Contrasting Effects of Increased and Decreased Dopamine Transmission on Latent Inhibition in Ovariectomized Rats and Their Modulation by 17β-Estradiol: An Animal Model of Menopausal Psychosis?

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Women with schizophrenia have later onset and better response to antipsychotic drugs (APDs) than men during reproductive years, but the menopausal period is associated with increased symptom severity and reduced treatment response. Estrogen replacement therapy has been suggested as beneficial but clinical data are inconsistent. Latent inhibition (LI), the capacity to ignore irrelevant stimuli, is a measure of selective attention that is disrupted in acute schizophrenia patients and in rats and humans treated with the psychosis-inducing drug amphetamine and can be reversed by typical and atypical APDs. Here we used amphetamine (1 mg/kg)-induced disrupted LI in ovariectomized rats to model low levels of estrogen along with hyperfunction of the dopaminergic system that may be occurring in menopausal psychosis, and tested the efficacy of APDs and estrogen in reversing disrupted LI. 17β-Estradiol (50, 150 μg/kg), clozapine (atypical APD; 5, 10 mg/kg), and haloperidol (typical APD; 0.1, 0.3 mg/kg) effectively reversed amphetamine-induced LI disruption in sham rats, but were much less effective in ovariectomized rats; 17β-estradiol and clozapine were effective only at high doses (150 μg/kg and 10 mg/kg, respectively), whereas haloperidol failed at both doses. Haloperidol and clozapine regained efficacy if coadministered with 17β-estradiol (50 μg/kg, an ineffective dose). Reduced sensitivity to dopamine (DA) blockade coupled with spared/potentiated sensitivity to DA stimulation after ovariectomy may provide a novel model recapitulating the combination of increased vulnerability to psychosis with reduced response to APD treatment in female patients during menopause. In addition, our data show that 17β-estradiol exerts antipsychotic activity.

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INTRODUCTION

Since description of schizophrenia as 'men illness' by Kraepeline (1919), scores of epidemiological and clinical studies have documented gender differences in schizophrenia. Women have a more favorable illness course during the reproductive years, characterized by later onset of symptoms, lower symptom severity, and better response to antipsychotic drug (APD) treatment (Agius *et al*, 2009; Angermeyer and Kuhn, 1988; Hafner, 2003; Hafner *et al*, 1989; Iacono and Beiser, 1992; Lindamer *et al*, 1997; Mortimer, 2007; Pregelj, 2009; Riecher-Rossler and Hafner, 2000; Salem and Kring, 1998; Seeman, 1982, 1986; Szymanski *et al*, 1995; Tamminga, 1997). In contrast,

menopause is associated with increased vulnerability to illness, elevated symptom severity, and reduced response to treatment (Horacek *et al*, 2006; Kulkarni *et al*, 1996, 2008b; Lane *et al*, 1999; Salokangas, 1995; Saugstad, 1989; Seeman and Lang, 1990). Increased symptom severity and reduced treatment response are associated also with low-estrogen phases of the menstrual cycle (Ereshefsky *et al*, 1991; Farina *et al*, 1981; Lane *et al*, 1999; Salokangas, 1995; Seeman, 1989; Simpson *et al*, 1990; Tamminga, 1997). These data have been captured in the estrogen hypothesis of schizophrenia that posits exacerbations of illness manifestations in women are related to low levels of estrogen (Hafner *et al*, 1989; Huber *et al*, 2004; Seeman and Lang, 1990). Accordingly, it has been suggested that exogenous estrogen on its own or combined with APDs may have therapeutic potential in schizophrenia, although this notion has been challenged (for recent reviews see Agius *et al*, 2009; Mortimer, 2007).

Ovariectomy (OVX)-induced hormonal decline in rats is considered to model decreased gonadal function during

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menopause (Adam *et al*, 2009; Daniel *et al*, 2006; Gurkan *et al*, 1986; LeBlanc *et al*, 2009; Rogers *et al*, 2009; Walf *et al*, 2009), and has been proposed specifically to model the hormonal state associated with predisposition to schizophrenia during menopause (Bosse and Di Paolo, 1995). Although many studies showed that OVX is associated with poorer behavioral and cognitive performance, including tasks considered relevant to schizophrenia such as social interaction and object recognition (Barnes *et al*, 2006; Frye, 2001; Frye *et al*, 2006b, 2007; Frye and Rhodes, 2006a; Paris and Frye, 2008), the study of the estrogen hypothesis using animal models of schizophrenia has been limited. Some support derives from studies showing that estrogen affects prepulse inhibition (PPI) (Gogos *et al*, 2009; Gogos and Van den Buuse, 2004; Koch, 1998; Vaillancourt *et al*, 2002; Van den Buuse and Eikelis, 2001), a measure of sensorimotor gating whose disruption is considered to model sensorimotor deficits in schizophrenia, as well as the response to the pro-psychotic drugs amphetamine and cocaine (Becker and Beer, 1986; Becker and Rudick, 1999; Earley and Leonard, 1978; Gibbs *et al*, 1998; Naik *et al*, 1978; Segarra *et al*, 2009).

