Original Article

Morphometric Plasticity of Nitric Oxide Containing Neurons in the Barrel Cortex of De-whiskered Rats

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Abstract

Background: The rodent somatosensory barrel cortex is an ideal model to examine the effect of experience-dependent plasticity on developing brain circuitry. Sensory deprivation such as whisker deprivation may affect neuroanatomical aspects of the brain during developmental processes. The present study designed to investigate the possible effects of whisker deprivation on the morphometric characteristics of NADPH-d positive neurons in the barrel field cortex of adolescent rats.

Materials and Methods: Pups were divided into the intact (n=4) and whisker-deprived groups (n=4). In whisker-deprived group, the total whiskers of subjects were trimmed every other day from postnatal day (PND) 0 to PND 60. NADPH-d histochemistry reaction was processed to quantitatively analyze the feature of NADPH-d containing neurons of barrel cortex.

Results: Our results showed that the number of NADPH-d positive neurons remained unchanged in whisker-deprived group compared to controls. The mean soma diameter, dendritic length and the number of 3rd order processes were significantly decreased in the whisker-deprived rats ($p<0.05$).

Conclusion: Our results indicate that postnatal whisker deprivation possibly alter NADPH-d/NOS neuronal features in the barrel cortex. The functional implications of these data may relate the plasticity of synaptic receptive field and developmental brain circuits.

Keywords: Barrel cortex, Nitric oxide, Plasticity, Whisker deprivation

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Introduction

Active touch is characterized by the movement of tactile sensory. While humans move their finger tips for tactile sensation, rodents relay on the mobile set of the whiskers arranged in five horizontal rows along their snots. The whisker functions in the daily activities of rodents, such as decision making and environmental exploration. The whisker representation part of the primary somatosensory cortex (wS1) of the rodents, is called barrel field cortex[1-3]. This area in rodents provides a useful model system for exploring details of cortical organization in mammalian brains. When the rat whisks an object, sensory signals project through the trigeminal brainstem system and thalamus to the corresponding barrel columns in the wS1[4-6]. Early deprivation of peripheral sensory information inputs from whiskers causes long-lasting changes in the structure and function of somatosensory system[7].

Nitric oxide (NO) is an intracellular messenger in all vertebrate modulating neural activity[8-10]. The nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) positive neurons are extensively present within the central nervous system[8]. The neural nitric oxide synthase (nNOS) is colocoloized in neuronal populations with NADPH-d containing neurons capable of producing NO[11,12]. Histochemical staining for NADPH-d is commonly used to evaluate the distribution and morphometric characteristics of NO containing neurons[9,13,14]. NADPH-d stained neurons present intense Golgi-like staining of the cell body and processes. Comprising about 0.5–2% of all cortical neurons, they are dispersed in different cortical layers of barrels field. This may indicate a possible role of NO in the processing of sensory integration[15]. General agreements exists that NADPH-d positive cortical neurons represent GABAergic local interneurons[16]. Previous studies demonstrated that unilateral whisker trimming during early postnatal life results in a considerable reduction of GABA-positive synaptic terminals in the corresponding barrel cortex[17]. In fact, total whisker trimming during this period has been shown to affect dendritic arborization patterns and spine morphology[18].

So far, the effects of total whisker deprivation on the morphometric features of NADPH-d positive neurons have not been investigated in the barrel cortex. Therefore, the quantitative evaluation of the density of cell bodies and dendritic banching of barrel field NADPH-d positive neurons was performed in adolescent rats de-whiskered from birth.

Methods

Animals

The experiments were performed in accordance with guidelines for caring and using of laboratory animals set forth by the research council at Shahid Beheshti University of Medical Sciences (Tehran, Iran). Two groups of neonatal male Wistar pups and their mother (during lactation) were housed under standard condition at 22–24°C under light/dark cycles (12h/12h); food and water were available ad libitum. The first group of pups (n=4) was bilaterally whiskers trimmed every other day from postnatal day (PND) 0 to 60. The second group of 4 control pups was kept whiskers intact.

Tissue preparation

At PND 60, pups were deeply anesthetized (100 mg/kg ketamin and 5 mg/kg xylazine) and perfused transcardially with 0.9% saline, followed by cold 4% paraformaldehyde in 0.1 M phosphate buffer saline (PBS, pH 7.4). The brains were postfixed in the same fixative for at least 24 h and cryoprotected in 30% sucrose at 4°C overnight. The blocks of brain tissue were cut on a cryostat at 50 µm thick coronal sections. The sections were collected in 0.1 M PBS (pH 7.4) for histochemical processing[19].

