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Social Dominance in Male Vasopressin 1b Receptor Knockout Mice

Heather K. Caldwell^{1,*}, Obianuju E. Dike¹, Erica L. Stevenson¹, Kathryn Storck², and W. Scott Young 3rd²

¹Department of Biological Sciences and the School of Biomedical Sciences, Kent State University, Kent, OH 44242

²Section on Neural Gene Expression, NIMH, NIH, DHHS, Bethesda, MD 20892

Abstract

We have previously reported that mice with a targeted disruption of their vasopressin 1b receptor gene, *Avpr1b*, have mild impairments in social recognition and reduced aggression. The reductions in aggression are limited to social forms of aggression, i.e., maternal and inter-male aggression, while predatory aggression remains unaffected. To further clarify the role of the *Avpr1b* in the regulation of social behavior we first examined anxiety-like and depression-like behaviors in *Avpr1b* knockout (*Avpr1b* $-/-$) mice. We then went on to test the ability of *Avpr1b* $-/-$ mice to form dominance hierarchies. No major differences were found between *Avpr1b* $-/-$ and wildtype mice in anxiety-like behaviors, as measured using an elevated plus maze and an open field test, or depression-like behaviors, as measured using a forced swim test. In the social dominance study we found that *Avpr1b* $-/-$ mice are able to form dominance hierarchies, though in early hierarchy formation dominant *Avpr1b* $-/-$ mice display significantly more mounting behavior on Day 1 of testing compared to wildtype controls. Further, non-socially dominant *Avpr1b* $-/-$ mice spend less time engaged in attack behavior than wildtype controls. These findings suggest that while *Avpr1b* $-/-$ mice may be able to form dominance hierarchies they may employ alternate strategies.

Keywords

Avpr1b; social behavior; aggression; anxiety-like behavior; depression-like behavior

Introduction

Within the central nervous system vasopressin (Avp) acts as a neuromodulator that can influence social behaviors, such as aggression, across species (for review see Albers and Bamshad, 1998; Albers et al., 1992; Caldwell et al., 2008a; Caldwell and Young 3rd, 2006; Keverne and Curley, 2004; Young, 1999). Two Avp receptors mediate the central actions of Avp: the Avp 1a receptor (*Avpr1a*) and the Avp 1b receptor (*Avpr1b*). Previous behavioral and pharmacological work has focused primarily on the role of the *Avpr1a* in the modulation

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*To whom correspondence should be addressed: Heather K. Caldwell, Kent State University, PO Box 5190, 121 Cunningham Hall, Kent, OH 44242. hcaldwel@kent.edu.

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of aggression. In hamsters, there is ample evidence that the Avpr1a is important for aggression (Caldwell and Albers, 2004; Ferris et al., 2006; Ferris et al., 1997; Ferris and Potegal, 1988). In mice, however, while Avp is important for aggression, it is not clear that it acts via the Avpr1a (Bester-Meredith and Marler, 2001; Everts et al., 1997). For instance, Avpr1a null mutant mice are as aggressive as wildtype controls (Wersinger et al., 2007). While these findings do not rule out a role for the Avpr1a in mouse aggression, since there could have been developmental compensation, they do suggest that the Avpr1a may not be critical for Avp-mediated aggression. Rather, in mice it appears that the Avpr1b is necessary for normal aggression.

After the cloning (Lolait et al., 1995; Saito et al., 1995) and pharmacological characterization (Blanchard et al., 2005; Griebel et al., 2002) of the Avpr1b, Avpr1b knockout (Avpr1b $-/-$) mice were generated (Wersinger et al., 2006; Wersinger et al., 2002). Avpr1b $-/-$ mice have normal olfaction, impaired social recognition, reduced social motivation, and reduced social aggression (Caldwell and Young, 2009; Wersinger et al., 2006; Wersinger et al., 2002; Wersinger et al., 2004). Initial studies found that in both resident-intruder and neutral-cage paradigms, male Avpr1b $-/-$ mice show fewer attacks and longer attack latencies compared to controls (Wersinger et al., 2002). Avpr1b $-/-$ mice have normal defensive behavior in that while they will actively protect their bodies from harm, they will not, however, initiate “defensive attacks.” Aggression is also reduced in Avpr1b $-/-$ mice that are approximately 50% *Mus musculus castaneus*, as measured by longer attack latencies and fewer attacks in a resident-intruder test compared to wildtype littermates (Caldwell and Young, 2009). These deficits in aggression are not limited to inter-male aggression as female Avpr1b $-/-$ mice display reduced maternal aggression (Wersinger et al., 2006). Given that males do not display control levels of classic “attack” behavior in any of the previous reports, it might be assumed that these animals are simply incapable of displaying any “attack” behavior. However, when placed into a cage with a cricket, Avpr1b null mutant mice will attack the cricket just as quickly as wildtype controls (Wersinger et al., 2006). There is also an increase in “attack” behavior upon repeated aggression testing, when animals are food deprived, or when they are competing for food (Wersinger et al., 2006). Taken together these studies point to a deficit not necessarily in “attack” behavior per se, but rather a deficit in appropriately responding to social stimuli.

