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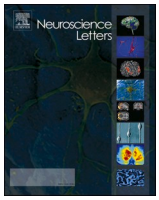

Ondansetron blocks fluoxetine effects in immature neurons in the adult rat piriform cortex layer II

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ABSTRACT

Neuronal structural plasticity gives the adult brain the capacity to adapt to internal or external factors by structural and molecular changes. These plastic processes seem to be mediated, among others, by the action of the neurotransmitter serotonin through specific receptors (5-HTRs). Previous studies have shown that the maturation of granule cells in the hippocampus is mediated by 5-HT3. In the present study, we wanted to check if the neural maturation in layer II piriform cortex is also mediated by 5-HT3. In the piriform cortex, in contrast to the hippocampus, there is no postnatal neurogenesis. All immature neurons (PSA-NCAM immunoreactive) were originated prenatally. Immature cells in this area begin as small cells (type I cells) that then mature to larger cells (type II cells), and finally, mature to principal cells (PSA-NCAM immunonegative). To study the role of 5HT3 in this population, we first demonstrated the presence of 5HT3 receptors on both type I and II cells. Then we increased serotonin concentration using chronic fluoxetine administration, producing a reduction in the number of type I cells and an increment of type II cells but not an induction in the final stage of maturation to principal cells, as shown by the higher number of immature cells than in controls. This effect was blocked by ondansetron (a 5 HT3 antagonist). In conclusion, serotonin induces the progression from type I cells to type II cells but not from the later to mature PSA-NCAM immunonegative neurons. This effect is mediated by 5-HT3 receptors present in the immature cells.

Significance statement

Neuronal maturation processes in the rat piriform cortex are sensitive to serotonin. In piriform cortex layer II, there is a population of immature neurons, generated prenatally. These immature neurons have different steps in the process of maturation: type I, type II, and mature excitatory neurons. Chronic fluoxetine treatment induces an increase in type II and a reduction of type I cells. Immature neurons in this region express receptor type 3 (5-HT3R) for serotonin. Administration of ondansetron (a 5-HT3 antagonist) blocks the effect of fluoxetine treatment.

1. Introduction

Structural plasticity can be defined as the ability to perform adaptive changes in the function of the central nervous system by morphological changes [1]. Structural plasticity is present from embryonic development to adulthood and includes cell proliferation, cell migration, growth and remodelling of axons and dendrites, and the formation of new synaptic contacts. In adults, structural plasticity is mainly restricted to specific regions, such as the hippocampus, piriform and prefrontal cortex, amygdala, and olfactory bulb [2]. Concretely, the piriform cortex contains a population of immature neurons in layer II expressing the plasticity markers polysialylated form of the neural cell adhesion molecule (PSA-NCAM) and doublecortin[3–5].

Several mood disorders are related to alterations in structural plasticity, particularly adult neurogenesis. One of them is major depression, which in some cases has been related to a decrease in the concentration of serotonin and, in animal models, to a reduction of adult hippocampal neurogenesis. In fact, many antidepressant treatments are based on the modulation of serotonin levels. Among them, one of the most prevalent is fluoxetine (commercially known as Prozac). Fluoxetine is a serotonin

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reuptake inhibitor, which generates an increase in the concentration of free serotonin [6]. Previous studies in our laboratory have demonstrated that fluoxetine induces changes in the expression of molecules related to structural plasticity, such as PSA-NCAM [7,8]. Chronic treatment with fluoxetine induces an increment of cell activity in cortical regions [9], as shown by c-fos expression, and increases neurogenesis in the subgranular zone (SGZ) [10]. Moreover, recent studies in our laboratory have demonstrated that the increased cell proliferation and neurogenesis observed in the SGZ are mediated by the ionotropic receptor 5-HT3 [11]. In that study, we observed the expression of this receptor in stem cells, amplifying neural progenitors and immature neurons [11].

In this study, we have tried to determine the effect of 5-HT3 on neuronal maturation in the adult rat piriform cortex. Piriform cortex layer II displays a population of immature neurons generated during the prenatal period (before E15) [3]. This population decreases with age [12] and is sensitive to processes that affect the olfactory bulb, such as bulbectomy [13]. Maturation of adult rat piriform cortex layer II immature neurons generates excitatory neurons [14]. Although this population of immature cells is restricted mainly to the piriform cortex in rodents, it does have an extensive distribution in the neocortex of primates [2], and therefore its modulation could be relevant.

