The effects of chronic stress on hippocampal adult neurogenesis and dendritic plasticity are reversed by selective MAO-A inhibition

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Short Report

The effects of chronic stress on hippocampal adult neurogenesis and dendritic plasticity are reversed by selective MAO-A inhibition

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Abstract

There is accumulating evidence that adult neurogenesis and dendritic plasticity in the hippocampus are neuroplastic phenomena, highly sensitive to the effects of chronic stress and treatment with most classes of antidepressant drugs, being involved in the onset and recovery from depression. However, the effects of antidepressants that act through the selective inhibition of monoamine oxidase subtype A (MAO-A) in these phenomena are still largely unknown. In the present study, adult neurogenesis and neuronal morphology were examined in the hippocampus of rats exposed to chronic mild stress (CMS) and treated with the selective reversible MAO-A inhibitor (RIMA) drug, pirlindole and the selective serotonin reuptake inhibitor (SSRI), fluoxetine. The results provide the first demonstration that selective MAO-A inhibition with pirlindole is able to revert the behavioural effects of stress exposure while promoting hippocampal adult neurogenesis and rescuing the stress-induced dendritic atrophy of granule neurons.

Keywords

Depression, pirlindole, fluoxetine, stress, neuroplasticity, hippocampus, neurogenesis

Introduction

Major depression is a highly prevalent mood disorder (Kessler and Walters, 1998) associated with a significant social and economic impact (Sheehan, 2002). However, the precise physiopathological mechanisms involved in the aetiology of this disorder and in the therapeutic actions of antidepressant (AD) drugs are still largely unknown (Berton and Nestler, 2006).

There is increasing evidence that the generation of new neurons and the dendritic reorganization of pre-existing neurons in the adult hippocampus are complementary neuroplastic phenomena involved not only in the onset of but also in the remission from depression (Mateus-Pinheiro et al., 2013; Snyder et al., 2011; Surget et al., 2011). The potentiation of adult neurogenesis in the dentate gyrus of the hippocampus has been extensively described with the administration of different classes of AD drugs, namely with non-selective monoamine oxidase (MAO) inhibitors (Malberg et al., 2000), tricyclic antidepressants (TCAs) (Sairanen et al., 2005) and selective serotonin reuptake inhibitors (SSRIs) (Santarelli et al., 2003). These observations suggest that the different pharmacological interventions targeting monoaminergic neurotransmission have a common effect in adult neurogenesis. However, the effects of ADs in the reversal of stress-induced morphological changes of granule neurons in the hippocampus have only been described with TCAs and SSRIs (Bessa et al., 2009a; Jayatissa et al., 2006; Surget et al., 2011). Thus, it remains to be established whether adult neurogenesis and dendritic reorganization of pre-existing hippocampal granule neurons after stress exposure are involved in the behavioural actions of selective reversible MAO-A inhibitors (RIMA).

In the present study, we evaluated the behavioural effects of the RIMA pirlindole (Macedo et al., 2011) and the SSRI fluoxetine in the chronic mild stress (CMS) animal model. In addition, we examined whether stress-induced changes in neurogenesis and neuronal plasticity within the hippocampus are influenced by these commonly used ADs. Our results demonstrate that, like fluoxetine, pirlindole is able to reverse the behavioural effects of stress exposure while potentiating hippocampal adult neurogenesis and rescuing the stress-induced dendritic atrophy of granule neurons.

Experimental procedures

Animals

Ninety-six male Wistar rats (Charles-River Laboratories, Barcelona, Spain), weight 300-400 g, age 2 months, were used in this study. Animals were housed (three per cage) under standard laboratory conditions (12 h light: 12 h dark cycle, lights on at 8 a.m., 22ºC, relative humidity 55%; free access to food and water). Animals

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were assigned to one of the six treatment groups. A control group (n=16) and five treatment groups exposed to CMS: vehicle (n=16), fluoxetine 10 mg/kg (n=16), pirlindole 5 mg/kg (n=16), pirlindole 15 mg/kg (n=16) and pirlindole 30 mg/kg (n=16). All procedures were carried out in accordance with the Portuguese national authority for animal experimentation, Direção Geral de Veterinária (ID: DGV9457) and in accordance with the guidelines for the care and handling of laboratory animals in Directive 2010/63/EU of the European Parliament and Council.

