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Social motivation is reduced in vasopressin 1b receptor null mice despite normal performance in an olfactory discrimination task

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Abstract

In this study, we characterized more thoroughly the social behavior of vasopressin 1b receptor null (V1bR−/−) mice. We confirmed that V1bR−/− males exhibit less social aggression than their wild-type (V1bR+/+) littermates. We tested social preference by giving male subjects a choice between pairs of soiled or clean bedding. In general, V1bR+/+ mice spent significantly more time engaged in chemoinvestigation of these social stimuli than V1bR−/− mice. Male V1bR+/+ mice preferred female-soiled bedding over male-soiled bedding, male-soiled bedding over clean bedding, and female-soiled bedding over clean bedding. In contrast, V1bR−/− males failed to exhibit a preference for any bedding. This difference in behavior is not explained by an anosmic condition as there were no differences between V1bR−/− and V1bR+/+ mice in their abilities to detect a cookie buried in clean bedding, or in their ability to perform in an operant conditioning task using a fully automated liquid dilution olfactometer. In the latter task, male V1bR−/− mice were fully capable of discriminating between male and female mouse urine. The latencies to learn this task did not differ between the two genotypes. Thus, a V1bR−/− male's ability to differentiate between male and female chemosensory cues appears no different than that of a V1bR+/+ male's. We propose that the V1bR plays an important role in social motivation, perhaps by coupling the processing, integration, and/or interpretation of chemosensory cues with the appropriate behavioral response.

Keywords: Vasopressin V3 receptor; Behavior; Chemosensory; Aggression

Introduction

Appropriate social behavior is critical for the evolutionary success of an organism. Without the proper regulation of social behavior, an animal may miss reproductive opportunities and risk injury through inappropriate aggression. The case for vasopressin (VP) playing an important role in social behavior has been strengthening over the past three decades (Albers and Bamshad, 1998; Bass and Grober, 2001; Dantzer, 1998; Dantzer et al., 1987; Ferris et al., 1984; Goodson and Bass, 2001; Hammock and Young, 2002; Insel et al., 1998: Moore and Miller, 1983; Pitkow et al., 2001; Semsar et al., 2001). One type of social behavior, aggression, is affected by VP in a variety of species (Bester-Meredith and Marler, 2001; Coccaro et al., 1998; Compaan et al., 1993; Delville et al., 1996), although the mechanism by which it does so remains unclear.

Vasopressin is thought to exert its behavioral effects by acting through its two well-characterized brain receptors, the vasopressin 1a receptor (V1aR) and the vasopressin 1b receptor (V1bR, also known as the vasopressin 3 receptor,

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V3R). Although VP clearly affects social behavior, the individual roles of the two receptors are less clear despite some early studies that used pharmacological agents that had preferences for one receptor over the other (Albers et al., 1986). Recently, specific receptor knockouts and other genetic manipulations have helped delineate which effects of VP are mediated by the V1aR and/or the V1bR. The V1aR has been shown to be especially important in the regulation of social memory (Bielsky et al., 2004; Landgraf et al., 2003) and pair bonding (Young et al., 1999). Targeted disruption of the V1bR markedly reduces male–male territorial aggression and maternal aggression (Wersinger et al., 2002, 2003), which may be considered social forms of aggression. Although a compound that specifically targets the V1bR has behavioral effects (Griebel et al., 2003; Holmes et al., 2003; Serradeil-Le Gal et al., 2003), its effects on social behavior have yet to be reported.

The mechanism by which disruption of the V1bR gene results in reduced aggression is still unknown. It is possible that VP directly activates the neural circuitry involved in the display of aggressive behavior. VP may activate the neural circuitry involved in social communication. For example, the rapid effect of intracranial infusion of VP on the induction of flank marking in hamsters is consistent with a direct activation of this reflex (Bamshad and Albers, 1996; Ferris et al., 1984). VP may also work by modifying another neurotransmitter system (i.e., serotonin or dopamine systems) that then directly impacts aggression (Ferris et al., 1997). In the mouse, VP might affect the perception of chemosensory cues provided by another mouse, so that in the V1bR−/− mouse there is a failure to associate a particular trait or event to a social stimulus and thus affect the social meaning assigned to the stimulus. The V1bR−/− mouse may not perceive chemosensory cues from a male intruder as threatening and therefore behaves no differently than if the cues were from a sibling, female, or juvenile.