The purpose of the present study was to determine whether or not Avpr1b $-/-$ mice could form social hierarchies even though their social forms of aggression are impaired (Wersinger et al., 2006; Wersinger et al., 2002). To this end, we first examined anxiety- and depression-like behaviors in Avpr1b $-/-$ and Avpr1b $+/+$ mice to assess any underlying differences in their “mood” that might impact aggression. We then went on to examine the formation and maintenance of social hierarchies in same-genotype groups of male Avpr1b $-/-$ and $+/+$ mice. Based on the previous work in these mice, we predicted that Avpr1b $-/-$ mice would be less adept at forming a dominance hierarchy than Avpr1b $+/+$ mice.

Methods

Animals

All experimental animals were generated from the C57BL/6J-129 SvJ hybrid RW4 embryonic stem cell line (Soriano et al., 1991). All of the experimental subjects were littermates from Avpr1b heterozygous crosses. Animals were weaned at 21 days and housed in same sex sibling groups. One group of mice (n=15 $+/+$ and n=14 $-/-$) were tested for anxiety- and depression-like behaviors. A second group of mice (n=24 $+/+$ and n=20 $-/-$) were used for social hierarchy testing. During all phases of testing animals were housed on a 12:12 light-dark cycle and provided with rodent chow and water ad libitum. All studies were conducted with the approval of the National Institute of Mental Health Animal Care and Use Committee and followed the NIH guidelines for the Care and Use of Laboratory Animals.

Genotyping

Mice were genotyped by PCR using sense Avpr1b $+/+$ primer (5'-ACCTGTAGATATTTGACAGCCCGG-3'), sense Avpr1b $-/-$ primer (5'-ACCCCTTCCCAGCCTCTGAGCCCAGAAGCGAAGG-3'), and a common antisense primer (5'-GAAACGGCTACTCTCTCCGATTCCAAAAGAAAG-3'), yielding 763 bp and 461 bp PCR products, respectively, for wildtype and knockout mice. Reactions were run with purified DNA isolated from tail clips. Both reactions are run in the same tube under the following conditions: $95^{\circ}\text{C} \times 4\text{min}$, ($95^{\circ}\text{C} \times 1\text{min}$, $60^{\circ}\text{C} \times 1\text{min}$, $72^{\circ}\text{C} \times 1\text{min}$) $\times 40$ cycles, $72^{\circ}\text{C} \times 5\text{min}$.

Anxiety- and Depression-like Behaviors

General—Behavioral tests were spaced at least one week apart and completed in the following order: open field, elevated plus maze, and forced swim test. All tests were conducted as previously described (Caldwell et al., 2006; Wersinger et al., 2007). Both the open field and elevated plus maze tests were conducted during the dark phase of the light cycle. For all tests, animals (average age at time of testing (days $\pm$ SEM) = 96.40 ± 5.06 for Avpr1b $+/+$ mice and 91.00 ± 4.22 for Avpr1b $-/-$ mice) were brought to the testing room just prior to lights out and were acclimated to the testing room, that was under dim red light illumination, for at least 15 minutes prior to the start of behavioral testing. Groups were compared using a one-way analysis of variance (ANOVA) with a p-value less than 0.05 considered statistically significant.

Novel Open Field and Elevated Plus Maze: Anxiety-like Behavior—The open field arena was made of clear Plexiglas and measured $45.5 \times 45.5 \times 30$ cm and was illuminated at approximately 200 lux. The open field was divided into an inner and outer square, with the inner square measuring 32×32 cm. Mice were placed into a corner of the open field and allowed to explore for 10 minutes. The percentage of time spent in the inner versus outer squares, latency to enter the inner square and distance traveled were determined using the Noldus EthoVision® video tracking system (Noldus Information Technology, Leesburg, VA).

Mice were placed into the center of an elevated plus maze for mice constructed of non-porous plastic with the following dimensions: height from floor- 38.75 cm, width of arms- 5 cm, height of walls- 15 cm, and length of each arm- 30 cm (San Diego Instruments, San Diego, CA). During the 5-minute test the open arms were illuminated at approximately 100 lux; the closed arms had a recorded illumination of 10 lux. The percentage of time the mice spent in the open arms relative to the time spent in the open and closed arms, as well as the total number of entries into the open and closed arms were determined using the EthoVision® video tracking system. Both the open field and elevated plus mazes were cleaned with 70% ethanol between each animal and the observer was blind to the genotype of the animals during testing.

Forced Swim Test: Depression-like Behavior—The forced swim tests were conducted as previously described (Caldwell et al., 2006). Briefly, a 19 cm diameter tank was filled 2/3 of the way with 23°C water. The mouse was gently placed into the water tank and videotaped for 6 min. The last 3 minutes (min 4–6) of the test were scored for immobility or swim frequency with a determination made every 5 seconds by an observer blind to the animal's genotypes. This method of sampling reduces the observer error associated with the transitions and we and others have consistently used this method when measuring depression-like behavior in the forced swim test (Caldwell et al., 2006; Holmes et al., 2002; Lee et al., 2008).

