

Modulation of BDNF expression by repeated treatment with the novel antipsychotic lurasidone under basal condition and in response to acute stress

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Abstract

It is known that long-term treatment with antipsychotic drugs (APDs) produces neuroadaptive changes through the modulation of different proteins that, by enhancing neuronal plasticity and cellular resiliency, may improve core disease symptoms. The aim of this study was to investigate the ability of chronic treatment with the novel antipsychotic lurasidone to modulate BDNF expression in hippocampus and prefrontal cortex, under basal conditions or in response to an acute stress, a major precipitating element in psychiatric disorders. By means of real-time PCR, we found that (1) chronic lurasidone treatment increases total BDNF mRNA levels in rat prefrontal cortex and, to less extent, in hippocampus; (2) the modulation of BDNF mRNA levels in response to acute swim stress in lurasidone-treated rats was markedly potentiated in hippocampus, and to less extent in prefrontal cortex, through the selective regulation of different neurotrophin isoforms. The increase of BDNF mRNA levels in prefrontal cortex was paralleled by an enhancement of mature BDNF protein levels. In conclusion, repeated exposure to lurasidone regulates BDNF expression, through a finely tuned modulation of its transcripts. This effect may contribute to the amelioration of functions, such as cognition, closely associated with neuronal plasticity, which are deteriorated in schizophrenia patients.

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Introduction

The treatment of schizophrenia relies primarily upon administration of antipsychotic drugs (APDs), which are divided in two main classes: (1) first-generation antipsychotics (FGAs), characterized by a prominent blockade of the dopamine D₂ receptors, which is associated with strong antipsychotic activity but also important side-effects such as extrapyramidal syndrome and hyperprolactinaemia, and (2) second-generation antipsychotics (SGAs) that show high affinity for different serotonin receptors as well as for other neurotransmitter systems. The receptor

profile of SGAs may contribute to their greater efficacy in treating negative symptoms and cognitive deterioration and it is associated with increased drug tolerability (Lieberman *et al.* 2008; Molteni *et al.* 2009b).

Although rapid control of schizophrenia symptoms by APDs depends on their ability to modulate neurotransmitter function in specific brain regions, it is now generally accepted that neuroadaptive changes taking place following repeated drug administration may be relevant for long-term stabilization and for the improvement of global functioning in schizophrenia patients. Within this context, evidence exists that SGAs may be superior to FGAs in the regulation of neuronal plasticity, presumably taking advantage of their multi-receptor profile that may lead to a stronger impact on disease symptoms, such as cognitive deficits, that are crucial for long-term disability

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(Calabrese *et al.* 2009; Lieberman *et al.* 2008; Molteni *et al.* 2009b).

In the present study we have investigated the regulation of neuronal plasticity in response to the novel antipsychotic lurasidone, which shares major pharmacodynamic features of SGAs (i.e. antagonism at dopamine D₂ and serotonin 5-HT_{2A} receptors) but it is also a potent antagonist of serotonin 5-HT₇ receptors (Meyer *et al.* 2009). Indeed blockade of 5-HT₇ receptors may improve cognitive functions, which are defective in schizophrenia (Guscott *et al.* 2005; Terry *et al.* 2008). To this end, it has been shown that lurasidone is able to revert MK-801-induced learning and memory deficit (Enomoto *et al.* 2008; Ishiyama *et al.* 2007). Preclinical observations have been confirmed by clinical studies that found a significant improvement in the treatment of acute schizophrenia as well as in cognitive functions, accompanied by a favourable side-effect profile (Ishibashi *et al.* 2010; Nakamura *et al.* 2009).

In order to address the impact of lurasidone on neuronal plasticity, we have investigated the expression of brain-derived neurotrophic factor (BDNF), a neurotrophin that plays a crucial role in brain plasticity and cellular resiliency. Evidence exists that schizophrenia patients show reduced expression of BDNF (Weickert *et al.* 2003) as well as decreased serum levels of the neurotrophin (Green *et al.* 2010). BDNF expression is also reduced in putative animal models of schizophrenia (Fumagalli *et al.* 2003, 2004; Roceri *et al.* 2002, 2004). It is thought that the reduced expression and function of BDNF may be particularly relevant for depressive symptoms and cognitive deficit: accordingly, amelioration of such deficit may occur through the modulation of BDNF after prolonged administration of psychotropic drugs (Calabrese *et al.* 2009; Duman, 2009; Lieberman *et al.* 2008; Molteni *et al.* 2009b).

Because long-term patient stabilization represents a key outcome after prolonged administration of APDs and since stress represents a major precipitating element for relapse and symptom exacerbation (Brown, 2011; Koenig, 2006), we also examined whether chronic lurasidone treatment could alter the rapid modulation of the neurotrophin in response to an acute stress, as a potential indication of coping ability under a challenging condition.

Materials and methods

Materials

General reagents and molecular biology reagents were purchased from Applied Biosystems (Italy),

Amersham Life Science (Italy), Bio-Rad Laboratories (Italy), Cell Signaling Technology (USA), Roche (Italy), Immuno Biological Laboratories, (Germany), Santa Cruz Biotechnology (USA) and Sigma (Italy).

Treatment

Adult male Sprague–Dawley rats (weighing 225–250 g at the start of the treatment) were used in the experiment. Animals were kept at constant temperature (22 °C) with a reversed 12-h light/dark cycle (lights on at 19:00 hours) and housed in groups of four with food and water available *ad libitum*. The rats were habituated to manipulation for at least 1 wk before starting the treatment and then randomly divided into four experimental groups:

Vehicle/sham ($n=15$): rats treated with vehicle and not subjected to swim stress.

Vehicle/swim ($n=10$): rats treated with vehicle and subjected to swim stress.

Lurasidone/sham ($n=15$): rats treated with 10 mg/kg lurasidone and not subjected to swim stress.

Lurasidone/swim ($n=10$): rats treated with 10 mg/kg lurasidone and subjected to swim stress.