We have recently tested the estrogen hypothesis using the latent inhibition (LI) model of schizophrenia (Arad and Weiner, 2008, 2009). LI is a cross-species selective attention phenomenon manifested as retarded conditioning to a stimulus that was pre-exposed (PE) without consequence before conditioning, and is commonly considered to index the ability to ignore irrelevant stimuli. Loss of LI induced in the rat by the psychotomimetic dopamine (DA) releaser amphetamine and its reversal by APDs is a well-established model of positive symptoms of schizophrenia (Lipska and Weinberger, 2002; Moser *et al*, 2000; Weiner, 2003; Weiner and Arad, 2009). We (Arad and Weiner, 2009) have shown that hormonal cessation after OVX led to loss of LI, which was restored after the administration of 17β-estradiol. Most interestingly, we found that OVX interfered with the efficacy of the typical APD haloperidol to restore LI, mimicking the reduced sensitivity to APD treatment seen in schizophrenic women during menopause. Furthermore, haloperidol regained efficacy when coadministered with a behaviorally inactive dose of 17β-estradiol, indirectly supporting an antipsychotic action of 17β-estradiol.

Here we sought to expand the OVX/LI model by testing the efficacy of 17β-estradiol, haloperidol, and clozapine to reverse amphetamine-induced disruption of LI in OVX rats. Given our previous finding that OVX reduced the efficacy of APDs, one major question of interest here was whether OVX would also reduce the efficacy of amphetamine. We hypothesized that amphetamine would not lose its efficacy to disrupt LI in OVX compared to sham rats. Conversely, APDs would be less effective in reversing amphetamine-induced LI disruption in OVX rats, but their action would be potentiated by concurrent 17β-estradiol treatment, which would also block amphetamine effect on its own. These outcomes would capture the combination of increased vulnerability to psychosis with reduced response to APD treatment that may be occurring during menopause in women vulnerable to psychosis, and support a direct antipsychotic (anti-amphetamine) action of 17β-estradiol.

MATERIALS AND METHODS

Animals

Female Wistar rats bred in our laboratory were housed 3–4 per cage under reversed cycle lighting (lights on 0700–1900 hours) with *ad lib* access to food and water. They were about 7 weeks old and weighing 155–267 g when submitted to OVX and approximately 3 months old and weighing 248–469 g when behavioral testing begun. All experimental protocols conformed to the guidelines of the Institutional Animal Care and Use Committee of Tel Aviv University, Israel, and to the guidelines of the NIH (animal welfare assurance number A5010–01, expires on 30 September 2011). All efforts were made to minimize the number of animals used and their suffering.

Ovariectomy

Rats were bilaterally ovariectomized under isoflurane (Nicholas Piramal, UK) anesthesia. After shaving the abdominal area, we made a midline incision through the skin and muscle layer. Fallopian tubes were ligated by a nylon thread, after which the ovaries were carefully removed. Sutures of muscle layer and skin were removed 10 days later. Rats were allowed additional 3 weeks of recovery after removal of the sutures, before the beginning of water restriction (see below). Within the 3-week recovery period, about a week after removal of sutures, vaginal smears were collected daily in the morning for 8 days in sham and OVX rats to confirm regular or discontinuation of estrous cycle. Phases of the estrous cycle were determined by the morphology of cells in the vaginal smear under a light microscope (Marcondes *et al*, 2002). Sham-operated controls underwent an identical surgical procedure without ovaries' removal. Only sham females with regular 4-day cycles in succession and OVX rats without estrous cycle were used for behavioral testing.

Latent Inhibition

LI was measured in a thirst-motivated conditioned emotional response procedure as described previously (Arad and Weiner, 2009). Water-restricted (23 h) rats were trained to drink in the experimental chambers for 15–20 min per day for 5 days. Water in the chambers was given in addition to the 1 h water in home cages. The LI procedure consisted of four stages given 24 h apart. *Pre-exposure*: with the bottle removed, PE rats received 40 or 50 tones (10 s, 80 dB, 2.8 kHz) 40 s apart, whereas non-pre-exposed (NPE) rats were confined to the chamber. *Conditioning*: with the bottle removed, all rats received one or two tone-shock (tone: 10 s, 80 dB, 2.8 kHz; shock: 1 s, 0.5 mA) pairings given 5 min apart. *Lick retraining*: rats were given a 15 min drinking session as in initial training. Data of rats that failed to complete 600 licks were dropped from the analysis. *Test*: rats were placed in the chambers with access to the bottle. When the rat completed 75 licks, the tone was presented for 5 min. Times to complete 25 licks before and after tone onset were recorded. Times to complete licks 76–100 were submitted to logarithmic transformation to allow parametric analysis of variance (ANOVA). Longer log times indicate stronger suppression of drinking. LI is defined as

shorter times to complete licks 76–100 after tone onset (weaker fear conditioning) of the PE compared to NPE rats.

