Enriched environment reverts somatostatin interneuron loss in MK-801 model of schizophrenia

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ABSTRACT

Dysregulation of inhibitory drive has been proposed to be a central mechanism to explain symptoms and pathophysiological hallmarks in schizophrenia. A number of recent neuroanatomical studies suggest that certain types of inhibitory cells are deficient in schizophrenia, including somatostatin-immunoreactive interneurons (SST+).The present study sought to use stereological methods to investigate whether the number of SST+ interneurons decreased after repeated injections of NMDA receptor antagonist MK-801 (0.5mg/kg), and to determine the effect of a limited exposure to an Enriched Environment (EE) in adult life on this sub-population of inhibitory cells. Considering that somatostatin expression is highly dependent on neurotrophic support, we explored the changes in the relative expression of proteins related to Brain Derived Neurotrophic Factor - Tyrosine Kinase B (BDNF-TrkB) signaling between the experimental groups. We observed that early-life MK-801 treatment significantly decreased the number of SST+ interneurons in the medial prefrontal cortex (mPFC) and the hippocampus (HPC) of adult Long Evans rats. Contrarily, short-term exposure to EE increased the number of SST+ interneurons in MK-801-injected animals, except in the CA1 region of the hippocampus, whereas this increase was not observed in vehicle-injected rats. We also found up-regulated BDNF-TrkB signaling after EE that triggered an increase in the pERK/ERK ratio in mPFC and HPC, and the pAkt/Akt ratio in HPC. Thus, the present results support the notion that SST+ interneurons are markedly affected after early-life NMDAR blockade and that EE promotes SST+ interneuron expression, which is partly mediated through the BDNF-TrkB signaling pathway. These results may have important implications, as SST+ interneuron loss is also present in the MK-801 pre-clinical model, and its expression can be rescued by non-pharmacological approaches.

Key words: NMDAR, BDNF-TrkB, medial prefrontal cortex, hippocampus

INTRODUCTION

Schizophrenia is a neuropsychiatric disorder, the etiology of which is still unclear, but neurodevelopmental processes are known to play a major role [1,2]. The aberrant maturation of interneurons has been proposed as a putative mechanism mediating deficits associated with schizophrenia [3,4]. Disturbances in the sub-populations of inhibitory GABAergic neurons that express parvalbumin (PV) and somatostatin (SST) have been consistently reported in schizophrenic subjects, these being the most predominant interneuron sub-types within the cerebral cortex [5-9]. Expression of the calcium-binding protein parvalbumin and the neuropeptide somatostatin does not overlap, so markers that target these proteins identify two independent interneuron populations. The reduction in SST mRNA expression in schizophrenia has been observed in *post mortem* studies by *in-situ* hybridization and real-time quantitative PCR, confirming that SST decreases more significantly than any other biomarker in the hippocampus and the medial prefrontal cortex [5-7]. It seems that these differences are already noticeable during neurodevelopment [10]. Furthermore, the total number and density of SST-expressing interneurons is also decreased [6].

N-methyl-D-aspartate receptor (NMDAR) hypofunction seems to be the most accepted hypothesis for explaining the symptomatology and pathophysiology of schizophrenia. Even though the mechanisms of dysregulated glutamatergic systems in humans are not completely established, studies conducted over the last two decades show that endogenous glutamate receptor antagonists, such as kynurenic acid, are increased in subjects with schizophrenia [11]. Chronic administration during neurodevelopment of NMDAR antagonists, such as dizocilpine (MK-801), are known to produce long-term disturbances on behavior, the expression of parvalbumin and synaptic plasticity in pre-clinical models [12-14], and therefore offer a suitable model to replicate some features of schizophrenia in rodents.

A large body of evidence suggests that Brain-Derived Neurotrophic Factor (BDNF) regulates the synthesis of neuropeptides, including somatostatin, *in vitro* [15-17] and *in vivo* [18,16].BDNF is a neurotrophic factor that binds to its high-affinity receptor Tyrosine Kinase B (TrkB). In addition to promoting neuronal survival, BDNF mediates activity-dependent synaptic plasticity and is a key signaling molecule in learning and memory processes [19,20]. It also mediates the activity-dependent maintenance of somatostatin expression from development through adulthood [21-24]. In schizophrenia, the mRNA levels of BDNF and its receptor TrkB are decreased in the prefrontal cortex [25,26], the hippocampus [27] and in plasma [28].Consistent with this, mice with genetically-engineered reductions in the expression of BDNF mRNA have significantly lower levels of cortical SST mRNA and protein [21]. Therefore, BDNF-TrkB signaling has been suggested to be an upstream event that contributes to decreased SST expression in schizophrenia [7], and the up-regulation of this pathway could potentially regulate SST+ interneuron abnormalities.