Drugs

The drugs used were fluoxetine (10 mg/kg; Kemprotec, Middlesbrough, UK) and pirlindole (5, 15 and 30 mg/kg; Grupo Tecnimede, Sintra, Portugal). Compounds were dissolved in sterile distilled water with sonication and administered intraperitoneally (1 mL/kg) to animals, daily at 20:00. During the last 3 weeks of CMS, animals were given daily injections with vehicle (n=16), fluoxetine 10 mg/kg (n=16), pirlindole 5 mg/kg (n=16), pirlindole 15 mg/kg (n=16) and pirlindole 30 mg/kg (n=16). The daily doses of AD drugs administered chosen were based on their therapeutic effects as described in the literature (Bruhwyler et al., 1997; Song et al., 2006).

Chronic mild stress

A slightly modified version of an unpredictable CMS protocol was used (Bessa et al., 2009b; Willner, 2005). It consisted of chronic exposure to unpredictable mild stressors (confinement in a restricted space for 1 h, placement in a tilted cage [30º] for 4 h, housing on damp bedding for 8 h, overnight illumination, food deprivation for 18 h followed by exposure to inaccessible food for 1 h, water deprivation for 18 h followed by exposure to an empty bottle for 1 h, overcrowding for 4 h, exposure to noise for 4 h, exposure to strobe lights for 4 h and reversed light/dark cycle for 48 h every 7 days) over 7 weeks (Supplementary Table 1).

Sucrose preference test

Anhedonia was assessed weekly during exposure to CMS using the sucrose preference test (SPT). Briefly, animals were allowed to habituate to the sucrose solution in three baseline trials of 1 h exposure to 1% sucrose solution or tap water, following 18 h of food and water deprivation 1 week before the CMS protocol, to establish baseline preference levels. To test sucrose preference, animals that were food- and water-deprived for 18 h (Supplementary Table 1) were presented with two pre-weighed bottles containing 1% sucrose solution or tap water for a period of 1 h. Sucrose preference was calculated according to the formula: sucrose preference = [sucrose intake / (sucrose intake + water intake)] X 100, as previously described (Bekris et al., 2005).

Forced swimming test

Depressive-like behaviour was evaluated in the forced swimming test (FST) on the last day of exposure to CMS. Twenty-four hours after a pre-test session (10 min), rats were placed in transparent glass cylinders (64 cm height and 22 cm diameter) filled with water (25ºC; depth 30 cm) for a period of 5 min. Test sessions were assessed using a camera connected to a video tracking system (Viewpoint, Lyon, France); the system automatically calculated immobility time and latency to immobility. Depressive-like behaviour was defined as an increase in time of immobility and a decrease in latency to immobility (Castagné et al., 2011).

Immunostaining procedures

For cell proliferation and cell phenotype analysis, eight animals from each experimental group were injected with a single dose of BrdU (100 mg/kg, Sigma-Aldrich, St Louis, USA) 24 h before sacrifice (Landgren and Curtis, 2011). At the end of the experimental procedures, animals were sacrificed under anaesthesia. Serial coronal sections (20 µm) were cut and stained for BrdU (1:50; Dako, Glostrup, Denmark). Sections were then double-stained with PSA-NCAM for neuroblasts (1:500; Millipore, Billerica, MA, USA) or GFAP for glial cells (1:200; Dako). Proliferation densities were estimated in the subgranular zone (SGZ) of the dentate gyrus as a ratio between the total number of immunostained cells and the area of the SGZ, using an Olympus BX51 optical microscope and *Newcast* software (Visiopharm, Hoersholm, Denmark). For each animal, eight sections were analysed. The double staining with neuronal (PSA-NCAM) or glial (GFAP) markers (von Bohlen, 2011) were performed using a confocal microscope (Olympus FV1000) and an optical microscope (Olympus BX51), respectively.