Predatory aggression is unaffected in V1bR−/− mice (Wersinger et al., 2003) indicating that the motor pathways associated with attack behavior are intact in these animals and can be activated. This observation also suggests that the deficit in aggressive behavior may be specific to social forms of aggression. An intact olfactory bulb seems important for the display of social aggression in mice (Denenberg et al., 1973; Rowe and Edwards, 1971). Bulbectomies may produce indirect effects on brain neurochemistry resulting in behavioral changes (Kelly et al., 1997). More recent experiments have used knockout models of the mouse Trp2 ion channel (Leypold et al., 2002; Stowers et al., 2002), which is found exclusively in the vomeronasal organ. These studies suggest that the absence of pheromonal signaling to the accessory olfactory bulb results in the complete absence of aggressive behavior in resident intruder paradigms (Leypold et al., 2002; Stowers et al., 2002). Despite the unproven hypothesis that the absence of aggression in Trp2−/− mice may be due to their inability to discriminate between male and female odors (Stowers et al., 2002), it is clear that olfactory function is critical for aggressive behavior in mice.

In our experiments, we sought to accomplish three goals. Our first goal was to reconfirm reduced aggression in V1bR−/− mice. Our second goal was to extend our characterization of social behavior in V1bR−/− mice to investigate preferences for chemosensory cues alone. Our final goal was to characterize more precisely the olfactory ability of V1bR−/− mice. We did this to determine whether altered preferences and decreased social aggression are the result of an impairment of olfaction or the inability to discriminate between male and female cues. In Experiment 1, we confirmed that aggression was reduced in V1bR−/− mice. In Experiment 2, we found that bedding preferences were altered in V1bR−/− mice. In Experiment 3, we showed that V1bR−/− mice were fully capable of discriminating male urine from female urine in an operant conditioning task.

Materials and methods

Subjects

The generation of the mutation has been described previously (Wersinger et al., 2002). All subjects were littermates of crosses using mice heterozygous for the mutation. The offspring were genotyped at weaning using PCR analysis of DNA isolated from tail clips. The parents of the subjects were a random mix of the C57/Bl6 and 129/6J strains. Males were group housed (three to four per cage) after weaning until used in the studies. Different groups of mice were used in each experiment. All experiments were conducted in accordance with the NIH guidelines for the care and use of animals.

Experiment 1

Aggression test

Aggression tests were repeated using the methodology reported in Wersinger et al. (2002), with two modifications: the age of the subjects (30 days older here) and the stimulus animal (BalbC here vs. 129SV before). All mice (n = 10 V1bR+/+ and n = 10 V1bR−/−) were singly housed on a 12:12-h light–dark cycle (lights on at 0600 h, EST) with food and water available ad libitum for at least 3 weeks before testing. The subjects were 90–120 days of age. Weight-matched, adult, gonad-intact male BalbC mice were used as stimulus animals. The subjects' home cages were not changed for at least 3 days before testing. Testing took place in the dark phase of the light cycle (between 1800 and 2400 h) under red light illumination using a modified resident–intruder paradigm. A stimulus male was added to the mouse's home cage. If no aggression was observed in the first 5 min, a latency of 300 s was recorded

and the test ended. Each subject received up to three separate tests, each separated by 48 h. Thus, a subject that never attacked received a latency score of 900s. Otherwise, the test was allowed to continue for 2 min after aggression was first observed. All interactions were videotaped using a Sony night shot digital video camera (Sony model DRV120) for analysis since many of the behaviors occur very quickly. The latency to attack, number of attacks, and number of tail rattles were recorded.