Drug and Hormone Administration

Amphetamine and APDs were administered intraperitoneally and 17β-estradiol was administered subcutaneously, all in a volume of 1 ml/kg, 30 (amphetamine and clozapine), 60 (haloperidol), or 120 (17β-estradiol) min before pre-exposure and conditioning stages. Amphetamine (Sigma, Israel) was dissolved in saline and administered at a dose of 1 mg/kg. Haloperidol (Johnson&Johnson, Belgium) was prepared from an ampoule containing 5 mg haloperidol in 1 ml solvent containing 6 mg lactic acid, diluted with saline, and administered at doses of 0.1 or 0.3 mg/kg. Clozapine (Novartis, Switzerland) was dissolved in 1 N acetic acid (1.5 ml/10 mg), diluted with saline, and administered at doses of 5 or 10 mg/kg. The doses of haloperidol and clozapine are routinely used in our LI studies (Arad and Weiner, 2009; Weiner et al, 1996b). 17β-Estradiol (Sigma) was dissolved in corn oil and administered at doses of 10, 50, and 150 μg/kg. These doses were chosen on the basis of behavioral literature and used in our previous study in OVX rats (Arad and Weiner, 2009; Galea et al, 2001; Gibbs et al, 1998; Nofrey et al, 2008; Van den Buuse and Eikelis, 2001; Walf and Frye, 2010). No-drug controls received the corresponding vehicle/s as follows: saline as amphetamine vehicle, saline solution containing 1% of lactic acid as haloperidol vehicle, saline solution containing 7.5% acetic acid as clozapine vehicle, and oil as 17β-estradiol vehicle. In all experiments, lick retraining and test sessions were conducted in a drug-free state.

Assessment of Estradiol Serum Levels

Serum estradiol levels produced by the four 17β-estradiol conditions were determined in separate groups of sham and OVX rats (n per group 7–8). Rats were given two injections 24 h apart (mimicking the injection protocol of LI).

Blood was taken by cardiac puncture (1 ml, without preservative). The blood was allowed to clot for 20–40 min and was centrifuged at 930 g (2000 r.p.m.) for 20 min. Afterward serum was collected and was assayed immediately.

17β-Estradiol serum levels were measured using an enzyme-linked immunosorbent assay kits (Cayman, Michigan, USA), based on manufacturer's instructions. Table 1 presents mean serum levels of estradiol following our administration regime in comparison to levels reported for different stages of the estrous cycle and pregnancy (Nequin et al, 1979; Shaikh, 1971).

Experimental Design

Experiment 1. In our previous study we showed that LI was absent in OVX rats (Arad and Weiner, 2009). Because here we intended to test whether amphetamine disrupts LI in OVX rats, we needed to create conditions under which OVX no longer disrupts LI, so that the effect of amphetamine could be manifested. As detailed by us elsewhere (Weiner, 1990, 2003; Weiner and Arad, 2009), the expression of LI is a function of the balance between the strength of pre-exposure and the strength of conditioning, so that increasing the strength of pre-exposure (eg, by increasing the number of stimulus pre-exposures) and/or decreasing the strength of conditioning (eg, by reducing the number of conditioning trials) are expected to promote the expression of LI. The aim of experiment 1 was to test whether such manipulations of pre-exposure and conditioning parameters would restore LI in OVX rats. In our previous study, OVX-induced disruption of LI was obtained using 40 pre-exposures and 2 conditioning trials. Here, we either increased the number of pre-exposures from 40 to 50 while keeping 2 conditioning trials or reduced the number of conditioning trials from 2 to 1 while keeping 40 pre-exposures, and compared LI under these two combinations to the previous combination of 40 pre-exposures and 2 conditioning trials. The experiment included 36 OVX rats divided into six experimental groups (n per group 6) in a 2 × 3 design with main factors of pre-exposure (NPE, PE) and pre-exposure-conditioning combination (40 pre-exposures and 1 conditioning trial; 40 pre-exposures and 2 conditioning trials; 50 pre-exposures and 2 conditioning trials). The latter combination that yielded LI in OVX rats was used in experiments 2–7.

Experiment 2 tested the capacity of amphetamine to disrupt LI in OVX rats. The experiment included 62 rats (32 sham, 30 OVX) divided into eight experimental groups (n per group 6–8) in a 2 × 2 × 2 design with main factors of pre-exposure (0, 50), gonadal status (sham, OVX), and treatment (0, 1 mg/kg amphetamine).

Experiment 3 tested the capacity of 17β-estradiol to reverse amphetamine-induced LI disruption in OVX rats. The experiment included 236 rats (119 sham, 117 OVX) divided into 32 experimental groups (n per group 6–8) in a 2 × 2 × 2 × 4 design with main factors of pre-exposure

Table 1 Serum Levels of Estradiol in Sham and OVX Rats After Administration of 17β-Estradiol at Three Doses

Gonads	E state						
	Control	Low E (10 μg/kg)	Metestrus	Medium E (50 μg/kg)	Proestrus	High E (150 μg/kg)	Pregnancy (GD 20–22)
Sham	48.7 ± 12.7	20.2 ± 3.2	20.6 ± 1.6	120.6 ± 14.0	142.2 ± 45.8	628.4 ± 95.0	628 ± 210
OVX	5.5 ± 0.6	17.9 ± 3.5	—	125.8 ± 15.7	—	660.4 ± 59.4	—

Mean (± SEM) of serum level of estradiol 2 h after second injection (given 24 h apart) of 0, 10, 50, or 150 μg/kg of 17β-estradiol, in sham and OVX rats.
For comparison, levels found by Nequin et al (1979) along the cycle (metestrus and proestrus) and by Shaikh (1971) on gestation days (GD) 20–22 are provided.

(0, 50), gonadal status (sham, OVX), treatment (0, 1 mg/kg amphetamine), and pretreatment (0, 10, 50, or 150 μg/kg 17β-estradiol).

