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Re-arrangements of gene transcripts at glutamatergic synapses after prolonged treatments with antipsychotics: A putative link with synaptic remodeling

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ABSTRACT

Objectives: The postsynaptic density (PSD) represents a site of dopamine-glutamate integration. Despite multiple evidence of PSD involvement in antipsychotic-induced synaptic changes, there are no direct head-to-head comparisons of the effects at the PSD of antipsychotics with different receptor profile and at different doses after chronic administration.
Methods: Molecular imaging of gene expression was used to investigate whether chronic treatment with first and second generation antipsychotics (haloperidol, asenapine and olanzapine) may induce changes in the expression levels of PSD transcripts involved in schizophrenia pathophysiology, *i.e. Homers*, *Shank1*, *PSD-95* and *Arc*.
Results: Genes' expression patterns were differentially modulated after chronic administration of typical and atypical antipsychotics as well as by the same compound administered at different doses. Antipsychotic treatment reduced gene expression in cortical regions, while *Homer1a* was still induced in striatum by haloperidol even after prolonged treatment. Moreover, chronic treatments appeared to cause a "de-recruitment" of brain regions demonstrated to be activated in acute treatments, with a prominent effect in the cortex rather than in striatum.
Conclusions: These results let hypothesize that prolonged antipsychotic treatment may trigger a set of plastic changes involving scaffolding and effector molecules causing a possible re-arrangement of PSD transcripts in brain regions relevant to schizophrenia pathophysiology.

1. Introduction

There is increasing interest for searching putative brain structural changes after chronic antipsychotic treatment, and to understand how antipsychotics with different receptor profiles and at different doses may impact brain plasticity (Ho et al., 2011; Vita et al., 2015). The postsynaptic density (PSD) is an attractive target for studying antipsychotic-induced changes after acute and chronic treatments. The PSD is an electron-dense thickness beneath post-glutamatergic synapses, where receptors, adaptors, and scaffolding proteins interact at the crossroads of dopamine-glutamate interaction (Iasevoli et al., 2013; Verpelli et al., 2012), and participate in synaptic plasticity processes (de Bartolomeis et al., 2014; Gao et al., 2013; Iasevoli et al., 2014).

PSD proteins have been involved in psychosis pathophysiology (de Bartolomeis et al., 2014; Dean et al., 2015; Hall et al., 2015), which is in agreement with the hypothesis that schizophrenia may be a disease of aberrant synaptic plasticity. Antipsychotic agents, the mainstay of schizophrenia pharmacological treatment, significantly modulate the expression and topography of PSD transcripts after acute administration in animal studies (de Bartolomeis et al., 2015; de Bartolomeis et al., 2013a; Fumagalli et al., 2008). However, a direct head-to-head comparison of the effects at the PSD of first and second-generation antipsychotics at different doses in a chronic paradigm is still lacking. To fill this gap, we evaluated the expression of key PSD transcripts, *i.e. Homer1a, Arc, Homer1b, PSD-95,* and *Shank,* after chronic administration of: i) the prototype first generation antipsychotic haloperidol, which has a relatively selective D2 receptor profile (Correll, 2010); ii) the prototype second generation antipsychotic olanzapine, which has a broad multi-receptor profile (Correll, 2010); and iii) the novel multi-receptor targeting antipsychotic asenapine, which holds a dopamine D1 and D2 receptor blockade ratio of approximately 1 (Shahid et al., 2009). In an acute paradigm of administration, increasing doses of these same

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antipsychotics have been reported to induce a progressive recruitment of cortical/sub-cortical regions (de Bartolomeis et al., 2015). However, molecular effects of antipsychotics may dramatically change according to the timing of administration (de Bartolomeis et al., 2016). Acute paradigms of antipsychotic administration may shed lights on adaptive and reactive synaptic mechanisms that follow an intense and punctual receptor perturbation. On the other hand, chronic antipsychotic paradigms may best recapitulate the synaptic changes occurring during or after prolonged treatments (Kontkanen et al., 2002), more closely mimicking real-world therapeutic approaches. Therefore, in the present study we tried to address the following questions:

1. Does chronic administration of antipsychotics with different receptor profiles induce changes in the expression of PSD transcripts differentially with respect to cortical/sub-cortical regions?
2. Do different doses of the same antipsychotic impact the topography of gene expression and therefore the recruitment of different cortical/sub-cortical circuitries?
3. Do antipsychotics with different receptor profile or different doses of the same antipsychotic affect the relative ratio of transcript expression of the Homer1 isomers (*i.e. Homer1a* and *Homer1b*), which have been described to exert opposite molecular effects (Kammermeier, 2008)?

The PSD molecules studied herein were chosen based on the fact that they have all been implicated in schizophrenia pathophysiology (de Bartolomeis et al., 2014; Fromer et al., 2014; Purcell et al., 2014), as well as demonstrated to be responsive to antipsychotic treatment (de Bartolomeis et al., 2013a; Iasevoli et al., 2011, 2010, 2009). Moreover, all transcripts studied herein are reported to directly or indirectly interact with each other and mainly mediate postsynaptic type 5 metabotropic glutamate receptor (mGluR5)-dependent signaling (Bertaso et al., 2010; Sala et al., 2005; Tu et al., 1999; Zhang and Lisman, 2012).