Experiment 2

Bedding preference

A new group of singly housed, socially experienced males were used to test for bedding preference. We chose to use bedding because it is easily controlled and contains many of the general sensory cues associated with the entire animal. The subjects were isolated for 14 days before testing. Social experience was given over the first 5 days of isolation. An adult, randomly cycling female was placed in each subject's home cage for 30 s, then removed. One minute later, an adult male was placed in each subject's home cage for 30 s. Experience was given during the dark phase of the light cycle (between 1800 and 2000 h). To test preference, males (n = 10 V1bR+/+ and n = 10 V1bR−/−) were placed into a clean Plexiglas™ testing cage (80 × 40 × 20 cm) with two small glass jars (7.5 cm diameter × 5.0 cm tall) full of bedding (*Care*FRESH, International Absorbents Inc., Bellingham, WA) placed in the left-front and right-front corners of the cage. The placement of the bedding was arbitrarily chosen and was not the same for all the subjects. The glass jars were fitted with a mesh lid so that the mice could not dig in the bedding. There was no bedding on the floor of the testing cage. Testing took place in the dark phase of the light cycle (between 1800 and 2400 h) under red light illumination. Mice were observed for 10 min. The number of approaches to and time spent investigating each bedding stimulus was recorded. The subjects were given three preference tests with a week in between each test. The choices were given to each subject in the same order: (1) female-soiled bedding vs. male-soiled bedding; (2) female-soiled bedding vs. clean bedding; and (3) male-soiled bedding vs. clean bedding. Soiled bedding was collected immediately before testing from cages containing four cycling females (female-soiled bedding) or three adult males (male-soiled bedding) that had not been cleaned for 3 days. Preference was measured by calculating the percent of total investigation time spent at each stimulus.

Hidden cookie test

Male mice (n = 8 V1bR+/+ and n = 8 V1bR−/− mice) were food-deprived overnight. A small cube (5 mm on each side) of Nutter Butter™ cookie (Nabisco) was buried beneath about 4 cm of clean woodchip bedding in a random location. The mouse was placed in the cage, again in a random location. The latency to locate the cookie was recorded. We defined finding the cookie as when the mouse held it in both paws.

Experiment 3

Olfactometer

Mice were trained and tested in a fully automated liquid dilution olfactometer (Knosys Instruments, Bethesda, MD) (Bodyak and Slotnick, 1999). Briefly, the apparatus consisted of a 20 × 15 × 13 cm Plexiglas™ box (operant chamber) with a glass tube affixed to the outside of one wall providing a port for odor delivery, exhaust, and water reward. Odors were delivered through the bottom of the glass tube and exhausted through the top with the aid of an exhaust fan. Water reinforcement was delivered through a stainless steel tube on the far side of the glass tube. A ventilation fan affixed to the wall opposite the glass tube served to continuously blow room air into the operant chamber. This provided a steady stream of fresh air for the animal and prevented test odors from leaking out of the delivery tube and into the operant chamber. All training and testing procedures were controlled, monitored, and recorded by means of a personal computer using software written in QBASIC.

Training for the operant task

Mice were trained using a go, no-go discrete trials operant conditioning procedure (Bodyak and Slotnick, 1999; Kelliher et al., 2003). An animal was trained to insert its nose into the odor delivery port, stay there until the odor was delivered, and then begin licking at the water reinforcement tube. The animal was rewarded with 5 μl of water for licking after the test odor (S+) was delivered. No water was dispensed after the control odor (S−) was delivered. During these experiments, an animal was given no more than one testing session a day. Each session was divided into blocks, a block consisting of 10 trials with the S+ odor and 10 trials with the S− odor. Licking for the S+ odor (hit) or not responding to the S− odor (correct rejection) was scored as correct choices. Not responding during an S+ trial (miss) or responding during an S− trial (false alarm) were scored as errors. At the end of each block, the number of correct choices out of 20 possible was recorded as a percentage of correct trials, yielding the performance accuracy.

Odor stimuli

Male and female urine were collected from C57/Bl6 mice using a metabolic chamber. We chose to use urine as the stimulus for two reasons. First, urine works very well in the olfactometer. Second, urine is likely to contain olfactory cues that mice use to assess socially relevant characteristics. We reasoned that if the animals could distinguish male urine from female urine, they would be able to discriminate bedding containing urine. Urine was pooled from 10 to 15 same sex mice over a single day and frozen in aliquots of 0.5 ml. On the day of testing, 0.5 ml of frozen urine was

diluted in 49.5 ml of distilled water with an end concentration of 1:100 being used for all detection and discrimination experiments. Urine odors in the saturation flasks were replaced every day. Odor concentration in this study refers to the concentration in the liquid phase of the flask—the exact odor concentrations at the odor delivery port is not known.

