experiment 3 exhibited high levels of attending, suggesting attending should have been reduced if Manning compound were effective. Manning compound did show an effect when co-administered ICV in a 5:1 or 1:1 ratio with AVT. Our sample sizes were small, but there was no apparent difference in mean attending between the 5:1 and 1:1 treatments. Pooling these treatments for statistical analysis provided a more powerful test of inhibitor effects on AVT-induced attending. This inhibition suggests that the AVT effects described here are mediated through an AVT receptor.
This study was performed in a controlled laboratory setting, but several recent studies in fishes suggest AVT is an important mediator of reproductive behavior in the natural environment. The activity of the AVT system, as measured by levels of AVT mRNA or numbers of AVT immunoreactive cells, is correlated with behavioral phenotype in several species. Grober and Sunobe (1996) found that when gobies (Trimma okinawae) undergo reversible sex change, there are correlated changes in the size of AVT producing cells in the forebrain. In the plainfin midshipman (Porichthys notatus), Foran and Bass (1999) found that the smaller non-territorial (sneaker) males have smaller sized but greater numbers of AVT immunoreactive cells in the preoptic area of the brain than larger nest guarding males. In bluehead wrasses (Thalassoma bifasciatum), the large territorial terminal phase males have greater hypothalamic AVT mRNA levels than females and nonterritorial males, but these levels rise rapidly with female-to-male sex change (Godwin, Sawby, Warner, Crews, and Grober, 2000; Grammer, 1998).

Until now, the strongest evidence that AVT and its mammalian analog AVP influenced reproductive behavior came from tetrapods (see references above). To our knowledge, this is one of the first two demonstrations in a fish that AVT induces a behavioral response that is clearly more than a result of smooth muscle activation (see also Salek, Sullivan, and Godwin, 2000; Semsar and Godwin, 2000). Moreover, this work suggests that AVT acts in the brain to produce this behavioral effect. Our findings build on the early work in killifish (Pickford, 1952; Wilhelmi et al., 1955; Pickford and Strecker, 1977) by demonstrating that AVT can also influence behavior in an advanced perciform teleost. The attending stimulated in white perch males injected with AVT is a
complex affiliative behavior that shows intriguing similarities to the affiliative behaviors mediated by AVP in mammals (e.g., Young et al., 1999). These similarities may point to a conserved behavioral role for AVP and AVT in vertebrates.
TABLE 7. Effect of ICV and IP AVT Injections on Courtship Behavior.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>N</th>
<th>Attend Early</th>
<th>Attend Late</th>
<th>Total Attending</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Injected IP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>6</td>
<td>17.6± 6.4</td>
<td>19.6± 4.3</td>
<td>18.6± 4.0</td>
</tr>
<tr>
<td>0.150 µg/g</td>
<td>7</td>
<td>23.4± 5.6</td>
<td>37.1± 4.0*</td>
<td>30.7± 4.8</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>5</td>
<td>16.8± 2.1</td>
<td>21.3± 3.9</td>
<td>19.0± 1.6</td>
</tr>
<tr>
<td>0.150 µg/g</td>
<td>7</td>
<td>13.3± 3.1</td>
<td>21.6± 5.3</td>
<td>17.5± 4.0</td>
</tr>
<tr>
<td>1.0 µg/g</td>
<td>8</td>
<td>28.5± 5.8</td>
<td>31.3± 4.1</td>
<td>30.0± 1.9</td>
</tr>
<tr>
<td>Experiment 3</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Saline/Saline</td>
<td>9</td>
<td>26.1± 5.0</td>
<td>34.0± 4.5</td>
<td>30.5± 4.2</td>
</tr>
<tr>
<td>Saline/AVT</td>
<td>10</td>
<td>29.0± 5.3</td>
<td>33.3± 4.8</td>
<td>31.1± 3.6</td>
</tr>
<tr>
<td>Manning/Saline</td>
<td>9</td>
<td>26.5± 5.1</td>
<td>35.8± 5.6</td>
<td>31.1± 4.0</td>
</tr>
<tr>
<td>Manning/AVT</td>
<td>9</td>
<td>28.5± 6.8</td>
<td>22.6± 4.6</td>
<td>25.5± 5.1</td>
</tr>
<tr>
<td><strong>Injected ICV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>4</td>
<td>22.6± 6.3</td>
<td>27.1± 5.1</td>
<td>24.9± 1.1</td>
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<tr>
<td>0.02 µg AVT</td>
<td>5</td>
<td>46.6± 5.2**</td>
<td>30.8± 4.8</td>
<td>35.7± 6.1</td>
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<tr>
<td>0.2 µg AVT</td>
<td>5</td>
<td>53.8± 4.5**</td>
<td>28.3± 4.1</td>
<td>41.0± 2.9</td>
</tr>
<tr>
<td>Experiment 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>0.2 µg AVT</td>
<td>5</td>
<td>45.6± 7.3</td>
<td>24.1± 4.6</td>
<td>34.9± 4.2</td>
</tr>
<tr>
<td>0.2 µg AVT/Manning</td>
<td>5</td>
<td>29.8± 4.6***</td>
<td>33.5± 6.8</td>
<td>31.6± 4.9</td>
</tr>
</tbody>
</table>

