



Short communication

The role of neurotensin in passive avoidance learning in the rat central nucleus of amygdala

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ABSTRACT

Tridecapeptide neurotensin (NT) acts as a neurotransmitter and/or neuromodulator and plays a role in learning and reinforcement. The central nucleus of amygdala (CeA), which is relatively rich in NT and neurotensin-1 receptors (NTS1), participates in the regulation of memory and learning mechanisms. The aim of this study was to examine the possible effect of NT and NTS1 antagonist (ANT) on passive avoidance learning after their microinjection into the CeA of male wistar rats. NT significantly increased the latency time. Effect of NT was blocked by ANT pretreatment. ANT in itself had no effect. Our results show that in the rat CeA NT facilitates passive avoidance learning via NTS1.

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Neurotensin (NT) is a tridecapeptide which is widely distributed in the central nervous system where NT acts as a neurotransmitter and/or neuromodulator [7,32]. NT was published to have the highest affinity to neurotensin-1 receptor (NTS1) [20,32]. It was shown that the central nuclei of the amygdala (CeA) is relatively rich in NT cell bodies, fibers and NTS1s [2,7,32]. Amygdala (AMY), as part of the limbic system, is involved in the regulation of learning, memory and fear related behaviour [16,17,22,23,29,30]. It was indicated before that intraamygdaloid neuropeptides like substance P and ghrelin enhance the passive avoidance learning [16,21,30]. We have published recently that NT microinjected into the CeA has spatial learning enhancing- and positive reinforcing effects via NTS1 [18,19]. In the present experiments we examined the effects of bilateral NT microinjections into the CeA on passive avoidance learning. NT was injected in two different doses which amounts were effective in our previous studies [17,18]. We used a specific non-peptide receptor antagonist SR 48692 to study the involvement of NTS1 in the effects of NT.

Sixty-eight adult male Wistar rats weighing 280–320 g at the beginning of the experiments were housed individually and cared for in accordance with institutional (Pécs University, Medical School) and international standards (National Institutes of

Health Guidelines for Laboratory Animals). Rats were kept in a temperature- and light-controlled room ($22 \pm 2^\circ\text{C}$; 12:12 h light–dark cycle with lights on at 6:00 a.m.). Standard laboratory food pellets (CRLT/N standard rodent food pellet, Charles River Kft, Budapest, Hungary) and tap water were available ad libitum. Daily food and water consumption and body weight were measured. All behavioural tests were done during the rats' daylight period between 08:00 and 12:00 h.

Rats were anesthetized i.p. by ketamine supplemented with diazepam (Calypsol and Seduxen, Richter Gedeon, Hungary, ketamine: 80 mg/kg body weight, diazepam: 20 mg/kg body weight). Animals were stereotactically implanted bilaterally with 22 gauge stainless steel guide cannulae, directed toward and 1 mm above the dorsal border of the CeA (coordinates relative to bregma: AP: -2.3 mm, ML: ± 4.1 mm, DV: -6.5 mm) according to the rats' stereotaxic atlas [33]. Cannulae were fixed to the skull with two stainless steel screws and dental acrylic. When not being used for injection, the guide cannulae were occluded with 27 gauge stainless steel obturators. Animals were allowed a minimum of 6 days postoperative recovery before experiments commenced, during which period they were handled daily.

A step-through avoidance paradigm was used in a two compartment passive avoidance apparatus. The experimental apparatus consisted of a large ($60\text{ cm} \times 60\text{ cm} \times 60\text{ cm}$), well illuminated (Tungsraflex, 100W) compartment and a small box ($15\text{ cm} \times 15\text{ cm} \times 15\text{ cm}$), painted black and having metal-grid floor for the delivery of electric shocks. Rats were habituated on the 1st day of the experiment when they were placed into the large

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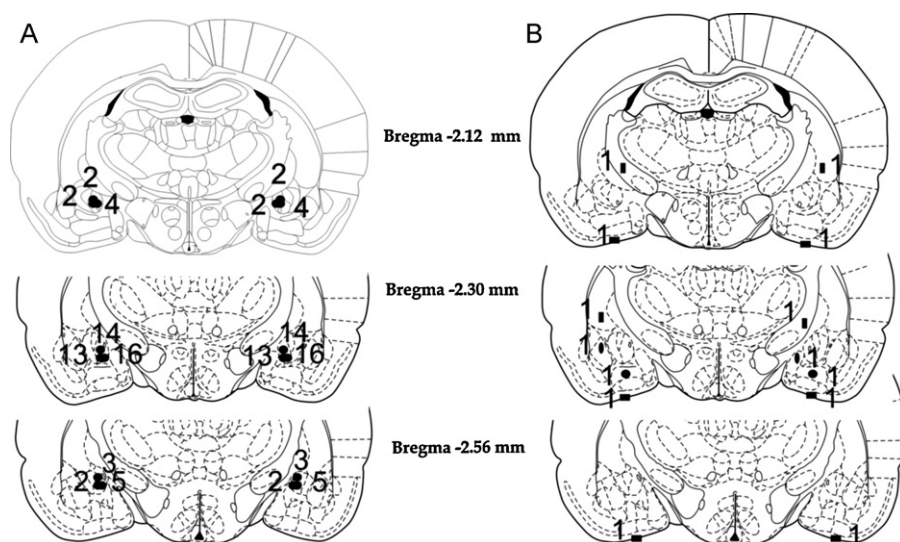