Detection and discrimination

V1bR−/− and V1bR+/+ male mice were first trained to discriminate between female urine (S+) and distilled water (S−). After animals were successfully trained on the apparatus, all mice were tested on three separate discrimination tasks. Mice were run in single 10 block (200 trials) sessions to examine whether they could distinguish among the following: female urine (S+) vs. distilled water (S−), female urine (S+) vs. male urine (S−), and, as a control for unsuspected cues, female urine (S+) vs. female urine (S−). In each of the tasks, the mean percentage of correct trials for the last three blocks (60 trials) was used to report performance accuracy. Latencies to perform were also recorded. An animal was considered able to perform a given task after two consecutive blocks with a 90% or greater performance accuracy.

Statistics

Group means in the aggression and hidden cookie tests were compared using a Mann–Whitney *U* test. Group means for the bedding preference data were compared using a two-way ANOVA followed by a Bonferroni post hoc analysis. Performance accuracy and latency were compared using a one-way ANOVA.

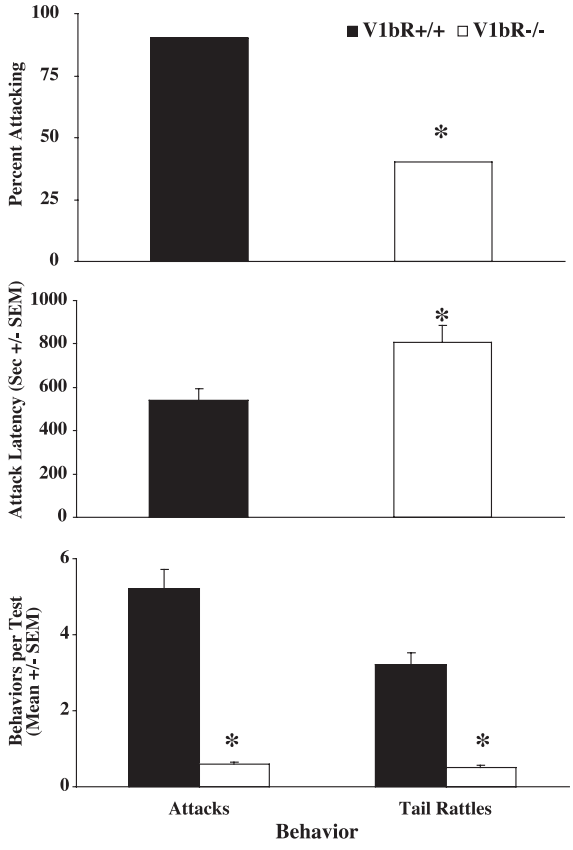

Fig. 1. V1bR−/− (n = 10) male mice are less aggressive than V1bR+/+ (n = 10) littermates in a resident–intruder paradigm. A significantly lower percentage of V1bR−/− mice displayed any aggressive behavior than V1bR+/+ mice (*P < 0.05; top panel). The mean latency (seconds ± SEM) to attack was significantly longer in V1bR−/− than V1bR+/+ mice (*P < 0.05; middle panel). The mean number (±SEM) of attacks per test and tail rattles per test was significantly lower in V1bR−/− than V1bR+/+ mice (*P < 0.05; bottom panel).

Results

Experiment 1

Aggression

As previously reported (Wersinger et al., 2002), a significantly lower percentage of V1bR−/− males displayed any attacks than V1bR+/+ males (40% vs. 90%; P < 0.05; Fig. 1, top panel). V1bR+/+ mice had a significantly shorter attack latency than V1bR−/− mice (540 ± 68 vs. 806 ± 49 s; U = 16.00, P < 0.01; Fig. 1, middle panel). In addition, V1bR+/+ males had a significantly greater number of attacks (5.2 ± 1.1 vs. 0.6 ± 0.3 attacks per test; U = 9.00, P < 0.01) and tail rattles (3.2 ± .3 vs. 0.6 ± 0.2 tail rattles per test; U = 1.50, P < 0.01; Fig. 1, bottom panel).