Given previous results relating the effect of fluoxetine, through 5-HT3 receptors, on structural plasticity in several brain regions (prefrontal, somatosensory cortices, and hippocampus), we wanted to analyze its putative role over the maturation in the adult rat piriform cortex layer II, a region where neuronal maturation is produced through adulthood but where cell proliferation is absent, allowing us to separate the effects of 5-HT3 on maturation from those on cell proliferation.

2. Material and methods

2.1. Animal treatments and histology

Twenty-four male Sprague-Dawley rats (4 months old, 320 ± 50 g, Harlan Iberica) were used in the experiments. All animal experimentation was conducted in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC). Rats were divided into four groups (n = 6) and treated once a day for 14 consecutive days, as follows: The first group received fluoxetine (10 mg/kg, intraperitoneal (i.p.), Sigma) and saline 30 min later. The second group received fluoxetine (10 mg/kg, i.p.) and the 5-HT3 receptor antagonist ondansetron (2 mg/kg, i.p., a generous gift of Glaxo-Smithkline) 30 min later. The third group received a saline injection and ondansetron (2 mg/kg, i.p.), 30 min later. Finally, the fourth group received two injections of saline, separated by 30 min. All drugs were dissolved in saline. The volume of all injections was adjusted to 500 μl. The day after the last injection, rats were transcardially perfused under deep anaesthesia with a saline solution followed by 4 % paraformaldehyde in sodium phosphate buffer 0.1 M, pH 7.4 (PB). After perfusion, brains were extracted and cryoprotected in 30 % sucrose in PB. Coronal sections (50 μm, 10 subseries per hemisphere) were obtained with a sliding microtome and stored at −20 °C in 30 % glycerol, 30 % ethylene glycol, and 40 % PB until used.

2.2. Characterization of the 5-HT3 receptor in immature neurons in the piriform cortex layer II

Double immunofluorescence was performed to characterize the expression of the 5-HT3 receptor in the immature neurons of the piriform cortex layer II. Control sections were used for the phenotypic characterization of 5-HT3 receptor expression. Tissue was processed 'free-floating' for immunofluorescence as follows: Briefly, sections were incubated for 1 min in an antigen-unmasking solution (0.01 M citrate buffer, pH 6) at 100 °C. After cooling down to room temperature, they were incubated for 1 h with 5 % normal donkey serum (NDS) (Jackson Laboratories) in PBS with 0.2 % Triton-X100 (Sigma) and then they were incubated overnight at room temperature with a combination of rabbit polyclonal IgG anti-5-HT3 receptor (1:250, Calbiochem) and mouse monoclonal IgM anti-PSA-NCAM (1:700, Chemicon). After washing, sections were incubated with donkey anti-rabbit IgG and donkey anti-mouse IgM secondary antibodies conjugated with Alexa 488 or Alexa 555, respectively.

All sections for fluorescent immunohistochemistry were mounted on slides and coverslipped using Dako Citomation mounting medium (Dako). Then, the sections were observed under a confocal microscope (Leica TCS-SP2). Z-series of optical sections from the piriform cortex layer II (1 μm apart) were obtained using sequential scanning mode. These stacks were processed using ImageJ software. Cells expressing the 5-HT3 receptor in the piriform cortex layer II were first identified using conventional fluorescence microscopy, and then, a stack of confocal images was taken to confirm the phenotype of these cells.

The companies of origin have previously tested the specificity of the antibodies. Moreover, their specificity in rat tissue has been confirmed previously [15]. Overnight incubation of the 5-HT3 antibody with an excess of its immunogenic peptide resulted in a total absence of 5HT-3 immunostaining in the piriform cortex, as previously reported in the hippocampus [11]. Additional controls for the immunohistochemical procedure were carried out in our laboratory by omitting the primary or secondary antibodies in each step of the immunohistochemical protocol.

2.3. Quantification of immature neurons (PSA-NCAM immunoreactive) in the piriform cortex layer II of adult rats

For the characterization of the effects of fluoxetine and ondansetron in adult rat piriform cortex layer II PSA-NCAM immunoreactive cells, sections were coded prior to processing; the immunostaining was done in all the samples in parallel to prevent the effects of the incubation process, and the code was maintained until finishing the cell quantification.