Rats were treated with lurasidone at a dose of 10 mg/kg or vehicle for 21 d. Lurasidone (provided by Dainippon Sumitomo Pharma Co. Ltd) was prepared by suspending the drug at a concentration of 10 mg/ml in a 1% hydroxyethylcellulose solution. The drug or vehicle (control) was administered per os (by gavage) in the amount of 1 ml/kg according to body weight.

All animal handling and experimental procedures were performed in accordance with the EC guidelines (EEC Council Directive 86/609 1987) and with the Italian legislation on animal experimentation (Decreto Legislativo 116/92).

Stress procedure

Twenty-four hours after the last drug administration, half of the animals were left undisturbed in their home cages (sham), while the other half were exposed to an acute stress, routinely used in our laboratory (Fumagalli *et al.* 2009; Molteni *et al.* 2009a), consisting of a 5-min forced swim stress. Animals were sacrificed 15 min after the end of the stress session: trunk blood was collected for the analysis of plasma corticosterone levels, while prefrontal cortices and hippocampi were dissected, frozen on dry ice and stored for later analyses.

Dissections were performed according to the atlas of Paxinos & Watson (1996). In detail, the prefrontal cortex was dissected from 2-mm-thick slices [prefrontal cortex defined as Cg1, Cg3, and IL subregions corresponding to plates 6–9 (approximately weight 8 mg)], whereas hippocampus (including both ventral and dorsal parts) was dissected from the whole brain.

RNA preparation for real-time reverse transcriptase–polymerase chain reaction (RT–PCR) and analysis of BDNF mRNA levels

Total RNA was isolated by single-step guanidinium isothiocyanate/phenol extraction using PureZol RNA isolation reagent (Bio-Rad Laboratories) according with the manufacturer's instructions and quantified by spectrophotometric analysis. Following total RNA extraction, the samples were processed for real-time RT–PCR to assess BDNF mRNA levels. A 2 μ g aliquot of each sample was treated with DNase to avoid DNA contamination. RNA was analysed by TaqMan qRT–PCR instrument (CFX384 real time system, Bio-Rad Laboratories) using the iScript™ one-step RT–PCR kit for probes (Bio-Rad Laboratories). Samples were run in 384-well formats in triplicate as multiplexed reactions with a normalizing internal control (36B4).

Probe and primer sequences of 36B4 were purchased from Eurofins MWG-Operon (Germany). The following TaqMan Gene Expression assays (Applied Biosystems) were used for total BDNF, its isoforms and Arc: Total BDNF: ID Rn02531967_s1; BDNF transcript I: ID Rn01484924_m1; BDNF transcript IIa: ID Rn00560868_m1; BDNF transcript IIb: ID Rn01484926_m1; BDNF transcript IIc: ID Rn0144925_m1; BDNF transcript III forward primer: ATGCTT-CATTGAGCCCAGTT and reverse primer: GTGGA-CGTTTGCTTCTTTCA; BDNF transcript IV: ID Rn01484927_m1; BDNF transcript VI: ID Rn01484928_m1, BDNF transcript IXa forward primer: TGGTGT-CCCCAAGAAAGTAA and reverse primer: CACGT-GCTCAAAAAGTGTCAG; Arc: ID Rn00571208_g1.

Thermal cycling was initiated with an incubation at 50 °C for 10 min (RNA retrotranscription) and then at 95 °C for 5 min (TaqMan polymerase activation). After this initial step, 39 cycles of PCR were performed. Each PCR cycle consisted of heating the samples at 95 °C for 10 s to enable the melting process and then for 30 s at 60 °C for the annealing and extension reaction. A comparative cycle threshold (C_t) method was used to calculate the relative target gene expression.

Protein preparation and analysis of signalling pathways

Individual brain structures (hippocampus or prefrontal cortex) from the different animal groups were homogenized in a glass–glass potter in ice-cold 0.32 M sucrose buffer (pH 7.4) containing 1 mM HEPES, 0.1 mM EGTA and 0.1 mM PMSF, in the presence of commercial cocktails of protease (Roche) and phosphatase (Sigma-Aldrich) inhibitors and the total homogenate was obtained. Total protein content was measured according to the Bradford Protein Assay procedure (Bio-Rad), using bovine serum albumin as calibration standard.

Protein analyses were performed on total homogenate. Equal amounts of protein were run under reducing conditions on SDS–polyacrylamide gels (14% SDS–PAGE for BDNF and 10% SDS–PAGE for the other proteins examined). The blots were blocked with 10% non-fat dry milk and then incubated with the following primary antibodies overnight at 4 °C: anti-BDNF polyclonal antibody (Santa Cruz Biotechnology, 1:500) which recognizes both the mature form of the neurotrophin (mBDNF; 14 kDa) and its precursor (pro-BDNF, 32 kDa); anti-TrkB polyclonal antibody (Santa Cruz Biotechnology, 1:500) which recognizes both forms of the receptor (full length 145 kDa, truncated 95 kDa); monoclonal anti-phospho-ERK1/2 (Santa Cruz Biotechnology, 1:2000); polyclonal anti- α -ERK1/2 (Santa Cruz Biotechnology, 1:10 000); monoclonal anti-Ser473-phospho-Akt (Cell Signaling Technology, 1:1000); monoclonal anti- α -Akt (Cell Signaling Technology, 1:1000). Membranes were then incubated for 1 h at room temperature with a peroxidase-conjugated anti-rabbit IgG (1:10 000 for TrkB and ERK1/2, 1:2000 for Ser473-phospho-Akt and 1:1000 for α -Akt) or with an anti-mouse IgG (1:2000 for phospho-ERK1/2) and immunocomplexes were visualized by chemiluminescence using the ECL Western Blotting kit (Amersham Life Science). Results were standardized using β -actin as control protein, which was detected by evaluating the band density at 43 kDa after probing the membranes with a polyclonal antibody (1:10 000, Sigma) followed by a 1:10 000 dilution of peroxidase-conjugated anti-mouse IgG (Sigma).

