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Research article

Rapid induction of granule cell elimination in the olfactory bulb by noxious stimulation in mice

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HIGHLIGHTS

- Electrical foot shock rapidly induced granule cell death in the mouse olfactory bulb.
- Granule cell death occurred during startle and fear responses.
- Granule cell death was inhibited by suppression of activity in the olfactory cortex.
- Olfactory cortical activity in startle and fear responses promotes the cell death.

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ABSTRACT

Elimination of granule cells (GCs) in the olfactory bulb (OB) is not a continuous event but is rather promoted during short time windows associated with the animal's behavior. We previously showed that apoptotic GC elimination is enhanced during food eating and subsequent rest or sleep, and that top–down inputs from the olfactory cortex (OC) to the OB during the postprandial period are the crucial signal promoting GC elimination. However, whether enhanced GC elimination occurs during behaviors other than postprandial behavior is not clear. Here, we investigated whether exposure to noxious stimulation promotes apoptotic GC elimination in mice. Mice were delivered a brief electrical foot shock, during and immediately after which they showed startle and fear responses. Surprisingly, the number of apoptotic GCs increased 2-fold within 10 min after the start of foot shock delivery. This enhancement of GC apoptosis was significantly suppressed by injection of the $GABA_A$ receptor agonist muscimol in the OC, despite these muscimol-injected mice showing similar behavioral responses by foot shock as control mice. These results indicate that GC elimination is promoted in foot shock-delivered mice within a short time period of startle and fear responses. They also indicate that OC activity plays a central role in the enhanced GC elimination during this period, as is also the case in GC elimination during the postprandial period.

1. Introduction

Extensive neuronal turnover occurs in the mouse olfactory bulb (OB) throughout life. Interneurons in the mouse OB are continually generated even in adulthood [1]. Among newly generated granule cells (GCs), the major GABAergic interneurons in the OB, only half are incorporated into the OB neuronal circuitry and live longer than one month; the other half are eliminated by apoptosis [1,2]. Preexisting old GCs are also gradually eliminated by apoptosis [3,4]. Extensive apoptotic GC elimination in mice therefore occurs even in the adult OB. This characteristic optimizes olfactory function by fine tuning and refining the OB neuronal circuitry [5].

Plastic changes in neuronal circuits in the individual animal often occur in association with the animal's behavioral state, such as the wake–sleep cycle [6]. We previously showed that the elimination of GCs in the OB is not a continuous event but is promoted during a short time window of food eating and subsequent rest or sleep, and that top–down inputs from the olfactory cortex (OC) to the OB during the postprandial period are the crucial signal promoting GC elimination [4,7,8]. Centrifugal axons from the pyramidal cells in the OC to the OB mostly terminate in the granule cell layer, and GCs receive these top–down excitatory synaptic inputs directly [9–11].

Abbreviations: OB, olfactory bulb; GC, granule cell; OC, olfactory cortex; APC, anterior piriform cortex; GCL, granule cell layer; LFP, local field potential; SPW, sharp wave.

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The identification of a specific time window for GC elimination raises the further question of whether enhanced GC elimination occurs only during the postprandial period, or during other behavioral states also. To sustain daily life, animals show a variety of behaviors. For example, animals exhibit aversive and protective behaviors to noxious stimulation from the environment. Such stressful experiences induce structural changes in neurons in the hippocampus, amygdala and prefrontal cortex [12], but little is known of their effect on OB neurons, particularly acute effects that may occur within minutes or hours of the experience.

Electrical foot shock is often applied to experimental animals as an unconditioned noxious stimulation [13]. Here, we delivered electrical foot shock to mice. We then examined whether this stimulation increased apoptotic GC elimination in the OB, and if so whether the enhanced elimination was dependent on neuronal activity in the OC, as is the case with GC elimination in postprandial period. Foot shock-delivered mice showed startle and fear responses during and immediately after shock delivery. GC elimination was very rapidly enhanced in mice which extensively showed these behavioral responses. Moreover, this enhanced GC elimination after shock delivery was inhibited by the suppression of neuronal activity in the OC.

2. Materials and methods

2.1. Animals

C57BL/6 male mice (10–11 weeks old; Japan SLC) were used for all experiments. They were housed individually under a 12-h light-dark cycle (lights on, 5:00–17:00). All experiments were conducted in accordance with the guidelines of the Physiological Society of Japan and were approved by the Experimental Animal Research Committee of the University of Tokyo.