Experiments 4 and 5 tested the capacity of the typical and atypical APDs, haloperidol (0.1, 0.3 mg/kg) and clozapine (5, 10 mg/kg), respectively, to reverse amphetamine-induced LI disruption in OVX rats. Because it is well documented that haloperidol and clozapine at the doses and injection protocol used here do not affect LI (Weiner and Feldon, 1987; Weiner *et al*, 1997; Weiner *et al*, 1996b), we did not use separate control (sham and OVX) groups for each APD dose but instead injected each dose to half of the controls. Both experiments included 20 experimental groups (*n* per group 6–8) in a $2 \times 2 \times 5$ design with main factors of pre-exposure (0, 50), gonadal status (sham, OVX), and treatment (vehicle, APD, amphetamine, amphetamine + low APD dose, amphetamine + high APD dose). Experiment 4 included 141 rats (71 sham, 70 OVX), whereas experiment 5 included 151 rats (77 sham, 74 OVX).

Experiments 6 and 7. Because amphetamine-induced disruption of LI in OVX rats was resistant to haloperidol (experiment 4) and showed reduced response to clozapine (experiment 5), here we tested whether the efficacy of haloperidol (0.1 mg/kg; experiment 6) and clozapine (5 mg/kg; experiment 7) in reversing amphetamine-induced LI disruption would be restored by their coadministration with an ineffective dose of 17β-estradiol (50 μg/kg). The experiments included 115 and 116, respectively, OVX rats divided into 16 experimental groups (*n* per group 7–8) in a $2 \times 2 \times 4$ design with main factors of pre-exposure (0, 50), treatment (saline, amphetamine), and pretreatment (vehicle, APD, 17β-estradiol, APD + 17β-estradiol).

Statistical Analysis

Times to complete licks 51–75 (before tone onset) and logarithmically transformed mean times to complete licks 76–100 (after tone onset) were analyzed with two-way ANOVA with main factors of pre-exposure and gonadal status (experiment 1); three-way ANOVAs with main factors of pre-exposure, gonadal status, and treatment (experiment 2, 4, and 5) or pre-exposure, treatment, and pretreatment (experiment 6 and 7); and a four-way ANOVA with main factors of pre-exposure, gonadal status, treatment, and pretreatment (experiment 3). In cases of significant interactions involving the factor of pre-exposure, LSD *post hoc* comparisons were used to assess the difference between the PE and NPE groups within each treatment condition.

RESULTS

There were no differences between the experimental groups in the times to complete licks 51–75 (A period; all *p*'s > 0.05) in any of the seven experiments (overall mean A periods were 8.19, 9.58, 7.92, 10.77, 10.04, 9.66, and 7.13 for experiments 1–7, respectively).

Experiment 1: LI in OVX Rats

Figure 1 presents the mean log times to complete licks 76–100 (after tone onset) of the PE and NPE OVX rats in the three experimental conditions. As shown by us previously,
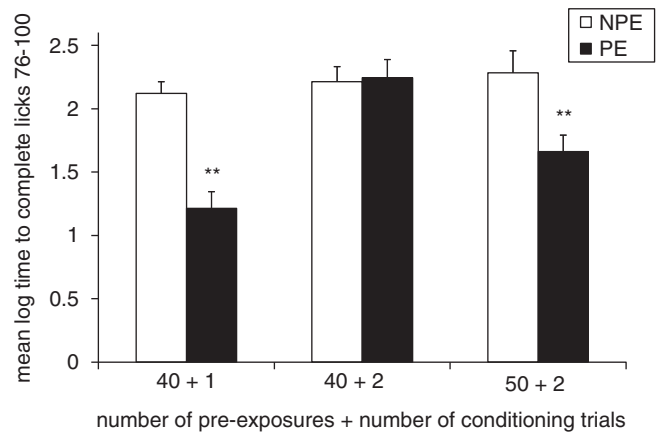

Figure 1 LI in OVX rats. Mean (± SEM) log times to complete licks 76–100 (after tone onset) of the pre-exposed (PE) and the non-pre-exposed (NPE) OVX rats in three pre-exposure/conditioning combinations (number of pre-exposures + number of conditioning trials): 40 pre-exposures and 1 conditioning trial (40 + 1); 40 pre-exposures and 2 conditioning trials (40 + 2); and 50 pre-exposures and 2 conditioning trials (50 + 2). Asterisks (**) indicate significant difference between the PE and NPE groups, namely, presence of LI (*p* < 0.01).

when 40 pre-exposures were followed by 2 conditioning trials (40 + 2), there was no difference in suppression between the PE and NPE groups, ie, no LI. In contrast, rats exhibited LI, ie, lower suppression of the PE as compared to the NPE group, when 40 pre-exposures were followed by 1 conditioning trial (40 + 1) or when 50 pre-exposures were followed by 2 conditioning trials (50 + 1). ANOVA yielded significant main effects of pre-exposure ($F_{(1,30)} = 20.88$, *p* < 0.01) and pre-exposure/conditioning combination ($F_{(2,30)} = 8.85$, *p* < 0.01), as well as their interaction ($F_{(2,30)} = 6.45$, *p* < 0.01). *Post hoc* comparisons confirmed the presence of LI in the 40 + 1 and 50 + 2 conditions (*p* < 0.01), but not in the 40 + 2 condition.

