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Effects of chronic fluoxetine treatment on the rat somatosensory cortex: Activation and induction of neuronal structural plasticity

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ABSTRACT

Recent hypotheses support the idea that disruption of normal neuronal plasticity mechanisms underlies depression and other psychiatric disorders, and that antidepressant treatment may counteract these changes. In a previous report we found that chronic fluoxetine treatment increases the expression of the polysialylated form of the neural cell adhesion molecule (PSA-NCAM), a molecule involved in neuronal structural plasticity, in the somatosensory cortex. In the present study we intended to find whether, in fact, cell activation and neuronal structural remodeling occur in parallel to changes in the expression of this molecule. Using immunohistochemistry, we found that chronic fluoxetine treatment caused an increase in the expression of the early expression gene c-fos. Golgi staining revealed that this treatment also increased spine density in the principal apical dendrite of pyramidal neurons. These results indicate that, apart from the medial prefrontal cortex or the hippocampus, other cortical regions can respond to chronic antidepressant treatment undergoing neuronal structural plasticity.

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Although the neurobiological bases of depression are not well understood, it has been proposed that dysfunction of the mechanisms of neuronal plasticity may be involved [3,6]. Moreover, antidepressant drugs may act by normalizing this neural plasticity [6,5,13]. Structural plastic processes, such as dendritic or spine remodelling, have been observed in animal models of depression [12,37] and after antidepressant treatment [10], specially in the amygdala, the hippocampus and the medial prefrontal cortex (mPFC) (see [24] for review). This structural remodelling may be mediated by changes in the expression of cytoskeletal proteins or cell adhesion molecules, such as the polysialylated form of the neural cell adhesion molecule (PSA-NCAM) [2,8,30]. In fact, the antidepressant fluoxetine, a serotonin reuptake inhibitor, increases the expression of PSA-NCAM in the mPFC, the hippocampal CA3 stratum lucidum and the visual and somatosensory cortices [38,39].

To date, there is no direct evidence that the somatosensory cortex is affected by depression, although animal models of this mental disorder show alterations in the physiology of this cortical region [35] and antidepressants modulate somatosensory-related functions when administered locally [15]. The finding of an altered expression of PSA-NCAM in the somatosensory cortex after chronic treatment with fluoxetine [39] has prompted us to study whether the structure and activity of this cortical region is also modified after antidepressant treatment.

We have used twelve male Sprague–Dawley rats (4 months old, 320 ± 50 g, Harlan Iberica), which were chronically injected intraperitoneally either with the antidepressant fluoxetine ($n = 6$, 10 mg/kg), or with saline solution ($n = 6$), during 14 days (once daily at 10.00 am). All animal experimentation was conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). Rats were perfused transcardially under deep chloral hydrate anaesthesia (chloral hydrate at 4%, 1 mL/100 g) with saline and then 4% paraformaldehyde in sodium phosphate buffer (PB 0.1 M, pH 7.4). After perfusion, the brains were extracted and stored in PB until used.

In order to study cellular activation in the somatosensory cortex the left hemisphere was cut into 50 μm thick sections with a freezing sliding microtome and immunohistochemically stained for the immediate early gene c-fos. Briefly, sections were incubated with 5% normal donkey serum (NDS) (Abcys) in PBS with 0.2% Triton-X100 (Sigma) for 1 h, and then overnight with rabbit polyclonal anti-c-fos K25 (1:2000; Santa Cruz Biotechnology, Inc.). After washing, sections were incubated for 30 min with biotinilated donkey anti-rabbit IgG (1:200; Jackson Immunoresearch), followed by an avidin-biotin-peroxidase complex (ABC, Vector Laboratories) for 30 min in PBS. Color development was achieved by incubating with 3,3′-diaminobenzidine tetrahydrochloride (DAB, Sigma) for 4 min. All the sections were coded to avoid any bias and,

