Central Neuropeptides Social Recognition, Social Preference and Social Fear in Rodents

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Abstract

Highly conserved brain neuropeptides, such as oxytocin (OT), vasopressin (AVP) and the newly described neuropeptide S, have the potential to regulate elementary social abilities, i.e. social approach and social preference, social fear as well as individual social memory. These social skills are essential for functioning social communication among conspecifics. To provide evidence for neuropeptide-dependent social abilities we established and further developed, respectively, various behavioral assays like the social recognition/social discrimination, the social preference and the social fear conditioning paradigms. The involvement of neuropeptides in these behaviors is investigated using central pharmacological manipulation and in vivo microdialysis.

Introduction

Besides of the general interest in social behavior regulation by neuropeptides, there is substantial interest in the regulation of abnormal sociability accompanying various psychopathologies including depression, bipolar disease, autism or anxiety disorders. To reveal the neurobiological underpinnings and to further understand the involvement of OT and AVP also from a translational perspective in human sociability, appropriate animal models are essential.

Here, we will describe our efforts to establish and employ rodent models of social behaviors resembling human behaviors including the social recognition (social discrimination paradigm), a test for social approach, as well as an experimental setup to test for social fear using a social fear conditioning paradigm in rats and mice where appropriate.

Methods and Paradigms

Social discrimination. The ability of adult male rats and mice to discriminate between a previously encountered (same) and a novel con-specific was tested in the social discrimination test according to Engelmann et al. [1] with some important modifications [2]. The social stimulus, i.e. a male juvenile, was introduced into the home cage of the experimental male rat or mouse for 4 min (acquisition period). After a defined inter-exposure interval (consolidation period), the same stimulus was re-introduced along with a novel conspecific for 4 min (retrieval period). The percentage of time investigating the same and the novel stimulus animal during the retrieval period was calculated. A lower investigation time directed towards the same versus the novel stimulus was interpreted as social recognition and memory.

Social preference. As recently described [3], adult male rats or mice were placed in a novel arena allowing a 30- sec habituation period. Then, a small empty wire-mesh cage (object stimulus) was placed at one side wall of the arena for 4 min (rats) or 2.5 min (mice). The empty cage was then exchanged by an identical cage containing an unknown male con-specific (social stimulus) for additional 4 min (rats) or 2.5 min (mice). Before each trial, the arena was cleaned with water containing a low concentration of detergent. In rats, all tests took place during the active phase starting one hour after lights off using an unknown male rat as social stimulus. Mice were tested in the early light phase (one hour after lights on) using an unknown male mouse as social stimulus.

Social fear conditioning. As described in great detail recently [4], social fear can be characterized using a novel social fear conditioning paradigm for mice. For social fear c*onditioning (day 1),* mice were placed in the

conditioning chamber and, after a 30-s adaptation period, an empty wire mesh cage was placed as a non-social stimulus near one of the short walls. The mice were allowed to investigate the non-social stimulus for 3 min, before it was replaced by an identical cage containing an unfamiliar male mouse. Unconditioned mice were allowed to investigate the social stimulus for 3 min. Conditioned mice were given a 1-s electric foot shock (0.7 mA, pulsed current) each time they investigated the social stimulus, defined by direct contact with the mouse. The time mice spent investigating the non-social stimulus served as a pre-conditioning measure of non-social anxiety. *Extinction (day 2 or 15).* To investigate whether conditioned mice displayed social fear and whether this fear could be extinguished, social investigation was assessed in the home cage 1 or 15 days after social fear conditioning for short-term and long-term social fear, respectively. *Extinction recall (day 3 or 16).* To investigate whether repeated exposure to social stimuli during extinction leads to a complete reversal of social fear, social investigation was assessed in the home cage 1 day after extinction. Extinction recall consisted of exposing the mice to six unfamiliar social stimuli for 3 min, with a 3-min inter-exposure interval.

Pharmacological manipulation. In order to manipulate the respective neuropeptide system, neuropeptide synthetic agonists or antagonists were infused either icv or directly into a brain target region via previously implanted guide cannulas (2 mm above the right lateral ventricle or bilaterally above the region of interest). Drugs were infused mostly 10 to 20 min before behavioral testing.