Social Dominance

As indicated previously under the "Animals" section, Avpr1b $+/+$ and $-/-$ males were generated from the mating of heterozygous animals and siblings were used for this study. Groups were formed by housing non-sibling same genotype animals together. Individuals

within each group were weight-matched (average age at time of testing (days $\pm$ SEM) = 161.88 $\pm$ 7.54 for Avpr1b $+/+$ mice and 168.00 $\pm$ 8.71 for Avpr1b $-/-$ mice) and the animals housed four per a cage in standard shoe box cages (L: 28.75cm; W: 18.75 cm; H: 12.5 cm) to produce 6 groups of Avpr1b $+/+$ and 5 groups of Avpr1b $-/-$ mice. At the time of group housing, mice within each group were shaved with one of four possible patterns to allow observers to differentiate one animal from another within the cage.

Animals were housed in a 12:12 photoperiod with lights out at 1500 h. The first 20 minutes after initial group housing the mice were observed to assure that no animals were injured. Then, on the day of group housing (Day 1), Day 2, and Day 4, each group was videotaped for four 10-minute sessions a day at 1100, 1300, 1500, and 1700 h. This allowed for two observation sessions during “lights on” and two observation sessions during “lights off”. Each animal within a group was then scored using a continuous scoring method for the frequency and duration of attack behavior that included lunges and bites, as well as the durations of non-attack aggression (pushing/chasing), social behavior (active investigation of another animal’s rostral area, body, or ano-genital region), non-social behavior (feeding, sleeping, or exploring the cage), grooming behavior (self-grooming and grooming others), defensive/submissive behaviors (fleeing, freezing, and defensive postures), and mounting behavior (pelvic thrusting). As an additional measure the amount of time animals spent lying within 1cm of another animal (huddling behavior) was quantified. Huddling behavior was scored separately as it is a subdivision of non-social behavior and may represent an individual animals’ tendency to interact in a passive way with other animals.

Mice were assigned a social rank based on a dominance index, which was calculated by the sum of the frequency of offensive behavioral elements (non-attack aggression, and attacks) minus the sum frequency of the defensive behavioral elements (defensive/submissive behaviors) (similar to that described in Reber and Neumann, 2008). Mice that had the highest dominance index, were designated as “Rank 1” animals that had the next highest dominance index were designated as “Rank 2”, the next “Rank 3” and lastly “Rank 4.” For each cage that displayed both offensive and defensive behavioral elements only one Rank 1, dominant, animal emerged; in several instances, all of the other animals of the cage had the same dominance index and were thus assigned as Rank 4. Groups in which none of the individuals in the group displayed any offensive or defensive behavioral elements were assigned to the “No Rank” group. On Day 1, since group sizes within ranks 2–3 were small (Rank 2: N=3 Avpr1b $+/+$ and N=2 Avpr1b $-/-$; Rank 3: N=3 Avpr1b $+/+$ and N=2 Avpr1b $-/-$), these two groups were pooled to form the “Ranks 2 & 3” group, and constituted a non-socially dominant group. For Days 2 and 4, so few animals were ranked that Ranks 1–4 were pooled and compared to No Rank animals between the genotypes. Observers blind to the animals’ genotypes completed the scoring and the ranking.

The behavioral data collected for each day were summarized and the data for Day 1 were separately analyzed using an analysis of variance (ANOVA) comparing the main effects of genotype, rank, and any interaction. For Days 2 and 4, behavioral differences were analyzed using an ANOVA comparing the main effects of genotype, grouping, as well as their interaction. If statistically significant interactions were found a post hoc test was performed to determine where the differences were. For each day the percentage of groups for each genotype that formed a social hierarchy were compared using Fisher’s Exact Test of Probability, and the distribution of the animal’s ranks between the two genotypes were compared using a two-sample Kolmogorov-Smirnov Z test. Lastly, a rank stability measure was made by the determining the percentage of animals keeping the same rank from Day 1 to Day 2 and from Day 2 to Day 3, these percentages were compared using Fisher’s Exact Test of Probability.

Results

Anxiety- and Depression-like Behaviors

Novel Open Field, Elevated Plus Maze, and Forced Swim Test—In the novel open field there were no statistically significant genotypic differences in the percentage of time spent in the inner or outer square, or in the distance traveled. In the elevated plus maze there were no statistically significant genotypic differences in the percentage of time spent in the closed or open arms or entries into the closed arms. However, Avpr1b $-/-$ mice showed fewer overall entries into the open arms ($F_{(1,27)}=4.14$, $p=0.04$) compared to Avpr1b $+/+$ mice, suggesting a possible increase in anxiety-like behavior. There were no statistically significant genotypic differences in the forced swim test as measured by percentage of immobility time. These data are summarized in Table 1.

