



## TNF- $\alpha$ Levels throughout the Critical Period for Experience-Dependent Plasticity in the Rat Primary Auditory Cortex

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### Abstract

Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) is likely to play a role in brain plasticity. To determine whether TNF- $\alpha$  levels change throughout a critical period of experience-dependent brain plasticity, we assessed these levels in the primary auditory cortex of rats before, during and after the critical period (at postnatal day 7, postnatal day 12 and at adulthood, respectively). TNF- $\alpha$  levels in the auditory cortex increased from before to after a critical period of brain plasticity. We suggest that TNF- $\alpha$  may play a role in the brain plasticity that occurs in the auditory cortex.

**Keywords:** TNF- $\alpha$ ; Brain plasticity; Auditory cortex; Critical period

**Abbreviations:** AMPA:  $\alpha$ -Amino-3-hydroxyl-5-methyl-4-isoxazole-propionate; AMPAR:  $\alpha$ -Amino-3-hydroxy-5-methyl-4-isoxazolepropionic Acid receptor; ANOVA: Analysis of Variance; BCA: Bicinchoninic Acid; ELISA: Enzyme-Linked Immuno Sorbent Assay; GABA<sub>A</sub> receptor:  $\gamma$ -Aminobutyric Acid  $\alpha$  Subunit Receptor; TNFR1: Tumor Necrosis Factor Receptor type 1; TNFR2: Tumor Necrosis Factor Receptor type 2; NF- $\kappa$ B: Nuclear Factor Kappa-light-chain-enhancer of Activated B cells; TNF- $\alpha$ : Tumor Necrosis Factor-alpha; ip: intra-peritoneal

### Introduction

Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) is a well-studied cytokine that acts as an important central mediator in the on-set of inflammatory cascade and exerts anti-tumoral activity [1]. TNF- $\alpha$  can mediate opposing effects within the central nervous system, such as neurotoxicity through the caspases pathway, or neuroprotection through NF- $\kappa$ B dependent gene transcription [2]. As a potent pro-inflammatory cytokine, TNF- $\alpha$  levels rise in the nervous system after insults or after exposure to exogenous signals such as bacterial and viral proteins [3]. Beside the pro-inflammatory actions and mechanisms, the neuromodulatory face of TNF- $\alpha$  starts to be revealed [4]. For example, TNF- $\alpha$  enhances glutamatergic synaptic transmission by increasing surface expression of neuronal AMPA receptors [5,6]. Several findings have been pointing for a role of TNF- $\alpha$  in synaptic plasticity, more specifically in homeostatic synaptic scaling. Synaptic scaling is a type of plasticity, which involves adjustments in the strength of all synapses on a neuron in response to prolonged changes in its activity. First, it was shown that TNF- $\alpha$  mediates homeostatic synaptic scaling in response to prolonged blockade of activity [7-9]. A more recent study suggested that TNF- $\alpha$  is critical for maintaining synapses in a plastic state in which synaptic scaling can be expressed [10]. Homeostatic synaptic scaling mediated by TNF- $\alpha$  participates in experience-dependent brain plasticity [11]. Kaneko et al. described that experience-dependent plasticity in the developing visual cortex involves a homeostatic increase in responses, which is dependent on TNF- $\alpha$  signaling. Critical periods are restricted early development time windows during which the central nervous system displays heightened plasticity in response to events occurring in the environment. To determine the onset and duration of the critical period of experience-dependent plasticity in the primary auditory cortex, rat pups were exposed to pure-tones at different postnatal ages. Profound and persistent alterations in sound representations in the primary auditory cortex were found only when

exposure occurred during postnatal day 11 (P11) to P13 [12], pointing this epoch as a window of critical period for plasticity in this cortical region [12]. Evidence point to a role of TNF- $\alpha$  in brain plasticity, but the modulation of endogenous TNF- $\alpha$  levels throughout a critical period of brain plasticity is not known. Here we document changes in TNF- $\alpha$  from before to after the critical period of experience-dependent plasticity in the primary auditory cortex. We assessed the endogenous TNF- $\alpha$  levels at different time-points around the critical period: before the critical period (P7), during the critical period (P12) and after the critical period (P30). Additionally, we measured the TNF- $\alpha$  levels in the frontal cortex at the same ages.

