Neuropeptide S facilitates spatial memory and mitigates spatial memory impairment induced by \(N\)-methyl-\(d\)-aspartate receptor antagonist in mice

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**ABSTRACT**

Neuropeptide S (NPS) is a recently discovered peptide shown to be involved in regulating arousal and anxiety. NPS receptor (NPSR) mRNA is expressed significantly in the major input and output regions of hippocampal formation, which are critical in the modulation of learning and memory. However, the role of NPS/NPSR system in regulating learning and memory is still unknown. Here, we use the Morris water maze (MWM) to determine the effects of NPS on spatial learning and memory following intracerebroventricular (i.c.v.) injection in mice. Our data show that i.c.v. injection of NPS facilitates spatial memory in the MWM without significant alteration of latency to the target and swimming speed. Furthermore, NPS (i.c.v.) mitigates spatial memory impairment induced by the selective \(N\)-methyl-\(d\)-aspartate receptor antagonist MK801. Taken together, our results firstly demonstrate that NPS facilitates spatial memory and mitigates MK801-induced spatial memory impairment in mice.

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Neuropeptide S (mouse) was synthesized by manual solid-phase synthesis using standard Fmoc chemistry as described in our previous report [15]. MK801 was purchased from Sigma.

Surgical implantation of cannula into lateral ventricle was conducted according to our previous report [15]. Mice (18–22 g) were anesthetized intraperitoneally (i.p.) with pentobarbital sodium (80 mg/kg), and placed in a stereotaxic apparatus. A vertical incision was made in the skin to expose the skull. A stainless steel guide cannula was implanted into lateral ventricle and was fixed with dental cement. Coordinates toward the bregma were L + 1 mm, A – 3 mm, and V + 3 mm. To prevent occlusion, a dummy cannula was inserted into the guide cannula. The dummy cannula protruded 0.5 mm from the guide cannula. After surgery, the animals were allowed to recover for at least 5 days, and during this period, mice were gently handled daily to minimize the stress associated with manipulation of the animals throughout the experiments.

Spatial learning and memory were detected in the MWM. Mice were trained to find a submerged platform (8 cm diameter, 1 cm below surface) in a circular pool (diameter, 100 cm; height, 35 cm) filled with milky water (depth, 20 cm; 20 ± 1 °C) [29]. External visual cues were placed around the pool to facilitate navigation of the animals. The platform was fixed at SW. Start positions alternated between N, E, SE, and NW in a pseudo-random fashion. Each mouse was placed in the water facing the wall of the pool and allowed to swim for 60 s to reach the platform. Mice failing to complete the task in 60 s were placed on the platform manually. All mice were allowed to rest there for 30 s, and then performed the next trial immediately. Mice performed four consecutive trials per day over a 4-day training period. The time to reach the target (escape latency) and the swimming path and speed for each mouse were recorded automatically using MWM Tracking System (TME, Chengdu, China). On the last day (day 5), a probe test was adopted. The platform was removed and each mouse started at NE and had to swim freely for 1 min. Memory retention was measured by quantifying the time spent in the target and opposite quadrants.

Mice received three different doses of NPS (0.1, 0.3 and 1 nmol) by intracerebroventricular (i.c.v.) injection, respectively, 10 min before the first training trial on days 1–4. Control animals received 2 μl artificial cerebrospinal fluid (aCSF) containing (in mM) 126.6 NaCl, 27.4 NaHCO3, 2.4 KCl, 0.5 KH2PO4, 0.89 CaCl2, 0.8 MgCl2, 0.48 Na2HPO4, and 7.1 glucose, pH 7.4. Mice of three groups (Saline-aCSF, MK801-aCSF and MK801-NPS group) were used to investigate whether NPS could affect MK801-induced spatial learning and memory impairments. Saline-aCSF group received saline (10 ml/kg, i.p.) and aCSF (2 μl, i.c.v.) 30 and 10 min before the first trial on days 1–4, respectively. MK801-aCSF group received MK801 (0.1 mg/kg, i.p.) and aCSF (2 μl, i.c.v.), MK801-NPS group received MK801 (0.1 mg/kg, i.p.) and NPS (1 nmol, i.c.v.) at matched time points, respectively. The dose of MK801 used to impair spatial learning and memory in the present study was based on the previous reports [9,10].

After completion of behavioral testing, mice were injected with methylene blue dye (2 μl) which was allowed to diffuse for 10 min. Then mice were decapitated, and their brains were removed and frozen. Gross dissection of the brain was used to verify the placement of the cannula. Only the data from those animals with dispersion of the dye throughout the ventricles were used.