Social Dominance

Day 1—In the first 20 minutes of grouping no attacks were observed; therefore, all groups were included in the behavioral study. On Day 1 there were no statistical differences between the genotypes in the percentage of groups that contained individuals that could be ranked based on the dominance index: 50% (3 out of 6) of the Avpr1b $+/+$ groups compared to 60% (3 out of 5) of the Avpr1b $-/-$ groups. There were no statistical differences in the distribution across ranks between Avpr1b $+/+$ and Avpr1b $-/-$ mice (Figure 1A). There were also no statistical differences in the percentage of individuals that kept the same tank from Day 1 to Day 2 within each genotype (Avpr1b $+/+$: 29.2%, 7 out of 24; Avpr1b $-/-$: 25%, 5 out of 20). There was a genotype $\times$ rank interaction in the frequency of attack behavior ($F_{(3,43)}=5.87$, $p<0.01$), the duration of attack behavior ($F_{(3,43)}=3.79$, $p<0.02$) and the duration of mounting behavior ($F_{(3,43)}=3.15$, $p<0.04$). Ranks 2 & 3 Avpr1b $+/+$ mice had more attacks (Figure 2A) and longer durations of attack (Figure 2B) than Rank 2 & 3 Avpr1b $-/-$ mice, as well as all Rank 4 and all No Rank groups. Further, Rank 1 Avpr1b $-/-$ mice engaged in a longer duration of mounting behavior (Figure 2C) compared to Rank 1 Avpr1b $+/+$ mice, as well as all Rank 4 and all No Rank groups. There was a main effect of rank in the durations of non-attack aggression ($F_{(3,43)}=7.23$, $p<0.01$), non-social behaviors ($F_{(3,43)}=3.77$, $p<0.02$) and defensive/submissive behavior ($F_{(3,43)}=3.65$, $p<0.03$). Animals designated as Rank 1 spent more time engaged in non-attack aggression compared to Ranks 2 & 3 and No Rank individuals. Rank 1 animals also spent less time engaged in non-social behaviors compared to No Rank individuals. Lastly, Rank 4 animals spent more time engaged in defensive/submissive behaviors than Rank 1 or No Rank animals. The behavioral data for Day 1 are summarized in Table 2.

Day 2—On Day 2 there were no statistical differences between the genotypes in the percentage of groups that contained individuals that could be ranked based on the dominance index: 66% (4 out of 6) of the Avpr1b $+/+$ groups compared to 40% (2 out of 5) of the Avpr1b $-/-$ groups. There were no statistical differences in the distribution across ranks between Avpr1b $+/+$ and Avpr1b $-/-$ mice (Figure 1B). There were also no statistical differences in the percentage of individuals within each genotype that kept the same rank from Day 2 to Day 4 (Avpr1b $+/+$: 45.8%, 11 out of 24; Avpr1b $-/-$: 70%, 14 out of 20). There was a genotype by rank interaction in the duration of social behaviors ($F_{(1,43)}=7.30$, $p<0.01$), with Ranks 1–4 Avpr1b $-/-$ mice demonstrating longer durations of social behavior than Ranks 1–4 Avpr1b $+/+$ mice and No Rank Avpr1b $-/-$ mice. There was a main effect of rank on the duration of attack ($F_{(1,43)}=7.73$, $p<0.01$), defensive/submissive behaviors ($F_{(1,43)}=7.08$, $p<0.02$), non-attack aggression ($F_{(1,43)}=6.16$, $p<0.02$), duration of huddling ($F_{(1,43)}=12.51$, $p<0.01$), and the frequency of attack ($F_{(1,43)}=14.11$, $p<0.01$). Ranks 1–4 mice displayed more attacks and longer durations of attack behavior, non-attack aggression, and defensive/submissive behaviors compared to No Rank mice. Also, No Rank mice spent more time huddling than Ranks 1–4 mice. The behavioral data for Day 2 are summarized in Table 3.

Day 4—By Day 4, there were no statistical differences between the genotypes in the percentage of groups that contained individuals that could be ranked based on the dominance index, with 17% (1 out of 6) of the Avpr1b $+/+$ groups and 40% (2 out of 5) of the Avpr1b $-/-$ groups. There were no statistical differences in the distribution across ranks between Avpr1b $+/+$ and Avpr1b $-/-$ mice (Figure 1C). Genotypic comparisons were made between groups that contained individuals that were ranked and no ranked groups. There were genotype $\times$ rank interactions in the frequency of attacks ($F_{(1,40)} = 9.54$, $p < 0.04$), the durations of attacks ($F_{(1,40)} = 8.07$, $p < 0.01$), non-attack aggression ($F_{(1,40)} = 8.06$, $p < 0.01$), mounting ($F_{(1,40)} = 6.56$, $p < 0.01$), non-social behaviors ($F_{(1,40)} = 10.04$, $p < 0.01$), social behaviors ($F_{(1,40)} = 11.92$, $p < 0.04$), defensive/submissive behaviors ($F_{(1,40)} = 18.16$, $p < 0.01$), and huddling ($F_{(1,40)} = 7.85$, $p < 0.01$). For the measures of attack frequency, and the durations of attack, non-attack aggression, mounting and defensive/submissive behaviors Ranks 1–4 Avpr1b $+/+$ mice engaged in more frequent or longer durations of these behaviors compared to both No Rank Groups. Only in the measure of defensive/submissive behaviors did Ranks 1–4 Avpr1b $+/+$ differ significantly from Ranks 1–4 Avpr1b $-/-$ mice. Ranks 1–4 Avpr1b $-/-$ mice had shorter durations of non-social behaviors and huddling than No Rank Avpr1b $-/-$ mice, longer durations of social behaviors compared to Ranks 1–4 Avpr1b $+/+$ mice and No Rank Avpr1b $-/-$ mice. A summary of the data from Day 4 can be found in Table 4.

