Urocortin II increases spontaneous parental behavior in prairie voles (*Microtus ochrogaster*)

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Abstract

Stress and anxiety play a role in many psychological processes including social behavior. The present study examines the effects of urocortin II (UCN II) on spontaneous parental behavior in adult prairie voles (*Microtus ochrogaster)*. UCN II was found to increase passive parental behavior in voles while not affecting any stress-related measures. Delineating the mechanism of this change will aid in our understanding of the regulation of parenting.

Keywords

Urocortin (UCN) II, also known as stresscopin-related peptide, is a 38 amino acid member of the mammalian corticotropin-releasing hormone (CRH) peptide family, which also includes CRH, UCN I, and UCN III [1;2]. CRH mainly binds to type 1 CRH receptors (CRH1), while UCN II and III bind primarily to type 2 CRH receptors, and UCN I binds to both (CRH2) [1]. Each of these hormones has distinctive distribution patterns in the central nervous system and the periphery, suggesting each peptide may have distinct behavioral and physiological effects, although all have been associated with anxiety [2–5]. In general, agonism of CRH1 receptors is posited to be anxiogenic and agonism of CRH2 receptors is posited to be anxiolytic [6].

UCN II, however, has produced mixed results in tests of anxiety. Central administration of UCN II increased anxiety-like behavior when given intracerebroventricularly (ICV) thirty minutes before a plus maze test [7;8]. It also caused increased anxiety when administered by osmotic minipump infusion during a novel object exploration task [9]. However, UCN II minipump administration caused no differences in open field behavior [9]. In another study, ICV UCN II caused no change in plus-maze behavior either ten minutes or one hour post-injection [10]; however, animals tested four hours post-administration showed a decrease in anxiety-like behavior. It is thus possible that the effects of UCN II on anxiety are dependent on the time-course of administration [6], with anxiogenic effects peaking earlier than anxiolytic effects. Administration of the glucocorticoid, dexamethasone, leads to an increase in UCN II mRNA, which has been suggested to mediate anxiolytic functions of UCN II [11]. Effects of CRH2 ligands on other aspects of behavior, particularly social behavior, are thus far little studied, as are the effects of peripheral administration on anxiety and social behaviors.

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Prairie voles are among the few mammals to exhibit the traits of social monogamy, including lifetime pair-bonds and male parental care [12;13]. Previous research in the prairie vole has chronicled the interaction of social behavior with anxiety and the HPA axis. Carter and colleagues [14] observed elevation in plasma corticosterone (CORT) due to separation from a social partner and a return to baseline values upon reunion with that partner. Furthermore, a reduction of CORT is seen in both male and female naïve voles within an hour of being paired with a vole of the opposite sex, and this effect is greater in males than females [15–17]. Increases in stress, facilitated by either a swim stressor or surgical stressor, have also been found to promote parental care in male prairie voles while having no effect on parental care in females [18;19]. We originally hypothesized that, because we were using relatively long timecourses, UCN II would be anxiolytic. Given this assumption, we predicted that UCN II would decrease spontaneous parental care in males while having only a slight—or no—positive effect on the parental care of females. Alternatively, if UCN II administration caused an increase in anxiety in voles, we would predict an increase in parental care in males and a decrease or no effect in females.

This is the first study to examine the effects of peripherally administered UCN II on parental care in males and females of any species. UCN II crosses the blood-brain barrier, the only member of the CRH peptide family to do so [20]. In order to dissociate the effects of UCN II on parenting behavior from those on general locomotion, we also examined locomotor variables in both the plus-maze and forced swim tests. Finally, we assayed corticosterone (CORT) to examine possible mediators for the effects of UCN II on behavior.

The prairie voles (*Microtus ochrogaster*) used in this study were descendants of a wild stock caught near Champaign, Illinois. Husbandry procedures are described in [18]. All test subjects had not been used in any previous experiments, were sexually naïve, and had never been exposed to pups. Because female prairie voles do not experience a spontaneous estrus cycle [21], stage of estrus was not considered in the study design. All subjects received treatment as adults at approximately 60–100 days of age.

