Neuroscience Letters 600 (2015) 22–27

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

Activation of hypothalamic oxytocin neurons following tactile stimuli in rats

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HIGHLIGHTS

- Stroking stimuli activated hypothalamic paraventricular oxytocin neurons.
- Rats emitted 50-kHz ultrasonic vocalizations during stroking stimuli.
- Pleasant stroking is thought to activate hypothalamic oxytocin neurons.

ARTICLE INFO

Article history:
Received 21 April 2015
Received in revised form 16 May 2015
Accepted 25 May 2015
Available online 29 May 2015

Keywords:
Oxytocin
Stroking
Touch
Fos protein
Ultrasonic vocalizations
Hypothalamus

ABSTRACT

Gentle touching or stroking has anxiolytic actions and contributes to the establishment of an intimate relationship between individuals. Oxytocin administration also has anxiolytic actions and facilitates social behaviors. In this study, we examined effects of stroking stimuli on activation of oxytocin neurons and emission of 50-kHz ultrasonic vocalizations, an index of positive emotion, in rats. The number of oxytocin neurons expressing Fos protein was increased in the hypothalamus, especially in the dorsal zone of the medial parvicellular part of the paraventricular nucleus. The number of 50-kHz ultrasonic vocalizations was also increased. These findings suggest that pleasant sensory stimuli activate hypothalamic oxytocin neurons.

1. Introduction

Gentle touching or stroking induces a pleasant sensation via activation of slow-conducting unmyelinated nerves [1,2]. Affective touch plays an important role in affiliative behaviors including parental care and pair-bonding to reduce anxiety [3], to induce analgesia [4] and to contribute to the establishment of an intimate relationship [5]. Information of this affective-motivational dimension of touch is processed in social brain regions including the medial prefrontal cortex, cingulate cortex, insula, and superior temporal sulcus [6]. However, subcortical regions that are activated by affective touch are largely unclear.

Rats emit ultrasonic vocalizations dependent on the their emotional states. In aversive situations such as during predator exposure, lower frequency vocalizations, 22-kHz vocalizations, are emitted [7]. In appetitive situations such as during social investigation and rough-and-tumble play, higher frequency vocalizations, 50-kHz vocalizations, are elicited [7]. Thus, 22-kHz and 50-kHz vocalizations have been used as indices of negative and positive states of rats, respectively [7].

Oxytocin, which is mainly synthesized in the hypothalamus and the bed nucleus stria terminalis (BNST), has been shown to be associated with social behaviors [8–15], to induce anti-stress or anxiolytic actions [16] and to induce analgesia [17]. Oxytocin is released from the neurohypophysis into the blood at birth and during lactation and acts on peripheral tissues. Oxytocin has also been shown to be released from dendrites or cell bodies as well as from axonal terminals of oxytocin neurons within the brain following appropriate stimuli [18]. Massage or non-noxious tactile stimulation induces an increase in plasma oxytocin [19–21]. However, the localization of oxytocin neurons activated by non-noxious sensory stimuli has remained to be identified.

In this study, we examined the emission of ultrasonic vocalizations, an index of emotional states, and expression of Fos protein, an index of neuronal activation, in oxytocin neurons of the hypothalamus and BNST following tactile stimuli in rats.

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http://dx.doi.org/10.1016/j.neulet.2015.05.055
0304-3940/

2. Methods

2.1. Animals

Twenty-four male rats (9 weeks old, Iar: Long–Evans, Institute for Animal Reproduction, Ibaraki, Japan) were housed individually under a 12:12 h light/dark cycle (lights on at 7:30 am) at 22 ± 2 °C and 55 ± 15% relative humidity. Food and water were available ad libitum. Animal experiments were carried out after receiving approval from the Animal Experiment Committee of Jichi Medical University and were in accordance with the Institutional Regulations for Animal Experiments and Fundamental Guidelines for Proper Conduct of Animal Experiments and Related Activities in Academic Research Institutions under the jurisdiction of the Ministry of Education, Culture, Sports, Science and Technology.

2.2. Massage-like stroking

Rats were placed on the experimenter's lap and given stroking stimuli on the dorsal part of their bodies by the experimenter's hand with a cotton glove at a speed of 8.76 ± 0.17 cm/s (mean ± SEM, $n = 8$) and a frequency of 0.62 ± 0.01 Hz (mean ± SEM, $n = 8$) for 5 min without immobilization of their bodies. The speed and frequency of the stroking stimuli were monitored by use of a video camera. The force applied by the manual-stroking stimuli was relatively mild and was estimated to be less than 250 mN/cm^2. Cotton gloves were used for stroking to reduce friction and to make stroking as smooth as possible.