Discussion

The results from this study support our prediction that Avpr1b $-/-$ mice would be less successful at forming dominance hierarchies than Avpr1b $+/+$ mice. While Avpr1b $-/-$ mice are able to form dominance hierarchies, some of the behaviors that are employed as well as the intensity of the aggression used by some animals appears to differ between the genotypes. On Day 1, the behaviors of Rank 1, dominant, Avpr1b $-/-$ mice do not differ significantly from Rank 1 Avpr1b $+/+$ mice with the exception of one behavioral measurement, mounting behavior. Rank 1 Avpr1b $-/-$ mice displayed more mounting behavior in the initial stages of hierarchy formation, Day 1, than any other group, including Rank 1 Avpr1b $+/+$ mice. On Day 1, Ranks 2 & 3 Avpr1b $+/+$ mice attacked more frequently and had longer attack durations than Ranks 2 & 3 Avpr1b $-/-$ mice, and all Rank 4 and No Rank Groups. These data suggest that Avpr1b $-/-$ mice that are not socially dominant display less aggressive behavior than wildtype controls that are not socially dominant. On Days 2 and 4, Ranks 1–4 Avpr1b $-/-$ mice display longer durations of social behaviors than Ranks 1–4 Avpr1b $+/+$ mice and No Rank Avpr1b $-/-$ mice, though, on Day 4 Ranks 1–4 Avpr1b $-/-$ mice spent less time engaged in non-social behavior and huddling compared to some of the groups. Also on Day 4, Ranks 1–4 Avpr1b $+/+$ mice had greater attack frequencies and longer durations of attack, non-attack aggression, mounting and defensive/submissive behaviors than all No Rank groups, suggestive that the hierarchies within Avpr1b $+/+$ mice may have taken longer to stabilize. Though, in measures of rank stability from Day 1 to Day 2 and from Day 2 to Day 4 there were no statistically significant differences between the two genotypes.

The data from Day 1 Ranks 2 & 3 mice are consistent with previous reports of reduced levels of social aggression in male Avpr1b $-/-$ mice. However, as seen in measures of aggression in Day 1 Rank 1 animals, aggression in Avpr1b $-/-$ mice can be increased under specific social conditions. Avpr1b $-/-$ mice show increased aggression, as measured by the latency and frequency of attacks, following food deprivation, competition, and social experience (Wersinger et al., 2006). The aforementioned data suggest that the neural circuitry critical for displays of aggression is intact in Avpr1b $-/-$ mice and subject to modulation. The differences in aggression between Avpr1b $-/-$ and Avpr1b $+/+$ mice cannot be attributed to olfactory deficits as Avpr1b $-/-$ have normal olfaction. However, Avpr1b $-/-$ mice do not show a preference for female-soiled bedding over male-soiled bedding (Wersinger et al., 2006;

Wersinger et al., 2002; Wersinger et al., 2004); suggestive that *Avpr1b* $-/-$ mice do not respond appropriately to behavioral stimuli.

Of note is that the most robust deficits in aggression in *Avpr1b* $-/-$ mice have been elicited in tests that rely on isolation-induced aggression (Caldwell and Young, 2009; Wersinger et al., 2006; Wersinger et al., 2002). This is significant because isolation-induced aggression is thought to be due to social stress (Guidotti et al., 2001; Matsumoto et al., 2005). As the *Avpr1b* gene is important in regulation of the hypothalamic-pituitary-adrenal axis, as it is highly expressed in the anterior pituitary and synergizes the *Avp* signal with the corticotropin-releasing hormone (*Crh*) signal to stimulate adrenocorticotrophic hormone (ACTH) release (Antoni, 1993), it would be easy to speculate that any changes in aggression are caused by a differential response to stress. Consistent with this hypothesis, *Avpr1b* $-/-$ mice do not show the normal reduction in ACTH following repeated restraint stress but continue to show high levels of plasma ACTH (Lolait et al., 2007; Volpi et al., 2004). Following some, but not all, an acute stressors, *Avpr1b* $-/-$ mice also have an attenuated ACTH response (Lolait et al., 2007; Stewart et al., 2008; Tanoue et al., 2004). If differences in stress responsiveness contribute to reduced aggression in *Avpr1b* $-/-$ mice it might be predicted that these mice would show increased anxiety-like and depression-like behavior, as these mood changes are stress-related disorders. *Avpr1b* antagonists have been studied for their ability to reduce anxiety- and depression-like behaviors in rodents (for review see Griebel et al., 2005; Serradeil-Le Gal et al., 2005) and even the reduced aggression seen in Syrian hamsters treated with the selective *Avpr1b* antagonist SSR149415 is thought to be secondary to reduced anxiety (Blanchard et al., 2005). The findings of this study do not support the hypothesis that changes in mood are responsible for the reduced aggressive behavior often observed in *Avpr1b* $-/-$ mice. While *Avpr1b* $-/-$ mice may have a slight increase in anxiety-like behavior, as measured by the number of entries into the open arms of the EPM, this is the only measure of anxiety-like behavior in which there was a genotypic difference. Further, this is the first time that differences in this measure have been found between the genotypes (Wersinger et al., 2002). The findings of no genotypic differences in depression-like behavior are consistent with other studies using these mice (Caldwell et al., 2006; Wersinger et al., 2002). Despite what seems to be normal anxietylike and depression-like behavior, *Avpr1b* $-/-$ mice do not display the appropriate behavioral response to social stimuli, though they are able to perceive a threat on some level since there is a rise in plasma cortisol following exposure to another male (Wersinger et al., 2002).