2.2. Electrical foot shock delivery

Electrical foot shock was delivered in a foot shock chamber (O'hara & Co., Japan). The floor of the chamber consisted of stainless steel rods, which were wired to a stimulator (Nihon Koden, Japan). Mice housed in home cages were transferred to the foot shock chamber and habituated to the environment for 2 h. They were then delivered a series of three electrical shocks (0.4 mA, 1 s) at 10-s intervals via the stainless steel floor rods. Following the foot shock, the mice were kept in the chamber for a further 3 min. Behaviors during the 3 min before and 3 min after the foot shock were recorded with video cameras and analyzed. Control mice were habituated to the foot shock chamber for 2 h but were not delivered the foot shock.

2.3. Categorization of mice behavior

Mice behaviors before and after the foot shock were categorized into five types: grooming, when mice showed grooming behavior; exploration, when mice were actively walking/running or were searching around by staying at one place and moving the head; rest/sleep, when mice stayed at one place calmly with the head dropping; freezing, when mice were completely motionless except for respiration with head elevating; alert, when mice took a lowered posture and hesitantly stepped forward and backward or when mice adopted a lowered posture by raising the head and extending the neck. Behavior was categorized in 1-s bins and charted on the graph.

2.4. Implantation of cannula for drug injection in freely behaving animals

Mice were anesthetized with medetomidine (0.5 mg/kg i.p.) and ketamine (37.5 mg/kg i.p.). A stainless steel 26 gauge guide cannula with a 31 gauge obturator was implanted into the unilateral anterior piriform cortex (APC) (1.5–1.8 mm anterior to the bregma, 2.0 mm lateral to the midline, 3.4–4.4 mm deep to the brain surface). The depth of the cannula in the APC was adjusted in layer II and optimized by monitoring centrifugal fiber-evoked field potential in the OB. A tungsten electrode bonded on the obturator was used as a stimulating electrode. The polarity of the evoked field potential in the OB by stimulating different depth of the APC was monitored. The electrode (and the cannula) was fixed at the depth of layer II of the APC where the polarity of the evoked field potential in the OB flipped over [14].

2.5. Muscimol injection

Muscimol was injected unilaterally in the APC through the implanted cannula. This experiment used 5 mM fluorescent dye-conjugated muscimol (M23400; Invitrogen) or 5 mM fluorescent dye (control vehicle) (D6117; Invitrogen) dissolved in PBS with 40% dimethyl sulfoxide (DMSO). At the end of the 2-h habituation to the foot shock chamber, the mouse was gently held by an experimenter and the drug or vehicle was injected at 0.05 μl/min for 10 min through a stainless-steel injector (31 gauge). The mouse was then returned to the chamber. Diffusion of injected dye-conjugated muscimol was examined in serial coronal sections through the OB to APC. Mice in which dye-conjugated muscimol diffused into the OB were excluded from analysis.

2.6. Immunohistochemistry

Following the 3 min of observation after foot shock, mice were deeply anesthetized by intraperitoneal injection of sodium pentobarbital and transcardially perfused with PBS followed by 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB), pH 7.4. The brains were postfixed in 4% PFA at 4 °C overnight and then transferred to 30% sucrose in 0.1 M PB. They were then embedded in OCT compound (Sakura Finetechnical), frozen at −80 °C, and sectioned coronally with a thickness of 20 μm with a cryotome.

Sections were preincubated with Tris-buffered saline containing 0.2% Triton-X (TBST), and then incubated with blocking buffer (TBST containing 10% of normal goat serum) for 60 min. Primary antibody was diluted with blocking buffer and applied to the sections overnight. The primary antibody used was rabbit polyclonal anti-active caspase-3 antibody (Cell Signaling Technology, #9661; 1:100). After rinsing with TBST, secondary antibody was diluted with blocking buffer and applied to the sections for 1 h. Secondary antibody was Alexa 488-conjugated goat anti-rabbit IgG antibody (Molecular Probes, A11034; 1:300). Sections were rinsed, stained with 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) (Nacalai Tesque; 2 μg/ml in PBS) for 10 min, rinsed again and then coverslipped with Prolong Gold Antifade Reagent (Invitrogen).

2.7. Cell counting

Coronal sections of OB from the rostral tip to the caudal end were selected at the rate of 1 in every 10 serial sections. Active caspase-3-labeled GCs in the granule cell layer were counted under a fluorescence microscope at ×200 magnification. The numbers were then summed and multiplied by 10 to estimate the total labeled number of GCs per OB.