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EFFECTS OF COMBINED NICOTINE AND FLUOXETINE TREATMENT ON ADULT HIPPOCAMPAL NEUROGENESIS AND CONDITIONED PLACE PREFERENCE

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Abstract—Adult neurogenesis occurs in mammals within the dentate gyrus, a hippocampal subarea. It is known to be induced by antidepressant treatment and reduced in response to nicotine administration. We checked here whether the antidepressant fluoxetine would inverse the decrease in hippocampal neurogenesis caused by nicotine. It is shown that repeated, but not a single injection of rats with fluoxetine was able to abolish the decrease in adult dentate cell proliferation produced by nicotine treatment. We measured the expression of several biochemical parameters known to be associated with neurogenesis in the dentate gyrus. Both drugs increased the expression of p75 neurotrophin receptor, which promotes proliferation and early maturation of dentate gyrus cells. Using the conditioned place preference (CPP) paradigm, we also gave both drugs in a context in which their rewarding properties could be measured. Fluoxetine produced a significant but less robust CPP than nicotine. A single injection of fluoxetine was found to reduce nicotine-induced CPP. Moreover, the rewarding properties of nicotine were completely abolished in response to repeated fluoxetine injections. Expression of nicotine-induced CPP was accompanied by an increase of phospho-CREB (cyclic AMP-responsive element-binding protein) and HDAC2 (histone deacetylase 2) expression in the nucleus accumbens. The data suggest that fluoxetine reward, as opposed to nicotine reward, depends on dentate gyrus neurogenesis. Since fluoxetine was able to disrupt the association between nicotine and the environment, this antidepressant may be tested as a treatment for nicotine addiction using cue exposure therapy.
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Key words: conditioned place preference, fluoxetine, histone deacetylase, neurogenesis, nicotine, phospho-CREB.

INTRODUCTION

The dentate gyrus (DG) in the hippocampal formation is one of the few brain structures in which neurogenesis has been demonstrated in adult mammals (Eriksson et al., 1998). Increased neurogenesis can be produced by a variety of treatments, including enriched environment (Brown et al., 2003), physical activity (van Praag et al., 1999), memory (Denny et al., 2014) or antidepressant drugs, including fluoxetine (Mendez-David et al., 2013). The neurogenic hypothesis of depression postulates that decreased production of new granule cells in the DG is linked to the pathophysiology of depression and that increased hippocampal neurogenesis is required for the behavioral effects of antidepressant treatment (Malberg et al., 2000; Santarelli et al., 2003). In contrast to the effect of antidepressants, decreased neurogenesis can be produced by drugs of abuse (Campbell et al., 2011), stress (Schoenfeld and Gould, 2012) or certain brain diseases (Danzer, 2012). For example, early studies have indicated that repeated exposure to nicotine impaired the long-term survival of adult-born neurons (Berger et al., 1998). These data were confirmed by studies using mutant mice lacking the two major nicotinic receptor subtypes (Campbell et al., 2011).

The neurobiological mechanisms by which antidepressants exert their effect are under reassessment. Recent data suggest that an additional mechanism used by the selective serotonin reuptake inhibitor (SSRI) fluoxetine to alleviate depression is by inhibiting the activity of the cholinergic system (Chau et al., 2011). Further arguments indicate a strong relationship between major depression and acetylcholine nicotinic receptors (Bertrand, 2005). Moreover, prevalence of nicotine dependence is significantly higher among individuals with mood disorders (Grant et al., 2004), probably because nicotine alleviates some negative cognitive features in afflicted individuals (Dani and Harris, 2005). Other data show that smokers present a greater risk of becoming depressed than nonsmokers (John et al., 2004), mostly during withdrawal periods. On the other hand, nicotine administration to freely moving mice was shown to produce

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Abbreviations: BrdU, bromodeoxyuridine; CPP, conditioned place preference; CREB, cyclic AMP responsive element binding protein; DAB, 3,3′-diaminobenzidine; DG, dentate gyrus; GFAP, glial fibrillary acidic protein; HDAC, histone deacetylase; i.p., intraperitoneal; NAc, nucleus accumbens; NeuN, hexaribonucleotide binding protein-3; $p75^{NTR}$, p75 neurotrophin receptor; PBS, phosphate-buffered saline; pCREB, phospho-CREB; PFC, prefrontal cortex; s.c., subcutaneous; VTA, ventral tegmental area.

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long-term potentiation (LTP) *in vivo* in the DG (Tang and Dani, 2009), implying that nicotine acts on the DG circuits. The effect may be direct or may occur through dopaminergic afferents from the ventral tegmental area (VTA), the latter establishing a functional link between reward centers and memory systems (Lisman and Grace, 2005).

Given that nicotine reduces adult hippocampal neurogenesis while fluoxetine increases it, we tested the hypothesis that fluoxetine would be able to reverse the decrease in DG neurogenesis caused by nicotine. **N**eurogenesis in the adult rodent brain was assessed by measuring bromodeoxyuridine (BrdU) labeling as well as expression of the cellular markers hexaribonucleotide binding protein-3 (NeuN) and glial fibrillary acidic protein (GFAP) (Kempermann et al., 1997; Bernabeu et al., 2006). Expression of p75 neurotrophin receptor ($p75^{NTR}$) was carried out as the receptor is expressed by adult dentate progenitor cells and it promotes proliferation and early maturation of DG cells in mice (Bernabeu and Longo, 2010). We also characterized the effects of combining both drugs on conditioned place preference (CPP) to evaluate whether fluoxetine modifies the CPP induced by nicotine. Nicotine-induced CPP has been well characterized (Le Foll and Goldberg, 2005; Pascual et al., 2009; Natarajan et al., 2011; Pastor et al., 2011), while fluoxetine-induced CPP has only been shown in initial studies (Collu et al., 1997).

Expression of proteins that were previously associated with nicotine-related conditioning was also analyzed. We measured the expression of phospho-cyclic AMP responsive element binding protein (pCREB) since enhanced phosphorylation of CREB is required for nicotine-induced CPP and reinstatement in rats (Pascual et al., 2009; Pastor et al., 2011). It is also involved in the regulation of adult neurogenesis in the DG (Nakagawa et al., 2002). Phospho-CREB binds the CREB-binding protein CBP, a transcriptional coactivator which possesses histone acetyltranferase (HAT) activity (Kalkhoven, 2004). In addition, histone deacetylase (HDAC) family members, especially HDAC2, are recognized as playing an important role in cognitive functions, inducing memory impairment when over-expressed (Guan et al., 2009). HDAC2 has been proposed to be involved in promoting synaptic plasticity underlying the preference for nicotine (Pastor et al., 2011). By removing acetyl groups from key histone residues, HDACs promote an inactive chromatin state, resulting in the silencing of downstream genes (Klose and Bird, 2006).

In the present study, we found that repeated, but not a single injection of rats with fluoxetine was able to abolish the decrease in adult dentate cell proliferation produced by nicotine treatment. A single fluoxetine injection was also found to reduce nicotine-induced CPP and the rewarding properties of nicotine were completely abolished in response to repeated fluoxetine injections.

EXPERIMENTAL PROCEDURES

Animals

Sixty-six adolescent male Sprague–Dawley rats (School of Pharmacy and Biochemistry, University of Buenos Aires), weighing 80–100 g (PN 25–26) at their arrival at the laboratory were housed in groups of four on a 12-h light/dark cycle with *ad libitum* access to food and water. Animals were handled for 5 min twice a day for 4 days prior to behavioral experiments. Adolescent rats were used since we and others previously found that they establish much stronger nicotine-induced CPP than adult rats (Natarajan et al., 2011; Pastor et al., 2011; Ahsan et al., 2014). All procedures involving animal care were conducted in compliance with national laws and policies, with the approval of the Ethics committee of the University of Buenos Aires.