Tissue was processed 'free-floating' for immunohistochemistry as follows: In brief, sections were incubated for 1 min in an antigen-unmasking solution (0.01 M citrate buffer, pH 6) at 100 °C. After cooling down to room temperature, they were incubated for 10 min in a solution of 3 % H2O2 and 10 % methanol in PBS to block endogenous peroxidase activity. After washing, sections were incubated for 1 h with 5 % normal donkey serum (NDS) (Jackson Laboratories) in PBS with 0.2 % Triton-X100 (Sigma) and incubated overnight at room temperature with mouse monoclonal IgM anti-PSA-NCAM (1:700, Chemicon). After washing, sections were incubated with donkey anti-mouse IgM biotinylated antibody (Jackson Laboratories, 1:250) for 2 h, followed by an avidin–biotin-peroxidase complex (ABC, Vector Laboratories) for 30 min in PBS. Color development was achieved by incubating with 0.05 % 3,3′ diaminobenzidine tetrahydrochloride (DAB, Sigma) and 0.003 % hydrogen peroxide in PBS for 4 min. PBS containing 0.2 % Triton-X100 and 3 % NDS was used for primary and secondary antibody dilutions.

The number of immature neurons in the piriform cortex layer II of the rat was estimated using a modified version of the fractionator method [16], as described before [17]. We counted cells covering 100 % of the sample area (piriform cortex layer II). The fractionator sampling scheme refers to the methodology of examining one out of every 10 brain sections. Thus, our modification of the optical dissector combined with 1:10 fractionator sampling is truly a modification of the optical fractionator method. A 1:10 parallel subseries of sections covering the whole rostral to caudal extension of this structure was viewed under an Olympus CX41 microscope. Cell somata were identified and counted with a 40X objective. Cells appearing in the upper focal plane were omitted to prevent cell overcounting. The volume of the different analyzed areas was determined for each animal using Cavalieri's principle [18]. Means were determined for each experimental group, and the data (after checking homoscedasticity and normality) were subjected to one-way ANOVAs followed by Student-Newman-Keels post hoc tests.

2.4. Cellular size of PSA-NCAM immunoreactive cells in piriform cortex layer II

The diameter of PSA-NCAM immunoreactive cells in the piriform cortex layer II was estimated in the four groups (control, fluoxetine, ondansetron, and fluoxetine-ondansetron). Images were taken using a digital camera (Olympus DP72) attached to a CX41 Olympus microscope. Images were taken using a 100x objective. Cellular diameter was measured using ImageJ software. Values were expressed as the mean diameter in microns ± S.E.M. Means were determined for each experimental group, and the data (after checking homoscedasticity and normality) were subjected to one-way ANOVAs followed by Student-Newman–Keels post hoc tests.

3. Results

We analyzed the presence of the 5-HT3 receptor in the immature neurons of adult rat piriform cortex layer II (Fig. 1). In this region, there were two populations of immature neurons: the small-size cell population (type I cells) and the large-size cell one (type II cells) (Fig. 1A). We analyzed the expression of the 5-HT3 receptor in both populations, and we observed that both type I and II cells display 5-HT3 receptor expression (Fig. 1B-C). The distribution of this receptor was similar in both cell types, being more abundant in the cell somata and proximal dendrites. However, the expression seemed higher in type I than in type II cells (Fig. 1B-C).

Then, we analyzed the effect of fluoxetine and ondansetron on the number of immature (PSA-NCAM immunoreactive) neurons in the adult rat piriform cortex layer II (Fig. 2). The distribution of PSA-NCAM-expressing neurons in layer II was similar across treatments, but the cell density was dependent on treatment (Fig. 2A–D). Chronic fluoxetine treatment induced an increase in the number of immature neurons (16780 ± 696 immature neurons in control rats vs. 18480 ± 491 immature neurons in the chronically fluoxetine-treated rats, $p < 0.05$). The rats treated with both fluoxetine and ondansetron displayed a number of immature neurons similar to control rats (15492 ± 371 immature neurons, n.s. to control and $p < 0.001$ to fluoxetine-treated rats). Finally, the rats treated chronically with ondansetron displayed a reduction in the number of immature neurons compared to control rats (13780 ± 397 immature neurons, $p < 0.01$ compared to control rats). Therefore, chronic fluoxetine treatment results in an increase in the number of immature neurons that is blocked by the co-treatment with ondansetron. In fact, treatment with ondansetron by itself reduces the number immature of neurons compared to controls.

We observed also differences in the size of the PSA-NCAM immature neurons in the adult rat piriform cortex layer II, being higher in the fluoxetine-treated group and smaller in the ondansetron-treated group. Cellular size in the immature neurons of piriform cortex layer II is related to the maturation state. Immature neurons remain in a tangled state (small size, type I), and during the maturation process, neurons pass to the transition state (larger size, type II), whereas when they complete maturation, they are no longer visible with PSA-NCAM