As same-sex mounting behavior in mice often indicates an olfactory deficit, it may be that in *Avpr1b* $-/-$ mice the mounting behavior reflects their more global problem of inappropriate response to social stimuli. Alternatively, displays of mounting behavior could be interpreted as a form of social intimidation that provides an alternate strategy for dominance hierarchy formation. Support for this comes from a study of defensive behavioral strategies in mice that were under chronic social stress that observed less mounting behavior by subordinate mice to mice compared to dominant mice (Reber and Neumann, 2008). Nonetheless, mounting behavior has not been seen in previous aggression tests nor was much seen on Days 2 or 4 of the present study (Caldwell and Young, 2009; Wersinger et al., 2003; Wersinger et al., 2002). One of the advantages of the current study over previous work was that aggression could be monitored over long periods of time. Generally, in isolation-induced aggression tests, the time for observation is less than five-minutes. It would be interesting to examine groups of mixed genotype to see if mounting behavior is observed when *Avpr1b* $-/-$ mice are housed with *Avpr1b* $+/+$ mice.

One pressing question is which brain areas are involved in the increased mounting behavior observed in Rank 1 *Avpr1b* $-/-$ mice and the decreased aggressive behavior observed in Ranks 2 & 3 *Avpr1b* $-/-$ mice on Day 1. The distribution of the *Avpr1b* mRNA may provide some

insight into its role. Of particular note is its expression by pyramidal cells in the CA2 field of the hippocampus. Avpr1b in this area is not affected by either restraint stress or adrenalectomy (Young et al., 2006). While the CA2 field is not very well studied, it does receive inputs from the entorhinal cortex (Bartesaghi and Gessi, 2004), an area important for the processing social odors (Mayeaux and Johnston, 2004; Petrulis et al., 2005). It has been suggested that the presence of the Avpr1b in CA2 of hippocampus may help to retrieve olfactory memories of social encounters and aid in the coupling of these olfactory memories with the appropriate behavioral response (Caldwell et al., 2008b; Young et al., 2006). If the CA2 field of the hippocampus is important to the deficits we have observed in the Ranks 2 & 3 Avpr1b $-/-$ mice, then future studies focusing on this neuroanatomical region will be critical for furthering our understanding of the role of the Avpr1b in the regulation of aggression.

In summary, these data continue to support our hypothesis that the Avpr1b gene helps couple chemosensory input with social awareness and response. Our observations also provide further support for the hypothesis that the Avpr1b gene is necessary for displays of normal social behavior that are likely independent of any underlying anxiety-or depressive-like behaviors. Furthermore, we have shown that Avpr1b $-/-$ mice can form dominance hierarchies even though there are some qualitative differences in the behaviors employed.

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Figure 1.

There were no differences in rank distribution on Day 1 (A), Day 2 (B), or Day 4 (C) of testing between wildtype (*Avpr1b* $+/+$) mice and vasopressin 1b receptor knockout (*Avpr1b* $-/-$) mice. By Day 4 only one group of *Avpr1b* $+/+$ mice and two groups of *Avpr1b* $-/-$ contained individuals that displayed any dominance behavior. Data are presented as the number of individuals at each rank. Within each day, the data were analyzed using a Kolmogorov-Smirnov Z test.

Figure 2.

On Day 1, Ranks 2 & 3 vasopressin 1b receptor knockout (Avpr1b $-/-$) mice displayed fewer attacks (A) and a shorter duration of attack behavior (B) than Ranks 2 & 3 vasopressin 1b receptor wildtype (Avpr1b $+/+$) mice. Also on Day 1, Rank 1 Avpr1b $-/-$ mice engaged in more mounting behavior (C) than Rank 1 Avpr1b $+/+$ mice. Data are displayed as means $\pm$ SEM. * indicates $p < 0.05$.

Table 1
Anxiety- and Depression-like Behavior

Male vasopressin 1b receptor knockout (Avpr1b $-/-$) mice were similar to wildtype (Avpr1b $+/+$) littermates in the open field, elevated plus, and forced swim tests. Though, in the elevated plus maze male Avpr1b $-/-$ mice show fewer entries into the open arms compared to male Avpr1b $+/+$ mice, these data may reflect a slight increase in anxiety-like behavior in Avpr1b $-/-$ mice.