On the basis of these results in all the following experiments (2–7), we used 50 pre-exposures and 2 conditioning trials.

Experiment 2: Effects of Amphetamine (1 mg/kg) on LI in Sham and OVX Rats

Figure 2 presents the mean log times to complete licks 76–100 (after tone onset) of the PE and NPE sham and OVX rats injected with amphetamine (0, 1 mg/kg). As can be seen, saline-injected sham and OVX rats exhibited LI, but LI was lost in both groups after amphetamine injection. ANOVA yielded a significant effect of pre-exposure ($F_{(1,54)} = 34.08$, *p* < 0.001) and a significant pre-exposure × treatment interaction ($F_{(1,54)} = 19.57$, *p* < 0.001). *Post hoc* comparisons confirmed the presence of LI in saline (*p* < 0.001), but not in amphetamine conditions.

Experiment 3: Effects of 17β-Estradiol (10, 50, or 150 μg/kg) on Amphetamine-Induced LI Disruption in Sham and OVX Rats

Figure 3 presents the mean log times to complete licks 76–100 (after tone onset) of the PE and NPE saline- or amphetamine-injected sham and OVX rats pretreated with

0, 10, 50, or 150 μg/kg of 17β-estradiol. As can be seen, saline-injected sham and OVX rats exhibited LI whereas amphetamine-injected sham and OVX rats did not exhibit LI. LI was restored in amphetamine-injected sham rats given 50 or 150 μg/kg of 17β-estradiol, whereas in OVX rats only the high 17β-estradiol dose reversed amphetamine-induced disruption of LI. On its own, 17β-estradiol disrupted LI at the low dose of 10 μg/kg in both sham and OVX rats but spared LI at the two higher doses. ANOVA yielded main effects of pre-exposure ($F_{(1,204)} = 55.024$,

$p < 0.0001$), treatment ($F_{(1,204)} = 11.296$, $p < 0.001$), and pretreatment ($F_{(3,204)} = 2.74$, $p < 0.05$), as well as a significant pre-exposure × gonadal status × treatment × pretreatment interaction ($F_{(3,204)} = 3.023$, $p < 0.05$). *Post hoc* comparisons confirmed the presence of LI in saline-injected sham and OVX rats given 0, 50, or 150 μg/kg 17β-estradiol; in amphetamine-injected sham rats given 50 or 150 μg/kg 17β-estradiol; and in OVX rats given 150 μg/kg 17β-estradiol (p's < 0.05), but not in the other conditions.

Experiment 4: Effects of Haloperidol (0.1, 0.3 mg/kg) on Amphetamine-Induced LI Disruption in Sham and OVX Rats

Figure 4 presents the mean log times to complete licks 76–100 (after tone onset) of the PE and NPE saline- or amphetamine-injected sham and OVX rats pretreated with 0, 0.1, or 0.3 mg/kg of haloperidol. As can be seen, saline-injected sham and OVX rats exhibited LI whereas amphetamine-injected sham and OVX rats did not exhibit LI. LI was restored in amphetamine-injected sham rats given both doses of haloperidol, whereas both doses were ineffective in amphetamine-injected OVX rats. On its own, haloperidol spared LI. ANOVA yielded significant main effects of pre-exposure ($F_{(1,121)} = 119.42$, $p < 0.001$), gonadal status ($F_{(1,121)} = 5.32$, $p < 0.05$), and treatment ($F_{(4,121)} = 4.99$, $p < 0.05$), as well as a significant pre-exposure × gonadal status × treatment interaction ($F_{(4,121)} = 2.75$, $p < 0.05$). *Post hoc* comparisons confirmed the presence of LI in saline-injected sham and OVX rats that received vehicle or haloperidol and in amphetamine-injected sham rats that received both doses of haloperidol (p's < 0.01), but not in the other conditions.
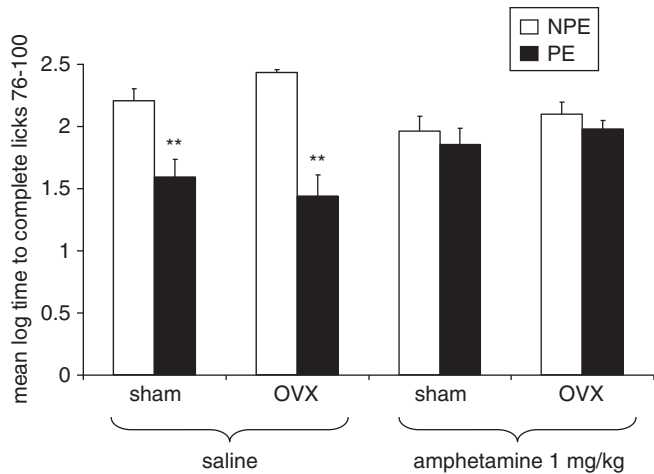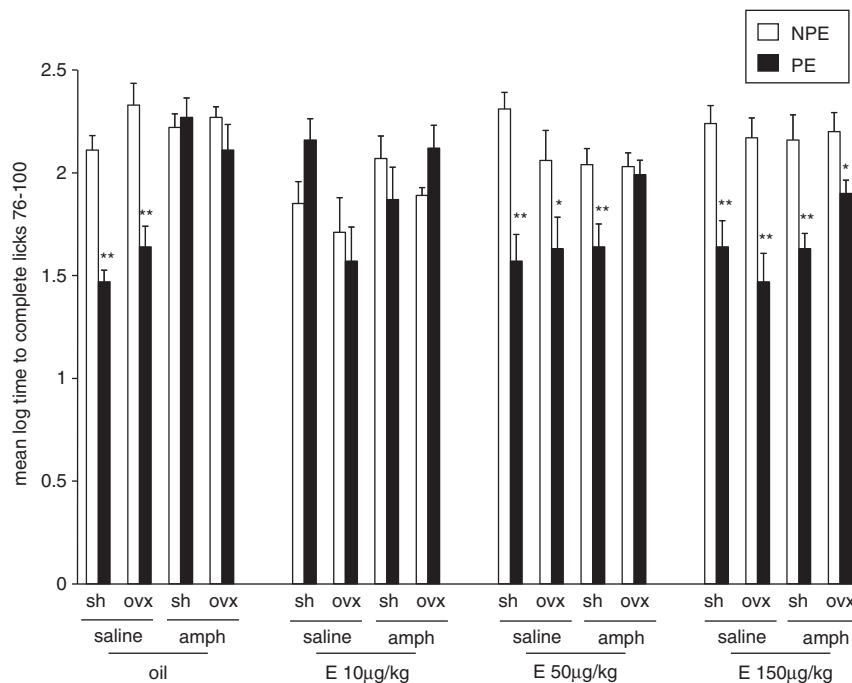