Analysis of plasma corticosterone levels

Samples of blood from each rat were collected in heparinized tubes. Plasma was separated by centrifugation (6500 g for 10 min) and corticosterone was determined by an enzyme-linked immunosorbent assay (ELISA) using a commercial kit according to the

Table 1. Effect of acute swim stress on hypothalamus–pituitary–adrenal axis responsiveness in vehicle- and lurasidone-treated animals

Experimental groups	Corticosterone levels (ng/ml)
Vehicle/sham	19 ± 2
Vehicle/stress	24 ± 1*
Lurasidone/sham	20 ± 1
Lurasidone/stress	28 ± 1 ^{§§}

Analysis of corticosterone plasma levels was performed on the blood from rats treated for 21 d with vehicle or lurasidone (10 mg/kg) which were subsequently exposed to forced swim 24 h after the last drug administration and sacrificed 15 min after the end of the stress session. Corticosterone levels are expressed in ng/ml and represent the average of at least nine animals for each experimental group.

* $p < 0.05$ vs. vehicle/sham (two-way ANOVA with single contrast *post-hoc* test).

^{§§} $p < 0.01$ vs. lurasidone/sham (two-way ANOVA with single contrast *post-hoc* test).

manufacturers' instructions (Immuno Biological Laboratories).

Statistical analysis

Changes produced by treatments were analysed using a two-way analysis of variance (ANOVA), with status (sham or acutely stressed animals) and treatment (lurasidone or vehicle) as independent variables. When appropriate, further differences between groups were analysed by single contrast *post-hoc* test. Changes produced by lurasidone on BDNF and TrkB protein levels were analysed by Student's *t* test. Significance for all tests was assumed at $p < 0.05$. For graphic clarity, optical densities from experimental groups were expressed and presented as mean percent of control group, i.e. the group that received vehicle ± standard error of the mean (S.E.M.) with each individual group comprising 5–15 samples.

Results

We first investigated whether chronic treatment with lurasidone may alter the responsiveness of the hypothalamus–pituitary–adrenal (HPA) axis in response to the acute swim stress. The analysis of circulating corticosterone levels indicated an overall effect of stress ($F_{1,38} = 15.796$, $p < 0.001$), with no effect of lurasidone ($F_{1,38} = 3.375$, $p > 0.05$) and no stress × treatment interaction ($F_{1,38} = 0.132$, $p > 0.05$). As shown in Table 1, stress significantly increased blood

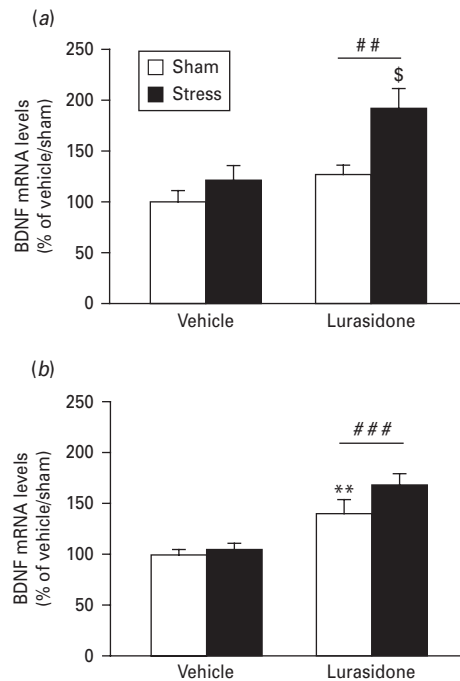


Fig. 1. Effect of an acute swim stress on brain-derived neurotrophic factor (BDNF) mRNA levels in hippocampus and prefrontal cortex of rats chronically treated with lurasidone or vehicle. Animals were treated for 21 d with vehicle or 10 mg/kg lurasidone. Twenty-four hours after the last drug administration half of the animals were exposed to a single 5-min swim stress and were sacrificed 15 min later. Total BDNF mRNA levels were measured in (a) hippocampus and (b) prefrontal cortex. The results, expressed as % of vehicle-treated rats not exposed to stress, represent the mean ± S.E.M. from at least eight independent determinations. ### $p < 0.001$ effect of treatment, two-way ANOVA; ## $p < 0.01$ effect of treatment, two-way ANOVA; § $p < 0.05$ vs. lurasidone/sham, two-way ANOVA with single contrast *post-hoc* test; ** $p < 0.01$ vs. vehicle/sham, two-way ANOVA with single contrast *post-hoc* test.

corticosterone levels by 27% in vehicle-treated rats and by 41% in lurasidone-treated rats, without statistically significant difference between lurasidone/stress and vehicle/stress animals ($p = 0.129$).

We next analysed total BDNF mRNA levels following lurasidone treatment and acute stress, alone or in combination. As shown in Fig. 1, repeated treatment with lurasidone modulated the expression of the neurotrophin under basal conditions and in response to acute stress. Within the hippocampus we found a significant effect of treatment ($F_{1,33} = 11.630$, $p < 0.01$) and stress ($F_{1,33} = 6.621$, $p < 0.05$). Total BDNF mRNA levels were only slightly increased by lurasidone treatment (+27%). Interestingly, while exposure of

