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High estrogen and chronic haloperidol lead to greater amphetamine-induced BOLD activation in awake, amphetamine-sensitized female rats

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ABSTRACT

The ovarian hormone estrogen has been implicated in schizophrenia symptomatology. Low levels of estrogen are associated with an increase in symptom severity, while exogenous estrogen increases the efficacy of antipsychotic medication, pointing at a possible interaction between estrogen and the dopaminergic system. The aim of this study is to further investigate this interaction in an animal model of some aspects of schizophrenia using *awake* functional magnetic resonance imaging. Animals receiving 17β-estradiol and haloperidol were scanned and BOLD activity was assessed in response to amphetamine. High 17β-estradiol replacement and chronic haloperidol treatment showed increased BOLD activity in regions of interest and neural networks associated with schizophrenia (hippocampal formations, habenula, amygdala, hypothalamus etc.), compared with low, or no 17β-estradiol. These data show that chronic haloperidol treatment has a sensitizing effect, possibly on the dopaminergic system, and this effect is dependent on hormonal status, with high 17β-estradiol showing the greatest BOLD increase. Furthermore, these experiments further support the use of imaging techniques in studying schizophrenia, as modeled in the rat, but can be extended to addiction and other disorders.

Introduction

Although the overall risk for developing schizophrenia in men and women is equal, the age of onset for women is delayed by three to four years compared to men (Castle et al., 1998; Saha et al., 2005). In addition, women show a second onset peak corresponding to the onset of menopause, which is characterized by a decline in circulating levels of estrogen (Grigoriadis and Seeman, 2002). Women receiving estrogen in addition to antipsychotic treatment show a better response than those with antipsychotic treatment alone (Akhondzadeh et al., 2003; Kulkarni et al., 1996, 2001). The outcome in women is overall superior to men, with cognitive and negative symptomatology being more prevalent in men, and thus implicating ovarian steroids in schizophrenia onset, symptomatology and treatment responsiveness (Grigoriadis and Seeman, 2002; Riecher-Rossler et al., 1994).

Haloperidol (HAL) is a typical antipsychotic, binding to dopaminergic D2 receptors (Seeman and Tallerico, 1998). Although it has been shown to be effective in reducing overall symptomatology in women with schizophrenia, HAL shows further symptom amelioration when paired with ethinyl estradiol, outlining the possible antipsychotic effects of estrogens (Akhondzadeh et al., 2003). Given the evidence implicating estrogen in schizophrenia pathophysiology, it is critical that the interaction between E2 and HAL treatment is further studied in patients, as well as animal models of aspects of schizophrenia. One such model is driven by the sensitizing effects of AMPH, whereby repeated exposure to AMPH leads to an increased behavioural response to subsequent, lower doses of AMPH (Featherstone et al., 2007; Schmidt and Beninger, 2006; Vezina, 1996); behaviours elicited by AMPH sensitization are thought to reflect the neurobiological substrate underlying some of the positive and cognitive symptoms of schizophrenia (Featherstone et al., 2007; Peleg-Raibstein et al., 2008; Tenn et al., 2003). For example AMPH-sensitized rats exhibit disrupted sensorimotor gating and lower binding of striatal [^{3}H]raclopride, as well as increased locomotor responses to AMPH; these effects were more pronounced if rats we treated for longer periods of time (i.e. 5 weeks) and higher doses of AMPH (Tenn et al., 2003). The process is time-dependent, such that motor effects are fully developed after a period of withdrawal (Paulson et al., 1991; Paulson and Robinson, 1995), and not immediately after AMPH treatment cessation. Furthermore, the

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sensitizing effects of AMPH last for at least one year after treatment cessation (Paulson et al., 1991), and possibly longer, with female rats showing greater rates of behavioural sensitization compared to males (Robinson, 1984; Robinson et al., 1982).

Links between dopamine (DA) and E2 have been well established, with E2 potentiating AMPH-induced behaviours and striatal DA release (Becker, 1990; Madularu et al., 2014; Peris et al., 1991), as well as increasing striatal DA receptor density and sensitivity (Hruska et al., 1982; Hruska and Silbergeld, 1980a, 1980b). Blood-oxygen-level dependent (BOLD) activation, an indirect measure of neuronal activity, is influenced by E2 (Sarvari et al., 2014), and is dose-dependent, with animals receiving high levels of E2 showing highest increase in AMPH-induced BOLD activation compared to those receiving low levels of the hormone (Madularu et al., 2015b). In a similar fashion, cocaine potentiates neuronal activity in E2-treated animals compared to ovariectomized (OVX) controls, but only after repeated exposure (Febo et al., 2005). In addition, the antipsychotic HAL differentially affects behavior in rats, with males showing increased sensitivity to the inhibitory effects of HAL compared to female rats (Van Hest et al., 1988). On the other hand, E2 and HAL were shown to have similar effects in reducing AMPH-induced locomotor activity in AMPH-sensitized rats (Madularu et al., 2014).