NADPH-d histochemistry

Sections were pre-incubated in 0.2% Triton X-100 in 0.1 M PBS (pH 7.4) for 20 min and then reacted for 1 h at 37°C in a solution containing 0.5 mg/ml b-NADPH (Sigma, Saint Louis, MO, USA), 0.6 mg/ml nitroblue tetrazolium (NBT, Sigma, Saint Louis, MO, USA), 0.3% Triton X-100 dissolved in 0.1 MPBS (pH 7.4). The histochemical reaction was monitored by inspecting sections under the microscope. At the end of the reaction, sections were rinsed in 0.1 M PBS (pH 7.4) and then mounted on gelatinized glass slides, counterstained with neutral red (0.1%) and coverslipped[19].

Counting procedure and image analysis

Sections were examined under the light microscope to localize cells exhibiting NADPH-d positive reaction. A camera lucida system using a 40x objective was used to determine the density and two-dimensional reconstruction of labeled cells. Eight randomly selected sections per animal were counted. The barrel cortex area was determined according to Paxinos and Watson atlas (2007)[20] and Nogueira-Campo et al. (2012)[15].

We randomly selected 160 NADPH-d positive neurons from each group. Cells were selected for reconstruction depending on their integrity of the dendritic arborization in a single histological section. Only cells with complete typically thin distal dendritic arborizations were included for analysis (Figure 1, C and D). Cells with artificially cut dendrites or apparently not fully reacted were not included in our results. Four morphometric parameters were quantitatively evaluated[21,22] in the reconstructed neurons: 1- soma diameter (µm) measured at the

maximal axis of cell body, 2- Number and length of dendrites (µm) per 1st, 2nd and 3rd order rows (Figure 1, C and D), and 3- The longest dendritic branch (µm).

Data analysis

Statistical differences between labeled cells in different groups were determined by student's t-test. The tests were performed using SPSS version 19.0 software. All data were expressed as mean±SEM. The level of significance was set at $p<0.05$.

Results

On the day of experiment, control (180±10 g) and whisker deprived (178±13 g) rats exhibited similar body weight gain. Quantitative analysis revealed a homogenous density of NADPH-d positive neurons throughout the boundaries of barrel cortex in whisker-deprived and control pups (Figure 2A). However, a significant decrease of the soma diameter was observed in whisker-deprived group compared to intact group ($p<0.05$; Figure 2B). Although, only the number of 3rd order dendritic branches was significantly reduced ($p<0.05$; Figure 2D), but the length of all 1st, 2nd and 3rd order branches was shorter in whisker deprived rats in comparison to control ones ($p<0.05$, $p<0.05$ and $p<0.01$, respectively; Figure 2E). The extension of longest dendritic processes shows 25% reduction in whisker deprived group compared to their control homologues ($p<0.01$; Figure 2C).

Discussion

Nitric oxide (NO) is involved in the control of cerebral blood flow, metabolism[23] and experience-dependent plasticity[24]. It has been reported that the cortical NADPH-d positive neurons co-localize with other substances such as GABA and calcium-binding proteins[25], parvalbumin[26] and somatostatin[27]. The results of the present study showed that neonatal whisker deprivation did not appear to affect the number of NADPH-d positive neurons in the barrel cortex. In consistent, unilateral nares occlusion did not affect the number of NADPH-d positive cells in the olfactory cortex[28]. Moreover, whisker trimming (PND1-PND56) had no effect on the density of parvalbumin-positive neurons in the barrel subfield of mice[29]. It has been also noted that the distribution pattern of NADPH-d neurons is comparatively similar to other subgroups of interneurons, such as GABA in the barrel cortex of small rodents[30]. Micheva et al. (1995) reported that unilateral sensory deprivation induces a decrease in the intracortical GABA inhibitory circuitry of barrel cortex[31].