2.3. Recording and analysis of ultrasonic vocalizations

Ultrasonic vocalizations recording was performed by use of a 1/4-inch measurement-grade microphone (Type 4158N, Aco, Tokyo, Japan) designed for measurements of sound pressure levels between 20 Hz and 100,000 Hz. The microphone was placed at a distance of approximately 30 cm from the rats and was connected to a sensor amplifier (SR-2200, Ono Sokki, Kanagawa, Japan) and an AD–DA converter (USB-6251, NI, Texas, USA). Acoustic data were recorded at a sampling rate of 200 kHz and with 16 bits by an Avisoft RECORDER (Version 4.2, Avisoft Bioacoustics, Berlin, Germany) and analyzed by Avisoft SASLab Pro (Version 5.2, Avisoft Bioacoustics). Spectrograms were generated with a fast Fourier transform length of 256 points and an overlap of 50%. The numbers of 50-kHz and 22-kHz vocalizations were counted manually according to previous reports [22,23].

2.4. Immunohistochemical detection of Fos protein in oxytocin-immunoreactive (-ir) neurons

Ninety minutes after termination of stroking or holding stimuli, the rats were anesthetized with Avertin (tribromoethanol; 200 mg/kg, i.p.) and perfused transcardially with heparinized saline (2 U/mL) followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 15 min. The brains were immediately removed from the skulls, post-fixed in 4% paraformaldehyde overnight, placed in 30% sucrose in 0.1 M phosphate buffer until they sank, and frozen in dry ice. The hypothalamic part of each frozen brain was sectioned coronally at 30 μm and processed for immunochemical detection of Fos protein and oxytocin as described previously with modifications [24]. In brief, sections were incubated with a rabbit polyclonal antibody raised against the Fos peptide sequence (Ab-5; Oncogene Science; Cambridge, MA, USA) diluted at 1:10,000 for 2 days at 4 °C. Immunoreactivity was visualized by sequential overnight incubation with peroxidase-labeled goat anti-rabbit IgG (diluted at 1:500; Vector Laboratories, Inc., Burlingame, CA, USA) at 4 °C and 3,3-diaminobenzidine tetrahydrochloride (DAB) with nickel sulphate. The sections were then processed for detection of oxytocin using an antibody against oxytocin (diluted at 1:1,000,000; Peninsula Laboratories International, San Carlos, CA, USA). After two days of incubation at 4 °C with the first antibody, sections were incubated with biotinylated anti-guinea pig IgG (diluted at 1:750; Vector Laboratories) for 2 h at room temperature and with avidin-biotinylated horseradish peroxidase complex (diluted at 1:50; Vector Laboratories) for 30 min. Oxytocin was visualized with DAB. Sections containing hypothalamic paraventricular nuclei (PVN) (14 sections), supraoptic nuclei (SON) (15 sections) or BNST (8 sections) were examined at intervals of 120 μm in each rat. Areas of the brain were identified according to brain atlases [25,26]

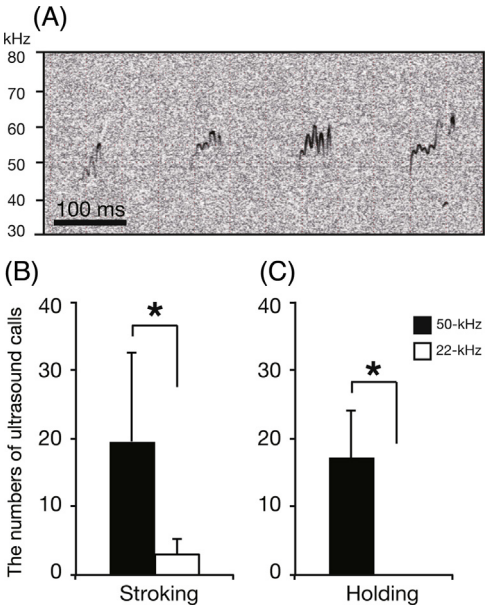

Fig. 1. Ultrasonic vocalizations during stroking and holding stimuli. A spectrogram of 50-kHz vocalizations in stroking group (A) and the numbers of 50-kHz and 22-kHz vocalizations during stroking (B) and holding (C) are shown. Rats emitted 50-kHz ultrasonic vocalizations more often than 22-kHz vocalizations during stroking and holding stimuli. *, $P < 0.05$. $n = 8$ per group.

2.5. Groups and experimental design

All rats received 5-min stroking stimuli once a day for 7 days. On the following day, rats were assigned to one of three groups: stroking group ($n = 8$), holding group ($n = 8$), and non-touch group ($n = 8$). In the stroking group, rats were placed on the experimenter's lap and given massage-like stroking for 5 min. In the holding group, rats were handled in the same manner as rats in the stroking group for 5 min without receiving stroking stimuli. In the non-touch group, rats were kept in their individual home cages. In the stroking and holding groups, ultrasonic vocalizations were monitored by use of an ultrasound microphone. After 5-min manipulation, rats were returned to their home cages.

Ninety minutes after termination of stroking or holding stimuli, the rats were perfused with 4% paraformaldehyde following deep anesthesia.

2.6. Statistical analysis

Data are expressed as means ± SEM. The numbers of 50-kHz and 22-kHz ultrasonic vocalizations were analyzed by Wilcoxon's signed rank test. The number of immunoreactive cells was analyzed by one-way ANOVA followed by the Tukey–Kramer post hoc test. $P < 0.05$ was considered statistically significant.