Animals received either an icv or a bilateral infusion into the region of interest of either synthetic OT/AVP (exogenous stimulation; rats: 0.01-0.1 µg), a selective receptor antagonist (blockade of endogenous release; rats: 0.1-0.75 µg, mice: 2-20µg), or vehicle via an infusion cannula.

Intracerebral microdialysis. For measurement of intracerebral oxytocin or vasopressin release during ongoing behavioral testing in the social discrimination paradigm we implanted a microdialysis probe stereotaxically in the respective target region (lateral septum). Two days later, five consecutive 30-min dialysates (3.3 µl/min, Ringer, pH 7.4) were collected during the social discrimination procedure: dialysates 1 and 2 were taken under baseline (undisturbed) conditions, dialysate 3 during the social memory acquisition period starting with the 4-min exposure to the first 3-week-old rat, and dialysates 4 and 5 were taken thereafter. Microdialysates were collected and vasopressin/oxytocin content was quantified using a highly sensitive radioimmunoassay.

Involvement of central neuropeptides in social behaviors

We provide further evidence for the a prominent role of OT and AVP in basic social behaviors, i.e. social approach and social recognition in male rats and mice, that are prerequisites for diverse social behaviors like aggression, sexual behavior, and pair bonding. Furthermore, we present a new model for measuring conditioned social fear in mice.

Naturally, male rats and mice demonstrate a preference for social stimuli over non-social stimuli. We demonstrated that the social preference behavior in both rats and mice is dependent on the endogenous OT system. Thereby, we provided first evidence for the involvement of endogenous OT in social approach behavior and affiliation towards a same-sex social stimulus in rodents, which is thereby not linked to reproductive behavior. We also demonstrated that social stressors, like acute social defeat, significantly impair social preference. Social defeat caused social avoidance in male rats when tested in the social preference paradigm, and synthetic OT was able to reverse such social deficits in rats, but could not further elevate social preference behavior in normal, undefeated rats.

Concerning the involvement of OT and AVP in rodent social cognition, we demonstrated that brain OT is required for the consolidation of social memory in male rats and mice. We demonstrate that OT receptor activation in the lateral septum is needed for social memory, whereas OT receptor activation in the medial amygdala is important for social memory for adult females only. This suggests a key role for the medial amygdala in mediating social memory depending on the biological relevance of the social stimulus.

Endogenous AVP release into the lateral septum of male rats was measured, accompanying social memory acquisition during social discrimination. This not only supports the involvement of septal AVP in male social

recognition, but also provides evidence that septal AVP release can also be triggered during a non-aggressive encounter.

We also measured AVP release within the lateral septum of postnatally stressed male rats (maternal separation) during social recognition. We found a blunted AVP release in response to the initial social stimulus during social recognition in these postnatally stressed rats. Control experiments, using non-social stimulation, proved that this blunted release was specifically related to the social stimulation. Finally, the impairment in social recognition was causally linked to the blunted AVP release during social memory acquisition, as administration of synthetic AVP enhanced social recognition abilities of stressed rats to the level of unstressed rats. These findings strongly imply a link between a deficient central AVP signaling and social cognition deficits in rats.

Neuropeptide S tested in the social discrimination and social preference paradigms had no effects on social behavior.

Using the social fear conditioning paradigm we can show that by administering electric foot shocks during the investigation of a con-specific (social fear conditioning), social investigation can be severely reduced for both short- and long-term periods. In contrast, other behaviors such as fear of novelty, general anxiety, depressive-like behavior, or locomotion were found unchanged, which is indicative of specific social fear as a result of social fear conditioning. Both diazepam and paroxetine were able to reverse social fear thus validating the model.

Thus, our models of social preference/social avoidance, and of social fear conditioning provide unique animal models for increasing our understanding of normal and dysfunctional social behaviours, in particular of social fear as element of social anxiety disorders and for developing and testing novel treatment options.

References

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