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Neuropeptide S interacts with the basolateral amygdala noradrenergic system in facilitating object recognition memory consolidation

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ABSTRACT

The noradrenergic activity in the basolateral amygdala (BLA) was reported to be involved in the regulation of object recognition memory. As the BLA expresses high density of receptors for Neuropeptide S (NPS), we investigated whether the BLA is involved in mediating NPS's effects on object recognition memory consolidation and whether such effects require noradrenergic activity. Intracerebroventricular infusion of NPS (1 nmol) post training facilitated 24-h memory in a mouse novel object recognition task. The memory-enhancing effect of NPS could be blocked by the β-adrenoceptor antagonist propranolol. Furthermore, post-training intra-BLA infusions of NPS (0.5 nmol/side) improved 24-h memory for objects, which was impaired by co-administration of propranolol (0.5 μg/side). Taken together, these results indicate that NPS interacts with the BLA noradrenergic system in improving object recognition memory during consolidation.

1. Introduction

Neuropeptide S (NPS) is a recently identified neuromodulator that selectively binds and activates Gs and Gq protein-coupled receptors NPSR (Reinscheid et al., 2005; Xu et al., 2004). According to the wide distribution of NPSR in the brain of rodents (Clark et al., 2011; Leonard & Ring, 2011; Xu, Gall, Jackson, Civelli, & Reinscheid, 2007), NPS/NPSR system is demonstrated to regulate multiple central functions, including wakefulness, stress and anxiety, locomotion, drug abuse, gastrointestinal functions, nociception and food intake (for a review see Guerrini, Salvadori, Rizzi, Regoli, and Calo (2010)). Central NPS is also shown to enhance spatial memory (Han, Yin et al., 2009), passive avoidance memory, as well as novel object-location and object-context recognition memory in rodents (Han et al., 2013; Lukas & Neumann, 2012; Okamura et al., 2011). Moreover, it is demonstrated that NPS attenuates expression of contextual fear memory and facilitates extinction of cued conditioned fear memory (Juengling et al., 2008; Meis et al., 2008).

Extensive evidence indicates that noradrenergic activation of the basolateral amygdala (BLA) modulates memory consolidation for high emotionally arousing experiences, such as inhibitory avoidance memory (Ferry, Roozendaal, & McGaugh, 1999; McGaugh, McIntyre, & Power, 2002; McIntyre, Power, Roozendaal, & McGaugh, 2003). Recently, the noradrenergic activity in the BLA was reported to be involved in the regulation of object recognition memory consolidation occurred under condition of lower arousal (Dornelles et al., 2007; Roozendaal, Castello, Vedana, Barsegyan, & McGaugh, 2008; Roozendaal, Okuda, Van der Zee, & McGaugh, 2006). The novel object recognition (NOR) task is a non-aversive learning paradigm which is based on the animals' spontaneous preference for the novel object. In this task, the role of noradrenergic system of the BLA in memory consolidation is similar to that in other tasks with high emotionally arousing training. For example, the post-training epinephrine infusion into the BLA immediately promotes object recognition memory during consolidation (Dornelles et al., 2007; Roozendaal et al., 2008). In contrast, the post-training infusion of β-adrenoceptor antagonist propranolol into the BLA impairs object recognition memory consolidation (Dornelles et al., 2007; Roozendaal et al., 2008).

Interestingly, NPS improves object recognition memory (Okamura et al., 2011), and NPSR mRNA is highly expressed in the BLA (Clark et al., 2011). Moreover, Central NPS-induced inhibitory avoidance memory enhancement is attenuated by propranolol injected intraperitoneally (ip) (Okamura et al., 2011). Here, we investigated whether intra-BLA injection of NPS improved object recognition

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memory and whether such effect of NPS involved in the noradrenergic system within the BLA.

2. Materials and methods

2.1. Animals

Male Kunming strain of Swiss mice was obtained from the Experimental Animal Center of Lanzhou University, China. Animals were housed in an animal room that was maintained at 22 ± 2 °C with a 12-h light: 12-h dark cycle. Food and water were available *ad libitum*. All the protocols in this study were approved by the Ethics Committee of Lanzhou University, China.

2.2. Surgical procedure

Surgical implantation of cannula into lateral ventricle was conducted according to our previous report (Han, Chang et al., 2009). Each mouse (20–24 g) was anesthetized with sodium pentobarbital (70 mg/kg; Sigma) and placed in a stereotaxic frame (Leica). According to the atlas of Paxinos and Franklin (2001), 9 mm 26-gauge stainless-steel guide cannulas, closed by stylets, were implanted over the lateral ventricle (0.5 mm posterior to bregma, 1.0 mm lateral to midline, 2.0 mm ventral to skull surface) or bilateral BLA (1.5 mm posterior to bregma, 3.1 mm lateral to midline, 4.0 mm ventral to skull surface). After surgery, mice were housed individually allowed to recover 5–7 days.

