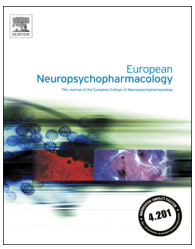
European Neuropsychopharmacology (2015) 25, 566–582



www.elsevier.com/locate/euroneuro

Progressive recruitment of cortical and striatal regions by inducible postsynaptic density transcripts after increasing doses of antipsychotics with different receptor profiles: Insights for psychosis treatment

Andrea de Bartolomeis[a,*], Felice Iasevoli[a], Federica Marmo[a], Elisabetta F. Buonaguro[a], Anna Eramo[b], Rodolfo Rossi[a], Livia Avvisati[a], Gianmarco Latte[a], Carmine Tomasetti[a]

[a]*Laboratory of Molecular and Translational Psychiatry, Department of Neuroscience, University Medical School of Naples Federico II, Italy*
[b]*Medical Affairs & Phase IV Clinical Affair. Lundbeck Pharmaceutical Services LLC, Deerfield, IL, United States*

Received 9 July 2014; received in revised form 5 January 2015; accepted 9 January 2015

KEYWORDS
Immediate-early genes;
Homer;
Asenapine;
Haloperidol;
Schizophrenia;
Bipolar disorder

Abstract
Antipsychotics may modulate the transcription of multiple gene programs, including those belonging to postsynaptic density (PSD) network, within cortical and subcortical brain regions. Understanding which brain region is activated progressively by increasing doses of antipsychotics and how their different receptor profiles may impact such an activation could be relevant to better correlate the mechanism of action of antipsychotics both with their efficacy and side effects.
We analyzed the differential topography of PSD transcripts by incremental doses of two antipsychotics: haloperidol, the prototypical first generation antipsychotic with prevalent dopamine D2 receptors antagonism, and asenapine, a second generation antipsychotic characterized by multiple receptors occupancy. We investigated the expression of PSD genes involved in synaptic plasticity and previously demonstrated to be modulated by antipsychotics: *Homer1a*, and its related interacting constitutive genes *Homer1b/c* and *PSD95*, as well as *Arc*, *C-fos* and *Zif-268*, also known to be induced

*Correspondence to: Laboratory of Molecular and Translational Psychiatry, Department of Neuroscience, University School of Medicine of Napoli "Federico II", Building 18, 3rd floor, 80131, Napoli, Italia.
Tel.: +39 081 7463673, +39 081 7463884; fax: +39 081 7462378; Mobile: +39 3662745592.
E-mail address: adebarto@unina.it (A. de Bartolomeis).

http://dx.doi.org/10.1016/j.euroneuro.2015.01.003

by antipsychotics administration. We found that increasing acute doses of haloperidol induced immediate-early genes (IEGs) expression in different striatal areas, which were progressively recruited by incremental doses with a dorsal-to-ventral gradient of expression. Conversely, increasing acute asenapine doses progressively de-recruited IEGs expression in cortical areas and increased striatal genes signal intensity. These effects were mirrored by a progressive reduction in locomotor animal activity by haloperidol, and an opposite increase by asenapine. Thus, we demonstrated for the first time that antipsychotics may progressively recruit PSD-related IEGs expression in cortical and subcortical areas when administered at incremental doses and these effects may reflect a fine-tuned dose-dependent modulation of the PSD.

1. Introduction

The acute administration of both typical and atypical antipsychotics has been demonstrated to modulate the transcription of multiple gene programs within peculiar regions of the forebrain, which are correlated with behavioral outcomes predictive of the efficacy and side effects profile of each drug (Andersen and Pouzet, 2001; de Bartolomeis et al., 2014b; de Bartolomeis and Tomasetti, 2012; Moy et al., 2004; Robbins et al., 2008). Thus, understanding which brain region or subregion is progressively activated by increasing doses of antipsychotics acutely administered and how their different receptor profiles may impact such an activation could be relevant to better correlate the mechanism of action of antipsychotics both with their efficacy and side effects. Despite being a potential translational issue, this phenomenon has been investigated very little so far.

