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Research report

Neonatal whisker clipping alters behavior, neuronal structure and neural activity in adult rats

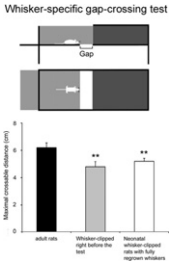
Yu-Fei Chu[a,b], Chen-Tung Yen[a,c,e], Li-Jen Lee[c,d,e,*]

[a] Graduate Institute of Zoology, National Taiwan University, Taipei, Taiwan
[b] Department of Mathematics and Science, National Taiwan Normal University, Taipei, Taiwan
[c] Graduate Institute of Brain and Mind Sciences, National Taiwan University, Taipei, Taiwan
[d] Graduate Institute of Anatomy and Cell Biology, National Taiwan University, Taipei, Taiwan
[e] Neurobiology and Cognitive Science Center, National Taiwan University, Taipei, Taiwan

HIGHLIGHTS

- We confirmed the effects of neonatal whisker clipping on tactile function in adults.
- Dendritic features in cortical neurons are sensitive to early sensory deprivation.
- c-Fos expressions in barrel cortex are influenced by early whisker clipping.

GRAPHICAL ABSTRACT



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ABSTRACT

Early experience plays critical roles during the development of sensory systems. For example, neonatal surgical manipulations of the whiskers in rodents lead to altered neural activity and behaviors later in life. However, while surgical procedures damage the sensory pathway; it is hard to examine the impact of whisker deprivation on adult animals. To address this issue, we performed a neonatal whisker clipping (WC0-3) paradigm, a non-invasive procedure, from the day of birth (P0) to postnatal day (P) 3, and examined behavioral performances in their adult age. With fully regrown whiskers, the WC0-3 rats exhibited shorter crossable distance than controls in a gap-crossing task, suggesting a defect in their whisker-specific tactile function. In their somatosensory cortex, the layer IV spiny stellate neurons had reduced dendritic complexity and spine density. After exploration in a novel environment, the expression of an activity-dependent immediate early gene, c-fos, increased dramatically in the somatosensory cortex. However, in WC0-3 rats, the number of c-Fos positive cells was less than those in control rats, indicating a fault in transducing sensory-related neural activity between cortical layers in WC0-3 rats. Together, our results demonstrate the roles of early tactile experience on the establishment of layer-specific excitatory connection in the barrel cortex. Early sensory insufficiency would leave long-lasting functional deficits in the sensory system.

1. Introduction

Early sensory experience plays an important role in shaping the structure and function of the nervous system [1–4]. The somatosensory system of rodents, for example, has been selected as a model system to study this subject in various aspects [5–15]. In rodents, the whiskers on the snout receive a variety of tactile information from the environment and the sensory information are transmitted

* Corresponding author at: Graduate Institute of Anatomy and Cell Biology, College of Medicine, National Taiwan University, No. 1, Ren-Ai Rd, Section 1, Taipei 100, Taiwan. Tel.: +886 2 23123456x88175; fax: +886 2 23915292.
E-mail address: ljlee@ntu.edu.tw (L.-J. Lee).

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to brainstem trigeminal nuclei, ventroposterior medial thalamic nucleus (VPM) and finally to the primary somatosensory (S1) cortex. The recipient layer IV spiny stellate cells preferentially orient dendrites toward thalamocortical afferents and form a specific cytoarchitectures termed "barrel" [16]. Each barrel receives the sensory information from one whisker on the controlateral snout thus is the functional unit in the S1. Neonatal manipulations of the whiskers or trigeminal nerve greatly affect the formation and maturation of barrel map [17–19], suggesting the significance of early sensory experience on the development of whisker-to-barrel system.

In rats, the thalamocortical afferents invade the developing cortex around the day of birth, postnatal day (P) 0 [20], and barrel pattern emerges in the S1 cortex around P3 [20–22]. During this specific short period of time, as the anatomical pattern appears, the thalamocortical connections and intra-cortical circuits are also establishing. The time window of the critical period for the development of whisker-to-barrel system has been determined by lesion studies, such as cauterization (burning) of whisker follicles or surgical damage on infraorbital nerve on rats and mice [17–19]. For example, cauterization of the row C follicles before the end of P3 results in shrinkage and fusion of the row C barrels in the contralateral cortex [23–25]. Infraorbital nerve section during P0 to P3 also produces fusion of the brainstem barrelettes and thalamic barreloids [26]. However, lesions after P4 do not lead to major structural alterations in the whisker-to-barrel system [27]. The critical window for the formation of whisker barrels is thus defined as P0–P3 in rodents (see [28]).

Lesion-induced structural plasticity during the formation of whisker-to-barrel system has been extensively studied in past decades; however, the impact of early sensory deprivation on the development of barrel map remains unclear. Damage to whisker follicles or lesion of infraorbital nerve could cause greater injuries to the nervous system than merely sensory deprivation. It may also produce a number of pathological side effects, such as cutting off the supply of trophic factors [29]. Most importantly, these damages are irreversible, thus the significance of early experience cannot be examined in adult animals. To preserve the entire whisker-to-barrel structures, a whisker clipping paradigm was developed [5]. The effects of early sensory deprivation on neural activity and behavior in adult animals can then be examined. Adult animals that had been whisker-clipped since the neonatal period showed significant anatomical, physiological and behavioral changes [5–8,10,12,14,30–37]. However, in most of these studies, chronic (weeks to months) whisker clipping protocols were favored. The period of whisker deprivation exceeded the critical period for the establishment of the whisker-to-barrel pathway.