Pharmacological treatment

Rats were injected intraperitoneally (i.p.) either acutely or repeatedly for 10 days (one injection per day, 'repeated treatment') with 10 mg/kg fluoxetine hydrochloride (Eli Lilly, Indianapolis, IN, USA) or an equivalent volume of phosphate-buffered saline (PBS). Nicotine tartrate (Sigma, St. Louis, MO, USA) was dissolved in PBS and administered subcutaneously (s.c.) at a dosage of 0.4 mg/kg in a volume of 1 ml/kg. This dosage is known to induce strong CPP in adolescent rats (Natarajan et al., 2011; Pastor et al., 2011; Ahsan et al., 2014). An equal volume of PBS was injected for the control condition. Indicated doses are based on the molecular weight of the free base. Bromodeoxyuridine (BrdU; Sigma, St. Louis, MO, USA) was dissolved in sterile 0.9% NaCl, filtered and injected i.p. at the dosage of 50 mg/kg. The various compounds were injected alone or in combination, according to the diagram shown in Fig. 1a. Animals were killed 1 day after the last injection (Pascual et al., 2009).

Conditioned-place preference (CPP)

Place conditioning was essentially performed in a three compartment box, as described previously (Pascual et al., 2009; Pastor et al., 2011). Briefly, home-made acrylic boxes were divided into two equally sized compartments (30 × 25 × 30 cm) separated by a smaller central chamber (12 × 21 × 21 cm) that had gray walls and a smooth plastic floor, with doors allowing access to the two lateral compartments. These two compartments had different visual, tactile and olfactory cues: one compartment had vertically striped black and white walls and a wire mesh floor above pine shavings; the other compartment had horizontal striped black and white walls and a bar-grid floor above cedar shavings. The apparatus was designed so that rats did not present any consistent bias for a particular chamber. During the habituation period, animals were handled twice a day for 5 days and were injected s.c. or i.p. with PBS to habituate them to the injections. A camera connected to a computer was placed approximately 1.2 m above the CPP boxes. During pretest and CPP test, rat behavior was recorded and videos were analyzed, first by direct observation and then using the Noldus Ethovision XT7 software (Noldus Information Technology, The Netherlands).

Pre-conditioning phase. Following the habituation period, animals were injected with PBS and placed in

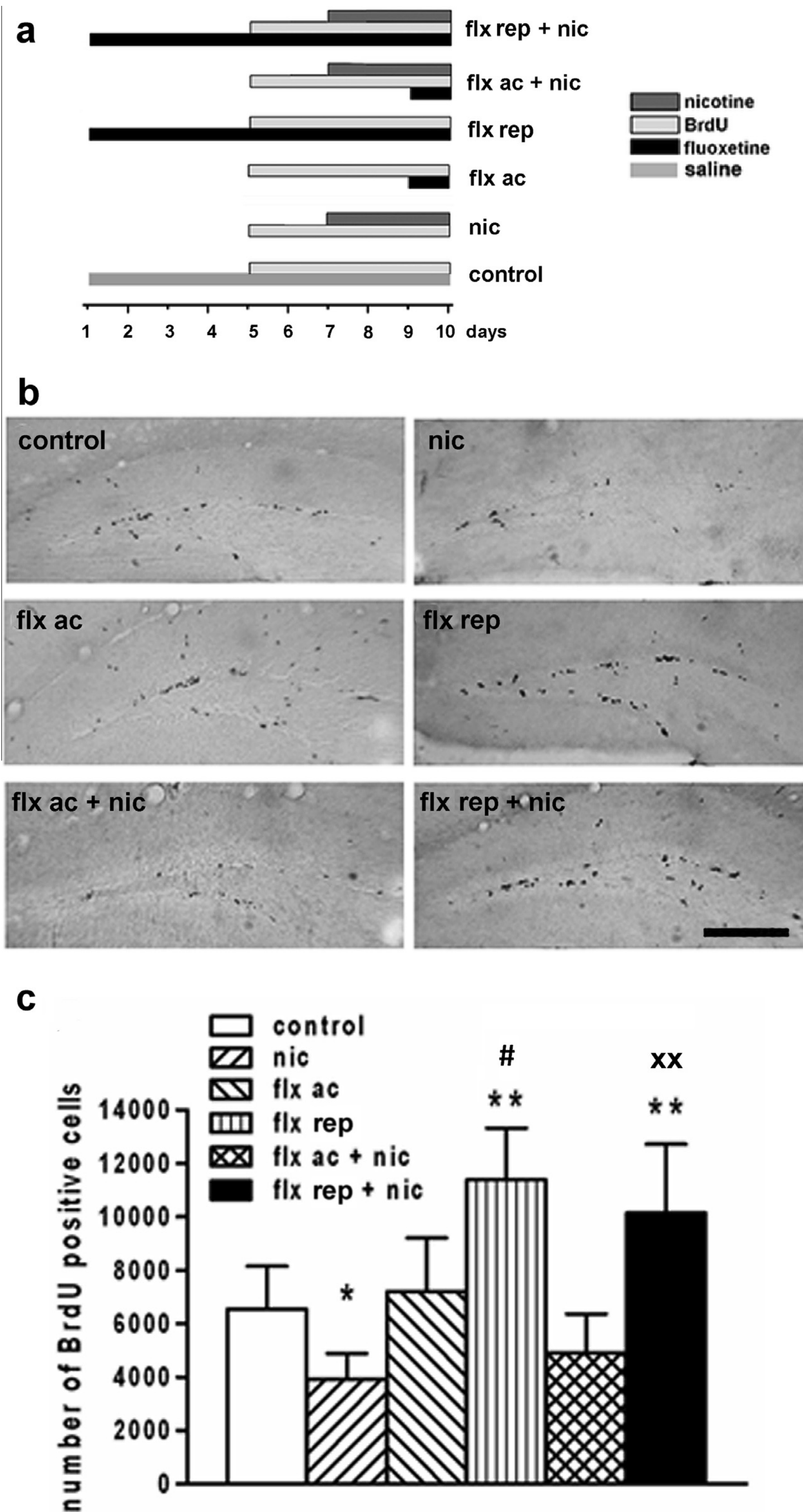

Fig. 1. Analysis of BrdU-positive cells in the DG. (a) schematic representation of the injection schedules used to evaluate the properties of newly formed cells in the DG. Rats were given injections of nicotine (s.c., 4 days), fluoxetine (i.p., 1 or 10 days), BrdU (i.p., 6 days) and saline (i.p., 10 days), as indicated; (b) photomicrographs showing BrdU-positive cells in coronal sections of the DG from rats treated with saline (control), nicotine (nic), acute fluoxetine (flx ac), repeated fluoxetine (flx rep), acute fluoxetine + nicotine (flx ac + nic) and repeated fluoxetine + nicotine (flx rep + nic). BrdU-positive cells are located in the subgranular zone adjacent to the DG. (c) Quantification of BrdU-positive cells in the various experimental groups of rats. Cell counts in right and left DG were averaged for each rat. Each bar represents mean ± SEM (n = 6–7 animals per group). $^{*}p < 0.05$, $^{**}p < 0.01$, in comparison with control rats; $^{\#}p < 0.05$, in comparison with acute fluoxetine group; $^{xx}p < 0.01$, in comparison with acute fluoxetine and nicotine treatment group (ANOVA followed by Scheffé *post hoc* test). Scale bar applicable to all micrographs, 300 µm.

the middle compartment with the doors open, which allowed them to roam freely from side to side for 10 min, and the time spent in each compartment was recorded. Placement was counterbalanced within each group.

Conditioning phase. At conditioning day 1, rats were injected with PBS and immediately exposed to the preferred compartment (door closed) for 20 min. At conditioning day 2, rats were injected with nicotine, fluoxetine or PBS according to the schedule shown in Fig. 6a, and immediately exposed to the non-preferred compartment (door closed). The entire procedure was repeated four times (8-day protocol).

Test phase. During the day following the last conditioning session, animals were tested in a drug free environment. They were placed in the middle compartment and allowed to explore the box for 10 min, the doors being open. The time spent in each compartment was recorded for each animal. Three control groups were established: two groups of animals were injected in their home-cage with nicotine for 4 days or with fluoxetine for 10 days before being killed. No pharmacological treatment was given to a third group.

Data analysis. Time spent in each compartment was converted into a preference score [Score (s) = time spent in the white compartment during test − time spent in the white compartment during pretest]. Preference scores were analyzed by a two-way ANOVA using nicotine (saline and repeated nicotine treatment) and fluoxetine (saline, single and repeated fluoxetine treatment) as independent factors, followed by Scheffé *post hoc* test. Results are expressed as mean ± SEM. Significance was set at $p \leqslant 0.05$.