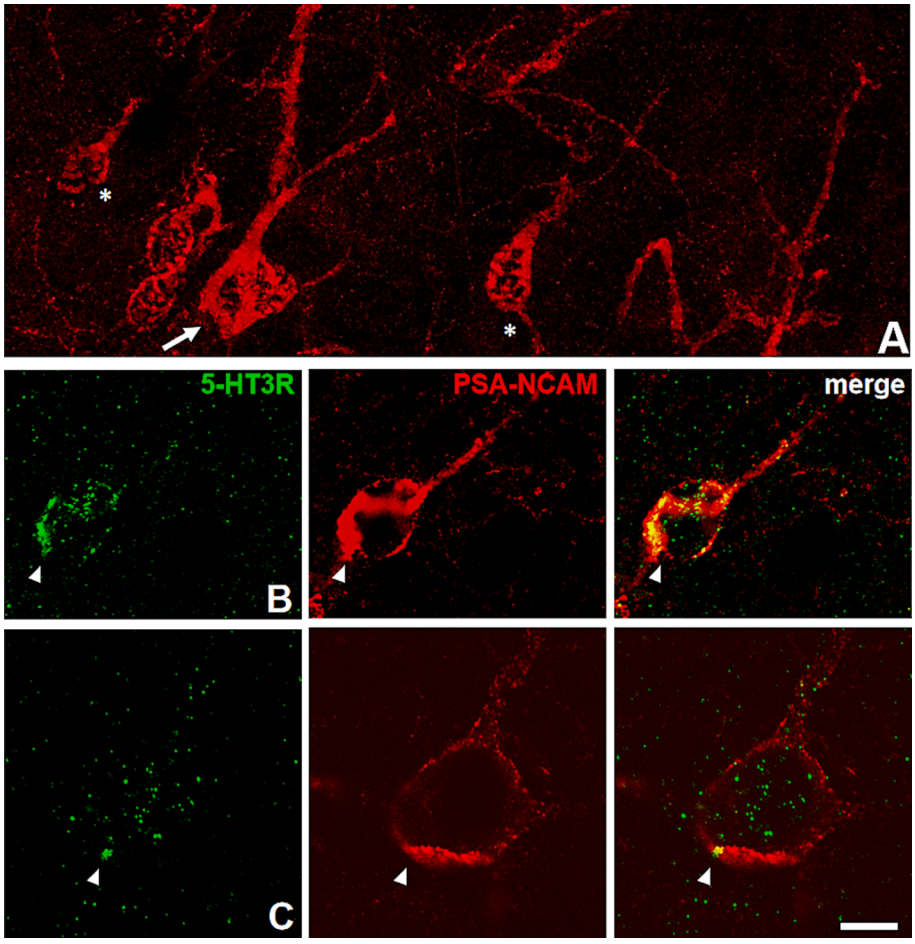

Fig. 1. Expression of the 5-HT3 receptor in immature neurons in the adult rat piriform layer II. A. Panoramic view of PSA-NCAM expression (immature neurons) in the adult rat piriform cortex. Observe the presence of two populations: the small cells (type I cells) marked with asterisk and the larger cells (type II cells) marked with arrows. B-C. Detail of colocalization between PSA-NCAM (red) and the 5-HT3 receptor (green) in type I (B), and type II (C) cells. Arrowheads indicate the presence of the 5-HT3 receptor. Scale bar represents 10 μm (A) and 5 μm (B-E).