Figure 2 Effects of amphetamine (1 mg/kg) on LI in sham and OVX rats. Mean (± SEM) log times to complete licks 76–100 (after tone onset) of the pre-exposed (PE) and the non-pre-exposed (NPE) sham and OVX rats injected with saline or 1 mg/kg amphetamine. Asterisks (**) indicate significant difference between the PE and NPE groups, namely, presence of LI ($p < 0.01$).

Figure 3 Effects of 17β-estradiol (10, 50, or 150 μg/kg) on amphetamine-induced LI disruption in sham and OVX rats. Mean (± SEM) log times to complete licks 76–100 (after tone onset) of the pre-exposed (PE) and the non-pre-exposed (NPE) saline- or amphetamine (amph)-injected sham (sh) and OVX (ovx) females, administered with 0, 10, 50, or 150 μg/kg of 17β-estradiol (oil, E 10 μg/kg, E 50 μg/kg, or E 150 μg/kg, respectively). Asterisks indicate a significant difference between the PE and NPE groups, namely, presence of LI (*$p < 0.05$; **$p < 0.01$).
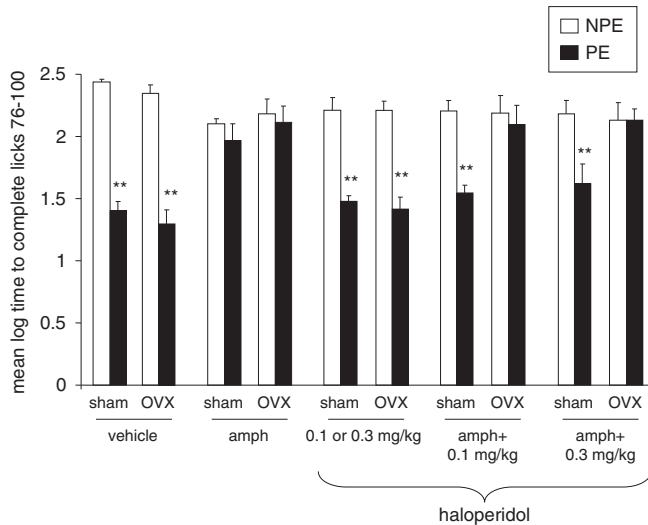
Figure 4 Effects of haloperidol (0.1, 0.3 mg/kg) on amphetamine-induced LI disruption in sham and OVX rats. Mean (± SEM) log times to complete licks 76–100 (after tone onset) of the pre-exposed (PE) and the non-pre-exposed (NPE) saline- or amphetamine (amph)-injected sham and OVX rats that received haloperidol (0.1 or 0.3 mg/kg to half of the group), amphetamine + 0.1 mg/kg haloperidol, or amphetamine + 0.3 mg/kg haloperidol. Asterisks (**) indicate significant difference between the PE and NPE groups, namely, presence of LI ($p < 0.01$).

Figure 5 Effects of clozapine (5, 10 mg/kg) on amphetamine-induced LI disruption in sham and OVX rats. Mean (± SEM) log times to complete licks 76–100 (after tone onset) of the pre-exposed (PE) and the non-pre-exposed (NPE) saline- or amphetamine (amph)-injected sham and OVX rats that received clozapine (5 or 10 mg/kg to half of the group), amphetamine + 5 mg/kg clozapine, or amphetamine + 10 mg/kg clozapine. Asterisks (**) indicate significant difference between the PE and NPE groups, namely, presence of LI ($p < 0.01$).