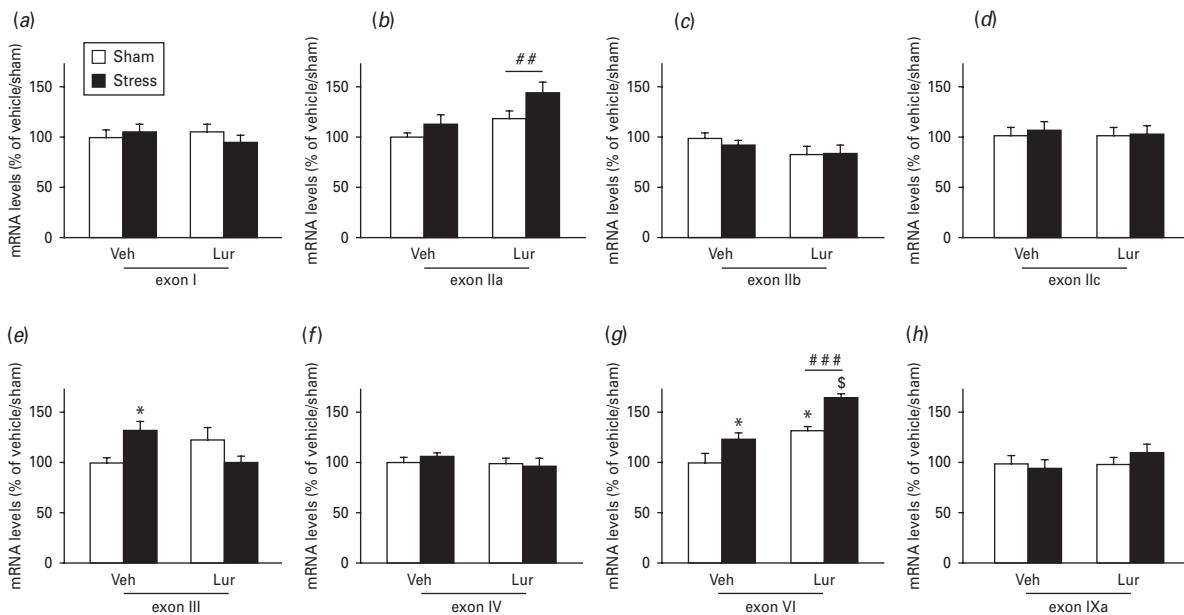


Fig. 2. Effect of an acute swim stress on the mRNA levels for different brain-derived neurotrophic factor transcripts in the hippocampus of rats chronically treated with lurasidone (Lur) or vehicle (Veh). The data represent the mRNA levels for (a) exon I, (b) exon IIa, (c) exon IIb, (d) exon IIc, (e) exon III, (f) exon IV, (g) exon VI and (h) exon IXa, expressed as a percentage of control values (vehicle/sham: unstressed animals treated with vehicle, set at 100%). Bar graphs are the mean \pm S.E.M. from at least nine independent determinations. ### $p < 0.001$ effect of treatment, two-way ANOVA; ## $p < 0.01$ effect of treatment, two-way ANOVA, * $p < 0.05$ vs. vehicle/sham, two-way ANOVA with single contrast *post-hoc* test; \$ $p < 0.05$ vs. lurasidone/sham, two-way ANOVA with single contrast *post-hoc* test.

vehicle-treated rats to acute stress produced a modest, but not significant elevation of neurotrophin expression (+21%, $p > 0.05$), its mRNA levels were increased in lurasidone-treated animals exposed to stress (+92%, vs. vehicle-treated animals) which was statistically significant with respect to lurasidone-treated animals ($p < 0.05$) (Fig. 1a). A similar effect was also observed in prefrontal cortex, where significant effects of treatment ($F_{1,49} = 18.062$, $p < 0.001$) and acute stress ($F_{1,49} = 4.939$, $p < 0.05$) were observed. In this brain structure, the expression of BDNF was significantly up-regulated by lurasidone (+41%, $p < 0.01$) and the effect was slightly potentiated by the acute swim stress (+69%, $p < 0.01$ vs. vehicle-treated animals) (Fig. 1b).

Based on the complex organization of the BDNF gene, which consists of 11 different transcripts driven by separate promoters (Aid *et al.* 2007), we decided to expand our analysis in order to establish which of these transcripts can be modulated by repeated lurasidone treatment and/or by acute stress. We thus analysed the expression of exons I, IIa, IIb, IIc (as a result of using alternative splice-donor sites within exon II), III, IV, VI, and IXa. Fig. 2 shows that, in the hippocampus, repeated lurasidone administration

produced a significant increase of exon IIa (treatment effect: $F_{1,39} = 12.196$, $p < 0.01$) and exon VI (treatment effect: $F_{1,38} = 25.916$, $p < 0.001$). The acute stress produced a slight increase of exon III (+32%, $p < 0.05$) and exon VI (+23%, $p < 0.05$) in vehicle-treated animals, whereas a more pronounced elevation of exon IIa (+44%, $p < 0.05$) and exon VI (+65%, $p < 0.05$ vs. lurasidone-treated rats) was observed in drug-treated rats exposed to the swim stress (Fig. 2).

With regard to prefrontal cortex (Fig. 3), we found that the exons contributing most to the changes of total BDNF were IIb (treatment effect: $F_{1,38} = 5.585$, $p < 0.05$), IIc (treatment effect: $F_{1,39} = 20.182$, $p < 0.001$) and, to a lesser extent, exon III (treatment effect: $F_{1,37} = 6.096$, $p < 0.05$) with no effect of acute stress. Interestingly, we found a significant increase of exon IV mRNA levels in animals treated with lurasidone and exposed to acute swim stress (+36%, $p < 0.01$), which did not alter its expression in vehicle-treated rats (Fig. 3).

We next measured the mRNA levels of the effector inducible early gene *Arc*, which is known to be up-regulated by neuronal activity and plasticity-inducing stimuli (Bramham *et al.* 2008) and may cooperate with BDNF in adaptive mechanisms leading to neuroplastic changes (Wibrand *et al.* 2006; Ying *et al.* 2002). Indeed,