Subjects received a 200 μl intraperitoneal injection containing either UCN II (30 μg/kg) or saline vehicle. This dosage and the two- and four-hour timecourses were chosen based on previous studies, especially those suggesting that UCN II may exert delayed effects on anxiety [10;22]. Upon injection, voles were placed into separate cages until their behavioral test.

Either two hours or four hours after injection, male and female voles received a 10-minute alloparental care test (measuring spontaneous parental care towards an unrelated pup) [23]. Forty-five minutes prior to testing, the test animal was placed in the testing apparatus and allowed to acclimate. The testing apparatus consisted of two small cages (same size as above), connected by a short, clear tube of approximately 3 inches in length. At the end of the acclimation period, voles were removed and a 1 to 3 day old pup was placed in the front cage. The adult subject was then placed in the tube, and behavior was recorded for 10 minutes on videotape. Tests were stopped immediately if any aggression was displayed towards pups, and pups were treated and returned to their parents. Following the alloparental care tests, subjects were returned to their original cage with their original cage-mate. Subjects were scored using Behavior Tracker 1.1 (www.behaviortracker.com) for positive pup-directed behavior including huddling over pups, pseudo-huddling (huddling without an arched back), non-huddling contact (any affiliative contact with the pup that is not huddling, pseudohuddling, retrieval, or licking), retrievals, and licking.

In a second set of animals, injections were administered as described above. Both male and female voles were tested for anxiety with a 5 minute elevated plus maze test (a mild stressor) at either 2 or 4 hours post-injection. The elevated plus maze (EPM) consisted of two closed

and two open arms, each 67 cm long at 5.5 cm wide, 1 m from the floor [24;25]. At the beginning of the test, each vole was placed in the neutral area located in the center of the EPM. The location of each subject (closed arm, open arm, or center) and the total number of entries into and arm was recorded for 5 minutes on laptop computer using Behavior Tracker 1.1. Subjects received a retro-orbital bleed 45 minutes following the test to allow for maximal elevation of stress hormones [26], and samples were assayed for CORT.

In a final set of animals, female prairie voles underwent a swim stressor (a moderate physical stressor) to examine the effects of UCN II on stress-related behaviors and CORT during a more stressful experience. Injections were given as described above. Voles were placed for 3 minutes in large plastic cages (20 cm × 25 cm × 45cm) that were filled with lukewarm water 2 hours after injection. The depth of water ensured that the voles could neither touch the bottom nor climb out of the cage. Voles were constantly monitored during the test for distress, but are good swimmers and no intervention was necessary. Behavior during the test was video-taped. Following the swim test, voles were placed back into their cages in proximity to heating lamps. Previous studies have detailed the response to this stressor in voles [15;16;26], and it consistently results in approximate doubling of CORT levels. The number of droppings was recorded following each test. The behaviors scored from videotape included floating, swimming, and struggling. Retro-orbital bleeds were administered 45 minutes after the swim test to allow for maximal elevations of stress hormones [26], and assayed for CORT.

CORT was assayed using a commercially available radioimmunoassay (MP Biomedicals, Irvine, CA) which was previously validated for prairie voles [26]. Samples were diluted to 1:2000 prior to the assay. Intra-assay CV's averaged 3.45%, and inter-assay CV's averaged 3.54%.

Based on evidence that they are under different neuroendocrine control [18;27–29], we grouped passive parenting behaviors (huddling and pseudohuddling) together, and active parenting behaviors (licking, non-huddling contact, and retrievals) together. For each set of behaviors we performed a MANOVA [30] with treatment, sex, timecourse, and a treatment by sex interaction as the fixed variables. Litter within pair was included as a random variable, but dropped when non-significant. All other variables were analyzed by mixed model ANOVA with fixed and random factors as detailed above; except for the swim test, which was performed on females only and therefore did not include sex as a fixed effect. Residuals were checked for normality and data transformed when necessary. When the overall ANOVA treatment effect was significant, post-hoc comparisons were carried out by least-squared means. All tests were two-tailed and significance was set to p = 0.05.

There was a significant effect of UCN II treatment on passive parental behavior (Wilk's Lambda = 0.89, $F_{2,73}$ = 4.65, p = 0.012; Figure 1), with UCN II-treated voles exhibiting increased passive parental behavior. No sex effects (Wilk's Lambda = 0.97, $F_{2,73}$ = 0.99, p = 0.376), timecourse effects (Wilk's Lambda = 0.94, $F_{2,73}$ = 2.36, p = 0.101), or sex by treatment effects (Wilk's Lambda = 0.95, F = 1.80, p = 0.172) were found.