(mean$\pm$SEM)		
<u>Open Field</u>	$+/+$	$-/-$
Time in inner square (%)	23.4 $\pm$ 3.5	23.5 $\pm$ 4.4
Time in outer square (%)	76.6 $\pm$ 3.5	76.5 $\pm$ 4.4
Distance traveled (cm)	2763 $\pm$ 140	2720 $\pm$ 152
<u>Elevated Plus</u>		
Time in open arms (%)	18.7 $\pm$ 3.0	14.0 $\pm$ 1.6
Open arm entries (#)	19.2 $\pm$ 2.2	12.2$\pm$2.4*
Closed arm entries (#)	24.4 $\pm$ 1.2	20.1 $\pm$ 1.8
<u>Forced Swim</u>		
Immobility time (%)	43.4 $\pm$ 5.2	38.7 $\pm$ 7.4

* indicates that $p < 0.05$ compared to Avpr1b $+/+$ mice.

Table 2

Behavioral Data from Day 1

On Day 1 there was a genotype $\times$ rank interaction in the frequency of attack and the durations of attack and mounting. There was main effect of rank the durations of non-attack aggression behaviors, non-social behaviors, and defensive/submissive behaviors.

Behavior (mean $\pm$ SEM)	Genotype	Rank 1	Ranks 2 & 3	Rank 4	No Rank
Attack Frequency (#) *Interaction: Ranks 2 & 3 Avpr1b +/+ > Ranks 2 & 3 Avpr1b -/- & all Rank 4 and No Rank	+/+	2.33 $\pm$ 0.88 (n=3)	4.5 $\pm$ 1.23* (n=6)	0.33 $\pm$ 0.33 (n=4)	0.00 $\pm$ 0.00 (n=12)
	-/-	6.0 $\pm$ 3.21 (n=3)	0.50 $\pm$ 0.50 (n=4)	0.00 $\pm$ 0.00 (n=5)	0.00 $\pm$ 0.00 (n=8)
Attack Duration (sec) *Interaction: Ranks 2 & 3 Avpr1b +/+ > Rank 2 & 3 Avpr1b -/- & all Rank 4 and No Rank	+/+	19.27 $\pm$ 9.13	22.26 $\pm$ 7.63*	1.32 $\pm$ 1.32	0.00 $\pm$ 0.00
	-/-	8.51 $\pm$ 8.13	0.49 $\pm$ 0.49	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
Non-attack Aggression (sec) *Rank: Rank 1 > all others	+/+	11.86 $\pm$ 2.98	4.33 $\pm$ 4.13	0.42 $\pm$ 0.42	0.00 $\pm$ 0.00
	-/-	8.56 $\pm$ 6.46	0.99 $\pm$ 0.99	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
Mounting (sec) *Interaction: Rank 1 Avpr1b -/- > all others & Rank 1 Avpr1b +/+ > Ranks 2 & 3, Rank 4, and No Rank	+/+	18.86 $\pm$ 10.25	1.97 $\pm$ 1.60	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
	-/-	122.47 $\pm$ 93.58*	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
Non-Social Behaviors (sec) *Rank: No rank > Rank 1	+/+	1822.46 $\pm$ 32.91	1919.99 $\pm$ 41.08	1919.55 $\pm$ 68.35	1994.74 $\pm$ 60.08
	-/-	1605.18 $\pm$ 143.51	1902.38 $\pm$ 111.56	1878.29 $\pm$ 186.07	2091.13 $\pm$ 50.37
Social Behaviors (sec)	+/+	274.71 $\pm$ 101.99	153.99 $\pm$ 45.52	78.62 $\pm$ 31.39	153.37 $\pm$ 39.35
	-/-	331.03 $\pm$ 69.69	220.18 $\pm$ 7.97	194.05 $\pm$ 108.19	138.57 $\pm$ 29.49
Defensive/Submissive Behaviors (sec) *Rank: Rank 4 > Rank 1 and No Rank	+/+	5.36 $\pm$ 5.36	14.52 $\pm$ 4.67	45.43 $\pm$ 18.49	0.00 $\pm$ 0.00
	-/-	0.28 $\pm$ 0.28	42.34 $\pm$ 15.10	74.73 $\pm$ 57.65	0.00 $\pm$ 0.00
Grooming Behavior (sec)	+/+	259.34 $\pm$ 107.07	279.65 $\pm$ 77.83	354.63 $\pm$ 81.80	251.70 $\pm$ 46.21
	-/-	332.54 $\pm$ 30.78	234.62 $\pm$ 91.45	252.92 $\pm$ 71.84	170.30 $\pm$ 45.57
Huddling (sec)	+/+	955.35 $\pm$ 226.91	916.62 $\pm$ 177.93	119.39 $\pm$ 159.15	1069.54 $\pm$ 103.91
	-/-	840.63 $\pm$ 745.12	304.01 $\pm$ 108.20	1107.03 $\pm$ 415.11	648.35 $\pm$ 154.50

* Indicates that p<0.05

Table 3
Behavioral Data from Day 2

On Day 2 animals of Ranks 1–4 within each genotype were pooled and compared to No Rank. There was a genotype $\times$ group interactions in the duration of social behavior and a main effect of rank in measures of durations of attack, defensive/submissive behavior, non-attack aggression, huddling, and the frequency of attack.