Structural analysis

For the 3D morphometric analysis, six animals from each treatment group were transcardially perfused with 0.9% saline and processed (Gibb and Kolb, 1998). Briefly, brains were immersed in Golgi-Cox solution for 21 days (Glaser and Van der Loos, 1981), transferred to a 30% sucrose solution and cut on a vibratome. Coronal sections (200 µm thick) were collected in 6% sucrose and blotted dry onto gelatin-coated microscope slides. They were subsequently alkalinised in 18.7% ammonia, developed in Dektol (Kodak), fixed in Kodak Rapid Fix, dehydrated and xylene-cleared before coverslipping. Dendritic arborisation and spine numbers and shape of granule neurons were analysed using a motorized microscope (Axioplan 2, Carl Zeiss) and Neurolucida software (Microbrightfield, Willinston, USA). A 3D analysis of the reconstructed neurons was performed using NeuroExplorer software (MicroBrightfield, Inc.). Forty neurons were studied for each animal and measurements from individual neurons were averaged for each animal. Several aspects of dendritic morphology were examined, namely, the total dendritic length, dendritic spine density, spine morphology and Sholl analysis to evaluate the spatial arrangement of dendritic material by quantifying the number of dendritic intersections at concentric 20-µm intervals from the soma (Harris et al., 1992).

Statistical analysis

After confirmation of homogeneity, appropriate statistical tests were applied to the data. Repeated measures ANOVA was used to analyse the results of the sucrose consumption test. One-way ANOVA was used to evaluate the impact of stress and the effect of ADs in further behavioural and structural data. Differences between groups were then determined by Tukey's honestly

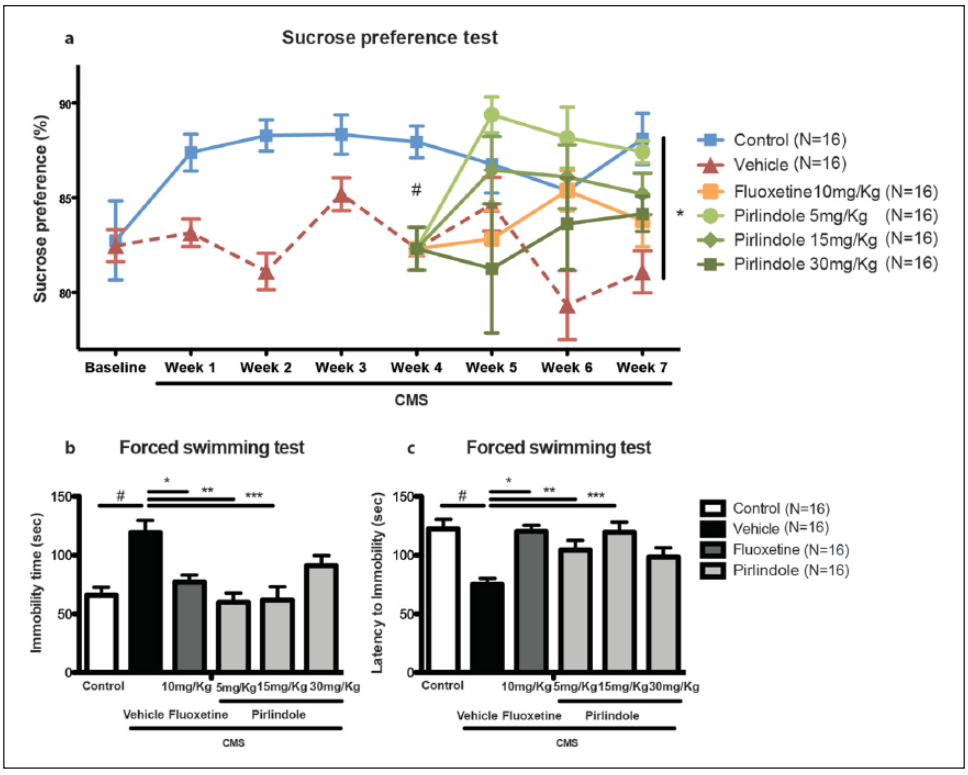

Figure 1. Behavioural effects of fluoxetine and pirlindole on hedonic and depressive-like behaviour. (a) Sucrose preference in the SPTs performed during CMS. #P=0.003, *P<0.001. (b) Immobility time and (c) latency to immobility time in the forced swimming test performed after CMS and drug administration. #P<0.001, *P<0.05; **P<0.05, ***P< 0.001. Data presented as mean + s.e.m.

significant difference test (Tukey HSD) post-hoc analysis. Statistical significance was accepted for P<0.05. Results are expressed as mean + s.e.m.