An Enriched Environment (EE) is an experimental paradigm that combines sensory stimulation, physical exercise and social interaction [29]. Enriched Environments have beneficial effects on cognitive functions and brain morphology, and EE-mediated changes are largely promoted by the synthesis and release of neurotrophic factors, such as BDNF [29]. Previous findings from animal studies suggest that environmental stimulation during early development or adolescence might be effective in altering the course of schizophrenia-like symptoms and associated neurobiological alterations [30-34]. However, the onset of symptoms in human patients with schizophrenia usually begins in late adolescence or early adulthood. We have previously reported that short-term exposure to EE during adulthood improved cognitive alterations, up-regulated parvalbumin expression and NMDARs sub-units, and restored hippocampal-prefrontal synaptic plasticity in rats injected with MK-801 during neurodevelopment [12,13]. In this work, we aimed to further extend our understanding of the neurorestorative role of EE on pathological hallmarks of schizophrenia. Therefore, we explored the effects of MK-801 and EE on the SST+ interneuron sub-population by stereological means.

MATERIAL AND METHODS

MK-801 administration

MK-801 [(5S,10R)-(+)-5-Methyl-10,11-dihydro-5H-dibenzo[a,d] cyclohepten-5,10-imine hydrogen maleate, Dizocilpine hydrogen maleate] was purchased from Sigma-Aldrich (Ref: M107, St. Louis, MO, USA). A dose of 0.5mg/kg was administered intraperitoneally to rat pups once daily from P10 to P20 diluted in 0.9% NaCl, as previously reported [12].

Experimental groups and housing conditions

Newborn Long Evans rats were assigned to four different experimental groups (n=12 per group), based on treatment regimen and housing conditions (Fig.1). Rat pups that received saline from P10 to P20 are referred to as "vehicle group" (VH), and those that received MK-801 injections as "MK-801 group" (MK-801). All groups were raised in standard laboratory conditions from P0 to P55 (500 mm x 280 mm x 140 mm cages), and an Enriched Environment was applied from P55 to P73 in the corresponding groups (one VH group and one MK-801 group). The Enriched Environment consisted in housing the animals in larger cages (720 mm × 550 mm × 300 mm) with free access to wheel-runners (voluntary exercise), increased sensory stimulation by using differently shaped and colored objects (e.g., shelters, tunnels and toys) that were changed every 2 days, and social interaction was promoted by housing 6 animals per cage.

All animals were maintained at 12-h light/dark cycle (lights on at 8:00am) with access to food and water *ad libitum*. All procedures were performed in accordance with the European Recommendations 2007/526/EC and were approved by the Ethical Committee on Animal Welfare of the University of the Basque Country (UPV/EHU).

Immunohistochemistry

The immunohistochemical studies were performed on 6 animals from each experimental condition. Serial coronal brain sections (50μm thick) were cut on a freezing microtome (Leica, Wetzlar, Germany). Free-floating sections were carried through antigen retrieval in sodium citrate buffer (pH 0.6) heated to 100º C for 10 minutes, washed two times for 5 min in 0.1M PBS, and incubated for 20 min in a solution of 3% hydrogen peroxide. After three washes, free-floating sections were blocked in 5% normal horse serum (NHS) in 0.1M PBS with 0.5% Triton X-100 (PBS-TX) during 1h, and incubated in blocking solution with the rabbit anti-somatostatin primary antibody (1:5000, Ref: T-4103, Peninsula Laboratories, San Carlos, CA) overnight at 4° C. The next day, sections were incubated with secondary antibodies (1:200, horse anti-rabbit IgG, Ref: PK-6200, Vector Laboratories) at room temperature in PBS-TX for 1h in 1:200 dilution. After washing three times for 5 min in 0.1M PBS, sections were incubated in Avidin-Biotin Complex (Vectastain Elite ABC kit, Vector Laboratories), and developed with DAB (Ref: D5637, Sigma-Aldrich). Finally, sections were mounted, air-dried, cleaned in Xilol for 2 hours, and cover-slipped with DPX mounting medium (Ref: 06522, Sigma-Aldrich, Spain).

Unbiased stereology

The number of somatostatin-immunoreactive cells in the mPFC and the dorsal hippocampus was estimated with unbiased stereology, as previously described [12]. Briefly, a Mercator Image Analysis system (Explora-Nova, La Rochelle, France) was used to count somatostatin-immunopositive cells using the Optical Fractionator approach. The section sampling factor (ssf) used in this study was 1/8 for the mPFC and 1/10 for the hippocampus. As the cytoarchitectural differences between prelimbic and infralimbic regions were not easily discernible, the azygous pericallosal artery was used to delineate the lower boundary of the limbic region, which partially includes the infralimbic cortex (IL) of the mPFC, and we refer to this region as the limbic area (PL/IL). The counting frame size was 80x80 μm in the mPFC and 50x50 μm in the hippocampus. The spacing between optical dissectors was 270x270 μm in mPFC and 50x50 in hippocampus. A guard zone of 5% was chosen.