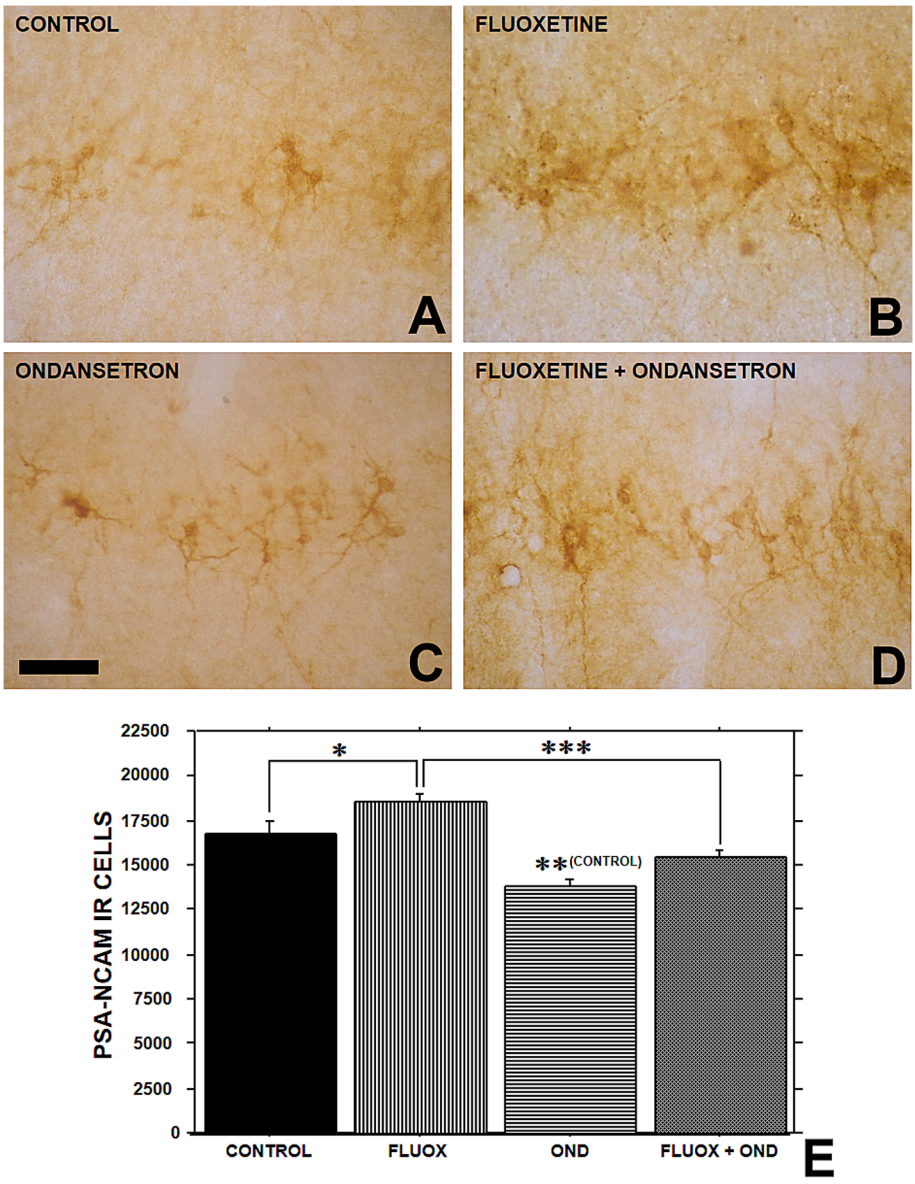

Fig. 2. Change in the number of PSA-NCAM immunoreactive neurons in the piriform layer II of adult rats after chronic fluoxetine treatment. A-D Representative images of the expression of PSA-NCAM in piriform layer II in the different groups analysed. E. Graph representing the number of cells expressing PSA-NCAM in the piriform layer II. Scale bar represents 50 μm. Data represent mean ± SEM (* $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$).

(Fig. 3A). We measured the diameter of the immature neurons in the adult rat piriform cortex layer II in the different groups (Fig. 3B). In the control group, we observed a mean diameter of 6.90 ± 0.41 μm. Chronic fluoxetine treatment induces an increase in the diameter of immature neurons (8.47 ± 0.32 μm, $p < 0.01$). The cotreatment of fluoxetine and ondansetron induces no changes in the diameter of immature neurons (7.04 ± 0.33 μm, n.s.). Finally, the chronic ondansetron treatment does not induce a significant reduction in the mean diameter of immature neurons (6.22 ± 0.33 μm, n.s.).

Since these changes likely originate from the shift in the proportions of the PSA-NCAM cells in the area, we estimated the total number of small (type I cells) and large (type II cells) PSA-NCAM immunoreactive cells in the adult rat piriform cortex layer II. There was a reduction in the number of type I cells after fluoxetine treatment (10767 ± 851 cells in control conditions vs. 4466 ± 555 cells after chronic fluoxetine treatment, $p < 0.001$) (Fig. 3C). Double chronic treatment (fluoxetine + ondansetron) did not induce changes compared to control conditions (10328 ± 554 cells after double treatment, n.s.), therefore reverting the effect observed of fluoxetine. Chronic ondansetron treatment alone was ineffective in increasing the number of type I cells (10335 ± 471). When we estimated the number of the type II cells, the results were opposite to those observed in type I cells (Fig. 3D). Chronic fluoxetine treatment induced an increase in the number of type II cells (2937 ± 887 cells in controls vs. 8624 ± 944 after chronic fluoxetine treatment, $p < 0.001$). Consistent with the data for type I cells, the coadministration of ondansetron blocks this increment (1807 ± 163 type II cells after double administration, n.s.). Chronic ondansetron treatment by itself did not reduce the number of type II cells in a significant manner (1493 ± 212 large immature neurons).