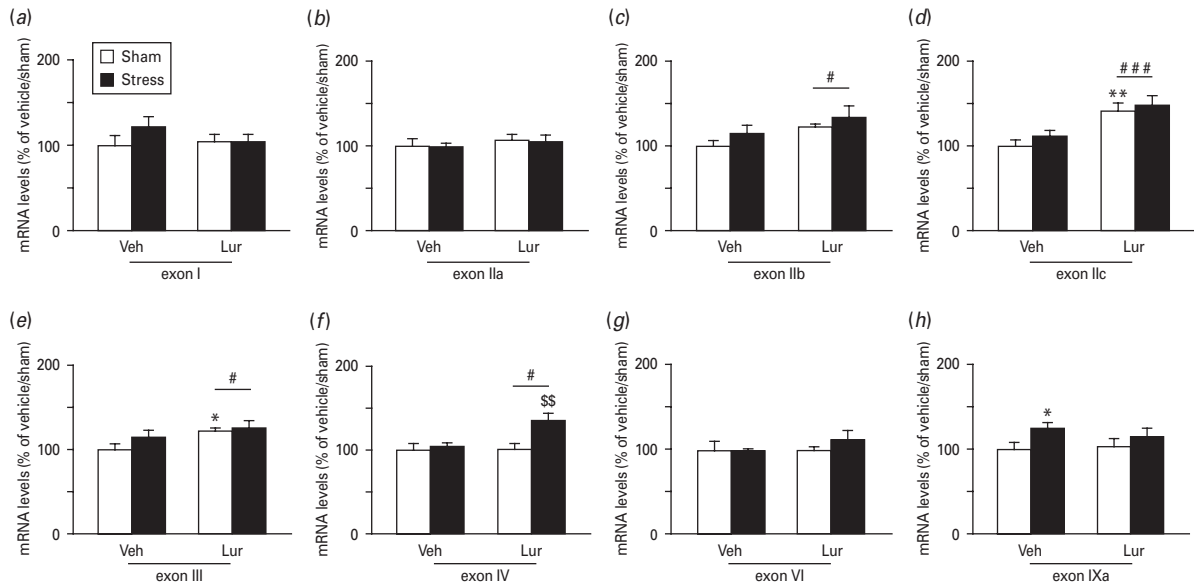


Fig. 3. Effect of an acute swim stress on the mRNA levels for different brain-derived neurotrophic factor transcripts in the prefrontal cortex of rats chronically treated with lurasidone (Lur) or vehicle (Veh). The data represent the mRNA levels for (a) exon I, (b) exon IIa, (c) exon IIb, (d) exon IIc, (e) exon III, (f) exon IV, (g) exon VI and (h) exon IXa expressed as a percentage of control values (vehicle/sham: unstressed animals treated with vehicle, set at 100%). Bar graphs are the mean \pm S.E.M. from at least nine independent determinations. ### $p < 0.001$ effect of treatment, two-way ANOVA; # $p < 0.05$ effect of treatment, two-way ANOVA; * $p < 0.01$ vs. vehicle/sham, two-way ANOVA with single contrast *post-hoc* test; * $p < 0.05$ vs. vehicle/sham, two-way ANOVA with single contrast *post-hoc* test; \$\$ $p < 0.01$ vs. lurasidone/sham, two-way ANOVA with single contrast *post-hoc* test.

Arc mRNA changes after repeated lurasidone treatment and acute stress resembled those observed for BDNF. Within the hippocampus (Fig. 4), we found a significant effect of treatment ($F_{1,38} = 38.106$, $p < 0.001$) and a significant treatment \times stress interaction ($F_{1,38} = 5.245$, $p < 0.05$). Accordingly, repeated lurasidone treatment significantly up-regulated the expression of Arc (+26%, $p < 0.05$ vs. vehicle-treated animals), an effect that was further enhanced in animals exposed to acute stress (+56%, $p < 0.001$ vs. vehicle-treated animals) (Fig. 4a). In prefrontal cortex, we observed a significant treatment effect ($F_{1,38} = 26.477$, $p < 0.001$), with Arc mRNA being up-regulated in lurasidone-treated rats (+48%, $p < 0.01$ vs. vehicle-treated animals), and a further enhancement in lurasidone-treated rats exposed to acute stress (+78%, $p < 0.001$ vs. vehicle-treated animals) (Fig. 4b).

After having established that chronic treatment with lurasidone regulates specific BDNF isoforms and alters their stress-induced transcription, we determined if such changes were paralleled by modifications of BDNF protein levels. As shown in Fig. 5, chronic lurasidone treatment does not affect the protein levels of the neurotrophin (both precursor and mature forms) in hippocampal extracts (Fig. 5a).

Conversely, in line with the significant up-regulation of its mRNA levels, chronic treatment with the anti-psychotic significantly increased the levels of mature BDNF in prefrontal cortex (+43%, $p < 0.05$ vs. vehicle-treated animals), without affecting the expression of proBDNF.

We also tried to establish if antipsychotic treatment could alter the expression of TrkB, the high-affinity receptor of BDNF. The receptor is expressed in two forms: the full-length, with tyrosine kinase activity, and a truncated form, which lacks the entire kinase catalytic region and may function as dominant negative to reduce BDNF signalling (Minichiello, 2009). However, as shown in Fig. 6, repeated exposure to lurasidone did not produce any significant change in the levels of full-length TrkB as well as for its truncated form, although a trend towards a reduction of the truncated form was observed in the hippocampus of lurasidone-treated rats (-13%, $p = 0.086$).

Finally, we decided to investigate whether chronic lurasidone treatment could affect the release of BDNF in response to the acute stress paradigm. To this end, we measured the activation of signalling pathways (Akt and ERK1/2), that lie downstream from TrkB (Minichiello, 2009), as an indirect measure of

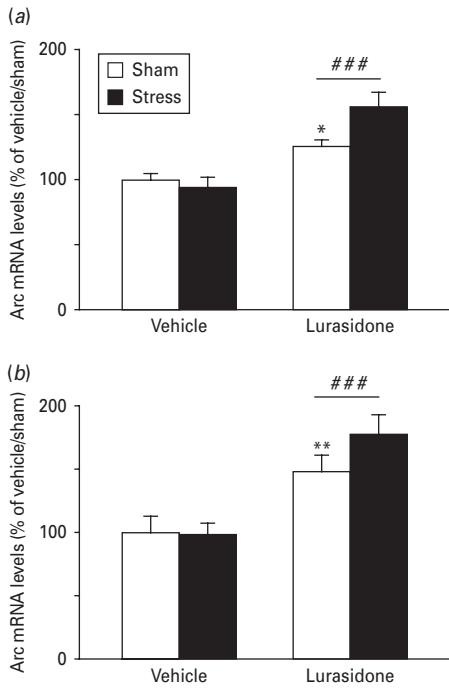


Fig. 4. Effect of an acute swim stress on Arc mRNA levels in rats chronically treated with lurasidone or vehicle. The data represent Arc mRNA levels in (a) hippocampus and (b) prefrontal cortex expressed as a percentage of control values (vehicle/sham: unstressed animals treated with vehicle, set at 100%). Bar graphs are the mean \pm S.E.M. from at least nine independent determinations. ### $p < 0.001$ effect of treatment, two-way ANOVA; ** $p < 0.01$ vs. vehicle/sham, two-way ANOVA with single contrast *post-hoc* test; * $p < 0.05$ vs. vehicle/sham, two-way ANOVA with single contrast *post-hoc* test.

neurotrophin release. As shown in Fig. 7, acute stress increased Akt phosphorylation (Ser473) in hippocampus ($F_{1,29} = 14.114$, $p < 0.001$) and prefrontal cortex ($F_{1,25} = 6.55682$, $p < 0.05$), an effect that was similar in vehicle- and lurasidone-treated rats in both brain regions. Repeated lurasidone treatment as well as acute stress did not produce any significant change on total level of Akt (Table 2 and 3). With respect to ERK1/2, we observed that their phosphorylation was not altered by either drug treatment or acute stress (Fig. 8) similar to the total protein levels in both brain regions (Tables 2 and 3).