2.3. NOR task

The procedure of NOR task was based on our previous report (Han et al., 2013), and that described by Okamura et al. (2011). Briefly, each mouse was tested in their home cage in a sound-attenuated room with somber lighting. The general procedure consisted of two sessions: a training trial and a retention phase carried out 1 day later, respectively. Each mouse was handled 3 min per day for three consecutive days prior to training. In the sample phase, two identical objects were placed in opposite sides of the home cage. The sample trial ended when mouse had explored two identical objects for a total of 5 or 10 s, as mice will remember the sample object 1 day later when the exploration time was 10 but not 5 s. In test session, a familiar object from the sample trial and a novel object were placed in the same locations as in the training phase. The test phase was ended when mouse had explored two objects for a total of 25 s, or after 5 min had passed, whichever came first. All objects were made of plastic or glass, similar in size (4–5 cm high) but different in color and shape. There were several copies of each object for use interchangeably. Throughout the experiments, the objects and the location of the objects were counterbalanced and randomly permuted. Objects were cleaned thoroughly between trials to ensure absence of olfactory cue. Exploration was defined as sniffing or touching the object with the nose and/or forepaws. Resting against or turning around object was not considered exploratory behavior. The time spent exploring each object was recorded by an observer blind to treatments. A discrimination index (DI) in the test phase was calculated as a percentage of the time spent exploring the novel object over the total time spent exploring both objects. A DI of 50% corresponds to the chance level and a higher DI reflects intact object recognition memory.

2.4. Drugs and infusions

NPS (mouse) was synthesized and purified as described in our previous report (Chang et al., 2005). NPS was dissolved in artificial CSF containing (in mM) 126.6 NaCl, 27.4 $NaHCO_3$, 2.4 KCl, 0.5 KH_2PO_4, 0.89 $CaCl_2$, 0.8 $MgCl_2$, 0.48 Na_2HPO_4, and 7.1 glucose, pH 7.4. NPS was infused into the lateral ventricle (1 nmol) or bilateral BLA (0.5 nmol/side) 5 min post training. Propranolol, bought from Sigma, was dissolved in saline and injected ip (2 mg/kg; 10 ml/kg) 15 min prior to training, or dissolved in artificial CSF and co-infused to the bilateral BLA (0.5 μg/side) with NPS (0.5 nmol/side).

The mice were infused consciously, and were gently handled. For intracerebroventricular (icv) infusion, the infusion cannula extended 0.5 mm beyond the tip of the guide cannula. Drugs or vehicle (2 μl) were infused over a period of 2 min via a 25 μl Hamilton syringe mounted on a microdrive pump (KD Scientific). For the bilateral BLA infusion, the infusion cannula extended 1 mm beyond the tip of the guide cannula. A total volume of 1 μl (0.5 μl/side) drug or vehicle was infused over a period of 5 min via two 10 μl Hamilton syringes mounted on a dual channels microdrive pump (KD Scientific). Infusion cannulae remained in place for 1 min after infusion to allow for drug diffusion.

2.5. Experimental design

In our previous reports, we have demonstrated that mice could discriminate the familiar and novel objects at a delay of 1 day when the total exploration time (TET) was 10 s but not 5 s during the training phase (Han et al., 2013). Thus, we determined whether NPS and propranolol could facilitate memory when TET was 5 s, and whether memory-enhancing effect of NPS could be blocked by propranolol. In addition, we investigated whether propranolol per se could impair memory when TET was 10 s.

First, two groups (vehicle, $n = 9$; NPS, $n = 9$) of mice were used to study whether icv injection of NPS could improve memory consolidation. Then, four groups (vehicle + vehicle, $n = 10$; propranolol + vehicle, $n = 11$; vehicle + NPS, $n = 11$; propranolol + NPS, $n = 10$) of mice were adopted to determine whether propranolol could block the memory-enhancing effect of NPS. In addition, two groups (vehicle, $n = 8$; propranolol, $n = 7$) of mice were employed to investigate whether propranolol per se could impair memory. Finally, three groups (vehicle, $n = 11$; NPS, $n = 9$; propranolol + NPS, $n = 10$) of mice were utilized to study whether NPS injected to the BLA could improve memory and whether co-infusion of propranolol could block such effect of NPS.

2.6. Histology

Mice were sacrificed by decapitation, and whole brains were removed and fixed in 4% paraformaldehyde overnight at 4 °C. Coronal sections (60 μm) were cut in a vibratome and stained with cresyl violet. Slides were observed under a light microscope to verify the cannula placements. Mice with infusion needle placements outside the lateral ventricular or BLA were excluded from experiment. A representative photomicrograph of a needle track terminating within the BLA is shown in Fig. 1.

2.7. Statistical analysis

Data were expressed as mean ± SEM. Statistical analysis was conducted using SPSS 17.0. One-sample *t*-test was used to determine whether DI differed from chance level (50%) for each group, depending to the result of the test for Normal distribution of the data. Differences between two groups were determined by unpaired Student's *t*-test, depending to the result of the test for Normal distribution of the data. Differences among more than two groups were determined by one-way ANOVA, and post hoc comparisons were done by Bonferroni-test. $p < 0.05$ was considered significant.