Experiment 5: Effects of Clozapine (5, 10 mg/kg) on Amphetamine-Induced LI Disruption in Sham and OVX Rats

Figure 5 presents the mean log times to complete licks 76–100 (after tone onset) of the PE and NPE saline- or amphetamine-injected sham and OVX rats pretreated with 0, 5, or 10 mg/kg of clozapine. As can be seen, saline-injected sham and OVX rats exhibited LI whereas amphetamine-injected sham and OVX rats did not exhibit LI. LI was restored in amphetamine-injected sham rats given both doses of clozapine, whereas only the high dose was effective in amphetamine-injected OVX rats. On its own, clozapine spared LI.

ANOVA yielded significant main effects of pre-exposure ($F_{(1,131)} = 143.36$, $p < 0.001$), gonadal status ($F_{(1,131)} = 3.96$, $p < 0.05$), and treatment ($F_{(4,131)} = 13.84$, $p < 0.001$), as well as a significant pre-exposure × gonadal status × treatment interaction ($F_{(4,131)} = 2.93$, $p < 0.05$). Post hoc comparisons confirmed the presence of LI in saline-injected sham and OVX rats that received vehicle or clozapine, in amphetamine-injected sham rats that received both doses of clozapine, and in amphetamine-injected OVX rats that received the high dose of clozapine (p's < 0.01), but not in the other conditions.

Experiment 6: Effects of Coadministration of Haloperidol (0.1 mg/kg) and 17β-Estradiol (50 μg/kg) on Amphetamine-Induced LI Disruption in OVX Rats

Figure 6 presents the mean log times to complete licks 76–100 (after tone onset) of the PE and NPE saline- or amphetamine-injected OVX rats pretreated with vehicle, 0.1 mg/kg haloperidol, 50 μg/kg 17β-estradiol or haloperidol
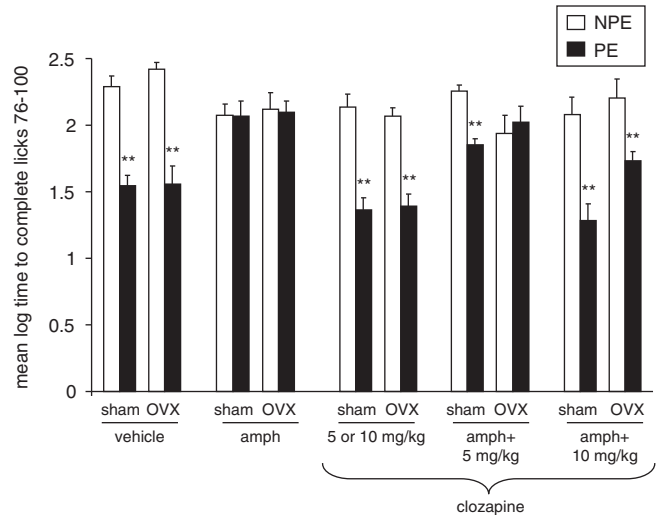
+ 17β-estradiol. As can be seen, vehicle-injected OVX rats exhibited LI whereas amphetamine-injected OVX rats did not exhibit LI. Administration of haloperidol or 17β-estradiol alone failed to restore LI in amphetamine-injected OVX rats, but LI was restored in rats that were coadministered with haloperidol and 17β-estradiol. On their own, haloperidol and 17β-estradiol spared LI. ANOVA yielded significant main effects of pre-exposure ($F_{(1,99)} = 126.53$, $p < 0.001$) and treatment ($F_{(1,99)} = 26.45$, $p < 0.001$), as well as a significant pre-exposure × treatment × pretreatment interaction ($F_{(3,99)} = 9.39$, $p < 0.001$). Post hoc comparisons confirmed the presence of LI in all four groups of saline-injected OVX rats, as well as in amphetamine-injected rats that received haloperidol + 17β-estradiol (p's < 0.01), but not in the other conditions.

Experiment 7: Effects of Coadministration of Clozapine (5 mg/kg) and 17β-Estradiol (50 μg/kg) on Amphetamine-Induced LI Disruption in OVX Rats

Figure 7 presents the mean log times to complete licks 76–100 (after tone onset) of the PE and NPE saline- or amphetamine-injected OVX rats pretreated with vehicle, 5 mg/kg clozapine, 50 μg/kg 17β-estradiol or clozapine + 17β-estradiol. As can be seen, vehicle-injected OVX rats exhibited LI whereas amphetamine-injected OVX rats did not exhibit LI. Administration of clozapine or 17β-estradiol alone failed to restore LI in amphetamine-injected OVX rats, but LI was restored in rats that were coadministered with clozapine and 17β-estradiol. On their own, clozapine and 17β-estradiol spared LI. ANOVA yielded significant main effects of pre-exposure ($F_{(1,100)} = 102.39$, $p < 0.001$), treatment ($F_{(1,99)} = 31.76$, $p < 0.001$), and pretreatment
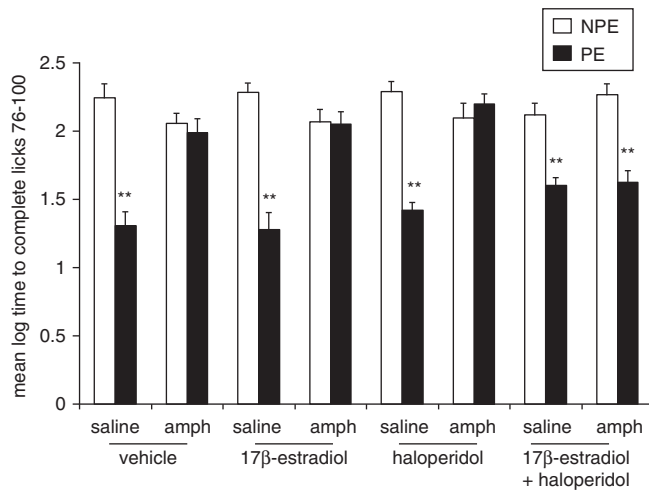
Figure 6 Effects of coadministration of haloperidol (0.1 mg/kg) and 17β-estradiol (50 μg/kg) on amphetamine-induced LI disruption in OVX rats. Mean (± SEM) log times to complete licks 76–100 (after tone onset) of the pre-exposed (PE) and the non-pre-exposed (NPE) saline- or amphetamine-(amph)-injected OVX rats administered with vehicle (vehicle), 50 μg/kg 17β-estradiol (17β-estradiol), 0.1 mg/kg haloperidol (haloperidol), or 17β-estradiol + haloperidol. Asterisks (**) indicate significant difference between the PE and NPE groups, namely, presence of LI ($p < 0.01$).

