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Research report

Development-dependent behavioral change toward pups and synaptic transmission in the rhomboid nucleus of the bed nucleus of the stria terminalis

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HIGHLIGHTS

- Juvenile C57BL/6J male mice exhibited the transition from parental to infanticidal behavior while growing.
- The paired-pulse ratio of evoked excitatory postsynaptic current in the BSTrh neurons differed between adult and 3-week-old mice.
- Synaptic maturation in the BSTrh may contribute to the behavioral transition during development.

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ABSTRACT

Sexually naïve male C57BL/6 mice aggressively bite unfamiliar pups. This behavior, called infanticide, is considered an adaptive reproductive strategy of males of polygamous species. We recently found that the rhomboid nucleus of the bed nucleus of the stria terminalis (BSTrh) is activated during infanticide and that the bilateral excitotoxic lesions of BSTrh suppress infanticidal behavior. Here we show that 3-week-old male C57BL/6 mice rarely engaged in infanticide and instead, provided parental care toward unfamiliar pups, consistent with observations in rats and other rodent species. This inhibition of infanticide at the periweaning period is functional because the next litter will be born at approximately the time of weaning of the previous litter through maternal postpartum ovulation. However, the mechanism of this age-dependent behavioral change is unknown. Therefore, we performed whole-cell patch clamp recordings of BSTrh and compared evoked neurotransmission in response to the stimulation of the stria terminalis of adult and 3-week-old male mice. Although we were unable to detect a significant difference in the amplitudes of inhibitory neurotransmission, the amplitudes and the paired-pulse ratio of evoked excitatory postsynaptic currents differed between adult and 3-week-old mice. These data suggest that maturation of the synaptic terminal in BSTrh that occurred later than 3 weeks after birth may mediate by the adaptive change from parental to infanticidal behavior in male mice.

1. Introduction

Rodents exhibit a transition in conspecific social behavior accompanied by physiological development and experience, and such transitions are exhibited by polygamous male in the company of female. For example, biting attacks on pups by sexually naïve male rodents is called male infanticide, which is an expression of sexual conflict [1,2]. However, infanticidal behavior is suppressed by cohabitation with female mice and ejaculation [3–7]. Further, father mice and mongolian gerbil care for their pups, for example, by retrieving pups to the nest as well as licking and crouching over the pups [6,8–10].

Parental behavior is exhibited by adult as well as juvenile rodents [11–14]. Juvenile male Djungarian hamsters and prairie voles retrieve pups to the nest [15,16]. Further, female rodents can mate and become pregnant shortly after delivery through the first maternal postpartum ovulation and siblings are born after approxi-

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mately three weeks. When older siblings live together, the survival of pups depends on the suppression of infanticide committed by older siblings, until the pups become independent from their parents. Actually, older siblings rarely commit infanticide, but care for younger siblings. These alloparental behavior decreases the level of anxiety of the pups even in their postgrowth point [17].

However, the motivation to care for pups seems to be getting less age-dependently. For example, young (postnatal day 24) rats exhibit parental behavior for the presented pups with shorter latencies than older rats [12]. Because the behavioral choice, parental or infanticidal, is influenced by sex hormones [18,19], sexual maturity is an important factor that shifts the behavioral pattern. However, behavioral ontogeny and related mechanisms remain unaddressed.

C57BL/6J male mice are known to exhibit the transition from infanticidal to parental behavior after mating and the birth of pups [6]. Recently, author's group analyzed the neural mechanism of this behavioral transition by using cFos expression as a marker of neural activity. We reported that posterior part of bed nucleus of the stria terminalis (BST) including the rhomboid nucleus of the bed nucleus of the stria terminalis (BSTrh) is a region of the brain that may be activated during and/or after infanticidal behavior of the sexually naïve mice [6]. Further, excitotoxic lesions of BSTrh suppressed the infanticidal behavior of sexually naïve male mice [10]. However, the physiological properties of BSTrh neurons and neuroplastic changes associated with this behavioral transition are unknown.

To fill these gaps in our knowledge, we investigated how juvenile male adult C57BL/6J mice behaved toward pups. We also performed the whole-cell patch clamp recording of brain slices that included BSTrh to compare the electrophysiological properties of these mice.

2. Material and methods

2.1. Animals

The Animal Experiment Committee of the RIKEN Brain Science Institute and Institutional Animal Care and Use Committee at Hokkaido University approved all animal experiments, which were conducted in compliance with the National Institute of Health guidelines for the care and use of laboratory animals. All C57BL/6J male mice were bred at the RIKEN Brain Science Institute and Graduate School of Pharmaceutical Sciences, Hokkaido University. Mice had *ad libitum* access to water and food, and they were maintained under a 12-h light/dark cycle with TEK Fresh Standard bedding (Harlan, Indianapolis, IN, USA). Weaning was conducted 28 days after birth, and male and female mice were then separated. Mice that were 21–27 days were selected from the dams' cages for behavioral and electrophysiological tests. Mice were grouped according to their ages indicated as postnatal (P)26, P43, and P51 for P25–26, P42–43, and P50–51, respectively.

