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# Haloperidol modulates midbrain-prefrontal functional connectivity in the rat brain

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**Abstract**
Dopamine $D_2$ receptor antagonists effectively reduce positive symptoms in schizophrenia, implicating abnormal dopaminergic neurotransmission as an underlying mechanism of psychosis. Despite the well-established, albeit incomplete, clinical efficacies of $D_2$ antagonists, no studies have examined their effects on functional interaction between brain regions. We hypothesized that haloperidol, a widely used antipsychotic and $D_2$ antagonist, would modulate functional connectivity in dopaminergic circuits. Ten male Sprague-Dawley rats received either haloperidol (1 mg/kg, s.c.) or the same volume of saline a week apart. Resting-state functional magnetic resonance imaging data were acquired 20 min after injection. Connectivity analyses were performed using two complementary approaches: correlation analysis between 44 atlas-derived regions of interest, and seed-based connectivity mapping. In the presence of haloperidol, reduced correlation was observed between the substantia nigra and several brain regions, notably the cingulate and prefrontal cortices, posterodorsal hippocampus, ventral pallidum, and motor cortex. Haloperidol induced focal changes in functional connectivity were found to be the most strongly associated with ascending dopamine projections. These included reduced connectivity between the midbrain and the medial prefrontal cortex and hippocampus, possibly relating to its therapeutic action, and decreased coupling between substantia nigra and motor areas, which may reflect dyskinetic effects. These data may help in further

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characterizing the functional circuits modulated by antipsychotics that could be targeted by innovative drug treatments.


## 1. Introduction

Schizophrenia is characterized by abnormalities in brain connectivity with the most pronounced and replicated changes in the prefrontal cortex (Fornito et al., 2012). The "dysconnection" hypothesis proposes faulty integration of functionally specialized brain circuits as being responsible for abnormal sensorimotor and cognitive processing in schizophrenia (Friston, 1998). Indeed, hyperconnectivity in the default mode network regions (medial prefrontal cortex and posterior cingulate cortex) both during rest and task (Whitfield-Gabrieli et al., 2009), as well as compromised/altered interactions between the prefrontal cortex, thalamus and striatum, have been observed in schizophrenic patients and/or their relatives (Meyer-Lindenberg, 2010). In addition, healthy carriers of a single nucleotide polymorphism in the ZNF804A gene associated with psychosis have abnormal interhemispheric prefrontal connectivity both during rest and working memory and emotional tasks (Esslinger et al., 2009, 2011; Rasetti et al., 2011). Positive symptoms of schizophrenia have been associated with increased connectivity within the frontomedial cortex and reduced connectivity within the right posterior cingulate, right frontal and right parietal cortices (Salvador et al., 2010).

At a neurochemical level, abnormal dopaminergic neurotransmission is hypothesized to be a major contributor to psychosis, since a number of antipsychotic compounds acting as $D_2$ receptor antagonists (or compounds whose mechanism includes a $D_2$ antagonist component) are effective in alleviating positive symptoms in schizophrenic patients (Laviolette, 2007). $D_2$ receptors are coupled to membrane-associated guanine nucleotide binding proteins – Gi/Go alpha subunits inhibiting adenylate cyclase activity (Sokoloff and Schwartz, 1995; Missale et al., 1998). $D_2$ receptors are mostly expressed in the dorsal striatum, nucleus accumbens, and substantia nigra (SN); they are also expressed in lower levels in the hypothalamus, amygdala, thalamus (although with a heterogenous expression between thalamic regions) and in the habenula, prefrontal, cingulate, temporal and enthorinal cortices (Missale et al., 1998; Charuchinda et al., 1987; Hall et al., 1996; Hurd et al., 2001). Three isoforms of $D_2$ receptors exist, $D_2^{Short}$, $D_2^{Long}$ and $D_2^{Longer}$ (Seeman, 2006). $D_2^{Short}$ act as presynaptic autoreceptors, while $D_2^{Long}$ are postsynaptic receptors (Usiello et al., 2000). Antipsychotics differ in their preference to binding either of these isoforms (Seeman, 2006), with such classical antipsychotic as haloperidol presumed to target $D_2^{Long}$ receptors (Usiello et al., 2000).

To better understand the pharmacodynamic consequences of successful $D_2$ receptor blockade, several studies have employed functional imaging methods to investigate brain functions modulated by haloperidol. Chronic treatment with haloperidol in humans elevates glucose utilization in the striatum and in anterior regions of thalamus, while suppressing it in the dorsolateral prefrontal cortex and anterior cingulate (Tamminga and Davis, 2007). These data are in good accordance with recent PET studies on regional cerebral blood flow in schizophrenic patients (Lahti et al., 2009) and structural magnetic resonance imaging studies in healthy volunteers showing striatal volume change highly correlating with motor impairment even following a single administration of haloperidol (Tost et al., 2010). Haloperidol challenge has also been demonstrated to modulate blood-oxygen-level-dependent (BOLD) signals evoked by sensorimotor stimuli. BOLD responses to a finger opposition task were reduced in the sensorimotor striatum, ipsilateral higher order motor cortices and contralateral cerebellum (Tost et al., 2006), and BOLD contrast was reduced in the middle occipital gyrus and enhanced in the lingual gyrus during visuo-acoustic stimulation (Brassen et al., 2003).

Despite the widespread use of haloperidol in clinical practice and the aforementioned imaging studies in humans, only few imaging studies of haloperidol have been performed in the rodent. Hagino et al. demonstrated decreased $T_2^*$-weighted signal intensity in the posterior caudate putamen (CPu), hypothalamus and perirhinal cortex of the rat after acute administration of 1 mg/kg haloperidol, and showed that this effect was enhanced by chronic treatment; however, only a single coronal slice was acquired in this study (Hagino et al., 1998). The same acute dose in rat has been shown to reduce cerebral glucose utilization in the prefrontal cortex but increase in the lateral habenula (Kurachi et al., 1993; Pizzolato et al., 1984). A slightly lower dose of 0.5 mg/kg reduced glucose utilization in the orbital cortex, hippocampal formation and septal area, substantia nigra reticulate, globus pallidus, amygdala, and ventral thalamus (Colangelo et al., 1997).

Task-based functional magnetic resonance imaging (fMRI), common in human studies, cannot be easily replicated in rodents due to the need for physical restraint in the scanner and the common use of anesthesia. In order to link findings in preclinical models to those in humans, translational imaging methods that probe baseline (resting) brain function and do not rely on explicit subject responses are of particular promise. One method of considerable current interest is task-free "resting state" fMRI (rsfMRI), in which low-frequency BOLD oscillations reveal functionally connected networks of brain regions in the absence of an explicit cognitive task. This approach is now well-established in humans (reviewed in Fox and Greicius, 2010) and reliable methods have recently been demonstrated in rodents (Lu et al., 2007; Pawela et al., 2008; Kannurpatti et al., 2008; Zhao et al., 2008; Hutchison et al., 2010; Kalthoff et al., 2011; Jonckers et al., 2011). Pharmacological modulation of functional connectivity has begun to be explored in humans (Upadhyay et al., 2010; Khalili-Mahani et al., 2012; Upadhyay et al., 2012), but has yet to be reported in the rodent. Until now the effects of haloperidol on brain connectivity have not been elucidated neither in humans or any preclinical species.

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