Western Blot

Medial prefrontal cortices and dorsal hippocampi from 6 adult Long Evans of each experimental condition were removed and homogenized in RIPA lysis buffer containing 50 mM Tris-HCl, 150 mM NaCl, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulphate, 1% Triton X-100, and 1.5% protease inhibitor cocktail (Ref: P3840, Sigma-Aldrich, Spain). Equal amounts of proteins were separated on SDS-polyacrylamide gels. Proteins were transferred onto PVDF membranes by semi-dry transfer in the Trans-Blot® Turbo™ Transfer System (Bio-Rad, Hercules, CA, USA). Membranes were incubated for 1 h in TBS buffer (100 mM Tris-HCl; 0.9% NaCl, 1% Tween 20, pH 7.4) containing 5% non-fat dried milk to block nonspecific binding sites. Blots were then incubated in the corresponding primary antibodies overnight: Brain-derived neurotrophic factor (rabbit anti-BDNF, 1:2000, Ref: NBP1-46750, Novus Biologicals, USA); Tropomyosin receptor kinase B (rabbit anti-TrkB (794), 1:1000, Ref: sc-12, Santa Cruz Biotechnology Inc, Spain); phosphorylated tropomyosin receptor kinase B (rabbit anti-pTrkB (Tyr 706), 1:250, Ref: sc-135645, Santa Cruz Biotechnology Inc, Spain); phosphorylated Akt (rabbit anti-pAkt

(Ser 473), 1:1000, Ref: 9271, Cell Signaling Technology Inc, USA); total Akt (rabbit anti-Akt, 1:1000, Ref: 9271, Cell Signaling Technology Inc, USA); phosphorylated extracellular signal-regulated kinase-1/2 (rabbit anti-phospho-p44/42 MAPK (Erk 1/2) (Thr 202/204), 1:1000, Ref: 9102, Cell Signaling Technology Inc, USA); total ERK1/2 (rabbit anti-p44/42 MAPK (Erk 1/2), 1:1000, Ref: 9102, Cell Signaling Technology Inc, USA). Actin was used as a loading control (rabbit anti-actin, 1:2000; Ref: A-2066, Sigma-Aldrich, St. Louis, USA). The following day, HRP-conjugated secondary antibody (anti-rabbit IgG [Ref:A-6164] or anti-mouse IgG [Ref:A-9044], 1:20.000, Sigma-Aldrich, Spain) was added for 1h at room temperature, and a chemiluminescence detection system (Ref: 34076, SuperSignal® West Dura Extended Duration Substrate, Fisher Scientific, Spain) was used to visualize the immunoreactive proteins. Images were acquired using the ChemiDoc™ XRS+ Imaging System (Bio-Rad, Hercules, CA, USA) and optical densities were quantified using Image Studio Digits 3.1 (LI-COR BioScience Biotechnology, Germany). The values are expressed as the relative change from the vehicle group.

Statistical analysis

Continuous data were analyzed for normality with Shapiro-Wilks. Group differences were assessed with Kruskal-Wallis ANOVA, followed by Dunn's post-hoc test. All computations were made using the SPSS software package (version 23.0, IBM, Spain), and differences with $p < 0.05$ were considered significant.

RESULTS

The number of SST+ interneurons decreased in the mPFC and the hippocampus in MK-801-administered animals and increased after adult housing in EE

The difference in the number of somatostatin (SST)-expressing interneurons in the mPFC (Fig.2a) and the HPC (Fig.2b) was statistically significant between groups in limbic areas of the mPFC (p=0.011) and in the CA1 and CA2/3 regions of the hippocampus (p=0.006). However, these differences were not statistically significant in the anterior cingulate (p=0.07) and dentate gyri (p=0.082), where decrements of 23% and 12% of SST+ interneurons were respectively observed in the MK-801 group with respect to the VH group (Fig.2). MK-801 administration during neurodevelopment decreased the number of SST+ interneurons by approximately 30% in the limbic region (p=0.004) and the CA1 (p=0.001) and CA2/3 (p=0.005) regions of the hippocampus compared to the VH group. Adult housing in EE was effective in increasing the number of SST+ interneurons in the MK-801+EE group, but those changes only reached statistical significance in PL/IL (p=0.041) and CA2/3 (p=0.01) when compared to the MK-801 group. In the remaining region, i.e., the CA1 area, we found a slight increase in the number of SST+ interneurons in the MK-801+EE group (12% compared to the MK-801 group) and it was estimated to be 15% lower than in the VH group (p=0.022). Intriguingly, EE did not alter the mean number of SST+ interneurons in the VH+EE group compared to VH (relative differences from 1 to 3%), suggesting that EE had a neurorestorative effect which promotes SST+ interneuron expression only in animals previously injected with MK-801.

Adult housing in EE up-regulated BDNF-TrkB signaling after MK-801 administration in early life

We used Western Blots to compare the relative expression of BDNF protein and its high-affinity TrkB receptor between groups (Fig.3). In the mPFC, BDNF peptide was significantly increased in the VH+EE group compared to the VH (p=0.003) and MK-801 (p<0.0001) groups as well as in the MK-801+EE group compared to the standard-housed MK-801 group (p=0.011) (Fig.3a). In this region, MK-801 animals showed decreased TrkB and pTrkB levels compared to the VH group, but these differences were non-significant (Fig.3b and c). Mean TrkB protein levels of MK-801-injected animals increased up to normal values after EE, being statistically different from the MK-801 group (p=0.004).

