Research report

Lateral septal vasopressin in rats: role in social and object recognition?

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Abstract

The capacity of male rats to remember familiar conspecifics is called social recognition. It is a form of short-term memory modulated by lateral septal LS vasopressin VP. The specificity of this phenomenon was studied by examining whether recognition of previously investigated objects is also under control of lateral septal VP. For social recognition male Wistar rats were confronted with juveniles for 5 min. Re-exposure to the same juvenile took place after 30 or 120 min, or with a different juvenile after 30 min. This procedure was duplicated for object recognition using a plastic food cup or a 50 ml Erlenmeyer flask. After these initial tests osmotic minipumps and brain cannulae were implanted, infusing VP receptor antagonist into the LS dPTyr(Et)AVP, 1 ng/0.5 μl/h, bilateral. Animals were re-tested for social and object recognition using 30 min re-test interval (same juvenile or object). We reproduced previous reports concerning social recognition; animals recognized juveniles after 30 min, not after 120 min and VP antagonist treatment blocked recognition. Testing for object recognition revealed a reduction in investigation time at the 30 min interval (same and different object), but not after 120 min. VP antagonist treatment was unable to block object recognition. The data suggest that, in contrast to social recognition, object recognition reflects a form of habituation, which is not under the control of lateral septal VP.

Keywords: Vasopressin; Lateral septum; Social recognition; Object recognition; V1-receptor; Habituation; Rat

1. Introduction

The research on social recognition and underlying mechanisms started in 1982 when Thor and Holloway proposed a test of social memory [40]. The test is based on the tendency of adult male rats to spend a great amount of time investigating novel juveniles. The test uses juveniles to exclude confounding effects of aggression and sexual behavior. When exposed to the same juvenile for a second time shortly after a first exposure, but within 1 h, a sharp drop in investigatory behavior will occur. This drop in behavior is absent when the adult is exposed to a different juvenile or when the interexposure time exceeds 1–2 h. This time related social investigation of conspecifics is considered to be an ethological model for short-term social memory [11,18,40].

Substantial research has been performed since to gain insight in the underlying mechanisms of social recognition. It has been shown that in the intact male rat vasopressin (VP) is the key modulator of this behavior [6,12,15,26,27,33]. Studies revealed that peripheral and central application of VP or related substances improves social memory. Application of VP receptor antagonists reduced the performance of adult rats to recognize previously met juveniles [4]. Several studies have implicated the lateral septum (LS) as a causally involved brain area [12,15,26], although very recently it has been shown that also the ventral and dorsal hippocampus is involved in social memory [42]. The VP containing fibers in the LS originate from the bed nucleus of stria terminalis (BNST) and the medial amygdala [8,9,13,14]. The released VP exerts its action through the postsynaptically located V1 receptor [29,38,41].

Next to the role of lateral septal VP in social recognition it is implicated in a diversity of behavioral and physiological functions. Since long it has been shown to play a role in learning and memory, in particular in avoidance behavior [7,24,43]. It has further been demonstrated to mediate antipyresis [10], hibernation [20,21], and, also relevant in a social context, septo-hypothalamic VP is involved in flank and scent marking [2,16,17]. More recently, it was shown to regulate paternal behavior in microtine rodents [3,44,45], reconfirming the differential functions of lateral septal VP.
In view of this variety of functions of lateral septal VP, one may question the specificity of VP in social recognition. The observed effects in a social recognition test may be due for example to more general effects on investigatory behavior. The present experiment will therefore measure object recognition in a paradigm identical to social recognition and it will test the effects of a VP receptor antagonist in the LS on both paradigms, thus answering two main questions. First, is the rat’s performance, as seen in juvenile recognition, confined to a social context or is it a general feature of investigatory behavior? Second, if so, does lateral septal VP play a role in object recognition as it does in social recognition?

2. Materials and methods

2.1. Animals

The subjects were male Wistar rats obtained from our own breeding facilities, weighing approx. 350 g at the beginning of the experiment. Initially, animals were housed in perspex cages in groups of five to eight animals. Two days prior to testing they were individually housed in standard cages (20 × 30 × 15 cm) with free access to water and lab chow on a 12:12 h light-dark cycle (lights off at 8.30 h), at a temperature of 19±2°C. Housing and all behavioral testing took place in the same room under red and dim light conditions.