Experiment 2

Bedding preference

Male- vs. female-soiled bedding (Fig. 2, top panel). There were significant effects of bedding and genotype and an interaction on the time spent investigating (x-axis, left) the stimuli [F(1,36) = 55.61, F(1,36) = 12.99, F(1,36) = 11.37, respectively; P < 0.01 for all effects] (Fig. 2). As expected, V1bR+/+ males spent a significantly greater percentage of investigation time at the female-soiled bedding than at the male-soiled bedding (t = 4.93, P < 0.015). V1bR−/− males, however, spent a similar percentage of investigation time at the female-soiled bedding and the male-soiled bedding. V1bR+/+ males spent significantly more time investigating the stimuli than V1bR−/− males (U = 0.00, P < 0.01). There were no effects of bedding or genotype, or an interaction on the number of approaches (x-axis, right) to the bedding stimuli.

Male soiled vs. clean (Fig. 2, middle panel). There were significant effects of bedding and genotype, and an interaction on the time spent investigating the stimuli [F(1,36) = 64.18, F(1,36) = 45.53, F(1,36) = 42.88, respectively; P < 0.01 for all effects]. V1bR+/+ males spent a significantly greater percentage of investigation time at the male-soiled bedding than at the clean bedding (t = 9.40, P <

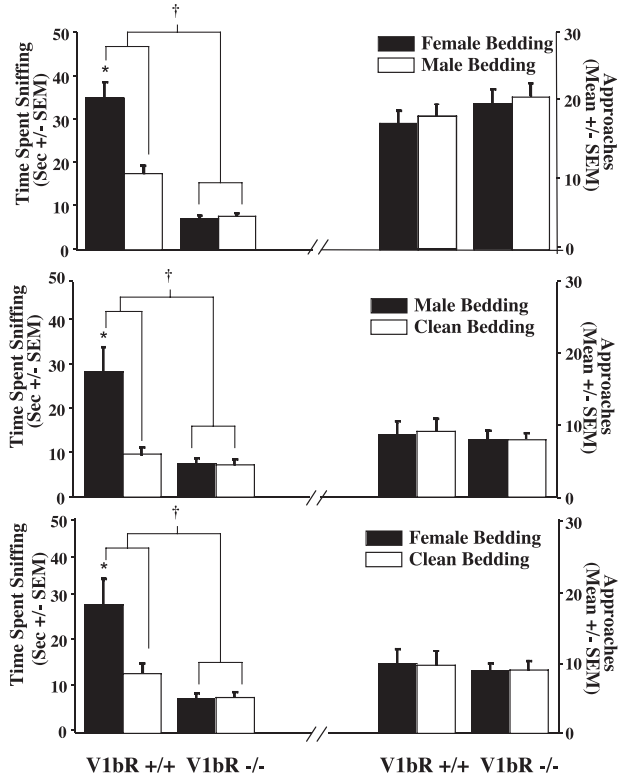

Fig. 2. Chemoinvestigatory behavior of soiled bedding by male V1bR+/+ (n = 10) and V1bR−/− (n = 10) mice. Amount of time investigating (seconds ± SEM) (left side of panels) and number of approaches (±SEM) (right side of panels) to female-soiled bedding vs. male-soiled bedding (top panel), male-soiled bedding vs. clean bedding (middle panel), or female-soiled bedding vs. clean bedding (bottom panel) are shown. On all tests, male V1bR+/+ mice spent significantly greater amount of time investigating bedding compared to V1bR−/− mice ($^{\dagger}P < 0.01$). Only male V1bR+/+ mice showed preferences for one stimulus over the other ($^{*}P < 0.01$).

0.01). V1bR−/− males, however, spent a similar percentage of investigation time at the male-soiled bedding and the clean bedding. V1bR+/+ males spent significantly more time investigating the stimuli than V1bR−/− males (U = 0.00, $P < 0.01$). There were no effects of bedding or genotype, or an interaction on the number of approaches (x-axis, right) to the bedding stimuli.