In the hippocampus, we also observed a significant increase in BDNF protein expression levels of enriched rats compared to their standard-housing litter-mates. More precisely, BDNF in the MK-801+EE group was significantly up-regulated compared to the standard-housed VH (p<0.0001) and MK-801 (p=0.012) animals. BDNF levels of VH+EE animals were also significantly up-regulated compared to the VH group (p=0.004) (Fig.3d). Although total protein levels of TrkB receptor were unchanged, animals reared in EE showed significantly more pTrkB than standard-housed ones, without variations between the VH and MK-801 groups (VH vs. MK-801+EE, p=0.022; VH vs. VH+EE, p=0.007; MK-801 vs. MK-801+EE, p=0.005, MK-801 vs. VH+EE, p=0.001) (Figs.3e and 3f).

Changes in TrkB transduction pathways: ERK and Akt

Abnormal ERK and Akt activity has been implicated in the pathogenesis of schizophrenia [35,36]. Therefore, we compared the relative ratio of phospho-Akt (pAkt) to total Akt and phospho-ERK (pERK) to total ERK. As shown in Figure 4, MK-801 caused a significant change in the phosphorylation levels of Akt in the mPFC (MK-801 vs. VH, p=0.012; MK-801 vs. MK-801+EE, p=0.04; MK-801 vs. VH+EE, p<0.0001), but not in the dorsal hippocampus (Figs.4a and 4b). Conversely, the phosphorylation level of prefrontal ERK was not disturbed in the MK-801 group (Fig.4c). Exposing animals to EE significantly increased the relative expression levels of pAkt and pERK in the hippocampus. In fact, EE reversed the MK-801-induced attenuation of pERK/ERK (p=0.001, Fig.4d), and the MK-801+EE group showed the highest levels of pAkt/Akt ratio in the hippocampus (VH vs. MK-801+EE, p=0.001; MK-801 vs. MK-801+EE, p=0.036, Fig.4b). Similarly, the prefrontal pERK/ERK ratio was significantly increased in MK-801+EE animals (MK-801+EE vs. MK-801, p=0.002; MK-801+EE vs. VH+EE, p=0.001), and EE reversed MK-801-induced increase in Akt phosphorylation in the mPFC (MK-801 vs MK-801+EE, p=0.04).

Thus, exposing MK-801-administered animals to EE in early adulthood promoted long-lasting up-regulation of BDNF-TrkB signaling, counteracting the effects induced by intraperitoneal injections of MK-801 in early life.

DISCUSSION

Stereology for unbiased cell counting of somatostatin-immunoreactive interneurons in the medial prefrontal cortex and the hippocampus revealed a neurorestorative role of EE following NMDA receptor blockade. MK-801 administration during early brain development produced a long-lasting decrease in the number of somatostatin-expressing interneurons that was reverted by adult housing in EE for a short period. Notably, the activity-dependent nature of EE up-regulated the BDNF-TrkB pathway in animals previously treated with MK-801, bringing forth BDNF-TrkB as a potential pathway by which SST+ interneurons may be rescued in the MK-801 animal model of schizophrenia.

Somatostatin interneurons are altered in schizophrenia

Several findings indicate that a deficiency in inhibitory signaling in schizophrenia leads to psychosis and cognitive impairment. Despite the convergent findings of altered interneuron markers in *post mortem* brains of patients with schizophrenia, animal research has been mainly focused on exploring the role of parvalbumin interneurons in sensorimotor gating and cognitive processes [37,38]. Even though previous studies suggest that parvalbumin interneurons are altered in schizophrenia, cellular disturbances in schizophrenia are complex and affect multiple interneuron sub-types [39,5,6]. More specifically, human mRNA and immunohistochemical studies also point to alterations in SST+ interneurons [10,40,6]. Somatostatin-expressing interneurons comprise 30 to 50% of GABAergic cells in the hippocampus and the neocortex [41] and are a prominent source of inhibition in neural networks. Studies in rodents have demonstrated that inhibition exerted by SST+ cells regulates motor activity, sleep, sensory processes and cognitive functions, and these interneurons have been involved in computations related to stimuli salience and behavioral relevance [42-44]. They also play an essential role in synaptic maturation of thalamocortical inputs to parvalbumin interneurons to enable feed-forward inhibition [45] and facilitate hippocampal-prefrontal synchrony and spatial encoding [46]. Therefore, the perturbation of somatostatin interneurons seems to be a key component of the cellular pathology in schizophrenia that could contribute not only to altered PV+ interneuron maturation, but also to psychotic and cognitive symptoms. However, it has been unclear whether this impairment is also present in pre-clinical models of schizophrenia. Arif et al. [47] first explored the effects of MK-801 on somatostatin. According to their results, 3 days after a single dose of 0.5mg/kg MK-801, SST mRNA and protein levels significantly decreased. Later, Perez-Rando et al. [48] demonstrated structural remodeling of spine and axonal boutons in hippocampal SST+ interneurons produced by a single injection of MK-801 (1 mg/kg) 24h prior to sacrifice.In response to chronic administration of MK-801 during early postnatal days, our data indicate that a dosing regimen faithfully mimics some behavioral, cognitive and neurobiological traits of schizophrenia in the long-term [14,49], and that the number of SST+ interneurons significantly decreased in a variety of sub-regions of the medial prefrontal cortex and the hippocampus. These results extend previous evidence of the actions of MK-801 on SST+ interneurons and demonstrate a persistent effect of chronic NMDAR blockade on the number of SST+ subpopulation. Moreover, according to our current and previous results [12], the relatively specific alterations of PV+ and SST+ interneurons found in schizophrenia seem to be also