2.2. Social recognition

Ten animals were tested for social recognition under basal, untreated condition. Male juveniles, 25–30 days old and housed in groups of six were used as social stimuli. The juveniles were kept individually 2 h prior to testing. All adult rats (n = 10) were tested in their home cage and exposed to three different settings of the test (4 days between each test session, semi-randomly applied). An initial 5 min exposure to a juvenile was followed by a second exposure to the same juvenile after 30 or 120 min, or a second exposure to a different juvenile (only after 30 min). Juveniles were kept individually between both exposures. All social encounters were videotaped with no experimenter present in the room during the test. Social investigatory behavior was scored by a trained observer, unaware of the test settings. Behavior directed towards the juvenile mostly consisted of anogenital sniffing, close following, and pawing. The amount of time animals spent on these behaviors (investigation time) gives a measure of social recognition or memory.

2.3. Object recognition

For the object related equivalent of social recognition we developed an experimental design comparable to the social paradigm in both time course and test settings. Two different objects were used instead of juveniles. A hole-board food cup made of gray PVC (depth and diameter: 4 cm) was used in the settings where the same object would be presented (after 30 and 120 min). A transparent Erlenmeyer, comparable in size, was used when a different object had to be presented to the rat (as in social recognition, only after 30 min). The objects were thoroughly cleaned with 70% alcohol 1 h before each test. Ten male rats (not tested in the social paradigm) were used for measuring object recognition. The tests were videotaped and analyzed as mentioned before. In a pilot experiment we made an inventory of the most important behaviors. In behavior directed towards the object we distinguished sniffing as an important component, next to object dragging, pushing and gnawing. Times spent on these behaviors were summed in the parameter object investigation.

2.4. Surgery

Twenty-four animals received bilateral brain cannulae aimed for the LS septum for infusing a VP antagonist using osmotic minipumps. This method was used to reduce stress of handling to a minimum during behavioral testing. Briefly, rats were halothane-anaesthetized and placed in a stereotaxic device. They received two 25 gauge stainless steel guide cannulae (implantation coordinates: 0.2 mm posterior to bregma, each 1.0 mm medio-lateral, depth of 2.2 mm from dura, extending 2.8 mm above dura [30]). These guides were secured to the skull with three stainless steel screws and dental cement. After the cement was hardened a short wire (diameter 0.12 mm) was inserted into the guides to close them and bone wax was used to cover the cannulae. The incision was closed and rats were allowed to recover. Before receiving the injector cannulae attached to osmotic minipumps all animals could recover for at least 6 days. One day before they obtained the actual cannulae, osmotic minipumps (Alzet, model 2002, 0.5 µL/h) were filled with either saline or the VP antagonist dP(Tyr(Et))AVP in a concentration of 2 ng/µL. A 10 cm length of polyethylene tubing was filled with the corresponding fluid and attached to the minipump. An air bubble was created at the free end of the tubing. The pumps were placed in small jars filled with saline with tubings extending above the fluid level, at 37°C overnight. In this way the pumps could reach a stable pumping rate and we could check the air bubble to see if the pumps functioned well. The rats were halothane-anaesthetized and received two minipumps placed subcutaneously. The tubings were length-adjusted and attached to the injector cannulae (0.15 mm inner diameter), which could easily be placed into the guide cannulae after removal of the bone wax and steel wire. This procedure ensured bilateral placement of the cannula tips into the lateral septum, 4.6 mm below the dura mater. The assembly was secured to the skull with dental cement and the incisions were closed.
again. Thirteen animals were infused with dPTyr(El)AVP, eleven with saline. All animals were allowed 7 days of recovery before behavioral testing.

2.5. Behavioral tests

After implantation of the cannulae the animals were tested for both object and social recognition. First, all rats were tested for object recognition. Animals were allowed 5 min to investigate the object followed by a second exposure to the same object 30 min later. After 1 day of rest all rats were tested for social recognition using the same paradigm (two exposures with 30 min interexposure time). Again, behavior of the animals was videotaped and analyzed afterwards. Before animals were sacrificed for brain histology they were used in additional behavioral testing, to be described elsewhere.