Data were expressed as mean ± S.E.M. The effects of agents on mean latencies to target and mean swimming speed were determined by ANOVA with repeated measures (MANOVA). ANOVA followed by Dunnett’s test was used to determine whether NPS played a role in memory. Student’s t-test was used to determine whether MK801 impaired memory, and to determine whether NPS affected such impairments induced by MK801. p < 0.05 was considered significance.

![Fig. 1.](image.png)

We found that mice treated with NPS (0.1–1 nmol, i.c.v.) had slightly shorter mean latencies (F(3,47) = 2.519, p = 0.073) in finding the platform throughout the 4-d training period (Fig. 1A). One day after completing the 4-day training period, the mice injected with NPS (0.1–1 nmol) dose-dependently prolonged and reduced the time in target (F(3,47) = 3.369, p < 0.05) and opposite (F(3,47) = 3.192, p < 0.05) quadrants, respectively (Fig. 1B). Further post hoc analysis indicated that mice treated with 1 nmol NPS spent significantly longer and shorter time in the target (<0.05) and opposite (<0.05) quadrants, respectively, compared to the mice treated with aCSF (Fig. 1B). NPS (0.1–1 nmol) injected by i.c.v. did not significantly change mice mean swimming speed (F(3,47) = 2.433, p = 0.070) during the 4-day training period (Fig. 1C). These data indicated that the mice treatment with NPS improved retention of spatial memory.

The mice of MK801-aCSF group had significantly longer mean escape latencies (F(1,27) = 7.707, p < 0.05) in finding the platform...
during training period, and spent significantly less and longer time in the target \((p<0.05)\) and opposite \((p<0.05)\) quadrants, respectively, on day 5 compared with the mice of Saline-aCSF group (Fig. 2). These results indicated MK801 (0.1 mg/kg, i.p.), the selective NMDAR antagonist, impaired mice spatial learning and memory.

Thus, we investigated whether 1 nmol NPS mitigated MK801-induced spatial learning and memory impairments. The mice of MK801-aCSF group had significantly longer escape latencies compared with the mice in Saline-aCSF group, and the mice in MK801-aCSF group spent significantly less and longer time in the target and opposite quadrants, respectively, compared with the mice in MK801-aCSF group. Results were presented as mean ± S.E.M. \((n=15, 14\) and \(14\) respective for the Saline-aCSF, MK801-aCSF and MK801-NPS group). \(*p<0.05\).

![Fig. 2. NPS (1 nmol, i.c.v.) mitigated spatial memory impairment induced by MK801 (0.1 mg/kg, i.p.) in the mice MWM. (A) The mice in MK801-aCSF group had significantly longer escape latencies compared with the mice in Saline-aCSF group, and (B) the mice in MK801-NPS group spent significantly less and longer time in the target and opposite quadrants, respectively, compared with the mice in Saline-aCSF group. The mice in MK801-NPS group spent significantly longer and slightly less time in the target and opposite quadrants, respectively, compared with the mice in MK801-aCSF group. Results were presented as mean ± S.E.M. \((n=15, 14\) and \(14\) respective for the Saline-aCSF, MK801-aCSF and MK801-NPS group). \(*p<0.05\).](image-url)

A great deal of evidence indicates that NMDAR activation plays an essential role in the acquisition of spatial learning and memory [16]. Pharmacological and genetic evidence indicate that blocking the function of NMDAR inhibits LTP, thereby impairing spatial learning and memory [16]. The results in present study also demonstrate that systemic injection of MK801, the selective NMDA receptor antagonist, significantly impairs spatial learning and memory. In addition, it has been reported that NPS has protective effects against the neurotoxic and psychotic symptoms produced by MK801 [22]. Thereby, we investigated whether NPS could mitigate spatial learning and memory impairments induced by MK801. Our present results indicate that central administration of NPS inhibits the spatial memory impairment induced by MK801. The spatial learning deficit induced by MK801 is also slightly inhibited by NPS. Therefore, we speculated that NPS might partial reversal the inhibition of LTP induced by MK801, thus mitigating MK801-induced memory impairment.

Furthermore, the mice centrally injected with NPS have similar swimming speed compared with vehicle control. The results indicate that NPS, inducing land-based hyperlocomotion activity [21,26,31], does not significantly affect the mouse swimming speed. Although NPS did not significantly affect swimming speed of our animals, we cannot rule out the effects of this compound on locomotion. Thus, further studies employing distinct animal models of memory are mandatory.

Collectively, our findings indicate that NPS plays a role in facilitating spatial memory, and mitigates spatial memory impairment. Such effects imply that NPS/NPSR system might be a new target for enhancement spatial memory or treatment spatial memory impairment.

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