Typical and atypical antipsychotics have been shown to reduce the hyperlocomotive effects of AMPH after acute, but not chronic administration in male rats (Samaha et al., 2007), however this was not the case for female rats (Madularu et al., 2014). Specifically, AMPH-sensitized female rats receiving E2 replacement showed reduced AMPH-induced locomotor activity in response to the typical antipsychotic HAL, but only when the drug was paired with high E2, and only after chronic treatment (i.e. 12 days; Madularu et al., 2014). Furthermore, high E2 treatment augmented the effects of chronic HAL in reducing nucleus accumbens DA release in sensitized rats (Madularu et al., 2014). Studies using MRI show that chronic (i.e. 8 weeks) HAL is associated with a significant decrease in brain volume and cortical gray matter in male rats (Vernon et al., 2011, 2012, 2014). We have recently found that a sensitizing AMPH regimen is associated with whole brain volume reductions in female rats, independent of hormonal status, albeit changes in hippocampal volumes were significant *before* AMPH exposure, with rats receiving a low E2 showing larger hippocampal volumes (Madularu et al., 2015a). In addition, continuous HAL administered chronically resulted in a further reduction in whole brain volume compared to acute treatment (i.e. 2 days) in AMPH-sensitized female rats (Madularu et al., 2015a). HAL was also shown to alter the midbrain-prefrontal connectivity in the rat brain, reducing connectivity between substantia nigra and the prefrontal cortex, ventral pallidum and posterodorsal hippocampus (Gass et al., 2013). Finally, HAL sensitivity has been shown to be affected by stress, where high intensity stressors reduce HAL responsiveness, while low intensity stressors elicit e sensitized response (Antelman et al., 1991, 1992).

Although there is ample evidence to support a link between DA and E2 in select brain regions and nuclei, less is known about the nature of this relationship at the level of entire systems, especially in response to antipsychotic treatment. We have shown that in rats, AMPH activates brain systems similar to those implicated in schizophrenia (Madularu et al., 2015b), however little is known about the effects of antipsychotic medication on BOLD response in these areas, with respect to E2 availability. Thus, the purpose of this study was to further investigate *whole brain* activity in response to AMPH as a function of E2 and antipsychotic treatment in a rat model of some aspects of schizophrenia, using awake fMRI. For this study, OVX, awake AMPH-sensitized female rats with high, low or no E2 replacement were scanned two (i.e. acute) and twelve (i.e. chronic) days into HAL treatment, and BOLD activation in response to a subsequent dose of AMPH was recorded. Based on earlier studies showing that E2 promotes reactivity to AMPH, a "dose-dependent" BOLD activity was expected, with animals receiving high E2 replacement showing the highest BOLD increase in areas previously shown to be affected by AMPH. In addition, these differences were expected to be mediated by HAL.

Methods

Animals

Thirty-six OVX Sprague Dawley rats (Charles River Laboratories, Wilmington, MA, USA) weighing 200–250 g (~two months old) were used for this study. Rats were pair-housed in cages located in a 21 °C with a 12-h light-dark cycle (lights off at 19:00 h), with ad libitum access to food and water. Testing, injections, surgical procedures and imaging were performed during the dark phase of the diurnal cycle, in semi-dark conditions. All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by Northeastern University's Institutional Animal Care and Use Committee.

Drugs

AMPH (1 mg/kg, or 0.25 mg/kg; Sigma-Aldrich, UK) was dissolved in 0.9% saline and administered intraperitoneally (IP); doses were selected based on studies involving behavioural sensitization to AMPH, as well as studies examining the efficacy of antipsychotics in response to an AMPH challenge (Madularu et al., 2014; Samaha et al., 2007). The sensitization regimen started two days after hormone replacement initiation (Fig. 1).

Rats were divided into three groups, with respect to hormone replacement: no E2 ($n = 12$), constant low E2 (Low E2; $n = 12$) and constant low plus phasic high E2 (High E2; $n = 12$). The E2 (low and high) groups were implanted subcutaneously with silastic capsules containing 5% 17-β estradiol in cholesterol two weeks post ovariectomy; each capsule (1 cm length) contained 0.4 mg 17-β estradiol (Almey et al., 2013; Mannino et al., 2005). High E2 rats also received a subcutaneous injection of 17-β estradiol every second day (20 μg/kg dissolved in sesame seed oil) in a volume of 0.5 mL/kg body weight, providing an intermittent phasic high dose. The No E2 and Low E2 groups also received an injection of sesame oil vehicle every second day as an injection control. These doses were chosen to mimic the levels of estrogen in estrous and proestrous young cycling rats (Madularu et al., 2014; Overpeck et al., 1978).

Haloperidol (HAL; 0.25 mg/kg/day; Sandoz Canada Inc., QC, Canada) was administered subcutaneously two weeks after AMPH sensitization as described in a previous study (Madularu et al., 2014), using fMRI-compatible Alzet osmotic minipumps (model: 2002, 14-day delivery, at a rate of 0.5 μL/h; Durect, Cupertino, CA, USA). This dose, when administered chronically, has been previously shown to result in rat striatal D2 receptor occupancy (Samaha et al., 2007, 2008) similar to effective antipsychotic doses in humans (Kapur et al., 2000). BOLD imaging was performed two and twelve days into HAL treatment.

Surgery

Rats were anesthetized using isoflurane, and a 0.5 cm incision was made in the nape region. For the E2 groups, silastic implants were placed subcutaneously, while No E2 rats received sham surgery (incision, but no implant). A similar procedure was applied implanting the HAL-containing minipumps (Madularu et al., 2014). All rats were administered analgesics (Anafen; 0.1 mL/rat, SC; Merial Canada Inc., Morgan Baie d'Urfe, QC, Canada), and local antibiotic ointment (By/Par Pharmaceuticals Inc., Brampton, ON, Canada).

AMPH sensitization

AMPH sensitization was induced following E2 replacement/sham surgery. All rats were administered daily injections of AMPH (1 mg/kg/day IP) for four consecutive days, as described elsewhere in