2.6. Histology

Two weeks after minipump implantation all animals were transcardially perfused with 50 ml of saline followed by 200 ml of 4% paraformaldehyde (pH = 7.4, 0.01 M phosphate buffer). The cannulae assembly was carefully removed from the skull and checked for irregularities. Brains were dissected and placed in 30% sucrose overnight at room temperature. Cryostat sectioning (30 μm) was followed by light microscopic examination of cannula placement.

2.7. Statistics

Results are expressed as means ± S.E.M. The data for untreated behavioral testing were analyzed using two-way ANOVA with repeated measures (2 exposures × 3 test settings, within-subject factors). Data obtained from the minipump implanted animals were submitted to one-way ANOVA with repeated measures (2 treatments × 2 exposures, between- and within-subject factors, respectively). Individual interval and treatment comparisons were made using post-hoc paired sample T-tests.

3. Results

During behavioral testing of the untreated animals one rat showed severe aggression towards the juvenile and was discarded from statistical analysis. Brain examination of the cannulated rats revealed improper placement of the cannulae in three animals. The minipump tubing had been obstructed in two animals during the experiment. Incomplete video registration during behavioral testing further reduced the number of saline- and VP antagonist-treated animals to eight and nine respectively.

3.1. Untreated testing

Results from the social recognition paradigm are summarized in Fig. 1. In all three test settings (30 and 120 min with the same juvenile and 30 min with a different juvenile) the adults spent about 180 s investigating (mainly anogenital sniffing) the juvenile during the first exposure. When confronted with the same juvenile (sj) 30 min later, a reduction in investigation time of about 40 s was found, suggesting that the adult recognized the juvenile as familiar. As expected, a 120 min delay between both exposures did not result in a decrease of investigation time. Also, no effect was found when a different juvenile (dj) was presented after 30 min. Statistical testing showed a significant effect of exposure, $F_{1,8} = 6.49, P = 0.034$, and test setting, $F_{2,16} = 6.49, P < 0.01$. No significant interaction effect was found (exposure × test setting, $F_{2,16} = 2.78$). Additional testing (post-hoc paired T-test) confirmed a decrease in investigation time only when the same juvenile is presented after 30 min (two-tailed, $P < 0.01$).

Behavior observed during object recognition mainly consisted of sniffing and manipulating the object. In parallel with social recognition we included all object-directed behavior in the parameter investigation. Results obtained for this parameter are illustrated in Fig. 2. During the first exposure the animals spent an equal amount of time investigating the object as was found in the social context. Initially, approx. 180 s were spent sniffing and manipulating the object in all three test settings (first exposure). On presenting the same object (so) a second time after 30 min there is a 40 s decrease in object investigation time (Fig. 2, Fig. 1. Results of social recognition in untreated, male rats. Each bar represents the mean (+ S.E.M.) duration of investigation of the juvenile on the first (left panel) and second exposure (right panel). The second exposure was carried out 30 or 120 min after the initial exposure introducing the same juvenile (sj) or a novel, different juvenile (dj). Re-exposure to the same juvenile after 30 min resulted in a significant decrease in investigatory behavior (+ $P < 0.01$ compared with first exposure).
object in untreated, male rats. Each bar represents the mean (+ S.E.M.) duration of investigation of the object on the first (left panel) and second exposure (right panel). Re-exposure was carried out 30 or 120 min after the initial exposure by placing the same object (so) or a different object (do) in the home cage. Re-exposure to the same or a different object after 30 min both resulted in a significant decrease in investigatory behavior (**P < 0.05 compared with first exposure).

so, second exposure). However, the same effect was observed when confronted with a different object (do) after 30 min. Using an interval of 120 min slightly reduced investigatory behavior. Indeed, ANOVA revealed a significant effect of exposure, F1,9 = 7.39, P = 0.024, but not of test setting, F2,18 = 0.29, or their interaction, F2,18 = 1.86. Post-hoc T-testing showed significant differences for presenting the same or a different object after 30 min (P = 0.011 and P = 0.021 respectively).

Additional statistical testing of the composing behaviors of object investigation revealed that the observed differences were mainly caused by significant differences in sniffing behavior (not illustrated here). Although considerable more time is spent on manipulating the object, ANOVA testing revealed no significance due to larger variability in these data. About two-thirds of investigating time is spent on gnawing, and to a lesser extent on dragging and some pushing.