present in this pre-clinical model, an observation that supports NMDAR hypofunction in early life as a relevant etiopathological mechanism of schizophrenia.

Enriched environment increases the number of somatostatin-positive interneurons in MK-801-injected animals

Chronic administration of NMDAR antagonists, such as MK-801, preferentially disturb the expression of PV+ and SST+ interneurons. The underlying mechanisms used by MK-801 to reduce the number of PV+ and SST+ interneurons are poorly understood, but the sub-unit composition of NMDAR could explain why these sub-types of interneurons could be more prone to being selectively blocked. Notably, NMDA receptor sub-unit NR2C is preferentially expressed in PV+ and SST+ interneurons, and NR2D in PV+ fast-spiking cells [50]. Since NMDARs containing NR2C and NR2D sub-units have low affinity to magnesium [51], MK-801 would have a higher probability of penetrating the channel pore of NMDARs with these sub-unit compositions. Intriguingly, both sub-types of interneurons share a common ontogenic origin in the medial ganglionic eminence, implying that disturbed developmental regulators might also play a role in humans [40]. However, our MK-801 dosing regimen in postnatal life coincided with the period when SST+ interneurons peak in number [52]. Thus, we believethat the NMDA receptor antagonist could have disturbed SST+ interneuron maturation in early life, an alteration that persisted throughout development into adulthood. From these findings, it was difficult to infer whether the decrease in the number of SST+ interneurons was due to an actual loss of SST+ cells or to a reduction in SST mRNA or protein expression. Considering that a substantial increase in SST+ interneurons was found after a brief exposure to EE in adulthood, a decrease of somatostatin expression would be the most likely explanation.

Even though somatostatin has long been recognized as an inhibitory neuropeptide expressed in an activity-dependent manner [53], the effect of increased sensory stimulation, physical exercise, social interaction or a combination of them has not been extensively explored. Gleichmann et al. [54] showed that housing the animals in an Enriched Environment plus running promoted an increase in the preprosomatostatin level measured by immunoblots that was otherwise decreased in cell cultures by 7 days of MK-801 treatment. Our data indicate that short-term adult housing in EE reversed the long-lasting actions of MK-801, except in the CA1 region of the hippocampus. This confirms the results from a previous work, in which we found that the number of PV+ interneurons in the CA1 region of the MK-801+EE group was significantly lower than in the VH group [12]. It remains to be determined if PV+ and SST+ interneurons in the CA1 area are particularly susceptible to MK-801-induced cellular damage and if NMDAR blockade in early development induces cell type-specific apoptosis, or whether EE exposure might not be enough to promote protein expression recovery in this particular region.

Up-regulation of the BDNF-TrkB pathway after Enriched Environment

In view of the contribution of EE during adulthood to the synthesis and release of BDNF in health and disease [55], we explored whether SST+ interneuron expression increase in the MK-801+EE group would be accompanied by increased BDNF-TrkB signaling. As opposed to previous works, we did not

observe BDNF-TrkB signaling decrease in MK-801 animals, but we found that in enriched groups, the relative activity of the BDNF-TrkB pathway was up-regulated in the medial prefrontal cortex and the hippocampus, being significantly higher in the MK-801+EE group compared to the MK-801 group. These results reveal the BDNF-TrkB signaling pathway as a potential mechanism by which somatostatin immunoreactivity was rescued after EE exposure. Nonetheless, these results are only correlational, and EE exerts its effects through several mechanisms, regulating multiple activity-dependent pathways. Therefore, we should be cautious in interpreting the causal relationship between the up-regulation of BDNF-TrkB signaling and the increase in the number of SST+ interneurons.

Several studies also indicate that activation levels of signaling pathways downstream of the TrkB receptor are altered in schizophrenia [36,56] and in response to NMDAR blockade [57-60]. These pathways regulate neuronal survival, differentiation, synaptic plasticity, and learning and memory processes. One of the best characterized is the ERK pathway, a cascade of kinases that present abnormal activity in frontal cortical areas of schizophrenic patients [36]. Consistent with earlier observations in rodents [57], we found that the pERK/ERK ratio decreased in the hippocampus but not in the frontal cortex in response to MK-801. Neuronal growth factors stimulate somatostatin gene expression by triggering CREB phosphorylation at serine 133, mainly through the activation of the ERK signaling pathway. However, ERK is known to be a downstream signal transduction pathway that is common to the NMDA and TrkB receptors. Therefore, CREB phosphorylation can also be induced by an elevation of intracellular calcium regulated by NMDARs [61]. We have previously reported that EE counteracts MK-801-induced decrease in NMDAR sub-unit NR1 expression [13], thus the NMDAR-ERK pathway could also contribute to somatostatin gene expression in this pre-clinical model.