2.2. Behavioral test

The pup retrieval-infanticidal behavioral test was performed according to the published procedures [6,10]. Briefly, P24–25, P41–42, and P49–50 mice were individually placed in new cages containing purified paper bedding (Alpha-Dri, Shepherd Specialty Papers, Watertown, TN, USA), and a cotton square (Nestlet, Ancare, Bellmore, NY, USA) was included to build the nest. After 1–2 days of habituation, the pup-exposure assay was performed once each day for 4 successive days for 30 min. Three pups aged 1–5 days were placed in the corner of the cage, except for the subject's nest. The latencies of the first pup sniffing, the retrieval of each pup, and infanticide (pup biting) were measured. If a pup screamed for 2 s or was visibly bitten, the experiment was terminated by removing all pups from the cage, and wounded pups were immediately euthanized. The behavioral pattern was scored according to procedures described in previous reports [6,10] with modifications as follows: 4 = all pups were retrieved, 3 = 1 or 2 pups were retrieved, 2 = no pup was retrieved, 1 = pup was attacked >3 min after placement in the test cage, and 0 = pup was attacked within 3 min after placement in the test cage. All behavioral tests were performed during the day.

2.3. Electrophysiological test

Mice aged 21–27 days were referred to as "3-week-old mice," and those aged 12–16 weeks were referred to as "adult mice." The protocols used for the electrophysiological experiments were modified according to previous studies [20,21]. Artificial cerebellar spinal fluid (ACSF: 126 mM NaCl, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 26 mM $NaHCO_3$, and 10 mM glucose, pH 7.3) and a cutting solution containing the same concentration of choline chloride instead of NaCl were prepared. Mice were anesthetized using pentobarbital (30 mg/kg, intraperitoneal), and perfused with an ice-cold cutting solution from their hearts. The brains were then removed and cut into 230-μm thick sections in the ice-cold cutting solution using a Leica VT1200 Semiautomatic Vibrating Blade Microtome (Leica Biosystems, Nussloch, Germany). Brain slices were stored in ACSF at 32 °C for 20–30 min and then at room temperature.

The recording chamber was perfused with ACSF at 2–4 mL/min at 32–34 °C. Brain slices were placed in the recording chamber, and the neurons were visualized using an IR camera (IR-1000, DEGE-MTI, IN, USA). Under 40 or 60 × magnification, the surfaces of brain slices were confirmed to lack an anterior commissure in the ventral region of BSTrh. A stimulating glass electrode with an approximately 0.5 MΩ tip was adjusted using a Microforge (Narishige Instruments, Tokyo, Japan), filled with ACSF. Recording glass electrodes with 4–9 MΩ tips were filled with an internal solution (132 mM K-gluconate, 3 mM KCl, 10 mM HEPES, 0.5 mM EGTA, 1 mM $MgCl_2$, 12 mM Na-phosphocreatine, 4 mM Mg-ATP, 0.5 mM Na-GTP, 0.2% biocytin, pH 7.3). For the experiments to compare the eEPSC amplitude (Fig. 3C and D, Fig. 4), we utilized cesium (Cs)-based internal solution (150 mM CsOH, 5 mM CsCl, 10 mM EGTA, 2 mM $MgCl_2$, 4 mM Na_2ATP, 10 mM HEPES, 0.5 mM Na-GTP, pH 7.3 adjusted with gluconic acid [22]) including 5 mM QX-314 (Sigma-Aldrich, St. Louis MO, USA). After forming the gigaohm seal, the membrane was penetrated using negative pressure. If a cell did not generate an overshoot phase after the application of a positive current or its access resistance was >30 MΩ, recording was not taken from that cell. Membrane potential values recorded with K-gluconate based internal solution were corrected for the liquid junction potential (K-based, 11 mV, Cs-based, 10 mV). To measure postsynaptic synaptic potentials or currents, picrotoxin, CNQX disodium salt, and MK-801 (Sigma-Aldrich) were added to the bath by changing the perfusion solution.

2.4. Statistical analysis

Two-tailed Fisher's exact test, the two-tailed Student's *t*-test, and two-way repeated measures ANOVA followed by a *post-hoc* test were used to analyze the data, as implemented in GraphPad Prism software 6 (GraphPad Software, Inc., La Jolla, CA, USA).

3. Results

3.1. Behavioral test

The behaviors of P26, P43, and P51 male mice were assessed for 4 consecutive days (Fig. 1A–C). Latencies to sniff pups did not sig-

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