Discussion

The data of the present study highlight the ability of chronic treatment with the novel antipsychotic lurasidone to modulate the expression and stress-responsiveness of the neurotrophin BDNF in

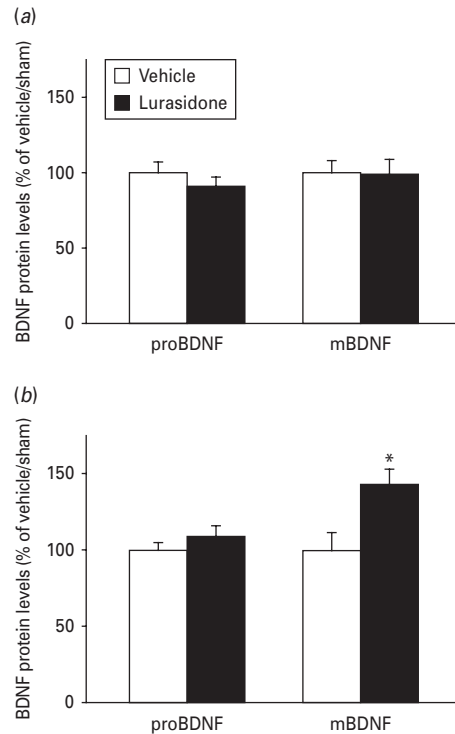


Fig. 5. Effect of chronic lurasidone treatment on brain-derived neurotrophic factor (BDNF) protein levels in rat hippocampus and prefrontal cortex. The levels of proBDNF (32 kDa) and mBDNF (14 kDa) proteins in (a) hippocampus and (b) prefrontal cortex, are expressed as percentage of control values (vehicle/sham: unstressed animals treated with vehicle, set at 100%). Bar graphs are the mean \pm S.E.M. from at least nine independent determinations. * $p < 0.05$ vs. vehicle/sham (Student's *t* test).

hippocampus and prefrontal cortex, two key brain regions for psychiatric disorders. First, total BDNF mRNA levels were significantly increased in prefrontal cortex after 21-d treatment with lurasidone, whereas a more limited elevation of neurotrophin expression was found in hippocampus. Notably, the increase in BDNF mRNA levels in prefrontal cortex was paralleled by an elevation of protein levels for mature BDNF, the form of the neurotrophin that has a protective role through the activation of TrkB receptors (Greenberg *et al.* 2009; Martinowich *et al.* 2007).

These results suggest that, at least under resting conditions, lurasidone may produce larger changes at cortical level. This effect appears to be unique when compared to other antipsychotic agents. In fact previous work has primarily investigated modulation of BDNF expression by APDs in hippocampus showing a consistent reduction after treatment with the

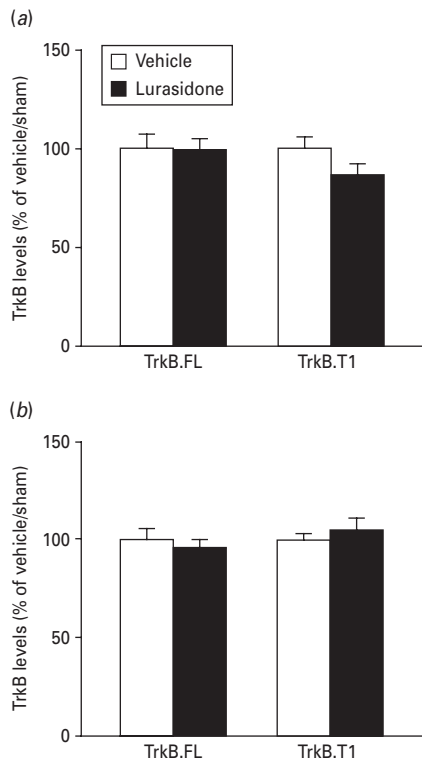


Fig. 6. Effect of chronic lurasidone treatment on TrkB protein levels in rat hippocampus and prefrontal cortex. The data, showing the full-length (TrkB.FL) or the truncated (TrkB.T1) form of the TrkB protein in (a) hippocampus and (b) prefrontal cortex, are expressed as a percentage of control values (vehicle/sham: unstressed animals treated with vehicle, set at 100%). Bar graphs are the mean \pm S.E.M. from at least nine independent determinations.

first-generation drug haloperidol, whereas a more variable picture emerged after administration of second-generation APDs (Molteni *et al.* 2009b). The increased BDNF expression observed in prefrontal cortex, and to less extent in hippocampus, after chronic lurasidone treatment is interesting in view of the neuroplastic properties of the neurotrophin that may sustain functions, such as cognition, associated with this brain structure. To this end, behavioural measurements indicate that lurasidone reverses MK-801-induced learning and memory impairments in rodent models (Enomoto *et al.* 2008; Ishiyama *et al.* 2007), evidence that finds preliminary support from clinical data (Meyer *et al.* 2009). Since lurasidone is a multi-target drug, with significant affinities for several neurotransmitter receptors, it is difficult to establish whether the significant changes in BDNF expression are the consequence of the occupancy at a given receptor or, rather, might be due to the high affinity to