Female soiled vs. clean bedding (Fig. 2, bottom panel). There were significant effects of bedding and genotype, and an interaction on the time spent investigating (x-axis, left) the stimuli [$F(1,36)$ = 14.33, $F(1,36)$ = 4.83, $F(1,36)$ = 4.95, respectively; $P < 0.01$ for all effects]. V1bR+/+ males spent a significantly greater percentage of investigation time at the female-soiled bedding than at the clean bedding (t = 3.13, $P < 0.01$). V1bR−/− males, however, spent a similar percentage of investigation time at the female-soiled bedding and the clean bedding. V1bR+/+ males spent significantly more time investigating the stimuli than V1bR−/− males (U = 9.50, $P < 0.01$). There were no effects of bedding or genotype, or an interaction on the number of approaches (x-axis, right) to the bedding stimuli.

Hidden cookie. There was no significant difference in the latency to find the hidden cookie between V1bR+/+ and V1bR−/− males (17.5 ± 3.1s vs. 20.3 ± 2.4s).

Experiment 3

Olfactometer

V1bR−/− and V1bR+/+ male mice were capable of detecting female urine in an operant training paradigm. Mice were considered to have learned the task after performing at 90% accuracy for two consecutive blocks. V1bR−/− and wild-type males had similar mean performance accuracies. Latencies to detect were also comparable between the V1bR−/− and V1bR+/+ mice, respectively. When tasked to discriminate between female and male urine, both V1bR−/− and V1bR+/+ mice were capable of discriminating between these two stimuli. V1bR−/− males had a mean performance accuracy of 95 ± 3.9% whereas V1bR+/+ males had a performance accuracy of 96 ± 4.6% (Fig. 3, top panel). Latencies for this discrimination task were also similar between V1bR−/− and V1bR+/+ mice blocks (Fig. 3, bottom panel). There were no

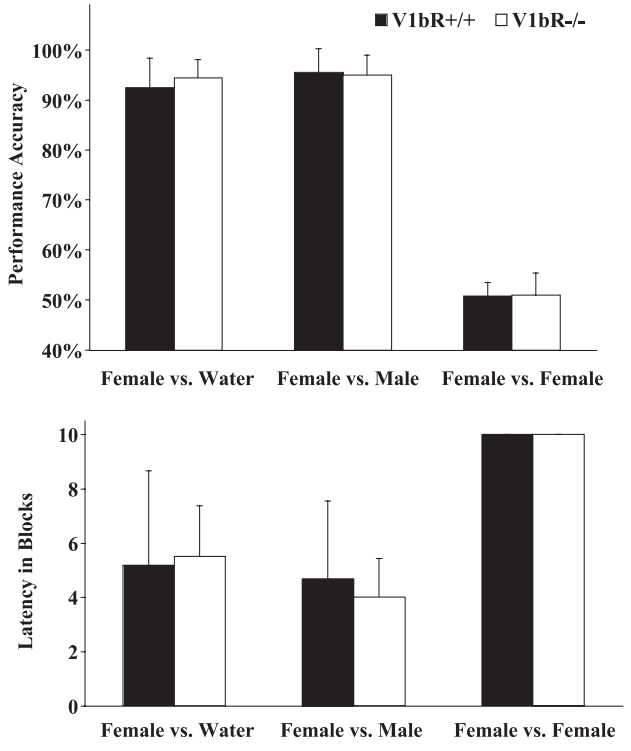

Fig. 3. V1bR−/− (n = 8) mice detect and discriminate between male and female urine in an operant testing paradigm as well as their V1bR+/+ (n = 8) littermates. In the top panel, performance accuracies (the percent of mean correct choices over the last three blocks of a ten block session) for both V1bR−/− (open bars), and V1bR+/+ (closed bars) mice were well above 90% for urine detection (female vs. water) and urine discrimination (female vs. male) tasks and at chance levels when both S+ and S− odors were from the same source (female vs. female). Note that the ordinate begins at 40%. In the bottom panel, latencies (mean number of blocks before the criteria of two consecutive 90% blocks had been met) were similar for both V1bR−/− and V1bR+/+ mice for detection, discrimination, and control tasks.

genotypic differences in latencies. To show that animals were in fact using odor cues alone to detect and/or discriminate stimuli, we tested whether any mice were capable of performing in the operant chamber when both S+ and S− odors were the same. Neither V1bR−/− nor V1bR+/+ male mice were able to discriminate between two batches of the same olfactory stimuli (Fig. 3), confirming that odor alone was the sensory cue being used for performance in these tasks.