Results

Behavioural results

The analysis of the SPT during the first 4 weeks of the CMS protocol revealed a significant decrease of sucrose preference in animals exposed to chronic stress treated with vehicle ($F_{1,94}$=9.147; P=0.003) confirming the induction of an anhedonic behavioural phenotype. During the last 3 weeks of the CMS protocol, a significant global effect of treatment was observed ($F_{5,90}$=5.964; P<0.001). Post-hoc analysis revealed significant differences between the animals treated with vehicle and animals treated with pirlindole 5 mg/kg (P=0.001). However, no significant differences were observed with fluoxetine or with higher doses of pirlindole (Figure 1a). Concerning behaviour in the FST, a significant increase in immobility time ($F_{1,32}$=23.839; P<0.001) and decrease in latency to immobility time ($F_{1,32}$=23.834; P<0.001) was observed in stress-exposed animals (Figure 1b,c). AD treatment proved to be a significant factor in recovery from depressive-like behaviour in the FST ($F_{4,80}$=7.433; P<0.001). Fluoxetine reversed the stress-induced behaviour in immobility time (P=0.012) and in latency to immobility time (P<0.001). Similarly, treatment with pirlindole induced a significant decrease in immobility time at dosages of 5 mg/kg (P<0.001) and 15 mg/kg (P<0.001) and an increase in latency to immobility time at dosages of 5 mg/kg (P=0.043) and 15 mg/kg (P<0.001). No significant effects were observed at a dosage of pirlindole 30 mg/kg.

Cell proliferation, neurogenesis and glial phenotype

Cell proliferation analysis (Figure 2a,b) revealed a non-significant decrease in the density of BrdU-positive cells in animals exposed to CMS ($F_{1,16}$=2.090; P=1.170). However, an overall effect of AD treatment was observed ($F_{4,40}$=2.697; P=0.046) with a significant increase associated with fluoxetine treatment (P=0.029) in comparison with vehicle-treated animals. Adult neurogenesis assessed by co-labelling of BrdU with neuroblast marker PSA-NCAM (Figure 2c,d) was significantly decreased with stress exposure ($F_{1,16}$=6.728; P=0.021). A significant effect of AD treatment was observed ($F_{4,40}$=5.235; P=0.002). Both fluoxetine (P=0.033) and pirlindole at all dosages tested (5 mg/kg, P=0.035; 15 mg/kg, P=0.005; 30 mg/kg, P=0.005), increased adult neurogenesis in the SGZ. No significant effects of stress exposure or AD treatment were observed in glial lineage in the SGZ as assessed by co-labelling of BrdU with glial cell marker GFAP (Figure 2e,f).

Structural analysis

The three-dimensional morphometric analysis of Golgi-impregnated neurons in the dentate gyrus (Figure 3) revealed that