Several studies using experience-dependent paradigms

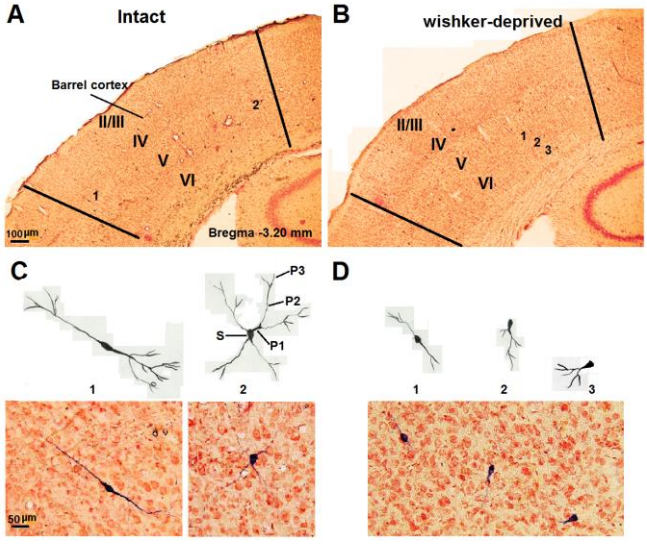

Figure 1. Representative photomicrographs of the barrel cortex in intact (A) and whisker-deprived (B) rats, indicating the location of selected NADPH-d positive neurons. C and D are camera lucida reconstructions of NADPH-d positive neurons in intact and whisker deprived rats with their corresponding photomicrographs, respectively. *Abbreviations:* P1-3; processes 1-3, S; soma.

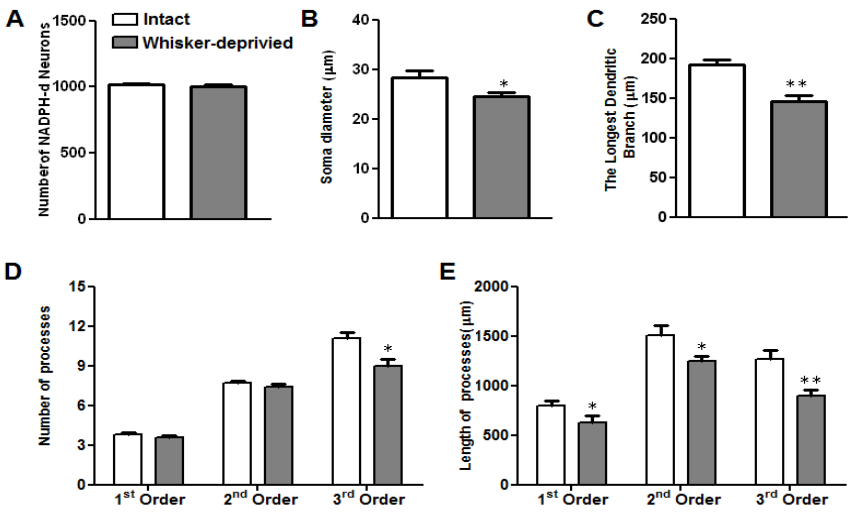

Figure 2. Quantitative morphological parameters of NADPH-d positive neurons in the barrel cortex: the number (A), soma diameter (B), the longest dendritic branches (C), the number of per 1st , 2nd and 3rdorders processes (D) and the length of dendrites per 1st , 2nd and 3rdorders (E). All data are mean ± SEM. (*p<0.05 and **p<0.01).

have demonstrated neocortical synaptic and anatomical plasticity following sensory deprivation[1,32-34]. In the present study, a significant reduction in *the soma diameter of NADPH-d positive cell was* observed in the whisker-trimmed rats. Moreover, these labeled neurons were smaller and less branched than those of intact rats. These results may confirmed by Chau et al. (2014) that demonstrated a significant increase in the spine density of pyramidal neurons in barrel cortex of the spared whisker barrels in contrast to reduced number of spines in deprived whisker barrels[35]. Decrease in cell body size or processes of NADPH-d positive neurons in the barrel cortical area may occurs due to the secondary effects of shorter thalamocortical inputs following whisker deprivation[34].

Axons, dendrites, and spines of neurons are highly dynamic structures[36]. Sensory deprivation might modify the connectional phenotype of cortical neurons by altering the relation between laminar fate and connectivity[28]. Decrease in the size and branching complexity of the dendritic trees of NADPH-d positive neurons may alter functional properties of cortical circuits in whisker-deprived rats[36,37]. A decline in the inhibitory control of excitatory neurons might explain the high rate of seizures found in sensory deprived animals[38].

Our results may suggest reduced dendritic receptive filed and possible synaptic impairment of barrel cortex nitritergic neurons following chronic whisker trimming from birth to adolescence period.

Conclusion

Smaller cell bodies and shorter dendritic ramifications may severely affect the biological activities of barrel NADPH-d containing neurons in long lasting whisker deprived rats. Further experimental studies will better explain the role of these neurons in response to the sensory experience dependency.

Acknowledgments

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Conflict of interest

None declared.

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