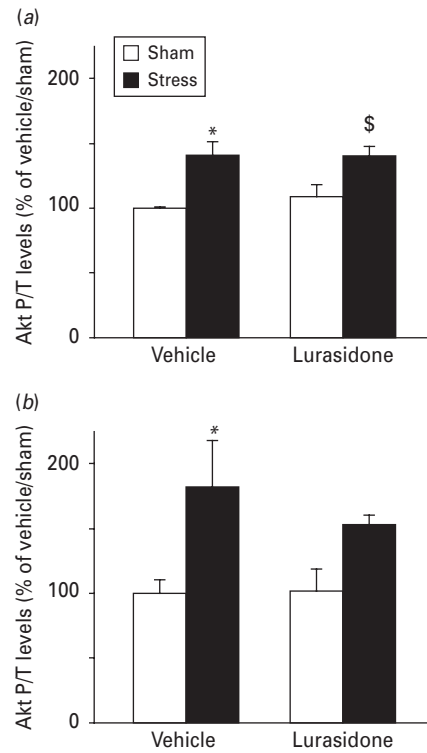


Fig. 7. Effect of an acute swim stress on the phosphorylation (Ser473) of Akt in (a) hippocampus and (b) prefrontal cortex of rats chronically treated with lurasidone or vehicle. The data, representing the ratio between phosphorylated and total (P/T) Akt levels in (a) hippocampus and (b) prefrontal cortex, are expressed as percentage of control values (vehicle/sham: unstressed animals treated with vehicle, set at 100%). Bar graphs are the mean \pm S.E.M. from at least five independent determinations. * $p < 0.05$ vs. vehicle/sham, two-way ANOVA with single contrast *post-hoc* test; $^{\$}$ $p < 0.05$ vs. lurasidone/sham, two-way ANOVA with single contrast *post-hoc* test.

5-HT₇ receptors, a major feature of lurasidone. Whatever the mechanism(s) involved, the modulation of BDNF expression seems to be a neat feature of lurasidone, as opposed to other SGAs which display a rather inconsistent degree of BDNF modulation (Molteni *et al.* 2009b), and it may contribute to its activity on cognitive function (Enomoto *et al.* 2008; Ishiyama *et al.* 2007).

One key aspect for the functional outcome of BDNF regulation in the brain is anatomical selectivity (Wang *et al.* 2008). Indeed, while overexpression of the neurotrophin may be depressogenic in nucleus accumbens and ventral tegmental area (Krishnan & Nestler, 2008), enhancement of BDNF expression and function in hippocampus or prefrontal cortex is antidepressive and it may facilitate neuroplastic

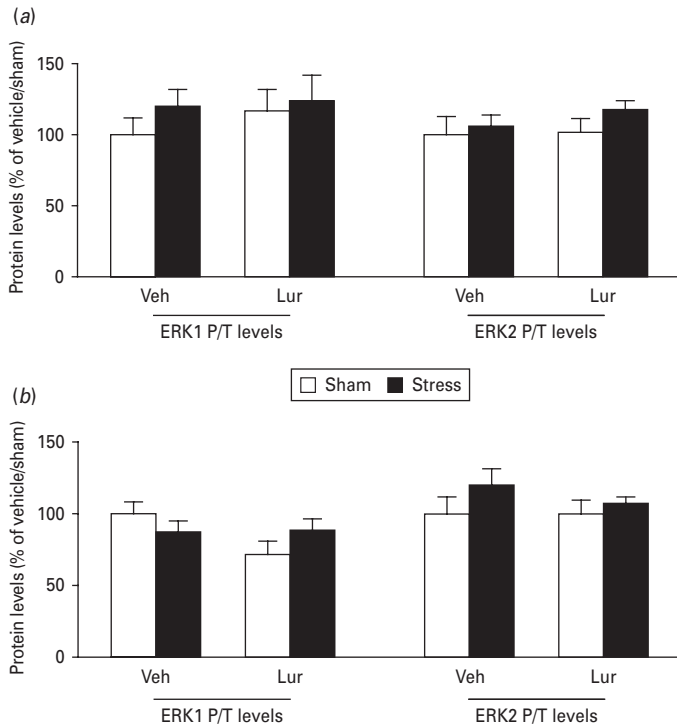


Fig. 8. Effect of an acute swim stress on the phosphorylation of ERK1 and ERK2 in hippocampus and prefrontal cortex of rats chronically treated with lurasidone (Lur) or vehicle (Veh). The data, representing the ratio between phosphorylated and total (P/T) ERK1/2 levels in (a) hippocampus and (b) prefrontal cortex, are expressed as percentage of control values (vehicle/sham: unstressed animals treated with vehicle, set at 100%). Bar graphs are the mean \pm S.E.M. from at least eight independent determinations.

Table 2. Protein expression levels of Akt, ERK1 and ERK2 in total homogenate from the hippocampus of animals exposed to repeated treatment with lurasidone or vehicle and then exposed to the acute swim stress

Experimental groups	Akt	ERK1	ERK2
Vehicle/sham	100 \pm 4	100 \pm 6	100 \pm 4
Vehicle/stress	100 \pm 5	100 \pm 9	104 \pm 8
Lurasidone/sham	95 \pm 3	83 \pm 4	93 \pm 3
Lurasidone/stress	96 \pm 4	94 \pm 3	103 \pm 3

The results, expressed as % of controls (vehicle-sham), represent the mean \pm S.E.M. of at least eight independent determinations.

mechanisms associated with cognition and coping (Martinowich *et al.* 2007). Hence the increase of BDNF expression after lurasidone treatment, under basal conditions or following stress exposure, should be viewed as a mechanism supporting neuronal plasticity, which is defective in psychiatric conditions.

The structure of the BDNF gene is quite complex with several 5' non-coding exons, each characterized by a separate promoter region triggering the tran-

Table 3. Protein expression levels of Akt, ERK1 and ERK2 in total homogenate from the prefrontal cortex of animals exposed to repeated treatment with lurasidone or vehicle and then exposed to the acute swim stress

Experimental groups	Akt	ERK1	ERK2
Vehicle/sham	100 \pm 9	100 \pm 7	100 \pm 5
Vehicle/stress	112 \pm 10	124 \pm 8	100 \pm 6
Lurasidone/sham	105 \pm 8	120 \pm 5	103 \pm 6
Lurasidone/stress	113 \pm 7	113 \pm 7	97 \pm 5

The results, expressed as % of controls (vehicle-sham), represent the mean \pm S.E.M. of at least eight independent determinations.

scription of a common 3' exon and encoding for the same protein (Aid *et al.* 2007). Although the functional consequences of multiple, differently regulated, BDNF transcripts are still elusive, specific exons may undergo different intracellular targeting. Hence, their analysis might add relevant information as to how antipsychotics may modulate specific functions related to BDNF expression.