To evaluate the significance of early whisker deprivation during the proposed critical period, P0–P3, we have recently used a short-term neonatal whisker clipping (WC) paradigm [38]. All the whiskers on the face of neonatal rat pups were bilaterally clipped from P0 to P3 and allowed for full regrowth. With fully regrown whiskers, neonatal whisker-clipped (WC0-3) adolescent rats exhibited impaired tactile function in the gap-crossing test. However, age-matched rats that had their whiskers cut only at P3 behaved normally in this test, suggesting the critical period for the development of whisker-specific tactile function is P0–P3 [38], in agreement with previous findings demonstrated by lesion methods. However, it is still not clear if the impaired tactile function in these WC0-3 adolescent rats persists into their adulthood.

The aim of the present study was to examine the effect of neonatal whisker clipping on behavior, neuronal structures and neural activity in adult rats. Adult WC0-3 rats were examined in several behavioral tests. Gap-crossing test was used to estimate their whisker-specific tactile function. Open field test was used to check the locomotor activity and thigmotaxis. Emotion-related behaviors were examined on an elevated plus maze and in a forced swim test. In the morphological aspect, Golgi–Cox impregnation was used to reveal the neuronal structure of layer IV spiny stellate neurons. Finally, in order to test if neonatal whisker clipping affect the neural activity in the barrel cortex, the quantity and distribution of cells expressing c-Fos, a neural activity-dependent marker, were measured. Our results clearly demonstrated that early tactile deprivation disturbs brain development and leaves long-lasting functional deficits in the nervous system.

2. Materials and methods

2.1. Subjects

Pregnant Wistar rats were obtained from BioLasco Taiwan Co., Ltd. All animal handling was in accordance with a protocol approved by the Institutional Animal Care and Use Committee of College of Medicine, National Taiwan University. Newborn male rat pups from eight litters were used in this study. Within the same litter, rat pups were randomly assigned into whisker-clipped (WC) and control (CON) groups. The pups of WC group received bilateral whisker clipping by a sterilized curved eye scissor twice a day from the day of birth (P0) to the third postnatal day (P3) and denoted as WC0-3 rats. All whiskers of WC0-3 rats, including supraorbital whiskers were clipped close to the surface of the whisker pads. The pups in CON group were handled the same way without the clipping of whiskers. After 4 days (P0–P3) of whisker clipping, the pups were kept untouched and the whiskers were allowed to re-grow. The sex-ratio was about 1:1 and was constant across litters. No abnormal mother-pup interactions were noticed during the lactation period. Pups were weaned and separated from the dam after 4 weeks of age and same-sex group-housed for another 8–10 weeks. Only male rats were used in the present study. All animals were housed in the Laboratory Animal Center of College of Medicine, National Taiwan University, under 12-h light/dark cycle with free access to food and water.

2.2. Behavioral tests

The animals were sequentially examined in gap crossing, open field, elevated plus maze and forced swim tests with 2 days intervals. All the animals were habituated in the testing environment for 30 min before each test. In total, 24 CON, and 29 WC0-3 rats were examined in these tests.

2.2.1. Gap crossing test

To examine the whisker-specific tactile function of rats, a gap-crossing test was used [39]. In brief, a gap-crossing apparatus was set up in the testing room with a fluorescent lamp placed on top of the apparatus as a light resource. One rat was placed in the bright side of the platform, and the nature of thigmotaxis would drive the animal to move into the dark sides, making it to cross the gap. The gap-crossing procedure was conducted in a series of increasing gap distance. Two attempts were allowed for a rat in a given distance and the cut-off time was 120 s. If the rat was not able to cross the gap within 120 s in two consecutive trials, the distance was recorded as the uncrossable distance, and the maximum crossable distance was thus determined. Besides CON and WC0-3 rats, another set of age-matched male Wistar rats ($n = 10$) were used as acute whisker-cut (aWC) rats. Right before the gap-crossing test, all the whiskers of aWC rats were clipped. Activities of rats in the gap-crossing apparatus were video-taped and reviewed.

2.2.2. Open field

The open field apparatus was designed as a 40 cm × 40 cm arena. Each rat was placed in the center of the open field and allowed to freely explore the novel environment. The locomotor activity of the rat was recorded for 15 min with a web camera on top of the arena. The behavioral profiles were analyzed by TopScan software (Clever System, Reston, VA, USA). The area of the open field was equally divided into 25 squares (8 × 8). The peripheral 16 squares were defined as the peripheral region whereas the central 9 squares were the central region. Parameters including total number of rears, total travel distance as well as travel distance in the center and peripheral regions were measured.

2.2.3. Elevated plus maze

The custom-made maze consists of two black plastic open arms (100 cm × 10 cm), crossed at right angle by two arms of the same dimensions enclosed by 40-cm high walls of the same material as closed arms. The maze is located 50 cm above the floor. The animals were tested by placing them on the central platform of the maze, facing one of the open arms, and letting them to move freely for 5 min. The behaviors were continuously videotaped by a video camera placed above the apparatus and then analyzed with TopScan software (Clever System). Total number of rearing, traveled distance and time spent in open arms, closed arms and central region were quantified.