Discussion

Our results further illustrate the social deficits of V1bR−/− mice. Although V1bR−/− mice can detect and discriminate between male urine and female urine, they do not show preferences for female-soiled bedding over male-soiled bedding. Thus, they do not respond to social cues appropriately. We therefore propose that motivation to investigate odors associated with social stimuli is markedly decreased or absent in V1bR−/− mice.

There are several explanations for our observation that V1bR−/− males do not investigate soiled bedding as robustly as V1bR+/+ mice. First, it is possible that that the mice are unable to extract and assign meaning to the relevant social cues from the stimulus. Another explanation is that V1bR−/− animals are not motivated by social cues. A simple anosmia would explain our results. However, the olfactometer data clearly demonstrate that the V1bR−/− subjects can detect and distinguish male urine from female urine. Thus, the lack of interest in soiled bedding does not reflect a major olfactory deficit. A previous study has shown that chemosensory cues from a conspecific male induces Fos-like immunoreactivity in the brains of V1bR−/− in a pattern no different than that in V1bR+/+ mice (Wersinger et al., 2002). The data from the olfactometer greatly strengthen these data. The olfactometer relies on a purely physiological motivation (thirst). Thus, we can separate the ability of the animal to distinguish male- and female-associated chemosensory cues from social motivation. This is not possible using preference tests.

V1bR+/+ mice prefer cues from female mice over male mice, a result consistent with previous reports (Mossman and Drickamer, 1996; Terranova et al., 2000; Wersinger and Rissman, 2000). Likewise, the V1bR+/+ mice are motivated to investigate social cues (either male- or female-soiled bedding) over bedding with no social cues. The V1bR−/− mice fail to show this social motivation. That is, they show reduced chemoinvestigatory behavior and no preference for any of the social stimuli. This is unlikely to be a result of reduced activity or a strict neophobia for two reasons: there were no deficits in open-field activity in V1bR−/− mice (Wersinger et al., 2002) and the number of approaches to the bedding stimuli was the same in V1bR−/− mice as in V1bR+/+ mice.

Given these and previous results, we propose that the V1bR gene is important for the display of social behavior that relies on olfactory input. V1bR−/− mice show deficits in social behaviors that rely on olfactory input, including aggression, social recognition (Wersinger et al., 2002), and bedding preference (present results). By contrast, sexual behavior appears normal (Wersinger et al., 2002). This is consistent with the observation that disruption of olfactory input tends to have mild or no effect on sexual behavior in the mouse (Bean, 1982; Champlin, 1977; Rowe and Smith, 1973). We propose that vasopressin and the V1bR act downstream from the detection process and have a cognitive rather than a sensory role.

These data suggest that the deficit exhibited by mice lacking a functional V1bR is specific to social motivation and does not reflect a global problem. We are confident that there is not a general "amotivational" syndrome present in the mice since V1bR−/− mice mate, search for a hidden cookie when they are food-deprived and perform the discrimination task. They are capable of associating an olfactory cue with a reward when it is not related to social motivation. It is likely that the processing of social chemosensory cues and the motivation underlying behaviors exhibited in response to these cues are utilizing completely different systems than the classical operant olfactory detection and learning paradigm needed for performing on the olfactometer. Our results would suggest that vasopressin, acting through the V1bR, is needed for processing of social cues and/or the normal behavioral response to these cues.

Of interest is that the social deficits exhibited by V1bR−/− null mice have parallels to those exhibited by patients suffering from autism (Baird et al., 2003; Tidmarsh and Volkmar, 2003; Tuchman, 2003). Neither autistic patients nor V1bR−/− mice respond appropriately to social cues. Autistic individuals, like the V1bR−/− mice, exhibit sexual motivation, although the degree to which it is or is not fully normal has yet to be completely described (Konstantareas and Lunsky, 1997; Realmuto and Ruble, 1999; Ruble and Dalrymple, 1993; Van Bourgondien et al., 1997). Further evaluation of impulsivity in V1bR−/− mice will help determine the appropriateness of this mouse as a model for human autism.

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