Immunohistochemistry

Tissue preparation. Animals were given an overdose of pentobarbital and were then perfused transcardially with 4% paraformaldehyde in PBS. The brains were removed, kept overnight at 4 °C in 30% sucrose, frozen in isopentane and stored at −20 °C. Coronal sections (50-μm thick) were obtained using a sliding freezer microtome and stored at 4 °C in 0.1 M phosphate buffer containing 0.005% sodium azide. For BrdU immunohistochemistry, DNA was denatured by incubating the free-floating sections in 50% formamide/50% 2× SSC (0.3 M NaCl/30 mM sodium citrate) at 37 °C for 2 h. Sections were then washed in 2× SSC, incubated in 2 N HCl for 30 min at 37 °C and rinsed in 0.1 M borate buffer (pH 8.5) for 15 min.

Antibodies. The following rabbit polyclonal antibodies were used: anti-BrdU antibody (Roche, Indianapolis, USA) diluted 1:1000 in 0.1 M TBS containing 0.1% triton and 5% normal donkey serum; anti-p75 antibody (Millipore, Massachusetts, USA) diluted 1:600; anti-pCREB antibody (Cell Signaling, Danvers, MA, USA) diluted 1:800; anti-HDAC2 antibody (Santa Cruz Biotech., Santa Cruz, CA, USA) diluted 1:500 and anti-GFAP antibody (Dako, Glostrup, Denmark) diluted 1:1000. Other antibodies were rat monoclonal anti-BrdU antibody (Accurate Scientific, Westbury, New York) diluted 1:500, or mouse monoclonal anti-NeuN antibody (Chemicon, USA) diluted 1:800; anti-GFAP antibody (Boehringer Mannheim, Indianapolis, USA) diluted 1:1000.

Immunohistochemistry. Immunohistochemistry was performed as described previously (Pascual et al., 2009; Pastor et al., 2011). Briefly, brain sections were incubated for 1 h in blocking solution (0.1 M phosphate buffer containing 0.1% triton X-100, 2% normal goat serum and 1 g/l bovine serum albumin) and then overnight at 4 °C with the primary antibody. Sections were then successively incubated with a biotinylated secondary antibody (Jackson Laboratories) for 2 h at room temperature, followed by an avidin–biotin-peroxidase complex (Vectastain ABC Kit, Vector, Burlingame, CA, USA). Staining was revealed with the chromagen 3,3′-diaminobenzidine (DAB) and H_2O_2. Slides were coverslipped with mounting medium (Vector Laboratories) and staining was observed by light microscopy using differential interference contrast, (Olympus (Olympus BX50 epifluorescence microscope). Images were acquired using a digital camera (Cool-Snap) and processed with Image-Pro Plus (Media Cybernetics Inc., Bethesda, MD, USA). Only general contrast enhancement and color level adjustment were improved; images were not otherwise digitally modified.

After the pretreatment for BrdU immunohistochemistry indicated above, sections were kept in blocking solution for 2 h, followed by incubation for 24 h at 4 °C with the primary antibody. Sections were then incubated for 2 h at room temperature with a secondary antibody raised in donkey (Cy3-conjugated for detecting BrdU and $p75^{NTR}$, Cy5-conjugated for detecting GFAP and FITC-conjugated for detecting NeuN). After washing the sections, slides were coverslipped with mounting medium for fluorescence (Vectashield, Vector Laboratories, Burlingame, CA, USA). Fluorescent signal was detected using a confocal microscope (Olympus FV1000, Japan) and the images were processed with Adobe Photoshop. Only general contrast enhancement and color level adjustment were improved; images were not otherwise digitally modified.

Quantification of DAB-labeled cells. The number of DAB-stained cells was determined within the subgranular layer of the hippocampus, defined as located within one-cell diameter from the granule cell layer boundary (for a full description of cell quantification method, see (Bernabeu and Longo, 2010). Cell counting was performed using a Plan-Apochromat 40X, NA: 1.25 with an oil immersion objective. Quantification of DAB-positive cells in the hippocampus and the nucleus accumbens (NAc) was performed by an investigator unaware of the identity of the samples, using Image-Pro Plus. The appropriate areas were imaged and immunoreactive cells were counted on five to six sections in both hemispheres

(Miller and Marshall, 2005; Pascual et al., 2009). Counts were averaged from squares of 0.5 mm^2 drawn randomly in the NAc core and shell. Statistical analysis was performed using a one-way ANOVA (number of positive cells as variation factor), followed by Scheffé *post hoc* test, when required.

Colocalization of BrdU-positive cells with cells expressing phenotype markers. Colocalization analysis was performed using a confocal microscope (Olympus, FV1000). Sections were optically sliced in the Z-axis at 4-µm intervals and fluorescence images were acquired for each marker. In each section, the number of BrdU-positive cells associated with the markers NeuN or GFAP was determined, as described before (Peterson, 1999). Seven to nine sections were assessed for a given DG, which corresponds to the number of sections required to fully span each DG (Bernabeu and Longo, 2010). For each animal, data from the right and left DG were averaged and the percentage of BrdU-positive cells associated with NeuN or GFAP was determined. Images obtained from individual optical slices were imported to Adobe Photoshop for the composition of figures.

RESULTS

Effect of fluoxetine and nicotine on the number of BrdU-positive cells in the DG

To determine whether nicotine and fluoxetine were affecting proliferation of new-born cells in the adult DG, rats were injected with 50 mg/kg BrdU during the last 6 days of the experiment. Nicotine was given for 4 days, the period required for inducing nicotine preference (see below) and fluoxetine was injected acutely or repeatedly for 10 days, as illustrated in Fig. 1a. Morphological assessment revealed that the number of BrdU-positive cells increased mainly in response to repeated fluoxetine injections, while some increase was also detectable when fluoxetine was co-administrated repeatedly with nicotine (Fig. 1b). Quantitative stereological analysis shows that repeated fluoxetine treatment elicited a 73% increase in BrdU-positive cells relative to control animals (Fig. 1c). The number of BrdU-positive cells was also significantly increased, by 55%, when the repeated fluoxetine treatment was accompanied with nicotine injection during the last 4 days. Nicotine treatment alone was found to decrease significantly, by 40%, the number of BrdU-positive cells.

Quantitative assessment of phenotype markers associated with BrdU-positive cells

Using the same groups of rats, immunodetection of the cell-specific NeuN and GFAP markers was performed in the DG to identify and quantify the amount of BrdU-positive cells expressing each marker (Fig. 2a). Six labeling combinations, when considering the three markers, could be found in the various sections. Cells displaying only one of each of the BrdU, NeuN or GFAP marker could be observed. BrdU-positive cells that also expressed NeuN or GFAP were detected. Quantitative analysis shows that the number of BrdU-positive cells expressing NeuN was increased 1.7-fold in the DG of rats treated repeatedly with fluoxetine compared to control rats (Fig. 2b); the increase reached only 1.3-fold in rats treated repeatedly with fluoxetine and nicotine. The number of BrdU-positive cells expressing GFAP was comparatively low and not affected by the various pharmacological treatments, as was the case for the cells that were only BrdU-positive. The data confirm that, at least at the time-point analyzed, cells newly synthesized in response to repeated fluoxetine exposure are predominantly neurons.