3.2. Treated testing

After being implanted with brain cannulae animals were re-tested for social and object recognition using two successive exposures to the same juvenile or object with a 30 min interval. Data for both behavioral paradigms are combined in Fig. 3. Social investigation is illustrated in the left panel and shows comparable levels (±180 s) for both saline- and antagonist-treated animals during the first exposure. During the second exposure the saline-treated rats decreased their amount of time investigating the juvenile to approx. 140 s. Instead, animals treated with VP antagonist increased their investigatory behavior compared to the first exposure to just above 200 s. Analysis of variance showed a significant effect of treatment, F1,15 = 8.92, P < 0.01, but no effect of exposure due to the diverging reactions of both treatment groups. The interaction (treatment × exposure) proved highly significant again, F1,15 = 14.99, P < 0.01, emphasizing the different reactions during the second exposure. Post-hoc T-testing confirmed the decrease and increase in investigation observed in respectively the saline- and antagonist-treated group (P < 0.05, two-tailed).

The data obtained for object recognition, illustrated in Fig. 3, showed a marked difference compared to the data for social recognition. During the first exposure saline-treated animals tended to spend more time investigating the object than antagonist-treated rats, although not significantly different. However, on the second exposure both treatment groups showed a decrease in behavior directed towards the object of approx. 40 s. Statistical analysis showed an effect of exposure, F1,15 = 23.32, P < 0.01, but no effect of both treatment and interaction between treatment and exposure. Post-hoc analysis proved that both treatment groups significantly decreased (P < 0.01) their time investigating the object after a 30 min interval. Performing ANOVAs for sniffing and manipulating proved again to be a significant factor only for exposure (F1,16 = 4.92, P = 0.04 and F1,15 = 7.85, P = 0.01 respectively). Post-hoc testing failed to reach significant levels.

When an animal is not directing its attention towards the juvenile or object it will spend most of its time explor-
ing its home cage. Therefore, data obtained for exploration should practically be the mirror image of the investigation data. Indeed, for social recognition, statistical testing revealed a significant effect of treatment and treatment × exposure \( (F_{1,15} = 8.49, P = 0.011; F_{1,15} = 13.32, P < 0.01) \). Post-hoc testing confirmed the significant increase and decrease in explorative behavior for saline- and antagonist-treated rats respectively. Exposure proved to be the only significant factor during object recognition \( (F_{1,18} = 4.77, P = 0.043) \). The saline-treated group exhibited a significant increase \( (P < 0.01) \).

Other behaviors which were scarcely observed are grooming, scanning the environment, immobility behavior, and even some burying of the object. Sometimes, upon presenting the juvenile, an animal became more aroused during the 5 min session, but no pattern emerged over successive observations. None of these behaviors revealed a significant effect over all test settings.

4. Discussion

As expected \([1,11,12,32,42]\), VP antagonism was able to effectively block social recognition of a previously exposed juvenile. In object recognition VP antagonism had no effect and thus seems to be independent of lateral septal VP receptors. However, it cannot be concluded, from the present results, that the lateral septum itself is not involved in object recognition. Myhrer \([28]\) showed that lesioning of this area reduces the animal’s preference to investigate a novel object compared to controls, probably due to insufficient processing of sensory information. It has also been shown that disruption of large parts of the septum \([34]\) results in an impairment of exploratory activity and habituation when displacing an object. These studies allow no conclusions to be drawn on the role of VP or its receptors in object investigation. Furthermore, the experimental designs of these studies make it difficult to compare them with the present study.