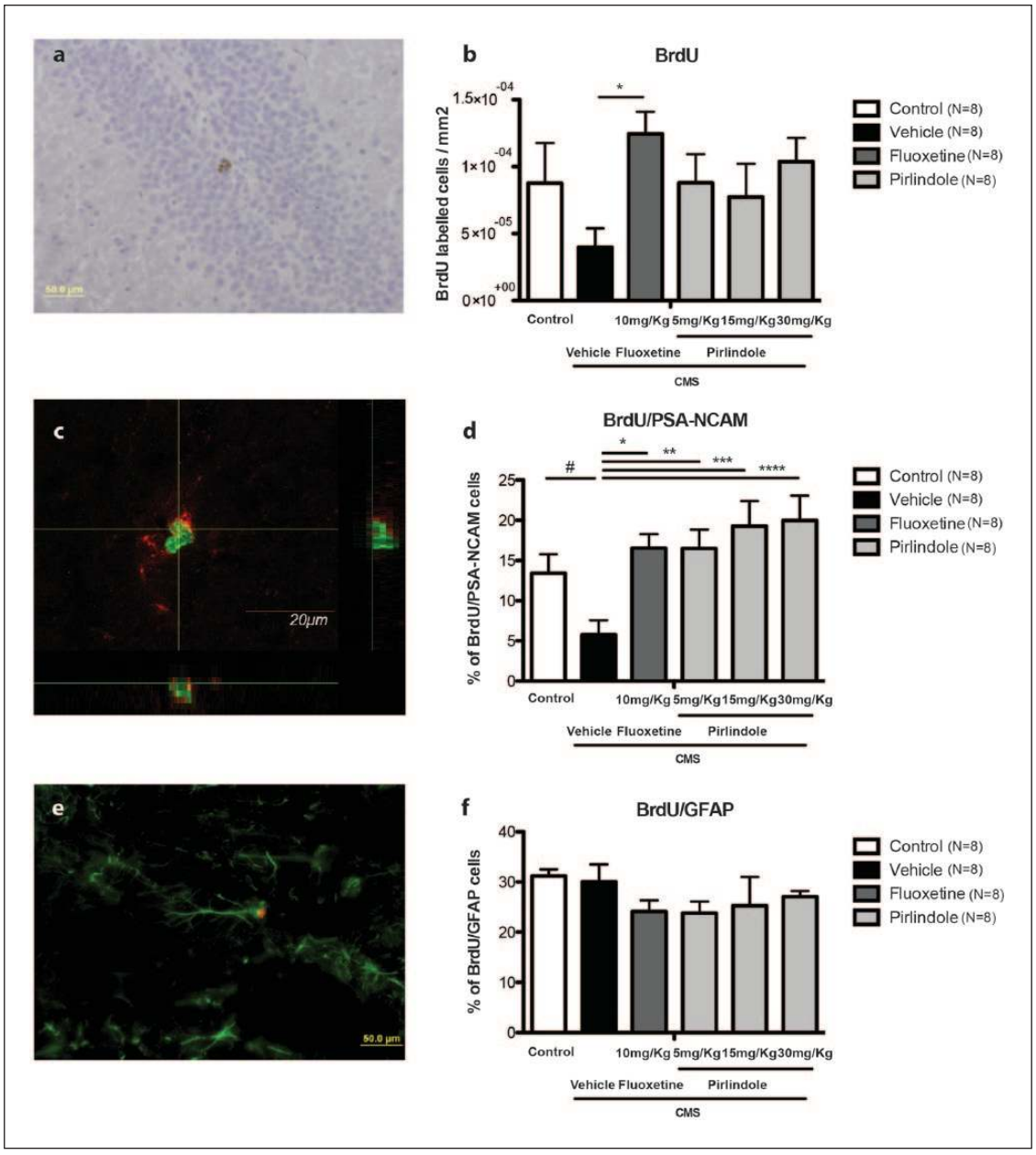

Figure 2. Cell proliferation, neurogenesis and glial phenotype. (a) Proliferative niche of BrdU-labelled cells in the subgranular zone (SGZ) obtained with optical microscopy. (b) The density of BrdU-labelled cells in the SGZ of the dentate gyrus. **P*<0.05. (c) Niche of newly formed neurons in the SGZ, obtained by confocal microscopy. Green: BrdU-positive cells; red: PSA-NCAM-positive cells. (d) The percentage of BrdU-immunopositive cells that were co-labelled with antibodies against PSA-NCAM in the SGZ. #*P*<0.05, **P*<0.05; ***P*<0.05, ****P*=0.005, *****P*=0.005. (e) Newly formed glial cells in the SGZ, obtained by optical microscopy. Green: GFAP-positive cell; red: BrdU-positive cell. (f) Percentage of BrdU-positive cells that were co-labelled with glial marker GFAP in the SGZ. Data represented as mean + s.e.m.

exposure to CMS induced atrophy in granule neurons, with a significant decrease in their total dendritic length ($F_{1,12}$=11.358; *P*=0.007). Importantly, this atrophic effect of chronic stress was reversed after administration of ADs ($F_{4,30}$=5.422; *P*=0.003). Both fluoxetine (*P*=0.002) and pirlindole at dosages of 30 mg/kg (*P*=0.040) significantly increased total dendritic length. No significant effects of stress exposure or antidepressant treatment were observed in spine densities, spine morphology or Sholl analysis (Supplementary Figure 1).