Finally, when we represent the number of PSA-NCAM immature neurons related to the diameter size (Fig. 3E), we observed that the control group presents two populations (around 6 and 9 μm). When we analysed the fluoxetine-treated group, we observed a reduction in the 6 μm group and an increase in the 9 μm groups. The group treated with fluoxetine and ondansetron displayed an increase in the 6 μm group.

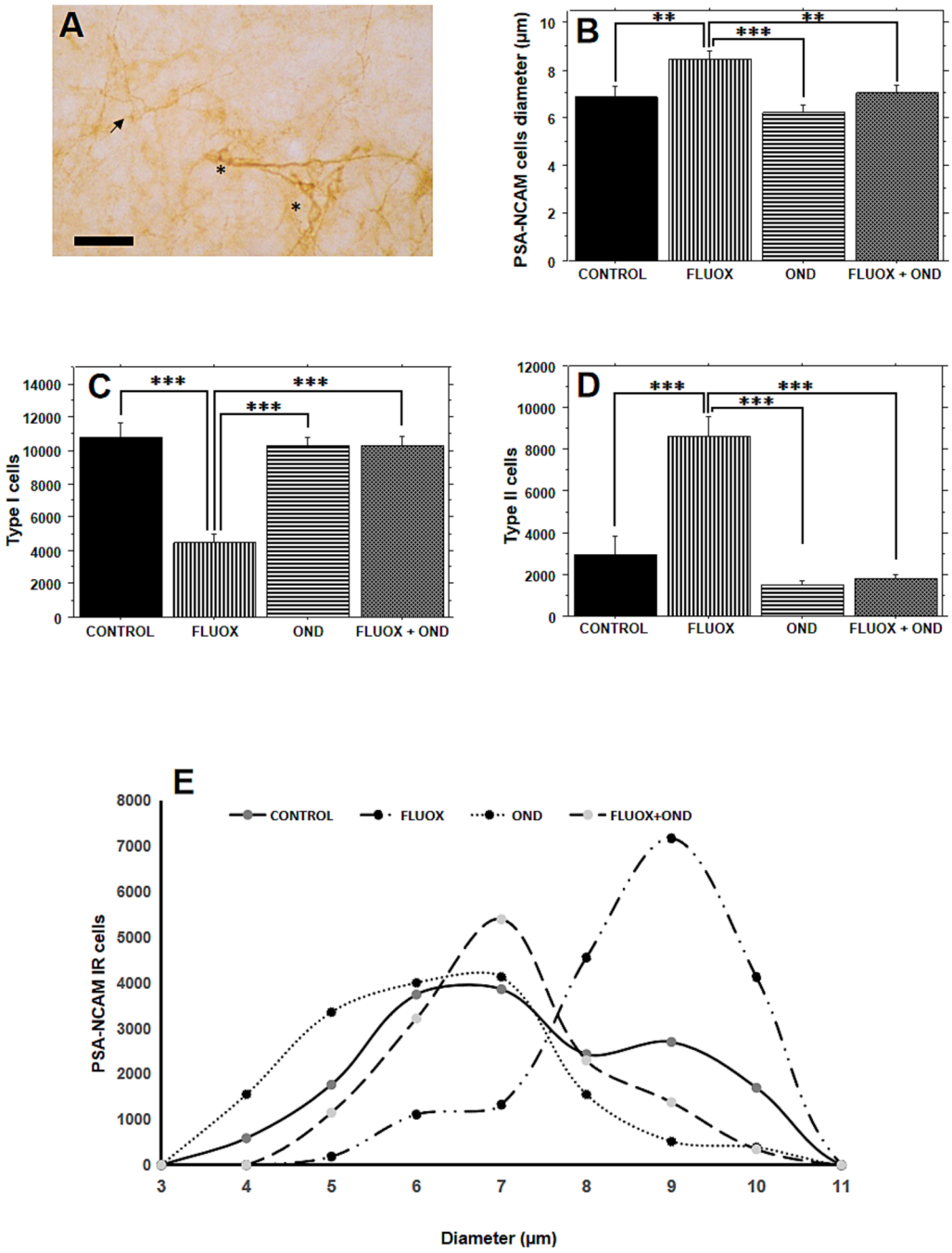

Fig. 3. Change in the size of immature neurons in the piriform layer II of adult rats after chronic fluoxetine treatment. A. Representative image of piriform layer II of an adult rat showing the two types of immature neurons: type I cells (asterisk) and type II cells (arrows). B. Graph representing the average of diameters of PSA-NCAM immature neurons in the piriform layer II in the different groups: control (CONTROL), fluoxetine (FLUOX), ondansetron (OND), and fluoxetine + ondansetron (FLUOX + OND). C. Graph representing the number of type I cells. D. Graph representing the number of type II cells. E. Graph representing the mean number of PSA-NCAM immature neurons for each group by diameter size. Scale bar represents 50 µm. Data represent mean ± SEM (** $p < 0.01$; *** $p < 0.001$).

