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Integration of neural networks activated by amphetamine in females with different estrogen levels: A functional imaging study in awake rats

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Summary Previous studies demonstrate that schizophrenia symptomatology in women is dependent upon estrogen levels. Estrogen has beneficial properties when administered in conjunction with antipsychotics, and estrogen also alters, in rats, dopamine neurotransmission, which is a common target of all antipsychotic medications, suggesting a possible interaction between the two. The aim of the current study was to investigate this possible interaction using functional magnetic resonance imaging in awake, female rats. Amphetamine-sensitized, ovariectomized rats receiving no, chronic low, or phasic high levels of estradiol replacement were used, and changes in blood-oxygen-level-dependent (BOLD) signal were recorded over time in response to an acute amphetamine injection. Increasing levels of estradiol enhanced BOLD activation in pathways previously known to be implicated in schizophrenia symptomatology, such as the mesocorticolimbic, habenular and olfactory pathways, as well as more widespread areas. We propose here the first comprehensive ''amphetamine activation map'' integrating brain regions where amphetamine-related BOLD activity is influenced by estrogen levels in sensitized female rats.

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1. Introduction

Schizophrenia symptoms are exacerbated in women when estrogen (E) levels are low, and women experience an improvement in symptoms during pregnancy when E levels fluctuate (Riecher-Rossler et al., 1994; Seeman and Lang, 1990). Women also show a worsening of symptoms after giving birth and/or experiencing menopause as E levels fluctuate and/or decline (Castle and Murray, 1993; Hafner, 2003). Thus it is suggested that E may play a role in schizophrenia onset and development in women. Women are also differentially responsive to antipsychotics; it has been consistently shown that women are more responsive to typical antipsychotics, and women with schizophrenia require increasing doses of antipsychotics upon menopause in order to maintain remission of symptoms (Seeman and Lang, 1990). Yet, we still know surprisingly little about the neurobiological effects of E in this regard.

Sensitization to amphetamine (AMPH) in rodents is a phenomenon where repeated, intermittent exposure to AMPH leads to an increased behavioral response to subsequent lower doses of the drug and has been widely used as a rodent model of some of the neurochemical and behavioral aspects of schizophrenia (for review, see Featherstone et al., 2007; Schmidt and Beninger, 2006; Vezina, 1996). In addition, AMPH sensitization is sex-dependent, such that female rats show increased locomotor activation in response to acute and repeated injections of AMPH compared to males (Forgie and Stewart, 1994). Ovariectomized (OVX) female rats with E replacement show higher levels of AMPH-induced locomotor activity during induction of sensitization compared to no-E controls (Forgie and Stewart, 1994) and E enhances sensitization to cocaine (Hu and Becker, 2003). We have shown that AMPH-sensitized, OVX rats show different behavioral and dopamine responses to a challenge injection of AMPH depending on their E replacement levels. That is, the antipsychotic, haloperidol, is effective in reducing locomotor activity in only those rats with high E replacement (Madularu et al., 2014). Although AMPH sensitization is not the only model of mimicking some aspects of schizophrenia in rodents (i.e. sensitization of the mesocorticolimbic dopaminergic system), this approach was chosen here because one of the purposes of this study, namely the possible interaction between dopamine and estradiol in schizophrenia is best suited to this model and much has been published on AMPH-sensitized rats.

A recent functional magnetic resonance imaging (fMRI) study showed that the prefrontal cortex (PFC) and ventral tegmental area (VTA) of OVX rats receiving E replacement yield robust AMPH-induced blood-oxygen-level-dependent (BOLD) signal increases compared to those without E replacement (Sarvari et al., 2014). An earlier study reported that OVX rats, with and without E replacement, show increased BOLD activation in the PFC, VTA, and nucleus accumbens (NAcc) in response to cocaine, with those with E replacement showing lower positive BOLD changes compared to those without (Febo et al., 2005). On the other hand, repeated cocaine administration increases BOLD signal changes in the NAcc, VTA and hippocampus, but only in those with 17β estradiol (E2: the most potent E during reproductive years) replacement. While much is known about the involvement of the PFC, NAcc and VTA in AMPH-sensitization models, there has been little attention paid to the integrated neural networks associated with, and independent of, the mesocorticolimbic dopamine system.

In terms of the interaction between E, dopamine and antipsychotics; the animal literature is divergent. On the one hand, E *enhances* dopamine release in response to AMPH (Becker, 1990; Becker and Cha, 1989; Peris et al., 1991), which would theoretically *worsen* schizophrenia symptoms. On the other, it *improves* behaviors similar to those observed in schizophrenia when combined with antipsychotics (Almey et al., 2013; Kulkarni et al., 2001; Madularu et al., 2014). Thus, it is important to examine widespread brain activation in response to AMPH under different E2 treatments to help clarify this issue.

With the advent of fMRI in awake animals it is now possible to offer a global perspective of changing brain function with high temporal and spatial resolution. When combined with 3D-segmented and annotated brain atlases as well as computational analysis, it is possible to reconstruct distributed and integrated neural circuits, or ''finger prints'' of brain activity (Ferris et al., 2014). To this end, here we use fMRI to corroborate previous findings on E-dependent effects of AMPH. Moreover, we can report the changing pattern of activation from 172 brain areas, showing a clear delineation of neural circuits involved in learning and memory, motivation and olfaction. This experiment was carried out in awake, OVX, female rats that had been sensitized to AMPH and had either no, low constant, or low constant plus phasic high E2 replacement.

2. Materials and methods

2.1. Animals

Twenty-seven Sprague Dawley rats (Charles River Laboratories, Wilmington, MA, USA) weighing 200—250 g (approx. 2—3 months old) were purchased already OVX and 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.

2.2. Drugs

AMPH (1 mg/kg, or 0.25 mg/kg; Sigma—Aldrich, UK) was dissolved in 0.9% saline and administered intraperitoneally (IP). These doses were selected based on previous studies inducing behavioral 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). AMPH sensitization commenced two days after hormone replacement.

Rats were divided into three groups, with respect to hormone replacement: no E2 ($n = 8$), constant low E2 (Low E2; $n = 9$) and constant low plus phasic high E2 (High E2; $n = 10$). The E2 (low and high) groups were implanted subcutaneously