Discussion

The results of this study reveal that the RIMA pirlindole is able to reverse stress-induced anhedonia and confirm the previously described AD effects of pirlindole in the FST (Bruhwyler et al., 1998). This concordance between the reversal of anhedonic behaviour in the SPT and the decrease in immobility in the FST, similar to the one observed with the SSRI fluoxetine, confirms the AD effects of pirlindole in this validated animal model of

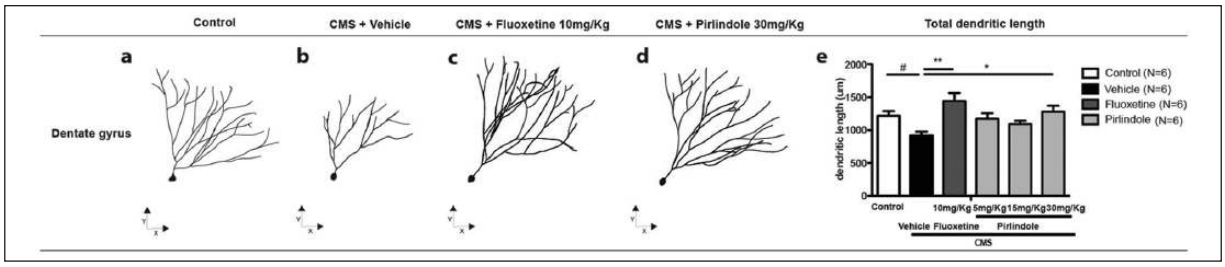

Figure 3. 3D morphometric analysis of Golgi-impregnated neurons using computer-assisted reconstructions of hippocampal granule neurons. Representative neurons of different experimental groups: (a) Control, (b) CMS+vehicle (c) Fluoxetine 10 mg/kg and (d) pirlindole 30 mg/kg. (e) Total dendritic length of neurons in the dentate gyrus of the hippocampus. #P<0.01, *P<0.05; **P<0.005. Data represented as mean + s.e.m.

depression (Bessa et al., 2009b). However, the fact that sucrose preference was evaluated after food deprivation does not exclude the possibility that metabolic demands may be involved in these behavioural results in the SPT. Importantly, the treatment with fluoxetine and pirlindole effectively restored the generation of new neurons in the SGZ without affecting glial cells and reversed the atrophic changes induced by chronic stress.

Interestingly, the results suggest that the effects of pirlindole on depressive-like behaviour are dose-dependent, with the lower dosages showing the ability to reverse the stress-induced changes in the sucrose preference and forced swimming test. However, the effects of this drug in the modulation of hippocampal adult neurogenesis are present at all the doses used. This observation is in accordance with the notion that the mood-improving effects of antidepressants are not exclusively dependent on the ability to modulate the generation of new hippocampal neurons (Bessa et al., 2009a).

The observation that the impact of CMS and ADs was significant on hippocampal neurogenesis but not on cell proliferation suggests that these processes may be differently regulated. This is in accordance with previous studies regarding the effects of CMS and ADs on the proliferation, differentiation and survival of newly born hippocampal cells (Lee et al., 2006; Mateus-Pinheiro et al., 2013). Furthermore, the fact that the highest dose of pirlindole failed to reveal significant AD effects while reversing the effects of stress on dendritic length, suggests a possible dissociation between the behavioural and neuroplastic effects of this drug.

In conclusion, this study provides the first demonstration that MAO-A selective inhibition reverses the deleterious neuroplastic effects of chronic stress in the hippocampus by restoring adult neurogenesis and by rescuing dendritic atrophy of granule neurons. Taking into account the fact that the subtype A of MAO preferentially metabolizes serotonin and noradrenaline (Syha and Schraven, 1990), these results further reinforce the notion that the modulation of monoaminergic neurotransmission is a critical factor for the neuroplastic effects of currently available antidepressant drugs.

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Conflict of interest

Filipe A, Pedroso P and Almeida S are employees of Grupo Tecnimede.

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