4. Discussion

In this study, we have observed that both types of immature neurons in adult rat piriform cortex layer II (type I and II cells) display 5-HT3 receptor expression. Chronic fluoxetine treatment results in an increase in the number of PSA-NCAM immunoreactive immature neurons in the adult rat piriform cortex layer II, which is neutralized by the co-treatment with ondansetron, whereas the treatment with only ondansetron reduces the number of immature neurons. After chronic fluoxetine treatment, immature neurons have a larger mean diameter. However, ondansetron can block this increase both in combination with fluoxetine and by itself. The estimation of the total number of both type I and II cells in the adult rat piriform cortex revealed that after chronic administration of fluoxetine, the number of type I cells decreases, whereas the number of type II increases. Chronic co-treatment with fluoxetine and ondansetron does not alter the number of type I or II cells. Finally, the chronic treatment with ondansetron does not induce a significant reduction of type II cells, whereas the number of type I cells does not change.

The expression of the 5-HT3 receptor has been previously described in the hippocampus, amygdala, and superficial layers of the cerebral cortex [15] and in the piriform and entorhinal cortices [19]. In previous

studies of our group, we have observed its expression in the prefrontal cortex (associated with plastic inhibitory-interneurons) [7] and in the dentate gyrus of the hippocampus [11], mainly in stem cells, in proliferating precursors, and, to a lesser extent, in immature neurons. That study showed the involvement of the 5-HT3 receptor in cell proliferation and neuronal maturation. In the current study, we have analyzed the effect of 5-HT3 in adult rat piriform cortex layer II, a region with an elevated presence of immature neurons [3,4]. Former studies using BrdU injections during gestation demonstrated that these neurons are generated during the prenatal period [3]. These immature neurons remain in a "standby mode" until they differentiate through the adult life of the animal [3,13], becoming excitatory-glutamatergic neurons [14]. Immature neurons in piriform cortex layer II appear either as type I cells (smaller) or as type II (larger) that are in a transforming state into mature excitatory neurons [3]. Therefore, the total number of immature neurons in this area can only diminish with time. This also applies to the total number of type I cells, and the higher numbers found in some of the groups can only represent a slower process of maturation than in controls. In our study, we observed 5-HT3 expression in both type I and type II cells, but the expression was more intense in the type I cells. This population of immature neurons does not renovate and therefore is reduced progressively as the rat gets old and the cells become non-PSA-NCAM mature neurons [12]. Previous studies in other regions have demonstrated the expression of 5-HT3 receptors in immature neurons such as those in the hippocampus [11] or sensory olfactory neurons [20].

After proving the presence of the 5-HT3 receptor in this population of immature neurons, the next step was to analyze the effect of 5-HT3 on the maturation of these neurons. For that, we used a chronic treatment with fluoxetine (increasing serotonin levels) and, since that would act on all 5-HT receptors, to test if the effect is mediated by 5-HT3 receptors, the co-administration of a specific antagonist for this type of receptor, ondansetron. We observed that the chronic fluoxetine treatment induces changes in this population of immature neurons. After fluoxetine treatment, we observed an increase in the total number of immature neurons, but the two populations of immature neurons were affected differently. There was a reduction in the type I cell population and a strong increase in the number of type II cells. Changes in the number of immature neurons in the adult piriform cortex have been previously observed due to genetic conditions such as Down syndrome [21] or after bulbectomy (a model of depression) [13]. The changes observed in this study reflect exclusively an alteration in neuronal maturation since there is not adult neurogenesis in this area. In our study, we have observed that fluoxetine reduces the number of type I cells and increases the number of type II cells. This fact can be interpreted as a partial maturation of immature neurons from the type I to the type II state. The fact that we observe a global increase in PSA-NCAM-positive neurons cannot be explained as an increase in neurogenesis, since immature neurons in piriform cortex layer II are generated in the prenatal period [3]. The increased number of cells expressing PSA-NCAM could be explained by several reasons. A possible explanation could be the fact that fluoxetine increases cell activity [9,22], and PSA-NCAM expression is sensitive to this increase in cell activity [7]. Cells with a sub-umbral level of expression could, because of the increase in the expression, be detected. In relation to these results, previous studies have demonstrated that chronic fluoxetine treatment increases expression of plastic molecules (such as PSA-NCAM) in a different population of PSA-NCAM immunoreactive neurons (inhibitory neurons in the prefrontal cortex) [7]. The number of PSA-NCAM-immunoreactive neurons in the adult rat prefrontal cortex was increased after chronic fluoxetine treatment [7]. Similar results have been observed in some regions of the neocortex [8]. Another possibility could be an alteration in the maturation rate of immature neurons in the piriform cortex. The different effect over the two populations of immature neurons could be related to the different expression of 5-HT3 receptors. Type I cells displayed a high expression of the receptor and are very sensitive to the increase in serotonin induced by fluoxetine. However, type II cells, with a lower expression of the receptor, are less sensitive to this increase in serotonin.