In the present study we addressed the following questions:

1. Are different cortical and striatal regions progressively "recruited" by increasing doses of antipsychotics?
2. Do different receptor profiles influence the recruitment of brain regions beyond dopamine receptors blockade?
3. Are cortical and subcortical regions activated by antipsychotics functionally correlated?

Thus, we imaged the differential expression of postsynaptic density (PSD) transcripts in cortical and subcortical brain regions induced by incremental doses of two antipsychotics: haloperidol, a first generation antipsychotic with prevalent dopamine D2 receptor blocking action and low affinity for D1 receptors, and asenapine, a novel antipsychotic holding multiple receptors occupancy and a blockade of D1 and D2 receptors ratio of approximately 1 (Shahid et al., 2009). A third group of animals was treated with olanzapine as a reference atypical antipsychotic with a multiple receptor profile other than D2 receptors (D2R) antagonism (Arnt, 1998). Olanzapine, indeed, displays binding affinity with antagonist activity at serotonin 5HT2a and 5HT2c receptors, which is considered prototypical of atypical antipsychotics, and has been reported to antagonize adrenergic and muscarinic receptors, other than histaminergic receptors, which may account for metabolic side effects of this compound (Wood et al., 2006). Olanzapine has previously been demonstrated to differentially induce immediate-early genes (IEGs) expression in rat forebrain (Iasevoli et al., 2010; Polese et al., 2002). All these compounds have been approved for treatment of schizophrenia and bipolar disorder (Hasan et al., 2013; Hasan et al., 2012; Potkin, 2011; Potkin et al., 2013).

We chose to investigate the expression of PSD genes that have been demonstrated to be responsive to typical and atypical antipsychotics treatment (Iasevoli et al., 2014a) and involved in synaptic plasticity process relevant for the pathophysiology of psychoses (de Bartolomeis et al., 2014a; Szumlinski et al., 2005), as well as for dopamine-mediated addiction behaviors (Obara et al., 2013). Thus, we studied the forebrain topography of *Homer*1a transcript, together with its interacting genes *Homer*1b/c and *PSD*95. *Homer*1a has been demonstrated to be induced by antipsychotics administration in an IEG-like fashion that tightly correlates with the D2Rs occupancy degree by different antipsychotics (Iasevoli et al., 2009). However, no systematic studies have been previously carried out using antipsychotics with different receptor profiles and at increasing doses in order to explore the putative differential effects on brain topography of *Homer* and related genes transcripts.

Homer genes encode for a family of PSD proteins including multiple constitutive isoforms (Homer1b/c, Homer2, Homer3) and two inducible forms (Homer1a and Ania3). Homer1b/c, by multimerizing through the COOH terminal, acts as multimodal scaffolding/adaptor between membrane surface receptors, such as mGluRs type I, and multiple molecules involved in glutamate-dependent signaling and Ca2+ regulation (*i.e.* Ryanodine Receptors, Inositol 1,4,5-trisphosphate receptors). Homer1a is truncated at COOH terminal and cannot multimerize. Homer1a is transcribed in IEG-like fashion in response to multiple stimuli, acting as a negative dominant by disrupting Homer1b/c oligomers and causing dramatic changes in glutamate signaling and Ca2+ homeostasis, therefore directly acting on synaptic plasticity and dendrites shaping (de Bartolomeis et al., 2014b).

We compared *Homer* expression with *C-fos*, an IEG well known to be modulated by typical and atypical antipsychotics (Polese et al., 2002), and with *Arc* and *Zif*-268, two IEGs also demonstrated to be responsive to antipsychotics, and involved in activity-dependent synaptic plasticity, as well as modulated by both dopaminergic and glutamatergic perturbations (Beauvais et al., 2010; Kumar et al., 2012; Unal et al., 2009).