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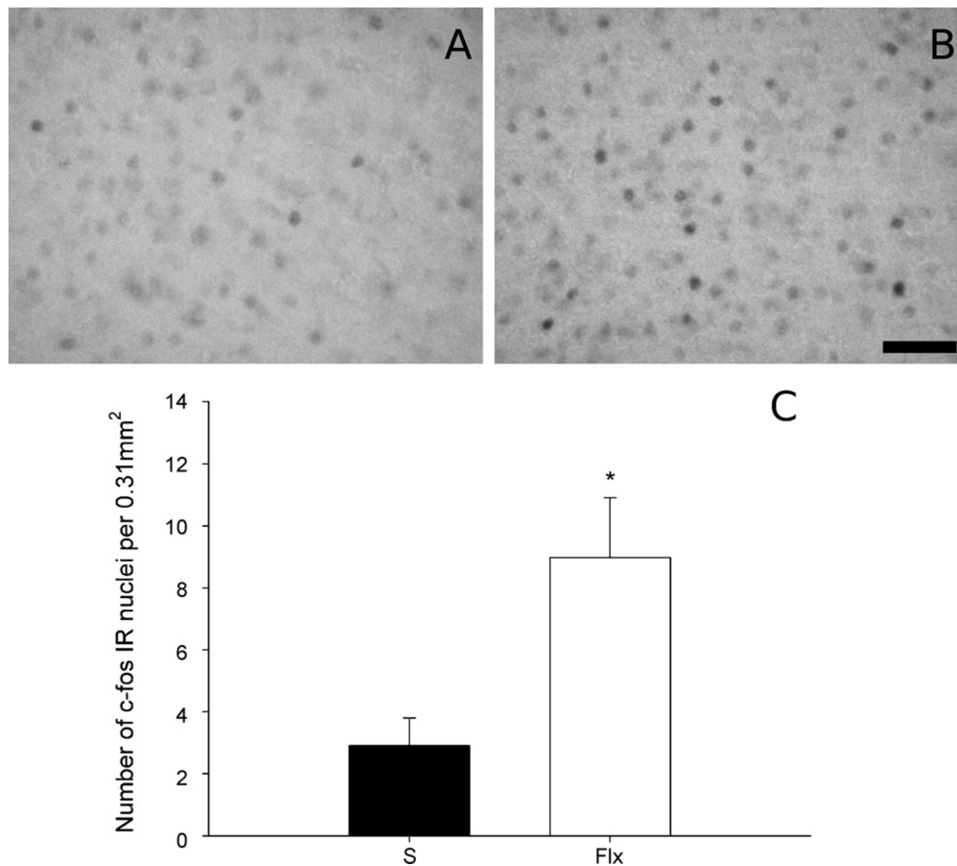

Fig. 1. A, B: Photographs showing coronal sections of c-fos immunoreactive nuclei in layers III to V of the somatosensory cortex of a control rat (A) and a rat treated for 14 days with fluoxetine (B). C: Graphs representing changes in the number of c-fos immunoreactivity nuclei in the primary somatosensory cortex after chronic fluoxetine treatment. Statistically significant (* 0.01), unpaired *t*-test. White bar represents control animals and black bar represents fluoxetine treated animals. Scale bar: 50 μm.

when processed, passed through all procedures simultaneously to minimize any difference from immunohistochemical staining itself. c-fos immunoreactive nuclei quantification was performed in two different sections containing the primary somatosensory cortex (between Bregma −2.80 mm and Bregma −3.10) in layers III, IV and V. Images were captured with a 40× objective under bright-field illumination, homogeneously lighted and digitalized using a CCD camera attached to the microscope. After background subtraction and histogram normalization, the region was selected using systematic criteria, and analyzing the whole image for the automated counting. Then the pictures were analyzed with Image J software (NIH) with a protocol for automated counting of stained nuclei based on the one described by [40]. Briefly, images were binarized using the same value of the gray histogram for all the images. Then the images were eroded and dilated. Objects smaller than 400 pixels were discarded.

In order to study spine density in pyramidal cells, the right hemisphere was processed for Golgi method. Tissue blocks (2.5–3 mm) were fixed with 3% potassium dichromate and 5% glutaraldehyde during 7 days and then impregnated with silver nitrate solution (0.75%) for 48 h. Then, the brains were cut into 150 μm-thick sections with a vibratome immersed in 70% ethanol, dehydrated with 100% ethanol and mounted with epoxy resin between two coverslips. To avoid any bias in the analysis, the slides were coded, and the code was not broken until the analysis was completed. Spine quantification was carried out in each animal in six pyramidal neurons from layers III, IV and V, which were randomly selected inside somatosensory primary cortex (S1) area. A total of 48 neurons were analyzed. In order to be suitable for dendritic spine analysis, neurons should follow these features: (i) they must display complete Golgi impregnation of the principal apical dendrite, (ii) the cell type must be identifiable and (iii) the minimum length of the apical dendrite must be 200 μm from the soma. The spine density was calculated in fragments of 50 μm length beginning from the soma.

In the somatosensory cortex c-fos expressing nuclei were widely distributed across all the layers, although they appeared to be less abundant in layer IV. This expression pattern is similar to that observed in other adjacent neocortical regions, although in these areas layer IV appeared more populated. Chronic fluoxetine treatment induced a, three fold, statistically significant increase ($p = 0.021$) in the number of c-fos immunolabeled nuclei in the primary somatosensory cortex (Fig. 1).

In both control and fluoxetine treated animals we observed that the dendritic spine density was lower in the proximal segment and that it increased progressively towards the apical extreme of the principal dendrite (Fig. 2A). There were significant differences in the spine density between the first and second segments ($p = 0.003$), the second and the third ($p < 0.0001$) and the third and the forth ($p = 0.042$), according to the distance from the soma.

After 14 days of fluoxetine treatment, we did not found differences in the dendritic spine density of the proximal segments (0–50 μm). However, significant differences were observed in the second ($p = 0.01$, 50–100 μm), the third ($p < 0.0001$, 100–150 μm) and the fourth segments ($p = 0.0006$, 150–200 μm) (Fig. 2A). Significant increases were also observed when considering the entire length of apical dendrites of pyramidal neurons in the primary somatosensory cortex ($p < 0.001$) (Fig. 2B).

The present results indicate that antidepressant treatment activates the somatosensory cortex and induces dendritic spine remodelling of pyramidal neurons in this cortical region, thus

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