To check if changes in PSA-NCAM expression and neuronal maturation due to fluoxetine, and subsequently, an increase in serotonin, are mediated by the 5-HT3 receptor, we co-administered fluoxetine and ondansetron chronically. As we pointed out previously, the chronic co-treatment with fluoxetine and ondansetron displayed similar results to the control group. The reduction in the observed increment of PSA-NCAM-positive cells after chronic fluoxetine, as well as the changes in the amount of type I or type II cells, indicate that the effect of the increment of serotonin in the process is exerted via 5-HT3 receptors. The treatment with only ondansetron proves that, at basal levels, the effect of serotonin through 5-HT3 is not enough to induce a significant change in maturation. These results suggest a pivotal role of the 5-HT3 receptor over 5-HT-induced maturation and PSA-NCAM expression. Previous studies by our group have demonstrated the role of the 5-HT3 receptor in the fluoxetine effect on neurogenesis and maturation in the hippocampus [11]. In this study, we have observed that the increased neurogenesis observed after chronic fluoxetine treatment is blocked when ondansetron is co-administered [11]. Our results in this report are in accordance with the previous one. Chronic fluoxetine treatment induces an increase in cell activity both in the neocortex [9] and in the piriform cortex [22] increasing the expression of PSA-NCAM [7,8]. The increase in expression of PSA-NCAM mediated by fluoxetine is blocked by ondansetron both in the adult rat prefrontal cortex [7] and in the adult rat hippocampus [11]. Finally, chronic administration of ondansetron leaves unaltered both populations of immature neurons.

It is interesting to note that although the increase of serotonin induces the maturation from type I to type II cells, it does generate an accumulation of immature cells. This maturation seems to be blocked at the intermediate stage (hence the higher total number of PSA immunoreactive cells), rather than resulting in a thorough maturation into the non-PSA expression stage. The final stage of maturation to mature cells is likely dependent on other factors than serotonin receptor activation.

Therefore, chronic fluoxetine treatment induces an increase in the expression of PSA-NCAM and increases the size of immature neurons in the adult piriform cortex layer II. The bigger size of immature neurons indicated a more advanced state of maturation (type I to type II). Immature neurons in the adult rat piriform cortex layer II express the 5-HT3 receptor in their membranes. The coadministration of ondansetron, which blocks this receptor, together with fluoxetine makes fluoxetine ineffective, demonstrating that the effect of fluoxetine is mediated by the 5-HT3 receptor, which is blocked by ondansetron. Finally, the single treatment with ondansetron was ineffective, leaving immature neurons in a similar state to control. In this report, we have shown the important role of the 5-HT3 receptor in neuronal maturation in the piriform cortex, a region without adult neurogenesis.

This population of neurons displays a delayed maturation that can be considered as "neurogenesis without division" [23]. The effect of fluoxetine on the maturation of these immature neurons corresponds to the general effect of fluoxetine on structural plasticity. In rodents, adult neurogenesis is abundant, but in other mammals, such as primates or humans, it is highly reduced. Although in rodents this population of immature neurons is restricted to the paleocortex, in other large-brained mammals it spreads over other cortical regions [24,25,26]. Modulation of the maturation of this population of cells could be of functional relevance for plastic events in humans and other large-brained mammals.

In conclusion, in a model in which adult neurogenesis is not present, we showed that the activation of the 5-HT3 receptor induces the maturation from type I to type II cells but does not induce the maturation from type II cells to mature cells. Therefore, the maturation for these cells can be broken into two consecutive steps with different regulation. It is possible the maturation of other types of immature neurons present in the adult brain follows a similar model.

CRediT authorship contribution statement

Marina Recatalá: Writing – review & editing, Visualization, Investigation. **Pablo Hidalgo:** Writing – review & editing, Investigation. **Juan Nàcher:** Writing – review & editing, Methodology, Conceptualization. **José Miguel Blasco-Ibáñez:** Writing – review & editing, Visualization. **Carlos Crespo:** Writing – review & editing, Formal analysis. **Emilio Varea:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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