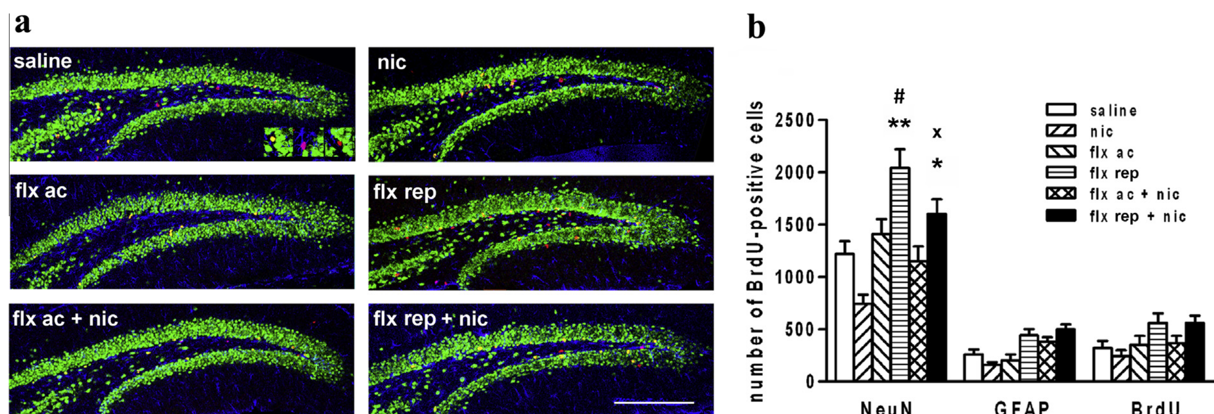

Fig. 2. Co-immunolabeling analysis of BrdU-positive cells expressing NeuN or GFAP in the DG. (a) Photomicrographs showing coronal sections immunolabeled using antibodies directed against BrdU (red), NeuN (green) and GFAP (blue). Cells positive for BrdU and NeuN appear yellow; cells positive for BrdU and GFAP appear pink. Sections were obtained from rats treated with saline, nicotine (nic), acute fluoxetine (flx ac), repeated fluoxetine (flx rep), acute fluoxetine + nicotine (flx ac + nic) and repeated fluoxetine + nicotine (flx rep + nic). The inset in the saline micrograph shows a high magnification illustrating the various patterns of cell labeling: BrdU (red), NeuN (green), GFAP (blue), BrdU + NeuN (yellow), BrdU + GFAP (pink). (b) Quantitative analysis of BrdU-positive cells expressing NeuN or GFAP marker in the same cells. Total cells in both DG were averaged for each animal. Each bar represents mean ± SEM (n = 6–7 animals per group). $^{*}p < 0.05$, $^{**}p < 0.01$, in comparison with control rats; $^{\#}p < 0.05$, in comparison with acute fluoxetine group; $^{x}p < 0.05$, in comparison with repeated fluoxetine group (ANOVA followed by Scheffé *post hoc* test). Scale bar applicable to all micrographs = 300 µm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Effect of fluoxetine and nicotine on pCREB, HDAC2 and p75^{NTR} expression

We next measured immunohistochemically the expression of the Ser133-phosphorylated form of CREB (pCREB) within cells located in the DG of the same experimental groups of rats (Fig. 3a). Again, the repeated fluoxetine treatment was found to significantly increase the number of pCREB-positive cells compared to the control group of rats (Fig. 3b). Contrary to what we observed for BrdU-labeled cells, the number of pCREB-positive cells in this group was not reduced by concomitant nicotine injections. Treatment with nicotine alone had no effect on pCREB expression.

Fig. 4a shows photomicrographs representing HDAC2 immunoreactivity observed in the molecular layer of the DG. Nicotine produced a significant stimulatory effect on HDAC2 expression in the hippocampus, which was however much weaker than the nicotine effect observed in structures of the reward system, such as the NAc or the prefrontal cortex (PFC), see also (Pastor et al., 2011). Quantitative analysis reveals that the number of HDAC2-immunoreactive cells in the DG was actually decreased in the group of rats repeatedly injected with fluoxetine when compared to the control group (Fig. 4b). In contrast, no such decrease was observed in the group of rats treated with fluoxetine and nicotine.

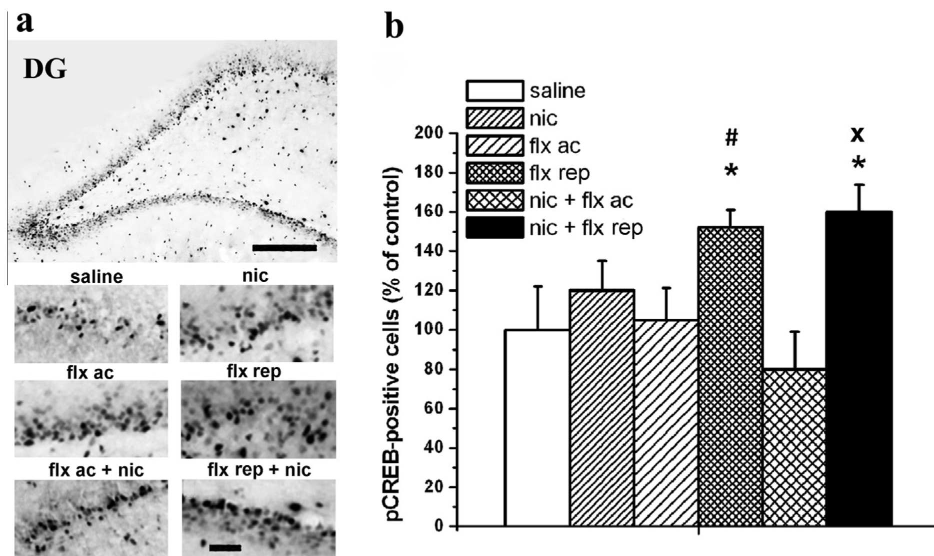

Fig. 3. pCREB expression in the DG. (a) Photomicrograph of pCREB labeling in the DG of rats treated with nicotine shown at low magnification; photomicrographs showing pCREB immunoreactivity in coronal sections of the DG from rats treated with saline, nicotine (nic), acute fluoxetine (flx ac), repeated fluoxetine (flx rep), acute fluoxetine + nicotine (flx ac + nic) and repeated fluoxetine + nicotine (flx rep + nic). (b) Quantification of pCREB immunoreactive cells in the various experimental groups of rats. Cell counts in the right and left DG were averaged for each rat. Each bar represents mean ± SEM (n = 6–7 animals per group). $^{*}p < 0.05$, in comparison with control rats; $^{\#}p < 0.05$, in comparison with acute fluoxetine group; $^{x}p < 0.05$, in comparison with acute fluoxetine and nicotine treatment group (ANOVA followed by Scheffé *post hoc* test). Scale bar = 100 μm for DG and 300 μm for the other micrographs.

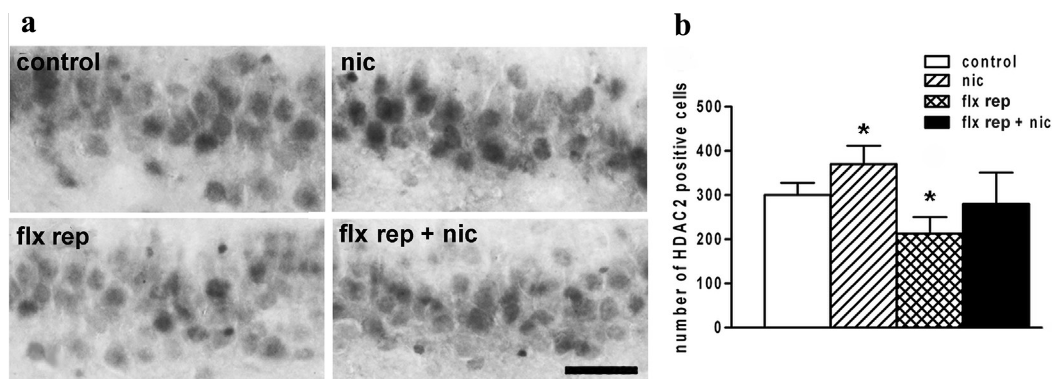

Fig. 4. HDAC2 expression in the DG. (a) Photomicrographs illustrating HDAC2 immunoreactivity in coronal sections of the DG from rats treated with saline (control), nicotine (nic), repeated fluoxetine (flx rep) and repeated fluoxetine + nicotine (flx rep + nic). (b) Quantification of HDAC2 immunoreactive cells in the various experimental groups of rats. The number of positive cells per section was averaged for each rat. Each bar represents mean ± SEM (n = 6–7 animals per group). $^{*}p < 0.05$, in comparison with control rats (ANOVA followed by Scheffé *post hoc* test). Scale bar applicable to all micrographs = 20 μm.