Treatment with UCN II did not significantly affect active parental behavior (Wilk's Lambda = 0.99, $F_{3,72}$ = 0.31, p = 0.818; Figure 2). However, there was a significant effect of timecourse (Wilk's Lambda = 0.87, $F_{3,72}$ = 3.57, p = 0.018) and sex (Wilk's Lambda = 0.76, $F_{3,72}$ = 7.45, p < 0.001; Figure 3). The sex by treatment interaction was not significant (Wilk's Lambda = 0.98, $F_{3,72}$ = 0.40, p = 0.754).

These effects were specific to parenting behavior, as UCN II did not affect anxiety or locomotor behavior in either the elevated plus-maze or the swim test. In the plus-maze, the most common measure of anxiety is a ratio of time spent in the open arm divided by the sum of the time spent in the open arm plus the closed arm. For this measure, treatment (F_1 = 0.8, p = 0.374), sex

($F_1 = 0.47$, $p = 0.498$), and timecourse ($F_1 = 1.71$, $p = 0.197$) were not significant. Total arm entries (a measure of locomotor behavior), was also not significantly affected by treatment ($F_1 = 0.01$, $p = 0.923$). For CORT collected following the plus-maze test, the overall ANOVA showed statistical significance ($F_{86} = 8.31$, $p < 0.0001$, $r^2 = 0.288$), with significant effects of sex ($F_1 = 18.5$, $p < 0.0001$), timecourse ($F_1 = 6.94$, $p = 0.01$), and a marginal sex by timecourse interaction ($F_1 = 3.4$, $p = 0.069$). However, UCN II treatment did not significantly affect CORT ($F_1 = 0.01$, $p = 0.933$). In addition, no significant effects of treatment on anxiety or locomotor variables were found in the swim test. Time spent swimming or struggling and number of wastes produced during the test were not significantly predicted by treatment or timecourse.

Our study has provided the first evidence that UCN II increases spontaneous parental behavior in prairie voles. Contrary to our prediction, this effect was not sexually dimorphic. UCN II administration did not affect locomotor behaviors, including passive floating during the swim test and total arm entries in the plus-maze [31], thus suggesting that the effects on passive parental behavior were specific and not due to a sedative effect. Future tests of conditioned taste aversion would be useful in confirming that these changes were also not due to a noxious effect of the treatment. Interestingly, UCN II also influenced parental behavior without having any significant effects on stress-related behaviors or CORT secretion. CRH2 receptors are found in many areas known to play a role in parental behavior, particularly the olfactory system and the periaqueductal grey [32;33]. In addition, prairie voles have been found to differ from non-parental meadow voles in expression of CRH2 in several regions implicated in social behavior such as the nucleus accumbens [32] and the lateral septum, whose plasticity for CRH2 could be mediating individual differences. Because these brain regions play a role in parental behavior and have altered expression of CRH2, UCN II may be enhancing parental behavior directly without activating stress systems.

UCN II may also directly affect other neuroendocrine hormones known to influence parental behavior. For example, previous research has chronicled the effects of CRH2 on serotonin systems which may indirectly affect parental behavior [34;35]. Furthermore, UCN II may have an effect on dopamine concentrations in the nucleus accumbens, a phenomenon previously illustrated with CORT [36].

It is also possible that UCN II exerts its effects through changes in peptide hormones such as oxytocin (OT). Manipulation of OT has previously been shown to affect passive parental behaviors, while manipulation of the related hormone vasopressin (AVP) may primarily affect active parental behaviors [18;37;38]. Arima and Aguilera [39] have observed CRH2 transcripts in the supraoptic nucleus and the paraventricular nucleus (where OT and AVP are produced), and more importantly have colocalized CRH2 mRNA with AVP and OT mRNA in the supraoptic nucleus. These findings suggest that CRH2 and its respective ligands may be playing a role in the hypothalamo-neurohypophyseal system, and thus on parenting behavior.