Additionally, we found that MK-801 administration significantly increased the phosphorylation level of Akt in the mPFC, which is in line with previous studies that used similar doses [58-60].Although the mechanism of increased Akt activity is still unclear, this pathway regulates the synthesis and insertion of $GABA_AR$ on the cell membrane and increases fast inhibitory neurotransmission [35,62]. Therefore, the activation of Akt could act as a compensatory mechanism to counteract the prefrontal hyperactivity induced by MK-801. Intriguingly, EE during adulthood decreased the pAkt/Akt ratio in the mPFC to normal values. It is worth mentioning that the relative activity of the Akt and ERK pathways were not modified in the mPFC of the VH+EE group.

Final remarks

These results extend previous research in pre-clinical models of schizophrenia based on NMDAR hypofunction and suggest that after MK-801 administration, SST+ interneurons decrease, in agreement with evidence in humans. As far as we know, this is the first study addressing the benefits of EE in promoting the expression of SST+ interneurons. A potential limitation of the current study is that the increase in the number of SST+ interneurons and BDNF-TrkB signaling after EE was a correlational finding, so, the effect of BDNF in somatostatin expression should be interpreted with caution.Moreover, although ERK and Akt are the two major pathways downstream of the TrkB receptor, we did not study other proteins involved in this pathway. While future studies are warranted to address these issues and the

behavioral significance of our findings, our results establish the foundation for the potential benefits of environmental intervention to overcome neurobiological alterations of schizophrenia. Translation of this paradigm to clinical therapy might not be straightforward, but according to our results, adolescents at high risk or first-episode of schizophrenia might benefit from physical exercise, social interaction and exposure to novel stimuli, and these activities could be implemented as a contributory therapy in clinical practice.