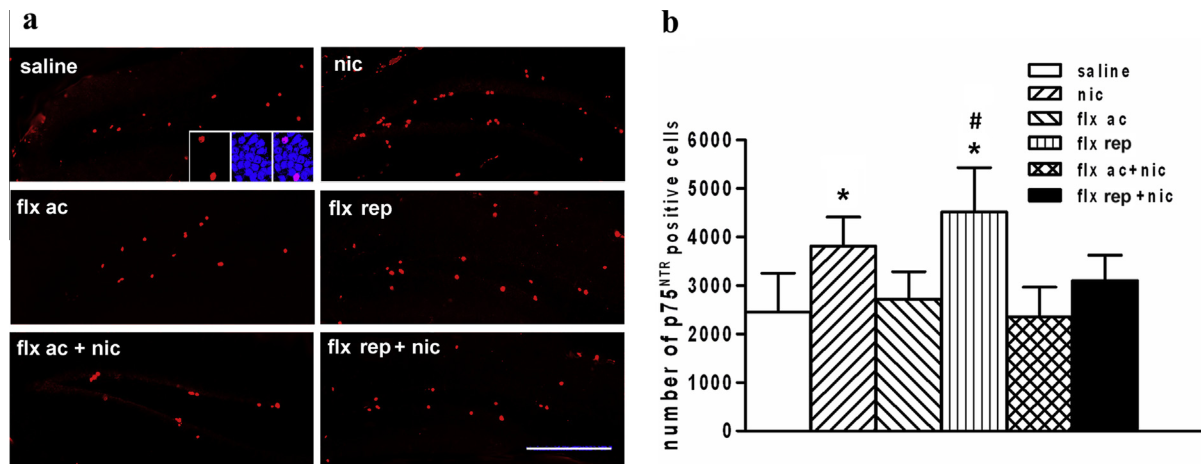

Fig. 5. p75^{NTR} expression in the DG. (a) photomicrographs illustrating p75^{NTR} immunoreactivity in coronal sections of the DG from rats treated with saline, nicotine (nic), acute fluoxetine (flx ac), repeated fluoxetine (flx rep), acute fluoxetine + nicotine (flx ac + nic) and repeated fluoxetine + nicotine (flx rep + nic). The inset in the saline micrograph shows a high magnification of p75^{NTR} staining (red, left), DAPI (blue, center) and co-localization of p75^{NTR} with DAPI (pink, right). (b) Quantification of p75^{NTR} immunoreactive cells in the various experimental groups of rats. Total cells in both DG were averaged for each rat. Each bar represents mean ± SEM (n = 5–6 animals per group). $^{*}p < 0.05$, in comparison with control rats; $^{\#}p < 0.05$, in comparison with acute fluoxetine group (ANOVA followed by Scheffé *post hoc* test). Scale bar applicable to all micrographs = 300 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

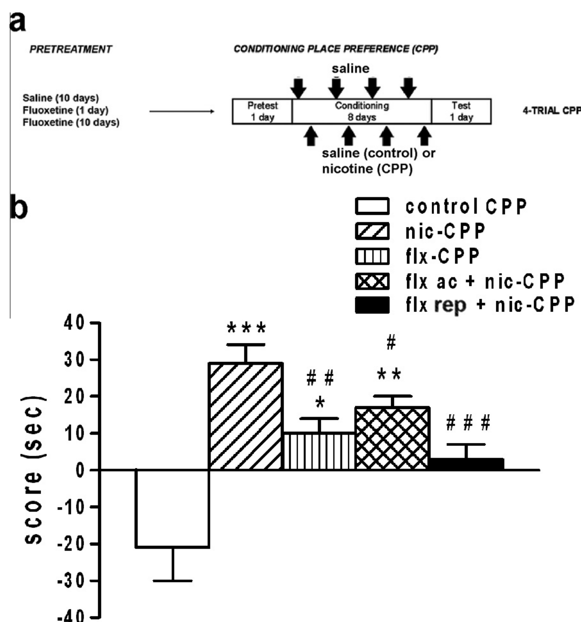

Fig. 6. CPP experiments induced by nicotine and fluoxetine in rats. (a) Schematic representation of the injection schedules used to induce CPP. Animals were pretreated with saline or fluoxetine and then submitted to nicotine-induced CPP. (b) Rats were injected s.c. with 0.4 mg/kg nicotine (nic-CPP) or i.p. with 10 mg/kg fluoxetine (flx-CPP). An equal volume of PBS was injected in the control condition. The fluoxetine-nicotine group of rats was injected with fluoxetine for one (flx ac + nic-CPP) or 10 days (flx rep + nic-CPP) before the nicotine-CPP (see methods for details). Bar graphs indicate preference scores (positive values) for the drug. Since nicotine was administered in the initially non-preferred compartment, the score was negative for the PBS control group. Each bar represents mean ± SEM (n = 5–7 animals per group). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, in comparison with the control group and $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, in comparison with the nicotine-induced CPP group of rats (ANOVA followed by Scheffé *post hoc* test).

We measured by immunofluorescence the expression of p75^{NTR} protein in the groups of rats described above. Many p75^{NTR} immunoreactive cells were detected in the subgranular zone of the DG (Fig. 5a). The quantitative analysis shown in Fig. 5b reveals that the number of p75^{NTR}-positive cells was significantly increased in the group of rats treated repeatedly with fluoxetine, and also in the group that was treated with nicotine, although to a lesser extent. Surprisingly, rats treated with fluoxetine and nicotine displayed no modification in the expression of p75^{NTR} in the DG compared to control animals.

Effect of fluoxetine and nicotine on CPP

To examine the effect of fluoxetine on the development of nicotine-induced place preference, we trained rats in a well-established biased CPP protocol (Fig. 6a). The results clearly show that the nicotine treatment we used throughout the present study (0.4 mg/kg given once a day for 4 days) was sufficient to induce an important CPP, as demonstrated previously (Pascual et al., 2009; Pastor et al., 2011). Using this protocol, fluoxetine injection also led to the development of a significant CPP, as reported previously (Collu et al., 1997; Subhan et al., 2000). Interestingly, an acute injection of fluoxetine was sufficient to significantly reduce the nicotine-induced CPP, while repeated injections almost completely prevented this preference (Fig. 6b).

pCREB and HDAC2 expression in the hippocampus and nucleus accumbens after nicotine-induced CPP

Numbers of pCREB- and HDAC2-immunoreactive cells were assessed in the DG, and also in the NAc of rats that had been subjected to CPP using nicotine, fluoxetine or a combination of both drugs (Fig. 7). Significant differences between experimental groups