Within the hippocampus major changes with lurasidone treatment, under basal conditions and, more importantly, following the acute stress can be observed on exons IIa and VI, which are among the transcripts that are targeted to dendrites (Chiaruttini *et al.* 2008) suggesting that, at least in hippocampus, lurasidone treatment may facilitate the transcription of a specific neurotrophin pool. This may occur to a certain extent also in prefrontal cortex where chronic lurasidone treatment enhances mRNA levels for exons IIb and IIc, with minor changes occurring under stress conditions.

Enhancing the synaptic pool of BDNF may represent a mechanism for the consolidation and strengthening of synapses; if this is the case, the effects of lurasidone may be important in light of the evidence that schizophrenia patients show several structural abnormalities such as shrinkage of cortical brain regions (Glantz & Lewis, 2000; Kolluri *et al.* 2005), accompanied by reduced expression of several markers of neuroplasticity including BDNF (Weickert *et al.* 2003).

While the effects produced by repeated exposure to lurasidone are indeed crucial in understanding its action in the long term, the possibility that this novel antipsychotic may modulate the response to a challenging event may even be more important, since it is known that coping with adverse events is impaired in schizophrenia patients (van Os *et al.* 2008). Analysis of BDNF transcripts revealed a peculiar modulation of exon IV transcription, which is increased only in the prefrontal cortex of lurasidone-treated animals exposed to acute stress. Exon IV represents the most abundant isoform in the brain and its transcription is activity-dependent (Pattabiraman *et al.* 2005): it may be inferred that prolonged exposure to lurasidone promotes activity-dependent exon IV transcription, which may represent a coping response to the challenging condition. It is known that there is an inverted U relationship between stress (and corticosteroids) and memory, with acute mild stressors producing a facilitation of mechanisms that may contribute to memory formation. Since rapid transcription of BDNF exon IV occurs in associative learning (Lubin *et al.* 2008) and based on the modulatory role of exon IV transcription in prefrontal cortex plasticity, it may also be inferred that the selective up-regulation of its expression in lurasidone-treated rats exposed to stress will contribute to functional modification at cortical level, which may lead to the enhancement of executive functioning (Enomoto, 2009). Moreover, since a critical role of promoter IV-driven BDNF transcription in GABAergic transmission has been shown (Sakata *et al.*

2009), the modulation of this neurotrophin transcript by lurasidone may also hold implications for the control of GABAergic function, which is impaired in schizophrenia (Lewis *et al.* 2005).

The observation that, in addition to lurasidone, the antidepressant duloxetine (Calabrese *et al.* 2010), the ADHD drug atomoxetine (Fumagalli *et al.* 2010), as well as mood stabilizers such as valproate and lithium (Yasuda *et al.* 2009) selectively enhance the mRNA levels of BDNF transcript IV suggests that its regulation may represent a converging mechanism relevant for the amelioration of mood and cognition that are defective in different psychiatric disorders.

It is worth noting that the effect produced by lurasidone on BDNF expression is mirrored by changes of the immediate early gene *Arc*, which also shows activity-dependent transcription and dendritic targeting (Dynes & Steward, 2007), suggesting a synaptic-driven effect produced by prolonged administration with lurasidone.

The neuroplastic changes set in motion by lurasidone at hippocampal level recapitulate, to some extent, the findings we have recently obtained using the antidepressant duloxetine in the same experimental paradigm (Molteni *et al.* 2009a). Indeed both drugs produced a limited increase of total BDNF mRNA levels *per se* (although through the regulation of different isoforms), with a clear stress-induced facilitation of BDNF transcription, which appears larger in lurasidone- than in duloxetine-treated animals. Moreover, both drugs cause a significant stress-induced modulation of exon VI mRNA levels suggesting that there may be some overlapping adaptive mechanisms peculiar of the two drugs, which would support and facilitate neuronal plasticity, an effect particularly relevant also for the amelioration of depressive symptoms (Calabrese *et al.* 2009; Duman, 2009; McClung & Nestler, 2008). The transcription of exon VI is also driven by glucocorticoids (Fuchikami *et al.* 2009; Molteni *et al.* 2009a); however, the analysis of corticosterone levels in response to stress did not highlight major differences between vehicle and lurasidone-treated rats, even though the elevation of plasma corticosterone levels in response to stress was larger in the lurasidone group. These data suggest that transcriptional changes in BDNF expression observed in the present study are not due a different responsiveness of the HPA system and are presumably the result of more complex synaptic mechanisms modulated by stress exposure.

Repeated lurasidone treatment does not alter the expression of the high-affinity BDNF receptor TrkB, although it is possible that changes in BDNF receptors

may be limited to the synaptic compartment and cannot be observed in the whole homogenate used in the present analysis. However, when we investigated stress-induced activation of signalling pathways connected with TrkB receptors, we could not detect any significant difference between vehicle and lurasidone-treated rats. Indeed the phosphorylation of Akt, an index of its activation, was similarly enhanced by stress in the two experimental groups confirming the effectiveness of the stress manipulation and suggesting that lurasidone is primarily affecting the transcription of BDNF and its stress sensitivity, without modulating the release of the neurotrophin. It should be borne in mind that, since these analyses have been carried out at a single time-point, we cannot rule out the possibility that lurasidone may affect the timing of stress-induced modification in signalling mechanisms.

In conclusion, we have here demonstrated that repeated exposure to the novel antipsychotic lurasidone alters BDNF transcription through a finely tuned modulation of BDNF isoforms. Our data suggest that adaptive changes produced by repeated treatment with lurasidone may contribute to the amelioration of functional capacities, closely associated with neuronal plasticity, which are deteriorated in patients with schizophrenia, bipolar disease and depression.

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Statement of Interest

None.

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