Fig. 1. Illustration of reconstructed injection sites. Correct bilateral injection placements are indicated as closed circles in the CeA on panel A ($n=61$). Incorrect injection placements are indicated on panel B ($n=7$). Brain structure diagrams of coronal sections are adapted from the stereotaxic atlas of Paxinos and Watson [33]. The numbers refer to anterior–posterior distance from bregma in mm. Identical symbols on panel B indicate coherent injection sites of bilateral injections. Numbers above symbols on panel A and B indicate number of animals.

compartment and were allowed a maximum time of 180 s to enter the dark compartment. On the following day animals were conditioned. Subjects were placed again into the illuminated compartment and latency to enter the shock box through a guillotine trap door was measured. After rats had entered the dark box, they were given electric foot shock three times, each for 1 s with weak (0.4 mA) electric current. Subsequently rats were removed from the apparatus and were microinjected bilaterally. Animals were injected in their home cage. The same rats were tested 24 h (Test 1) and 1 week after (Test 2) conditioning and the latency of entering the shock box was recorded. When the animal had not entered the shock box till the end of the trial the maximum value was given (180 s). The freezing and rearing behaviour of animals were also examined. Data were recorded and evaluated by means of the Noldus Ethovision System (Noldus Information Technology, The Netherlands).

In the first experiment (experiment 1) rats were microinjected bilaterally with 100 ng (54.6 pmol) or 250 ng (136.6 pmol) NT (Sigma–Aldrich Co., N3010). In a separate second experiment (experiment 2), application of 100 ng NT was repeated and the treatments were completed with 35 ng (60 pmol) ANT, (NTS1 antagonist SR 48692, gifted by Sanofi–Synthelabo Co.) or 35 ng ANT 15 min prior 100 ng NT microinjection (ANT+NT). In this report all the doses mentioned are meant to be the dose per side value. The volume of microinjections was 0.4 μ l/side. Vehicle in the same volume was applied for control animals. Solutions were microinjected for 1 min into the CeA through a 30 gauge stainless steel injection needle extending 1 mm below the tips of the implanted guide cannulae. Injector was attached to a 10 μ l Hamilton syringe (Bonaduz, Switzerland) via a polyethylene tube (PE10). The Hamilton syringe was operated with a Cole Parmer automatic minipump (IITC Inc. Life Sciences Instruments, USA). Needles were left in place for an additional 1 min after infusion to allow diffusion into the CeA. For details of procedures, solutions and group assignments of injections, see László et al. [18].

At the end of experiments rats received an overdose of Calypsol and Seduxen mixed in the ratio of 4:1 and were transcardially perfused with isotonic saline followed by 10% formalin solution. After 1 week of postfixation brains were frozen, cut into 40 μ m serial sections and stained with Cresyl-violet. Injection sites were reconstructed according to the stereotaxic atlas of the rat brain [33].

Only data from rats with correctly placed cannulae were analyzed. The track of cannulae and the tips were determined on the basis of existence of debris and moderate glial proliferation. Seven of the 68 operated rats were excluded from data analysis (Fig. 1.). Among these rats, in 3 cases, the cannula tips were symmetrically entered into the liquor space at the basis of the brain. In 2 cases cannula tips located laterally or medially and 1 mm above the AMY, so injections were made in the caudate–putamen on one side and in internal capsule on the other side. In 1 case cannula tips were placed laterally or medially to the target area, so injections were made in the lateral and basolateral AMY or in the medial AMY nucleus. In 1 case cannula tips were symmetrically located 1 mm below the target area, so bilateral injections were made in the basomedial AMY. Behavioural data concerning these incorrect and diverse placements were not enough to draw far-reaching conclusions.

All results are expressed as a mean $\pm$ S.E.M. Data were evaluated by two-way ANOVA (GraphPad InStat for Windows) followed by Tukey post hoc test if the treatment and/or the treatment $\times$ time interaction were significant in ANOVA. The statistical rejection criterion was set at $p < 0.05$ level.

Experiments started from the 6th postoperative day. By this time the body weight of the animals reached the preoperative level and it showed continuous increase. The statistical evaluation of the first experiment indicated that the intraamygdaloid microinjection of NT resulted in considerable alteration of learning in passive avoidance paradigm (Fig. 2.). By ANOVA the effect of time ($F=8.984$, $p < 0.01$) and the effect of treatment ($F=6.917$, $p < 0.05$) were significant but the time $\times$ treatment interaction ($F=2.051$, $p > 0.05$) failed to be significant. The post hoc analysis indicated that bilateral administration of 100 ng NT into the CeA significantly increased the latency time 24 h (Test 1) and 1 week (Test 2) after conditioning [Conditioning vs. Test 1 ($q=6.22$, $p < 0.01$), Conditioning vs. Test 2 ($q=4.909$, $p < 0.05$)]. On the other hand, control animals [Conditioning vs. Test 1 ($q=0.6555$, $p > 0.05$), Conditioning vs. Test 2 ($q=0.5868$, $p > 0.05$)] and 250 ng NT treated group [Conditioning vs. Test 1 ($q=3.006$, $p > 0.05$), Conditioning vs. Test 2 ($q=2.558$, $p > 0.05$)] showed only learning tendency.

In experiment 2 the effect of NTS1 antagonist was studied (Fig. 3.). ANOVA showed significant effect on time ($F=4.705$, $p < 0.01$), treatment ($F=8.676$, $p < 0.01$) and time $\times$ treatment interaction ($F=2.525$, $p < 0.05$). When groups were compared by post

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