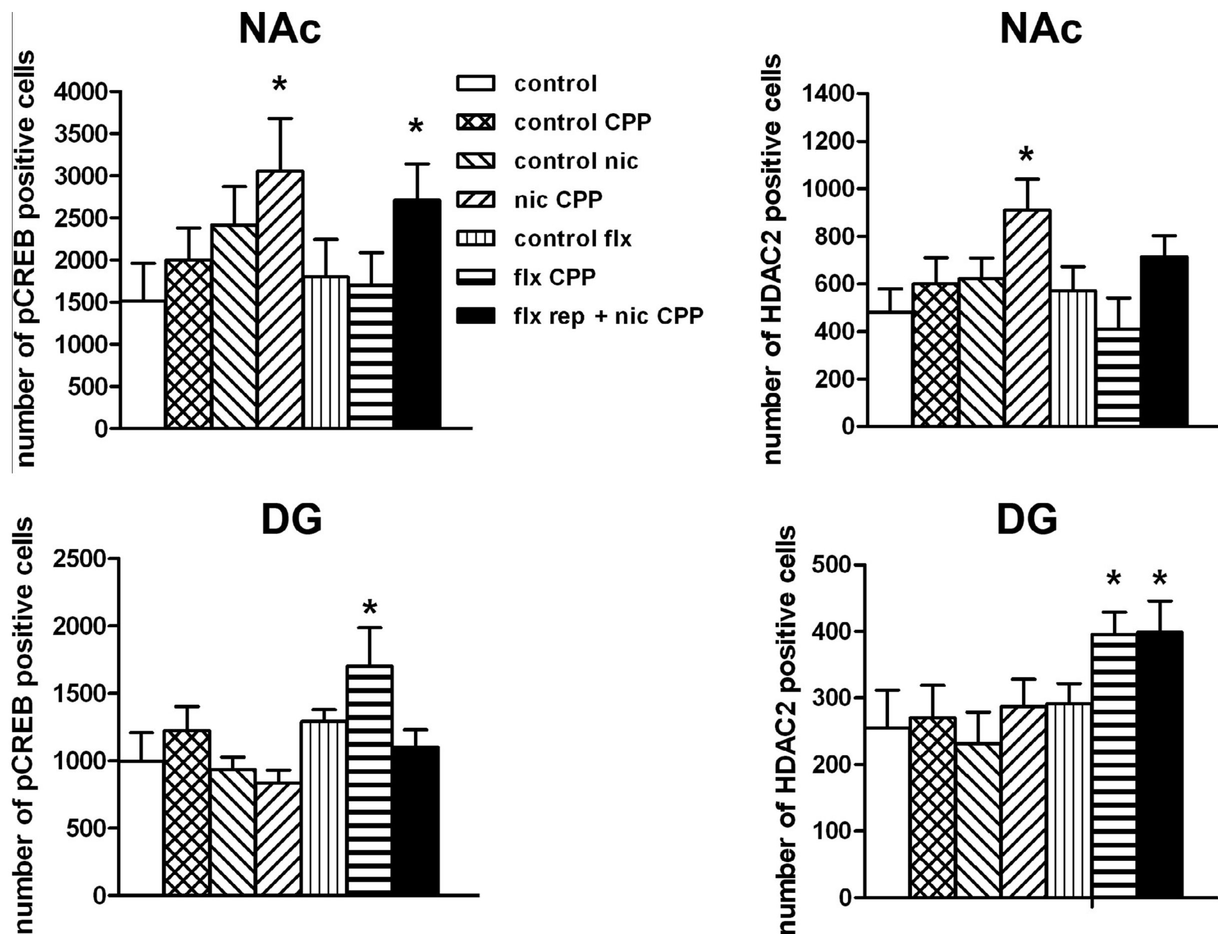

Fig. 7. Quantification of pCREB and HDAC2-positive cells in the DG and NAc of rats submitted to CPP. Animals from the various CPP experimental groups were analyzed for the expression of pCREB or HDAC2 in the NAc and the DG. Nic-CPP, nicotine-induced CPP; flx-CPP, fluoxetine-induced CPP; flx + nic-CPP, rats showing nicotine-induced CPP and treated repeatedly with fluoxetine. Three control groups were established: a group with no pharmacological treatment and two groups of rats treated repeatedly with nicotine and fluoxetine in their home-cage. Total cells in both DG were averaged for each rat. Each bar represents mean ± SEM (n = 5–7 animals per group). $^{*}p < 0.05$ in comparison with the control group of rats (ANOVA followed by Scheffé *post hoc* test).

could be established (DG: $F_{4,22} = 10.2$, $p < 0.0001$; NAc: $F_{4,22} = 6.45$, $p < 0.0021$). In the NAc, the number of pCREB-positive cells was increased in the group in which CPP was induced by nicotine. It was also significantly increased in the group injected with fluoxetine before the nicotine-induced CPP experiment. In contrast, an increase in pCREB expression was only noticed in the DG from rats subjected to fluoxetine-induced CPP, and was abolished in the group of rats treated with both drugs (Fig. 7). The expression of HDAC2 was increased in the NAc of rats in which CPP was induced by nicotine, but the effect was not significant in the group injected repeatedly with fluoxetine before the nicotine-induced CPP experiment. In the DG, we found an increase in the number of HDAC2-immunoreactive cells in rats subjected to fluoxetine-induced CPP that did not differ from that found in animals treated with both drugs (Fig. 7).

Number of BrdU-positive cells in the DG of rats subjected to fluoxetine- and nicotine-CPP

Finally, considering that repeated administration of nicotine and fluoxetine had opposite effects on the number of BrdU-positive cells, we determined the number of newly formed DG cells in animals subjected to fluoxetine- and nicotine-induced CPP. Fig. 8 shows that nicotine-induced CPP was not accompanied with the proliferation of progenitor cells in the DG, in contrast to fluoxetine-induced CPP, in which we noticed a big increase in the number of BrdU-positive dentate cells. When fluoxetine was administered before nicotine-induced CPP, no change in the number of BrdU-positive cells was found.

DISCUSSION

We report here that the decrease in adult hippocampal cell proliferation induced by nicotine was abolished when animals were also treated repeatedly with fluoxetine. Fluoxetine, which by itself induced a less robust CPP than nicotine, reduced nicotine-induced CPP in response to a single injection, and completely abolished the rewarding properties of nicotine after repeated exposure.

Effect of fluoxetine and nicotine on DG neurogenesis

The precise effect of fluoxetine on DG newly formed cells is still a matter of debate, given that it depends not only on

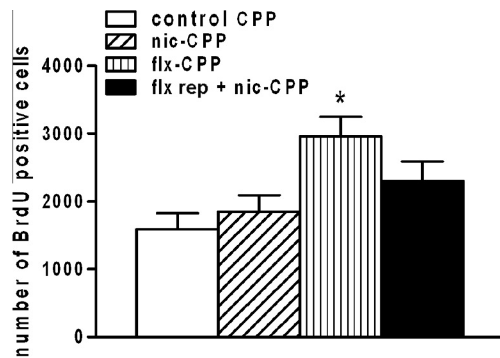

Fig. 8. Quantification of BrdU-positive cells in the DG of rats subjected to CPP. Animals from the following experimental groups were used: nic-CPP, nicotine-induced CPP; flx-CPP, fluoxetine-induced CPP; flx rep + nic-CPP, rats showing nicotine-induced CPP and treated repeatedly with fluoxetine. The number of BrdU-positive cells was determined in the DG. Each bar represents mean ± SEM (n = 5–7 animals per group). $^{*}p < 0.05$, in comparison with control rats (ANOVA followed by Scheffé *post hoc* test).

the species considered, but also on the type of treatment (Lee et al., 2001). Early studies demonstrated that repeated fluoxetine treatment was needed to induce DG neurogenesis (Malberg et al., 2000); however, a 6-week treatment was found to actually decrease it (Ohira and Miyakawa, 2011). In addition, recent data indicate a differential sensitivity of the adolescent brain as compared to the adult brain, with reduced neurogenesis observed in the latter (Klomp et al., 2014). Using adolescent rats, we found that fluoxetine was able to increase BrdU incorporation into the DG granular cells, and also to differentiate the majority of newborn cells into a neuronal phenotype. The effect was very robust, since nicotine was not able to inhibit it, even when administered repeatedly. This is of special interest when considering that nicotine has been reported to decrease neurogenesis (Campbell et al., 2011), as we have also found here. Early studies indicate that nicotine signaling may be pivotal for the development of adult-born neurons, since repeated exposure to nicotine impaired their long-term survival (Campbell et al., 2011).

To further characterize the effects of the two drugs, we analyzed the expression of $p75^{NTR}$. It appears that nicotine only increased the expression of $p75^{NTR}$ while fluoxetine increased $p75^{NTR}$ together with pCREB. We previously demonstrated that $p75^{NTR}$ is expressed by adult dentate progenitor cells and promotes proliferation and early maturation of DG cells in mice (Bernabeu and Longo, 2010). On the other hand, after 14 days $p75^{NTR}$ expression decreased in response to nicotine administration, whereas trkA expression was amplified (Hernandez and Terry, 2005). We show that nicotine exposure increased $p75^{NTR}$ expression, but failed to generate additional neurons in the DG. The $p75^{NTR}$ was proposed to act as a regulator of fluoxetine-stimulated adult hippocampal neurogenesis since $p75^{NTR-/-}$ mice displayed reduced neurogenesis. Furthermore, antidepressant administration increases the number of BrdU-positive cells without affecting the NeuN/BrdU ratio in DG cells (Colditz et al., 2010). Interestingly, $p75^{NTR}$ increases cell survival when associated with trkA, while inducing cell death when associated with sortilin (Chao, 2003). Although both drugs increased $p75^{NTR}$ expression in the DG, the differential associative functionality of $p75^{NTR}$ might help to explain why nicotine promotes a decrease in DG neurogenesis, while fluoxetine induces cell survival.

We report here that nicotine did not increase the amount of pCREB in the DG when administered for four consecutive days. A similar result was found when nicotine was given over a period of 3–10 days, resulting in reduced viability of progenitor cells in the DG (Jang et al., 2002; Scerri et al., 2006). Conversely, CREB phosphorylation followed by CREB-induced gene expression is produced by most antidepressants, including fluoxetine (Tiraboschi et al., 2004). Our results are in line with those findings. In addition, similarly to what we observed for neurogenesis itself, nicotine was not able to modulate the CREB phosphorylation provoked by fluoxetine, indicating that CREB activation by fluoxetine is a very robust effect. This observation also underlines the importance of CREB phosphorylation and activation in neurogenesis.