### Materials and Methods

All experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of California, San Francisco. Twenty-three female Sprague Dawley rats were studied. They were pre-medicated with atropine sulfate (0.02 mg/kg) to minimize bronchial secretions and dexamethasone (0.2 mg/kg) to prevent brain edema. They were then anesthetized with pentobarbital (35-60 mg/kg) and supplemental doses of pentobarbital (10 mg/kg, i.p.) were given if necessary. The right auditory cortex and the frontal cortex were exposed and auditory cortical responses were recorded with parylene-coated tungsten microelectrodes in a shielded, double-walled sound chamber. After the overall boundaries of the right primary auditory cortex were determined, the rats were deeply anaesthetized. The primary auditory cortex and the frontal cortex were rapidly dissected, frozen in dry ice and stored at -80°C until processing. Cortical fragments were lysed and acidified, and total protein concentrations were determined using the BCA assay. TNF- $\alpha$  was quantified using an ELISA kit (BD OptEIA™ Mouse TNF ELISA Kit II, San Jose, USA) as per the manufacturer's protocol.

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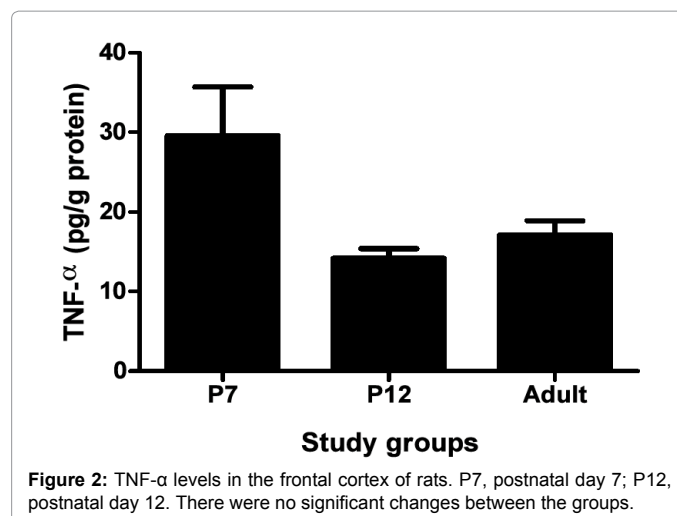
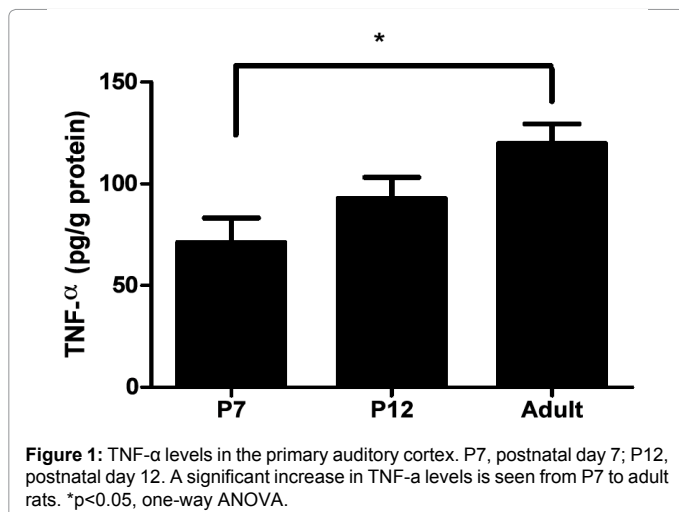
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## Results

