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Research article

Experience-dependent regulation of tissue-type plasminogen activator in the mouse barrel cortex

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HIGHLIGHTS

- Tissue-type plasminogen (tPA) activator is expressed in the mouse barrel cortex.
- Sensory deprivation increases tPA in the barrel cortex.
- Sensory restoration doesn't return tPA to control levels.
- tPA links changes in sensory experience with changes in the cortical environment.

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ABSTRACT

It has been suggested that tissue-type plasminogen activator (tPA), a serine protease, plays a key role in regulating the extracellular matrix core proteins, thereby impacting the structural plasticity in the cerebral cortex. Much is known about its role in regulating plasticity in the visual cortex. However, its permissive role has not been demonstrated to generalize to other cerebral cortical areas. By utilizing a combination of immunofluorescent histochemistry and confocal microscopy, we demonstrate that endogenous tPA is indeed present in the somatosensory cortex, and its expression is experience-dependent. Chronic sensory deprivation induced by whisker trimming from birth for one month leads to increased tPA immunoreactivity in all layers of the barrel cortex. Furthermore, tPA immunoreactivity remains high even after sensation has been restored to the mystacial pad (by allowing whiskers to grow back to full length for one month). Our results suggest that tPA levels in the cerebral cortex are regulated by sensory experience, and play a key role in regulating structural remodeling in the cerebral cortex.

1. Introduction

Tissue-type plasminogen activator (tPA) is a serine protease, and has been shown to provide a permissive role for plasticity. In the visual system, tPA is developmentally regulated, with its levels peaking around the critical period, and decreasing progressively into adulthood [27]. Furthermore, tPA levels are also activity-dependent; monocular deprivation spanning the developmental critical period is associated with a significant increase in tPA activity, and such increase is regulated by glutamate decarboxylase-65 (GAD65) [17]. tPA has also been implicated in the regulation of synaptic plasticity. Application of tPA accelerates the dynamics of dendritic spines [20], whereas genetic deletion of tPA prevented monocular deprivation-induced changes of dendritic spine density in the visual cortex [16], and prevented stress-induced loss of dendritic spines in the hippocampus [22]. In other systems, it has been shown that tPA is upregulated in the nucleus accumbens following exposure to nicotine [19], and in spinal cord injury models, tPA can facilitate motor improvement following injury [1].

Despite the thorough investigations in the systems mentioned above, the role of this tPA-enabled experience-dependent plasticity has been unexplored in the somatosensory system. Due to its well defined anatomical pathways and the ease of peripheral manipulations, the whisker-to-barrel system has been used as a model system to study cortical development as well as activity-dependent plasticity in general [21,26,9] and is therefore well suited for investigation of experience-dependent tPA expression in the somatosensory cortex. Another advantage of using the

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whisker-to-barrel system is its accessibility and painless sensory restoration (via whisker regrowth) following prolonged deprivation. Sensory restoration, in many cases, was able to provide partial recovery in dendritic spine dynamics and densities [28,5], but did not recover other indicators of anatomical plasticity, such as extracellular matrix core proteins and myelination ([18,29], Barrera et al., 2013). It is not well understood how sensation restoration following prolonged sensory deprivation may influence endogenous tPA levels in the previously affected cortical areas. Due to its role of providing enhanced permissibility of neuroplasticity, elucidating how tPA expression is regulated by developmental age and sensory inputs is an important question. Here, for the first time we show that tPA is present in the barrel cortex, and sensory experiences profoundly affect the level of this pivotal bioactive enzyme.

2. Methods

2.1. Experimental animals and chronic sensory deprivation

The selection and treatment of experimental animals (CD-1 mice of either sex (Charles River Laboratories) were followed as previously described [18,6,5] and in accordance with the Queens College, CUNY Institutional Animal Care and Use Committee and NIH guidelines. Briefly, animals were arbitrarily assigned at birth to be in control, unilaterally trimmed, or bilaterally trimmed conditions. For all trimmed animals, their whiskers were manually clipped by precision microscissors every other day for the animals' first 30 postnatal (P) days. Brief administration (~1 min) of anesthesia (isoflurane, Aerrane) was used from postnatal day 14 and on to prevent the animals from moving during trimming as well as to minimize handling-induced stress. All animals were exposed to the same conditions such as bedding texture, food and water source, maternal influence, anesthesia, handling, etc. For the P31–60 whisker regrow groups (sensory restoration condition), we performed ear punches at postnatal day 31 for the purpose of identifying which animals belong to the sensory restoration conditions so we could discern experimental animals from their control littermates at postnatal day 60 when their whiskers have regrown to full length. P30 controls, and P0–30 unilaterally and bilaterally trimmed animals were sacrificed and perfused at P30. P60 controls, P31–60 whisker regrowth animals were sacrificed and perfused at P60. (For detailed schematic description of the animal conditions, see Fig. 2A).