Controversial results have been reported concerning the effects of HDAC inhibition on DG neurogenesis. Some groups found that neurogenesis was stimulated by treatment with HDAC inhibitors (Kim et al., 2009; Yoo et al., 2011), while others found it was decreased (Tsankova et al., 2007; George et al., 2013). HDAC2 is highly expressed in the hippocampus and is known to negatively regulate memory formation (Guan et al., 2009). Fluoxetine was found to repress HDAC2 expression in the hippocampus, suggesting that it affects chromatin remodeling by increasing the acetylation levels of histones. Nicotine produced a significant stimulatory effect on HDAC2 expression in the hippocampus, which was weaker than the effect we observed in the NAc or PFC (Pastor et al., 2011). When nicotine was administered together with fluoxetine, HDAC2 expression reached a level corresponding approximately to the algebraic sum of each effect considered separately, suggesting that both drugs share a common target in the mechanism by which HDAC2 expression is regulated.

Effect of fluoxetine and nicotine on CPP at the behavioral and molecular levels

Several experiments, including ours using nicotine, have shown that the hippocampus regulates the activity of the mesocorticolimbic pathway (Lisman and Grace, 2005), suggesting that it is able to modulate the effects of drugs of abuse. Also, the hippocampus-VTA/NAc loop has been shown to be activated during memory consolidation and in addiction (Lisman and Grace, 2005). We therefore tested the effects of both drugs separately and combined, using CPP, a behavioral test which is used to analyze the appetitive and rewarding properties of drugs. Both nicotine and fluoxetine induced a positive CPP, with nicotine displaying a much stronger rewarding effect. This was expected, considering that nicotine is a very addictive compound in man and fosters strong CPP in rats (Pascual et al., 2009; Pastor et al., 2011). Whether fluoxetine actually promotes reward is under debate, as illustrated by the fact that its effects in the CPP paradigm

are not consistent (Collu et al., 1997; Subhan et al., 2000). The data shown here are nevertheless in favor of fluoxetine producing reward. Moreover, it was able to eliminate the nicotine-induced CPP, especially when given repeatedly. This is in line with the fact that fluoxetine was shown to act as a nicotinic receptor antagonist (Arias et al., 2010) and also that repeated fluoxetine treatment decreases extracellular acetylcholine levels in the NAc (Bertrand, 2005).

To characterize CPP experiments at the molecular level, the expression of pCREB and HDAC2 was analyzed in the NAc, a structure associated with reward, and in the DG, a structure mainly involved in memory consolidation (Lisman and Grace, 2005). The idea was to measure these parameters in response to drugs in an experimental paradigm where their rewarding properties could be evaluated. These results can be compared with those obtained in animals simply injected with the drug (Fig. 7 *vs.* Figs. 3 and 4). In the NAc, nicotine-induced CPP was accompanied with an increase in both parameters, similarly to what we found earlier (Pascual et al., 2009; Pastor et al., 2011), whereas no marker was modified in the group of rats that displayed CPP triggered by fluoxetine. When rats were treated with both drugs, pCREB levels were increased, suggesting that CREB activation in the NAc plays a major role during nicotine-induced CPP. In the DG, it was the group of rats in which CPP was induced by fluoxetine that displayed an increase of both pCREB and HDAC2 expression. However, only HDAC2 level was increased in the DG of rats treated with both drugs, suggesting that reduced acetylation levels of histones participate to the mechanism by which rats develop CPP in response to nicotine. It is noteworthy that animals which displayed fluoxetine-induced CPP and rats treated repeatedly with fluoxetine showed similar levels of pCREB but opposite levels of HDAC2 (compare Figs. 3b and 4b with Fig. 7), further suggesting that HDAC2 activity is involved in the mechanisms by which a drug is associated with a given environment (Malvaez et al., 2010; Pastor et al., 2011).

Finally, we determined the number of newborn cells in the DG of animals that exhibited nicotine- or fluoxetine-induced CPP in order to establish an eventual role of neurogenesis in the rewarding properties of these drugs. Nicotine-induced CPP was not accompanied with BrdU incorporation in the DG, whereas animals with fluoxetine-induced CPP showed an increase in BrdU labeling. Fluoxetine-induced BrdU-positive cells may therefore be related to the rewarding properties of the drug, which is not the case for nicotine. This would also mean that fluoxetine reward, but not nicotine reward, is under the control of hippocampal activity.

CONCLUSIONS: SIGNIFICANCE OF EXPERIMENTAL FINDINGS

Our data present an additional mechanism by which the hippocampus may control the reward system. Hippocampal neurogenesis has recently been shown to be associated with degradation or loss of established memories, since increasing neurogenesis following learning was sufficient to induce memory loss (Akers et al., 2014). We clearly show that nicotine reduced neurogenesis in the DG, and in the meantime produced a rather strong CPP. This is also the case of the stronger stimulant cocaine (Brown et al., 2010). By reducing DG neurogenesis, both stimulants may lessen memory loss, thus strengthening memory consolidation. Since there is an obvious memory component in the induction of CPP, the process would result in increased place conditioning. A similar reasoning may apply to drug addiction which also comprises a memory component. Conversely, fluoxetine increases dentate neurogenesis, while producing a much weaker CPP than nicotine. According to the same line of thought, fluoxetine was not able to produce a strong CPP because of the memory loss it induced as a result of increased DG neurogenesis.

Repeated fluoxetine treatment was able to abolish nicotine-induced CPP, probably by altering the association between the drug and the environment in which it was given. Since this association represents a key component in drug addiction, one may propose developing a pharmacological strategy based on antidepressants to disrupt nicotine preference from associated environmental cues. Along this line of thinking, it is noteworthy that some data, although they are limited or conflicting, provide evidence that the antidepressants fluoxetine and selegiline are effective in certain subgroups of tobacco smokers (Schnoll and Lerman, 2006).

CONFLICT OF INTEREST

The authors of this paper do not have any conflict of interests to report.

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REFERENCES

Ahsan HM, de la Pena JB, Botanas CJ, Kim HJ, Yu GY, Cheong JH (2014) Conditioned place preference and self-administration induced by nicotine in adolescent and adult rats. Biomol Ther (Seoul) 22:460–466.

Akers KG, Martinez-Canabal A, Restivo L, Yiu AP, De Cristofaro A, Hsiang HL, Wheeler AL, Guskjolen A, Niibori Y, Shoji H, Ohira K, Richards BA, Miyakawa T, Josselyn SA, Frankland PW (2014) Hippocampal neurogenesis regulates forgetting during adulthood and infancy. Science 344:598–602.

Arias HR, Feuerbach D, Bhumireddy P, Ortells MO (2010) Inhibitory mechanisms and binding site location for serotonin selective reuptake inhibitors on nicotinic acetylcholine receptors. Int J Biochem Cell Biol 42:712–724.

Berger F, Gage FH, Vijayaraghavan S (1998) Nicotinic receptor-induced apoptotic cell death of hippocampal progenitor cells. J Neurosci 18:6871–6881.

Bernabeu R, Yang T, Xie Y, Mehta B, Ma SY, Longo FM (2006) Downregulation of the LAR protein tyrosine phosphatase receptor is associated with increased dentate gyrus neurogenesis and an increased number of granule cell layer neurons. Mol Cell Neurosci 31:723–738.

Bernabeu RO, Longo FM (2010) The p75 neurotrophin receptor is expressed by adult mouse dentate progenitor cells and regulates neuronal and non-neuronal cell genesis. BMC Neurosci 11:136–147.

Bertrand D (2005) The possible contribution of neuronal nicotinic acetylcholine receptors in depression. Dialogues Clin Neurosci 7:207–216.

Brown J, Cooper-Kuhn CM, Kempermann G, Van Praag H, Winkler J, Gage FH, Kuhn HG (2003) Enriched environment and physical activity stimulate hippocampal but not olfactory bulb neurogenesis. Eur J Neurosci 17:2042–2046.

Brown TE, Lee BR, Ryu V, Herzog T, Czaja K, Dong Y (2010) Reducing hippocampal cell proliferation in the adult rat does not prevent the acquisition of cocaine-induced conditioned place preference. Neurosci Lett 481:41–46.

Campbell NR, Fernandes CC, John D, Lozada AF, Berg DK (2011) Nicotinic control of adult-born neuron fate. Biochem Pharmacol 82:820–827.