FIGURES AND FIGURE LEGENDS
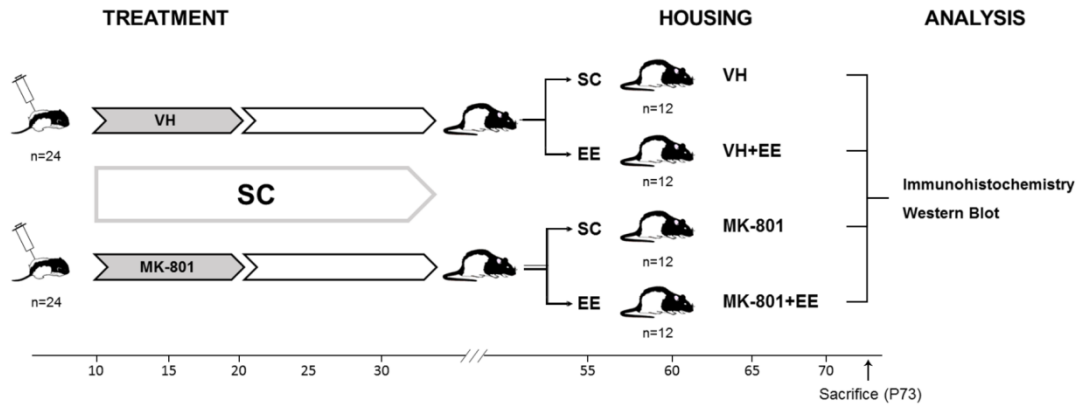
Fig.1. Experimental design. Diagram showing animal groups, drug injection schedule, timing of Enriched Environment housing and animal sacrifice (n=12 per group). EE: Enriched Environment; MK-801 group corresponds to animals who received intraperitoneal MK-801 injections (0.5 mg/kg) between P10 and P20; SC: standard conditions; VH: animals injected with saline.
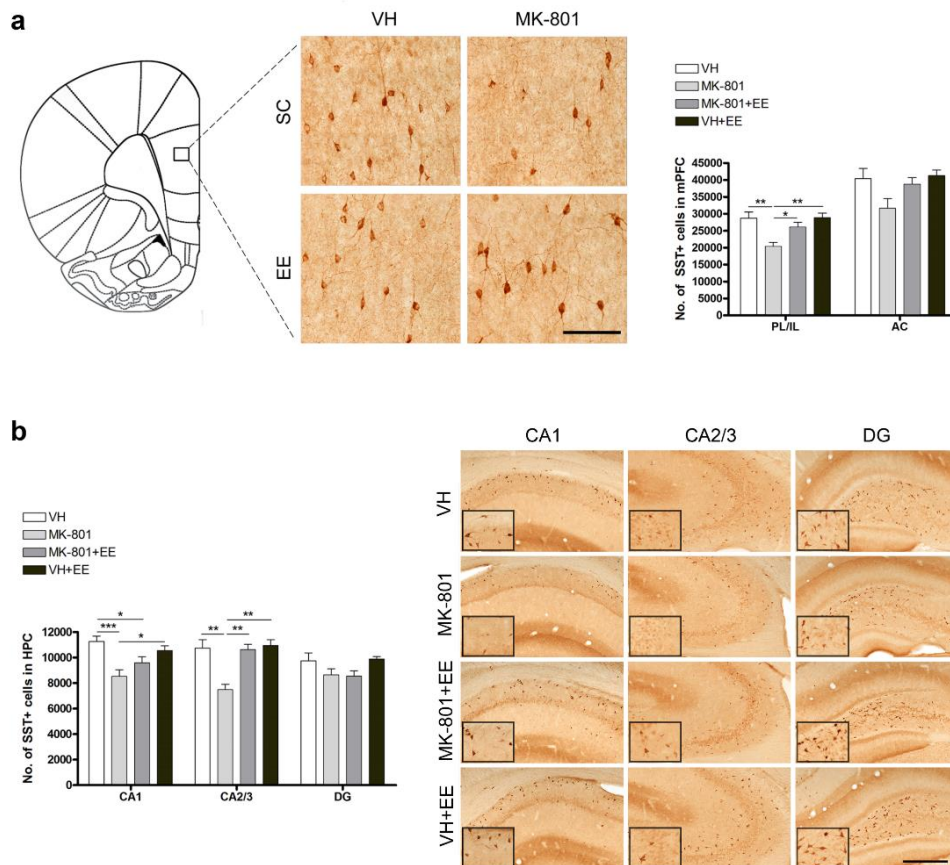
Fig.2. Effects of postnatal exposure to MK-801 and enriched environment on the number of interneurons immunoreactive to somatostatin in medial prefrontal cortex (mPFC) (A) and hippocampus (B) with the corresponding representative images. AC, anterior cingulate cortex; CA1, *cornusammonis*1 of hippocampus; CA2/3, *cornusammonis*2/3 of hippocampus; DG, dentate gyrus of hippocampus; EE: Enriched Environment; MK-801: MK-801-injected group (0.5mg/kg); PL/IL, prelimbic/infralimbic cortex; SC: standard housing conditions; VH: vehicle-injected group. Data are represented as mean ± SEM. *p<0.05; **p<0.01; ***p<0.001. Scale bar mPFC: 200 μm. Scale bar hippocampus: 600 μm.
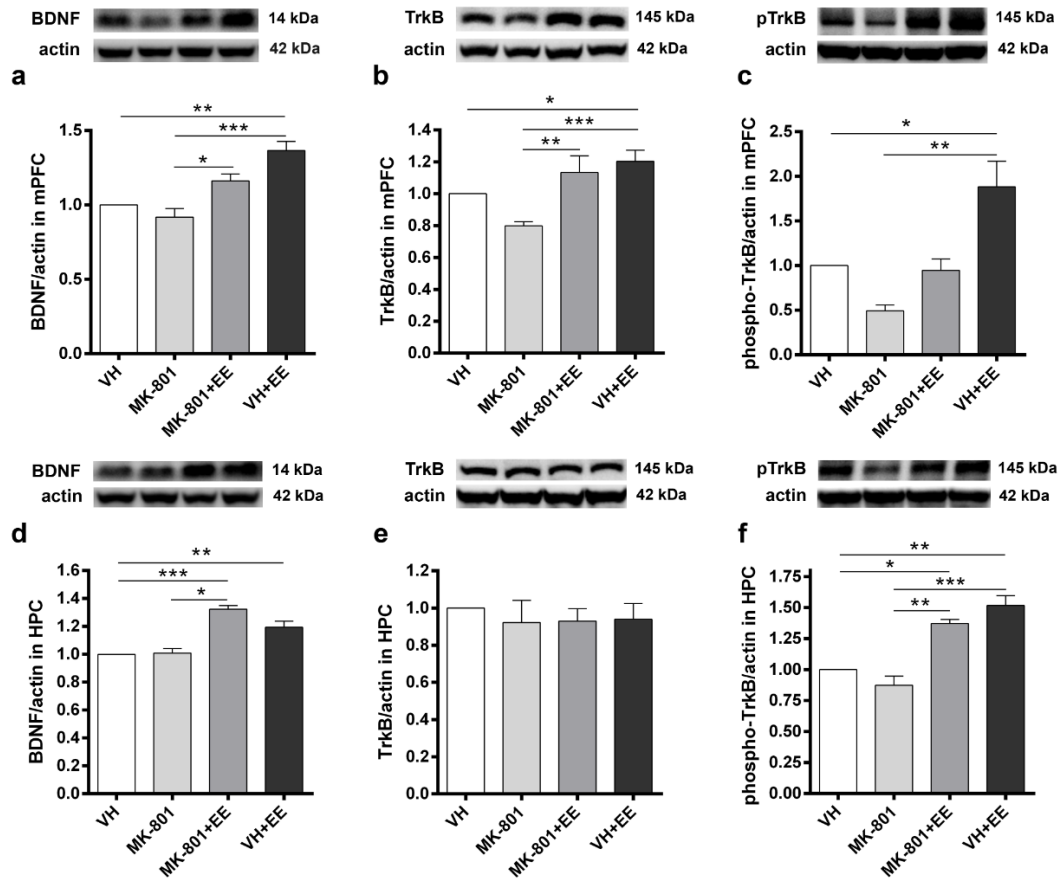