2.2. Immunofluorescence staining

Animals for immunofluorescence analysis were anesthetized with an interperitoneal injection of euthanizing agent (0.1 ml, Euthasol, Virbac AH, Inc.). When the animal was non-responsive to a noxious stimulus (toe pinch), it was transcardially perfused with saline followed by 4% paraformaldehyde in 0.01 M phosphate buffer (PB). The retrieved brain tissues were kept in fix overnight, followed by 30% sucrose embedding, and sectioned with a vibratome in room temperature (maximum amplitude, minimum speed, coronal plane, 50 μm per slice). Slices were collected in 0.01 M phosphate-buffered saline (PBS; pH 7.4). Free-floating sections were rinsed in 0.01 M PBS three times, 10 min each, then non-specific binding were blocked using 5% normal rabbit serum (Sigma–Aldrich) containing 0.5% Triton X-100 dissolved in 0.01 M PBS for one hour. The sections were incubated in sheep-antimurine tPA antibody IgG fraction (catalogue number SASMTPA–GF, Molecular Innovation Inc., dilution ratio 1:300) for three nights overnight at 4 °C in 0.01 M PBS. After rinsing in 0.01 M PBS, the brain slices were submerged in a secondary antibody, biotinylated rabbit anti-sheep (Jackson Immuno Research, dilution 1:200) dissolved in 0.01 M PBS and 2% normal rabbit serum. Following the secondary antibody incubation, slices were rinsed in 0.01 M PBS and incubated in fluorescein dichlorotriazine (DTAF)-conjugated streptavidin in the dark (excitation: 520 nm, Jackson Immuno Research, dilution 1:500). Lastly, the brain tissues were counterstained with Hoechst (Sigma–Aldrich, dilution 1:10000, final solution 0.12 μg/ml) for 15 min, followed by extensive rinsing (6 times, 10 min each) in 0.01 M PBS, briefly submerged in distilled water, mounted on fluorescence-free slides, air-dried, coverslipped with VectorShield (Vector Laboratory), and sealed with nail polish. For the purpose of assuring the primary antibody was targeting the desired antigen [tissue plasminogen activator], we also performed two forms of negative control staining: (1) negative control staining study without primary antibody, as well as (2) pre-absorbing the primary antibody with over-saturating levels of recombinant mouse tPA (Molecular Innovations Inc.). The first type of negative control is to ensure the observed fluorescent signals are not due to non-specific binding of the secondary antibodies and/or fluorescently labeled streptavidin, and the second type of negative control is to ensure the primary antibody is indeed targeting the desired antigen. By pre-binding the primary antibodies with over-saturating levels of exogenous tPA, the primary antibodies were thus neutralized and cannot further bind to tPA in the brain sections. Neither of these negative control staining yielded any tPA immunoreactive cells.

2.3. Optical densitometric quantification of immunofluorescence

Immuno-reactive tPA cells are visualized with a Leica TCS-SP5 (Leica Microsystems Wetzlar GmbH, Wetzlar, Germany) confocal microscope under 20× (Numerical Apperature (NA) 0.85) with excitation parameters for DTAP (excitation: 520 nm, 'green') and Hoechst (excitation: 405 nm, 'blue'). The brain slice was first inspected under the blue laser channel to determine the location of the barrels and the cortical laminae, followed by viewing in the green (tPA) channel. Image exposure, laser power (16%), field of view size, photomultiplier (PMT) frequency-sensitivity, and the digital magnification factors were all internally consistent (held constant) for the purpose of pre-standardizing the tissue background luminosity prior to densitometry analyses, in a manner similar to previously described studies [10,14]. The gain was adjusted to match the background fluorescence level to an arbitrary number of 30.00 (Leica TCS-SP5), with acceptable deviation of ±0.5% (~29.85–30.15). To avoid significant photobleaching, the immunofluorescence photomicrographs were collected within 3 min after their initial exposure to the laser. The absolute value of the tissue background luminosity (area devoid of immunonfluorescence staining) was further confirmed by directly measuring the pixel intensity using the Neurolucida software (under luminosity measurements). Photomicrograph snapshots were converted to grayscale images (Photoshop 7.0), in which pixel values representing the intensity of staining were expressed in a grayscale (0–255). The overall luminosity and relative optical density (ROD) of barrel cortex (layers II–VI) were measured and assessed using the software Densita (MBF Bioscience Inc.) as previously described [2]. Laminae were defined based on cell density and size determinations using the Hoechst labeled tissue. We derived the optical density index (ODI) of a specific cortical layer by taking the ROD of that cortical layer and divided by ROD of the corresponding white matter of the same slice (due to the finding that there are extremely few tPA-immunopositive cells residing in the white matter).

2.4. Statistical analyses

We sampled four to five brain slices per animal, and calculated the averaged ODI of one animal as an individual case and then