Attending early was 15 minutes post injection; Attending late was 65 minutes post injection. Total attending was early and late together. * T - test comparing to Saline (0.9% NaCl) p < 0.001, ** T - test comparing to Saline p < 0.05. *** One tailed T - test comparing 0.2 µg AVT to both Manning compound (0.2 µg -1.0 µg) and AVT (0.2 µg) in a single injection p = 0.05. Experiment 3 Manning and AVT given at a dose of 1.0 µg/g bodyweight.
TABLE 8. Effect of ICV and IP AVT Injections on Androgen Levels in Males.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>N</th>
<th>Testosterone</th>
<th>11-Ketotestosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Injected IP</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>6</td>
<td>0.61± 0.14</td>
<td>1.56± 0.25</td>
</tr>
<tr>
<td>0.150 µg/g</td>
<td>7</td>
<td>0.71± 0.09</td>
<td>1.51± 0.21</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>5</td>
<td>0.35± 0.12</td>
<td>0.95± 0.19</td>
</tr>
<tr>
<td>0.150 µg/g</td>
<td>7</td>
<td>0.43± 0.08</td>
<td>1.25± 0.26</td>
</tr>
<tr>
<td>1.0 µg/g</td>
<td>8</td>
<td>0.67± 0.16</td>
<td>1.94± 0.56</td>
</tr>
<tr>
<td>Experiment 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline/Saline</td>
<td>9</td>
<td>0.68± 0.18</td>
<td>1.49± 0.37</td>
</tr>
<tr>
<td>Saline/AVT</td>
<td>10</td>
<td>0.48± 0.10</td>
<td>1.25± 0.26</td>
</tr>
<tr>
<td>Manning/Saline</td>
<td>9</td>
<td>0.36± 0.09</td>
<td>0.72± 0.27</td>
</tr>
<tr>
<td>Manning/AVT</td>
<td>9</td>
<td>0.37± 0.07</td>
<td>1.19± 0.29</td>
</tr>
<tr>
<td><strong>Injected ICV</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>4</td>
<td>0.40± 0.05</td>
<td>0.66± 0.07</td>
</tr>
<tr>
<td>0.02 µg AVT</td>
<td>5</td>
<td>0.12± 0.05</td>
<td>0.39± 0.16</td>
</tr>
<tr>
<td>0.2 µg AVT</td>
<td>5</td>
<td>0.40± 0.09</td>
<td>0.82± 0.37</td>
</tr>
<tr>
<td>Experiment 5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2 µg AVT</td>
<td>5</td>
<td>0.59± 0.23</td>
<td>1.00± 0.41</td>
</tr>
<tr>
<td>0.2 µg AVT/Manning</td>
<td>5</td>
<td>0.74± 0.40</td>
<td>1.55± 0.41</td>
</tr>
</tbody>
</table>

Hormone assays revealed no significant differences in T or KT levels within experiments. Values are expressed in ng/ml. See caption on Table 7 for experimental details.
REFERENCES


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