The Homers1 are scaffolding and adaptor proteins associated with schizophrenia and depression-like behaviors and including constitutive forms (*i.e.* Homer1b/c) and an inducible early gene form (Homer1a) (de Bartolomeis and Iasevoli, 2003; Newell and Matosin, 2014; Serchov et al., 2015; Shiraishi-Yamaguchi and Furuichi, 2007; Wagner et al., 2015). PSD-95 is a membrane-associated guanylate kinase (MAGUK) scaffolding protein interacting with N-Methyl-D-Aspartate (NMDA) receptor NR2 subunit and shaker-type potassium channels (Chen et al., 2015). Shank is a PSD protein that has been associated to schizophrenia and autism spectrum disorders (Sala et al., 2015), and demonstrated to be instrumental in physically and functionally coupling of NMDA receptors and mGluR5 at the PSD (Hwang et al., 2005). Finally, Arc (activity-regulated cytoskeletal protein, also known as Arg-1) is a master regulator of synaptic plasticity, involved in long-term potentiation and both mGluR and NMDA receptor-dependent forms of long-term depression (Guzowski et al., 2000; Park et al., 2008).

2. Experimental procedures

2.1. Animals

Male Sprague-Dawley rats (mean weight 250 g) were obtained from Charles River Labs (Lecco, Italy), housed and let to adapt to human handling in a temperature and humidity controlled colony room and maintained under a 12-hour light/dark cycle with *ad libitum* access to laboratory chow and water. All procedures were in accordance with the NIH Guide for Care and Use of Laboratory Animals (NIH Publication no. 85-23, revised 1996) and were approved by local Animal Care and Use Committee. All efforts were made to minimize animal number and suffering.

2.2. Drug treatment

Asenapine powder (gently supplied by H. Lundbeck A/S, Copenaghen, Denmark), olanzapine powder (Sigma-Aldrich, Milan, Italy), and haloperidol injectable solution (Lusofarmaco, Italy) were all dissolved in saline solution (NaCl 0.9%). All solutions were adjusted to physiological pH value and injected i.p. at a final volume of 1 ml/kg. Rats were randomly assigned to one of the following treatment groups (n = 5 for each group): vehicle (NaCl 0.9%, VEH); haloperidol 0.25 mg/kg (HAL0.25); haloperidol 0.5 mg/kg (HAL0.5); haloperidol 0.8 mg/kg (HAL0.8); asenapine 0.05 mg/kg (ASE0.05); asenapine 0.1 mg/kg (ASE0.1); asenapine 0.3 mg/kg (ASE0.3); olanzapine 2.5 mg/kg (OLA). All drugs were given at behaviorally active doses, based on previous works (Huang et al., 2008; Iasevoli et al., 2010; Marston et al., 2009; Shahid et al., 2009; Tomasetti et al., 2007). The above-listed drugs were administered once a day for twenty-one consecutive days. All animals received the drugs in one injection a day. Care was taken to carry out injections at the same hour of the day, every day. Animals were sacrificed by decapitation 90 min after the last injection, brains were rapidly removed, quickly frozen on powdered dry ice and stored at −70 °C prior to sectioning. Serial coronal sections of 12 μm were cut on a cryostat at −18 °C through the forebrain at the level of the middle-rostral striatum (approx. from Bregma 1.20 mm to 1.00 mm), using the rat brain atlas by Paxinos and Watson (2007) as an anatomical reference. Sections were thaw-mounted on to gelatin-coated slides, and stored at −70 °C for subsequent analysis.

2.3. In situ hybridization

All procedures for radioactive *in situ* hybridization were carried out according to previously published protocols (Ambesi-Impiombato et al., 2003; Buonaguro et al., 2016; de Bartolomeis et al., 2016). Details for oligodeoxyribonucleotide probes have been listed in Table 1. A complete description of methods has been provided as Supplementary material.

Image analysis of autoradiographic signal was carried out within outlined ROIs in correspondence of the cortex, caudate putamen, and nucleus accumbens (Fig. 1). ROIs were selected based on data describing functional and anatomical correlation between cortical and striatal subregions (Willuhn et al., 2003; Steiner and Gerfen, 1993). Our previous studies have demonstrated that these ROIs are key motor-related and limbic-related regions for evaluating gene expression, with putative translational value (Buonaguro et al., 2016; de Bartolomeis et al., 2016, 2013b). ANOVA (One-way Analysis of Variance) was used to analyze treatment effects. The Student-Newman-Keuls (SNK) post-hoc test with the Bonferroni correction was used for groups' comparison. In all tests, significance was set at $p < 0.05$. To calculate the *Homer1a*/*Homer1b* ratio, signal intensity of *Homer1a* and *Homer1b* mRNA expression by each antipsychotic compound was normalized on values of vehicle mRNA expression (de Bartolomeis et al., 2013b). The Student's *t*-test was then used to compare normalized *Homer1a vs. Homer1b* mRNA levels by each antipsychotic in each ROI.

Table 1
Probes for *in situ* hybridization histochemistry.

Probe	cDNA length (bp)	cDNA position	mRNA	Gen-Bank#
Homer1a	48	2527–2574	*Homer1a*	U92079
Homer1b/c	48	1306–1353	*Homer1b/c*	AF093268
Arc	45	789–833	*Arc*	NM019361
Shank1	48	2757–2804	*Shank1*	AF131951
PSD-95	48	225–269	*PSD-95*	M96853