Fig.3. Protein levels for assessing BDNF-TrkB signaling (n=6 per group) in (a,b,c) mPFC and (d,e,f) dorsal hippocampus. Western blots (upper panels) and quantification (histograms) of band densities for (a,d) BDNF (b,e) TrkB receptor and (c,f) phosphorylated TrkB receptor. Histograms represent optical densities obtained for each primary antibody compared to actin, expressed as the ratio relative to VH animals. Data are represented as mean ± SEM. *p < 0.05; **p < 0.01; ***p < 0.001.
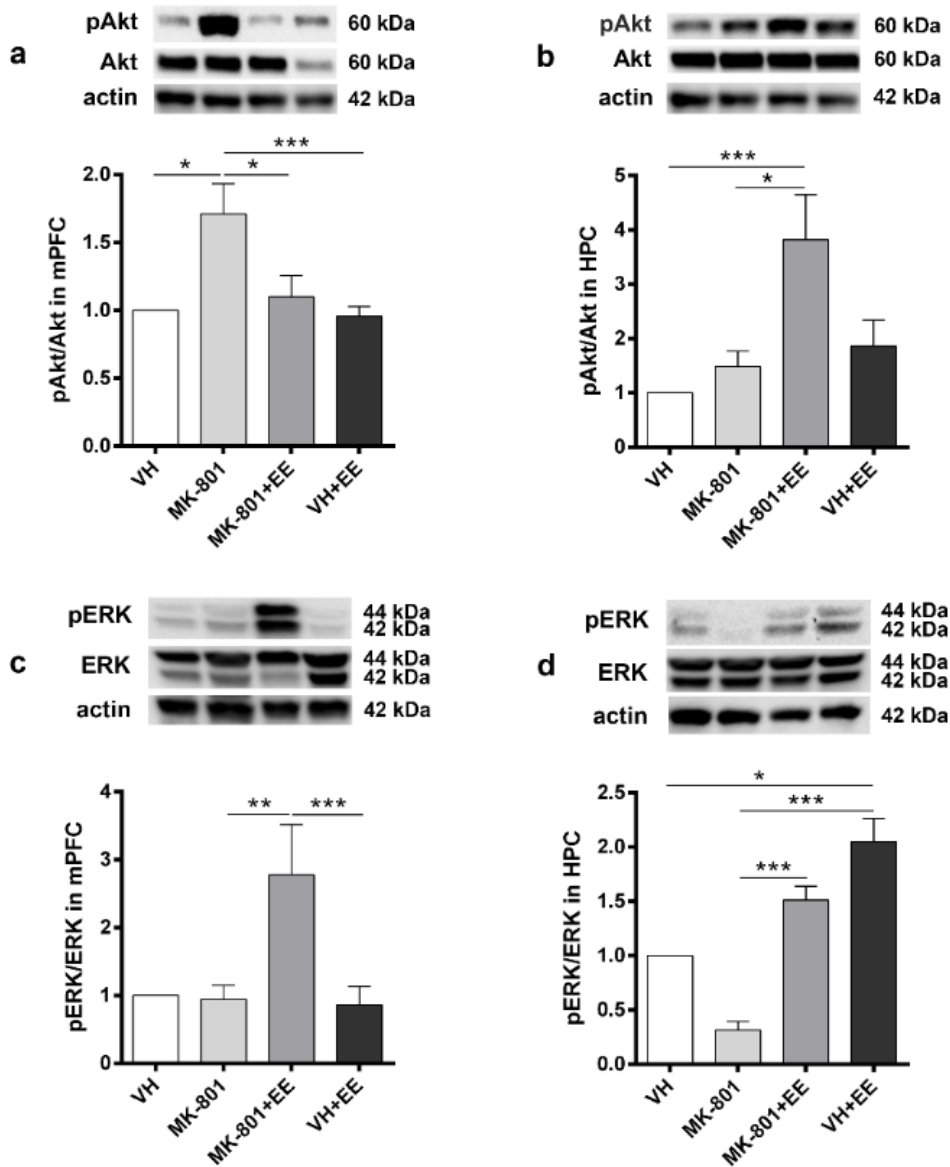
Fig.4. Relative activation of Akt and ERK1/2 pathways in animals housed in standard conditions and after adult enriched environment (EE) medial prefrontal cortex (mPFC) and hippocampus (HPC). Western blots (upper panels) and quantification (histograms) of band densities for the relative levels of (a,b) phospho-Akt (pAkt) to total Aktand (c,d) phospho-ERK1/2 (pERK) to total ERK. Histograms represent optical densities obtained for each primary antibody compared to actin, expressed as the ratio relative to VH animals. Data are represented as mean ± SEM. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

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