One may question to what extent the two tests measure the same phenomenon of familiarity or recognition. Clearly, both tests involve some form of short-term learning and memory processes. In both paradigms untreated animals showed a time related behavioral change. Exposure to the familiar juvenile or object within 30 min of the first exposure proved to reduce investigatory behavior. A 2 h delay between exposures gave no differences between first and second exposure in both test settings. However, there was a distinct difference between both paradigms when presenting an unfamiliar juvenile or object after 30 min. There is no reduction in investigation when confronted with a different juvenile, but introducing a different object did cause a reduction in investigation time. From this result it may be argued that two different forms of memory function were observed. The results obtained for juvenile investigation clearly match data obtained from previous studies \([5,11,18,36,42]\). The presence (or absence) of reduction in behavior reflects the recognition of a familiar conspecific and this has been interpreted as a form of social memory. Somewhat surprisingly, the reduction in object directed behavior seemed to be independent of the object used during the first exposure. This result might be explained in terms of habituation, a relatively simple form of memory function. Previous studies involving habituation to newly introduced novel objects reported increase in object exploration \([28,35,39]\). In most of these experiments, however, behavioral testing was performed in either a large, Plexiglas cage or an open field or hole board, but never in the animal’s home cage. Furthermore, animals were allowed to divide their attention to both novel and known objects. In the present experiment every test was performed in the home cage with only the novel object present. Using this procedure resulted in a clear distinction between behavioral reaction to either a different juvenile or an object.

It has been found that olfactory cues are a major component in social recognition, since the juvenile can also be replaced by presentation of soiled bedding or urine on the first exposure \([5,11,18,31,36]\). Johnston et al. \([22]\) have studied the nature of the scents which hamsters use for individual discrimination and recognition. These animals were shown to discriminate between individual volatile chemical scents when using urine, feces and flank gland secretions. In rats, however, social recognition depends mainly on licking and closely following of the anogenital area of the juvenile. The lack of sniffing at distance suggests that rats use non-volatile chemical cues for recognition, sensed by the vomeronasal organ \([5,11,18,31]\). Sensory information is routed via the accessory olfactory bulbs and amygdala to the BNST \([37]\), where the lateral septal VP fibers originate \([14]\) and mediate social recognition. The objects used in the present experiment are devoid of almost any (species specific) smell and therefore lack the characteristics of most olfactory cues. Thus, it is tempting to suggest that object recognition is not under the control of the neural network implicated in social recognition. This assumption is strengthened by the data which show that object recognition is not mediated by lateral septal VP receptors \([14]\). If there is a role of object odor in this paradigm, it might be sensed through the main olfactory system.

When the results from both paradigms are compared it is important to notice that there is a lively interaction between juvenile and adult rat. Part of the behavior of the resident will be influenced by the reactions of the juvenile. In object recognition, of course, there is no response from the object. This, in combination with the lack of olfactory cues, may explain the rapid habituation during the presentation of a novel object at the second exposure. It remains to be established whether the time course of memory processes in object recognition are different from that of social recognition. Dependent on the species, social
memory lasts for approx. 1 h [11,18]. Object recognition, at least in the present paradigm, might have a shorter time course. However, it would be a challenge to be able to discern this fact from habituation processes.

The method used in this study to chronically infuse test solutions into the lateral septum did not interfere with the ability to perform in the recognition paradigms. In both antagonist-treated and untreated sessions rats spent about 180 s investigating the juvenile or object (compare Figs. 1 and 2 with Fig. 3). Bluté and Dantzer [4] found a decrease in performance on the first exposure after implantation of a pumping device in the lateral ventricle. This decrease may have been caused by disturbance of cerebroventricular flow and ventricular lining [19]. They reported a partial blockade of social recognition after chronic antagonist treatment whereas in the present study a complete blockade was found. However, their rats had received the antagonist (i.c.v.) for 3–4 weeks before behavioral testing commenced. We applied VP antagonist locally and started testing after 7 days. This may explain the small differences in results obtained in the two studies.

Most studies investigating the role of VP and its antagonists on social recognition use acute injections of fluids (peripheral and i.c.v.) right after the first exposure [6,12,23,27,31,32,42]. The group of Landgraf and Engelmann utilizes new techniques (microdialysis and antisense oligonucleotides) to study the role of VP in social recognition [15,25,26]. As in the present study, their VP related substances are applied before the first exposure. Still, the effects are comparable with results from the acute experiments. These results strengthen the idea that, in the intact male rat, lateral septal VP is not necessary for the induction of investigatory behavior itself. It does play an important role in the storage and retrieval of social relevant information. This role of VP fits well to its assumed role in the dorsal and ventral hippocampus on memory processes [1,7,24,42,43].

References

[26] R. Landgraf, R. Gersberber, A. Moutkowski, J.C. Probst, C.J. Wotjak, F. Holsboer, M. Engelmann, V1 vasopressin receptor antisense oligodeoxynucleotide into septum reduces vasopressin bind-