Behavior	Genotype	Ranks 1–4	No Rank
Attack Frequency (#)	+/+	2.25 $\pm$ 0.62 (n=16)	0.00 $\pm$ 0.00 (n=8)
<i>*Rank: Ranks 1–4 > No Rank</i>	–/–	1.88 $\pm$ 0.74 (n=8)	0.00 $\pm$ 0.00 (n=12)
Attack Duration (sec)	+/+	7.81 $\pm$ 2.23	0.00 $\pm$ 0.00
<i>*Rank: Ranks 1–4 > No Rank</i>	–/–	15.80 $\pm$ 10.43	0.00 $\pm$ 0.00
Non-attack Aggression (sec)	+/+	28.82 $\pm$ 9.68	0.00 $\pm$ 0.00
<i>*Rank: Ranks 1–4 > No Rank</i>	–/–	10.25 $\pm$ 6.74	0.00 $\pm$ 0.00
Mounting (sec)	+/+	17.34 $\pm$ 7.08	0.00 $\pm$ 0.00
	–/–	3.01 $\pm$ 3.01	0.00 $\pm$ 0.00
Non-Social Behaviors (sec)	+/+	1524.45 $\pm$ 114.64	1474.64 $\pm$ 146.60
	–/–	1488.27 $\pm$ 129.91	1836.90 $\pm$ 106.44
Social Behaviors (sec)	–/–	206.28 $\pm$ 56.64	407.02 $\pm$ 149.20
<i>*Interaction: Ranks 1–4 Avpr1b –/– > Ranks 1–4 Avpr1b +/+ and No Rank Avpr1b –/–</i>	–/–	495.72$\pm$111.63*	181.26 $\pm$ 85.32
Defensive/Submissive Behaviors (sec)	+/+	38.74 $\pm$ 13.11	0.00 $\pm$ 0.00
<i>*Rank: Ranks 1–4 > No Rank</i>	–/–	21.25 $\pm$ 13.43	0.00 $\pm$ 0.00
Grooming Behavior (sec)	+/+	576.56 $\pm$ 85.13	518.34 $\pm$ 122.16
	–/–	365.70 $\pm$ 75.52	381.84 $\pm$ 63.98
Huddling (sec)	+/+	1266.43 $\pm$ 88.12	1529.55 $\pm$ 127.97
<i>*Rank: No Rank > Ranks 1–4</i>	–/–	835.99 $\pm$ 215.73	1534.63 $\pm$ 125.62

* Indicates that $p < 0.05$

Table 4
Behavioral Data from Day 4

On Day 2 animals of Ranks 1–4 within each genotype were pooled and compared to No Rank. There were genotype $\times$ rank interactions for all behavioral measurements, with the exception of grooming

Behavior	Genotype	Ranks 1–4	No Rank
Attack Frequency (#) <i>*Interaction: Ranks 1–4 Avpr1b +/+ > all No Rank groups</i>	+/+	1.25±0.75* (n=4)	0.00±0.00 (n=20)
	–/–	0.25±0.16 (n=8)	0.00±0.00 (n=12)
Attack Duration (sec) <i>*Interaction: Ranks 1–4 Avpr1b +/+ > all No Rank groups</i>	+/+	5.10±4.23*	0.00±0.00
	–/–	0.33±0.33	0.00±0.00
Non-attack Aggression (sec) <i>*Interaction: Ranks 1–4 Avpr1b +/+ > all No Rank groups</i>	+/+	15.91±12.88*	0.00±0.00
	–/–	1.40±1.09	0.00±0.00
Mounting (sec) <i>*Interaction: Ranks 1–4 Avpr1b +/+ > all No Rank groups</i>	+/+	0.80±0.80*	0.00±0.00
	–/–	0.00±0.00	0.00±0.00
Non-Social Behaviors (sec) <i>*Interaction: Ranks 1–4 Avpr1b –/– < Avpr1b –/– No Rank</i>	+/+	1951.02±97.40	1598.63±101.98
	–/–	1368.07±204.49*	2020.16±106.62
Social Behaviors (sec) <i>*Interaction: Ranks 1–4 Avpr1b –/– > Avpr1b –/– No Rank & Ranks 1–4 Avpr1b +/+</i>	+/+	72.08±13.96	280.22±61.17
	–/–	482.44±136.71*	73.72±17.81
Defensive/Submissive Behaviors (sec) <i>*Interaction: Ranks 1–4 Avpr1b +/+ > all other groups</i>	+/+	36.10±21.20*	0.00±0.00
	–/–	0.00±0.00	0.00±0.00
Grooming Behavior (sec)	+/+	319.01±68.20	521.15±67.98
	–/–	547.77±188.06	306.13±101.43
Huddling (sec) <i>*Interaction: Ranks 1–4 Avpr1b –/– < No Rank Avpr1b –/–</i>	+/+	1659.90±195.98	1791.46±75.35
	–/–	1031.62±241.11*	1964.90±65.66

* Indicates that $p < 0.05$