Though our findings did not find significant direct effects of UCN II on stress hormones, UCN II did increase passive parental behavior which in turn itself reduces stress, at least in males of this species [19]. Ultimately, our findings implicate peripherally administered UCN II and CRH2 in passive parental behavior in the prairie vole. This effect is mediated without directly influencing CORT levels or anxiety. Though previous research has implicated CRH2 in anxiety and related stress endocrinology, we found that peripherally administered CRH2 had no effect on any of the stress measures we utilized. Our results support CRH2's role in parental behavior, with the exact mechanism of this phenomenon to be delineated in the future.

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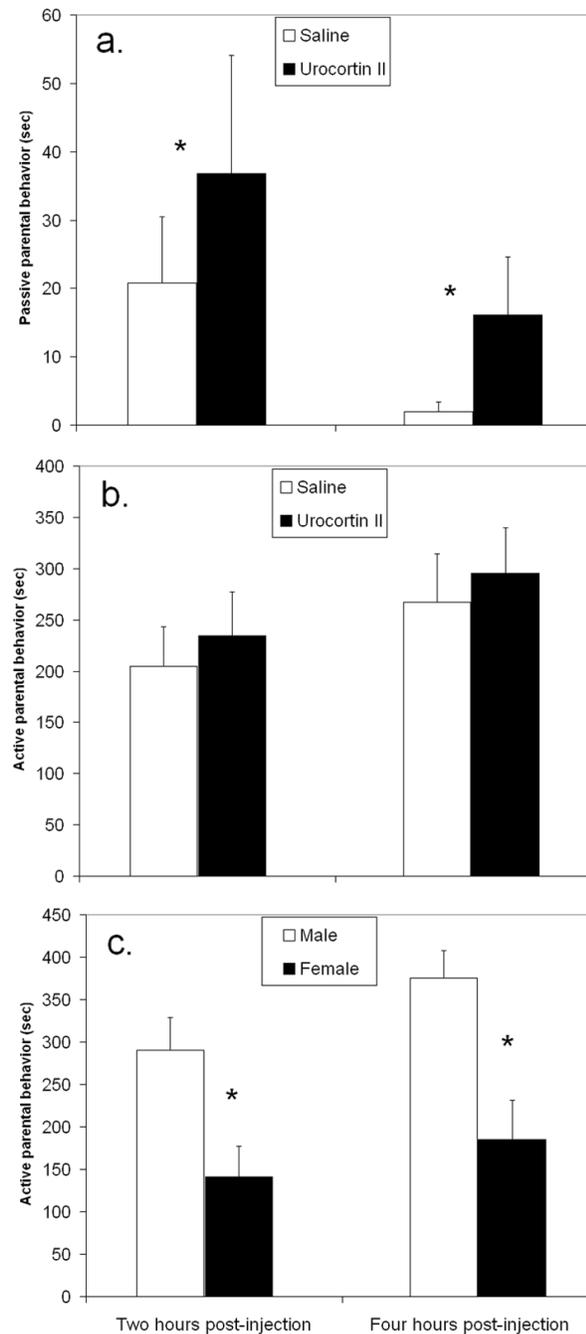
Figure 1.
a. Time spent in passive parental behavior (seconds, means ± standard errors) by treatment in the 2 hour (Urocortin II n = 20, Saline n = 20) and 4 hour (Urocortin II n = 21, Saline n = 18) timecourses. Treatment differences were statistically significant (Wilk's Lambda = 0.89, $F_{2,73}$ = 4.65, p = 0.012).
b. Time spent in active parental behavior (seconds, means ± standard errors) by treatment in the 2 hour (Urocortin II n = 20, Saline n = 20) and 4 hour (Urocortin II n = 21, Saline n = 18) timecourses. Treatment differences were not statistically different (Wilk's Lambda = 0.99, $F_{3,72}$ = 0.31, p = 0.818).

c. Time spent in active parental behavior (seconds, means ± standard errors) by sex in the 2 hour (Male n = 21, Female n = 19) and 4 hour (Male n = 20, Female n = 19) timecourses, combining both UCN II and Saline-treated animals. Males spent significantly more time in active parental behavior than females (Wilk's Lambda = 0.76, $F_{3,72}$ = 7.45, p < 0.001).