To specifically collect primary auditory cortical samples, we first functionally determined the boundaries of primary auditory cortex using the following criteria: (1) primary auditory neurons generally have a continuous, single-peaked, V-shaped receptive field; (2) characteristic frequencies of the primary auditory neurons are tonotopically organized with high frequencies represented rostrally and low frequencies represented caudally; and (3) External boundaries are characterized by nonresponsive sites and/or responsive sites with no well-defined pure tone-evoked response [13]. Using these criteria, we were able to precisely delineate the area within primary auditory cortex and collect respective cortical fragments for analysis. We assessed TNF- $\alpha$  levels in the primary auditory cortex at P7 (before the critical period), P12 (during the critical period) and P30 (after the critical period) (Figure 1). We found that mean TNF- $\alpha$  levels in the primary auditory cortex increase throughout the critical period, from 71.13 pg/g protein (SD=29.63; N=6) at P7, to 92.84 pg/g protein (SD=23.16; N=5) at P12, and to 119.8 pg/g protein (SD=23.58; N=6) at P30. One-way ANOVA analysis showed significant differences between the groups (overall  $F=5.36$ ;  $p<0.05$ ). Post hoc analysis (Bonferroni) showed that TNF- $\alpha$  levels in the P7 group were significantly lower than at P30 ( $p<0.05$ ), while no significant difference was found between P7 vs P12 and P12 vs P30. To assess whether the changes observed in the primary auditory cortex were specific for this structure or occurred globally in the brain, we measured endogenous TNF- $\alpha$  levels in the frontal cortex during the same time-points (Figure 2). Overall, the profile of changes in TNF- $\alpha$  levels in the frontal cortex did not recapitulate the changes observed in the primary auditory cortex. In contrast to the increase observed in the auditory cortex, TNF- $\alpha$  levels in the frontal cortex showed a tendency to decrease over the same time window: they went from 29.56 pg/g protein (SD=10.62; N=3) at P7 to 14.14 pg/g protein (SD=2.16; N=3) at P12, and to 17.08 pg/g protein (SD=3.13; N=3) in P30. Although, one-way ANOVA analysis showed no significant difference between the groups (overall  $F=4.74$ ;  $p>0.05$ ). It is noticeable that mean TNF- $\alpha$  levels measured in the frontal cortex were always lower than 30 pg/g protein, while in the primary auditory cortex they were higher than 70 pg/g protein.

## Discussion

We observed that cortical endogenous levels of TNF- $\alpha$  were significant higher at adulthood compared to P7 in the primary auditory



cortex, displaying a TNF- $\alpha$  increase from before to after the critical period for experience-dependent plasticity in this region. It was previously suggested that TNF- $\alpha$  maintains synapses in a plastic state in which synaptic scaling can be expressed [10]. Furthermore, synaptic scaling mediated by TNF- $\alpha$  plays a role in potentiation of responses that occurs during neural plasticity induced in the binocular zone of the developing visual cortex by monocular visual deprivation [11]. Different from the plasticity induced by sensory deprivation that occurs in the visual cortex, the experience-dependent plasticity in the auditory cortex is induced by sensory exposure [12]. Brain plasticity induced by visual deprivation or sound exposures have been related to an increase in cortical inhibition mediated by GABA receptors [14,15]. This increase in inhibition is compatible with the induction of homeostatic synaptic scaling, which is dependent on TNF- $\alpha$  signaling [9]. Our observation that endogenous levels of TNF- $\alpha$  specifically increase in the auditory cortex from before to after the critical period suggests for a role for TNF- $\alpha$  in experience-dependent brain plasticity in the auditory cortex. One could argue that the changes in TNF- $\alpha$  might be merely related to the general development. However changes in TNF- $\alpha$  levels in the frontal cortex showed a different profile over the time-window studied. In contrast to the increase in TNF- $\alpha$  levels observed in the auditory cortex from before to after the time-window of critical period of (P7 and adults, respectively), the frontal cortex showed (if any) a tendency of decrease in TNF- $\alpha$  levels. Interestingly, overall TNF- $\alpha$  levels in the auditory cortex were more than 2 times higher than the levels in the frontal cortex. A limitation to this study is that we do not know so far if these changes in TNF- $\alpha$  are relevant to the physiology of auditory cortex, since we did not perform any "loss-of-function" experiment. Additional studies are needed to address this question. In conclusion, we found that TNF- $\alpha$  levels in the auditory cortex of rats increases from before to after the critical period for experience-dependent plasticity in the primary auditory cortex. We speculate that TNF- $\alpha$  can play a role in brain plasticity mechanisms in the auditory system.

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