Chao MV (2003) Neurotrophins and their receptors: a convergence point for many signalling pathways. Nat Rev Neurosci 4:299–309.

Chau DT, Rada PV, Kim K, Kosloff RA, Hoebel BG (2011) Fluoxetine alleviates behavioral depression while decreasing acetylcholine release in the nucleus accumbens shell. Neuropsychopharmacology 36:1729–1737.

Colditz MJ, Catts VS, Al-menhali N, Osborne GW, Bartlett PF, Coulson EJ (2010) P75 neurotrophin receptor regulates basal and fluoxetine-stimulated hippocampal neurogenesis. Exp Brain Res 200:161–167.

Collu M, Poggiu AS, Pani L, Serra G (1997) Fluoxetine-induced conditioned place preference: a preliminary study. Synapse 25:309–311.

Dani JA, Harris RA (2005) Nicotine addiction and comorbidity with alcohol abuse and mental illness. Nat Neurosci 8:1465–1470.

Danzer SC (2012) Depression, stress, epilepsy and adult neurogenesis. Exp Neurol 233:22–32.

Denny CA, Kheirbek MA, Alba EL, Tanaka KF, Brachman RA, Laughman KB, Tomm NK, Turi GF, Losonczy A, Hen R (2014) Hippocampal memory traces are differentially modulated by experience, time, and adult neurogenesis. Neuron 83:189–201.

Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn AM, Nordborg C, Peterson DA, Gage FH (1998) Neurogenesis in the adult human hippocampus. Nat Med 4:1313–1317.

George S, Kadam SD, Irving ND, Markowitz GJ, Raja S, Kwan A, Tu Y, Chen H, Rohde C, Smith DR, Comi AM (2013) Impact of trichostatin A and sodium valproate treatment on post-stroke neurogenesis and behavioral outcomes in immature mice. Front Cell Neurosci 7:123.

Grant BF, Hasin DS, Chou SP, Stinson FS, Dawson DA (2004) Nicotine dependence and psychiatric disorders in the United States: results from the national epidemiologic survey on alcohol and related conditions. Arch Gen Psychiatry 61:1107–1115.

Guan JS, Haggarty SJ, Giacometti E, Dannenberg JH, Joseph N, Gao J, Nieland TJ, Zhou Y, Wang X, Mazitschek R, Bradner JE, DePinho RA, Jaenisch R, Tsai LH (2009) HDAC2 negatively regulates memory formation and synaptic plasticity. Nature 459:55–60.

Hernandez CM, Terry Jr AV (2005) Repeated nicotine exposure in rats: effects on memory function, cholinergic markers and nerve growth factor. Neuroscience 130:997–1012.

Jang MH, Shin MC, Jung SB, Lee TH, Bahn GH, Kwon YK, Kim EH, Kim CJ (2002) Alcohol and nicotine reduce cell proliferation and enhance apoptosis in dentate gyrus. Neuroreport 13:1509–1513.

John U, Meyer C, Rumpf HJ, Hapke U (2004) Smoking, nicotine dependence and psychiatric comorbidity–a population-based study including smoking cessation after three years. Drug Alcohol Depend 76:287–295.

Kalkhoven E (2004) CBP and p300: HATs for different occasions. Biochem Pharmacol 68:1145–1155.

Kempermann G, Kuhn HG, Gage FH (1997) More hippocampal neurons in adult mice living in an enriched environment. Nature 386:493–495.

Kim HJ, Leeds P, Chuang DM (2009) The HDAC inhibitor, sodium butyrate, stimulates neurogenesis in the ischemic brain. J Neurochem 110:1226–1240.

Klomp A, Vaclavu L, Meerhoff GF, Reneman L, Lucassen PJ (2014) Effects of chronic fluoxetine treatment on neurogenesis and tryptophan hydroxylase expression in adolescent and adult rats. PLoS One 9:e97603.

Klose RJ, Bird AP (2006) Genomic DNA methylation: the mark and its mediators. Trends Biochem Sci 31:89–97.

Le Foll B, Goldberg SR (2005) Nicotine induces conditioned place preferences over a large range of doses in rats. Psychopharmacology (Berl) 178:481–492.

Lee HJ, Kim JW, Yim SV, Kim MJ, Kim SA, Kim YJ, Kim CJ, Chung JH (2001) Fluoxetine enhances cell proliferation and prevents apoptosis in dentate gyrus of maternally separated rats. Mol Psychiatry 6(610):725–728.

Lisman JE, Grace AA (2005) The hippocampal-VTA loop: controlling the entry of information into long-term memory. Neuron 46:703–713.

Malberg JE, Eisch AJ, Nestler EJ, Duman RS (2000) Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J Neurosci 20:9104–9110.

Malvaez M, Sanchis-Segura C, Vo D, Lattal KM, Wood MA (2010) Modulation of chromatin modification facilitates extinction of cocaine-induced conditioned place preference. Biol Psychiatry 67:36–43.

Mendez-David I, Hen R, Gardier AM, David DJ (2013) Adult hippocampal neurogenesis: an actor in the antidepressant-like action. Ann Pharm Fr 71:143–149.

Miller CA, Marshall JF (2005) Molecular substrates for retrieval and reconsolidation of cocaine-associated contextual memory. Neuron 47:873–884.

Nakagawa S, Kim JE, Lee R, Malberg JE, Chen J, Steffen C, Zhang YJ, Nestler EJ, Duman RS (2002) Regulation of neurogenesis in adult mouse hippocampus by cAMP and the cAMP response element-binding protein. J Neurosci 22:3673–3682.

Natarajan R, Wright JW, Harding JW (2011) Nicotine-induced conditioned place preference in adolescent rats. Pharmacol Biochem Behav 99:519–523.

Ohira K, Miyakawa T (2011) Chronic treatment with fluoxetine for more than 6 weeks decreases neurogenesis in the subventricular zone of adult mice. Mol Brain 4:10.

Pascual MM, Pastor V, Bernabeu RO (2009) Nicotine-conditioned place preference induced CREB phosphorylation and Fos expression in the adult rat brain. Psychopharmacology (Berl) 207:57–71.

Pastor V, Host L, Zwiller J, Bernabeu R (2011) Histone deacetylase inhibition decreases preference without affecting aversion for nicotine. J Neurochem 116:636–645.

Peterson DA (1999) Quantitative histology using confocal microscopy: implementation of unbiased stereology procedures. Methods 18:493–507.

Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, Weisstaub N, Lee J, Duman R, Arancio O, Belzung C, Hen R (2003) Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science 301: 805–809.

Scerri C, Stewart CA, Breen KC, Balfour DJ (2006) The effects of chronic nicotine on spatial learning and bromodeoxyuridine incorporation into the dentate gyrus of the rat. Psychopharmacology (Berl) 184:540–546.

Schnoll RA, Lerman C (2006) Current and emerging pharmacotherapies for treating tobacco dependence. Expert Opin Emerg Drugs 11:429–444.

Schoenfeld TJ, Gould E (2012) Stress, stress hormones, and adult neurogenesis. Exp Neurol 233:12–21.

Subhan F, Deslandes PN, Pache DM, Sewell RD (2000) Do antidepressants affect motivation in conditioned place preference? Eur J Pharmacol 408:257–263.

Tang J, Dani JA (2009) Dopamine enables in vivo synaptic plasticity associated with the addictive drug nicotine. Neuron 63:673–682.

Tiraboschi E, Tardito D, Kasahara J, Moraschi S, Pruneri P, Gennarelli M, Racagni G, Popoli M (2004) Selective phosphorylation of nuclear CREB by fluoxetine is linked to activation of CaM kinase IV and MAP kinase cascades. Neuropsychopharmacology 29:1831–1840.

Tsankova N, Renthal W, Kumar A, Nestler EJ (2007) Epigenetic regulation in psychiatric disorders. Nat Rev Neurosci 8:355–367.

van Praag H, Kempermann G, Gage FH (1999) Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. Nat Neurosci 2:266–270.

Yoo DY, Kim W, Nam SM, Kim DW, Chung JY, Choi SY, Yoon YS, Won MH, Hwang IK (2011) Synergistic effects of sodium butyrate, a histone deacetylase inhibitor, on increase of neurogenesis induced by pyridoxine and increase of neural proliferation in the mouse dentate gyrus. Neurochem Res 36:1850–1857.

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