Figure 7 Effects of coadministration of clozapine (5 mg/kg) and 17β-estradiol (50 μg/kg) on amphetamine-induced LI disruption in OVX rats. Mean (± SEM) log times to complete licks 76–100 (after tone onset) of the pre-exposed (PE) and the non-pre-exposed (NPE) saline- or amphetamine-(amph)-injected OVX rats administered with vehicle (vehicle), 50 μg/kg (17β-estradiol), 5 mg/kg (clozapine), or 17β-estradiol + clozapine. Asterisks (**) indicate significant difference between the PE and NPE groups, namely, presence of LI $p < 0.01$.

($F_{(3,100)} = 3.38$, $p < 0.05$), as well as a significant pre-exposure × treatment × pretreatment interaction ($F_{(3,100)} = 6.21$, $p < 0.001$). *Post hoc* comparisons confirmed the presence of LI in all four groups of saline-injected OVX rats, as well as in amphetamine-injected rats that received clozapine + 17β-estradiol (p's < 0.01), but not in the other conditions.

DISCUSSION

In this study, we showed that (1) amphetamine disrupted LI in OVX rats as it did in sham-operated controls; (2) compared to sham controls, reversal of amphetamine-induced LI disruption in OVX rats required higher doses of 17β-estradiol and clozapine and was resistant to haloperidol; (3) coadministration of low dose of clozapine and haloperidol with an ineffective dose of 17β-estradiol restored LI in amphetamine-injected OVX rats; and (4) 17β-estradiol at medium and high doses reversed amphetamine-induced LI disruption in sham rats.

Compared to our previous study centered on OVX-induced disruption of LI (Arad and Weiner, 2009), in this work the LI protocol has been changed so that OVX no longer disrupted LI and an effect of amphetamine could be shown. In experiment 1, as in the work by Arad and Weiner (2009), no LI was evident in OVX rats if 40 pre-exposures were followed by 2 conditioning trials. However, raising the number of pre-exposures to 50 or reducing the number of conditioning trials to 1 led to emergence of LI in these rats. Nofrey *et al* (2008) have also found LI in OVX rats. The fact that OVX effects on LI can be influenced by changing procedural parameters indicates that hormonal level has no role in the acquisition of LI, but exerts a modulatory influence on its expression. Given the well-documented
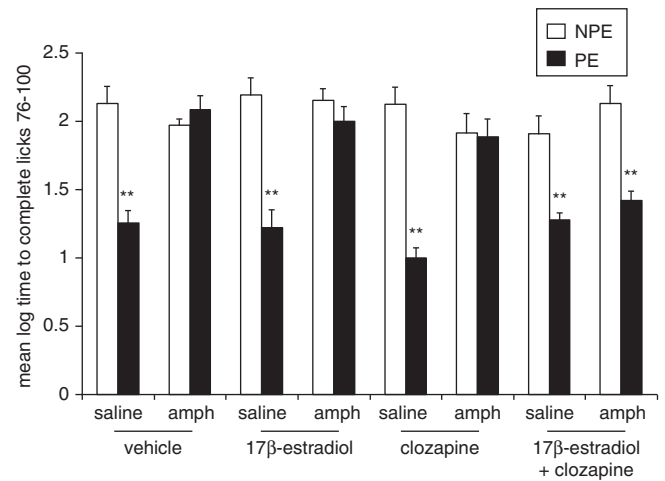
capacity of estrogen to modulate DA neurotransmission (Becker, 1999; Bourque *et al*, 2009; Chavez *et al*, 2010; Dluzen and Horstink, 2003; Hughes *et al*, 2009; Morissette *et al*, 2008), it is noteworthy that DA manipulations also exert only a modulatory influence on the expression of LI (Weiner, 2003).

Under conditions yielding LI in OVX rats, amphetamine disrupted LI as it did in sham controls. Disruption of LI reflects a selective attention deficit, whereby animals lose the capacity to ignore the irrelevant stimulus, and is also observed in amphetamine-treated humans, as well as in high-schizotypal humans (Braunstein-Bercovitz *et al*, 2002; Gray *et al*, 1992b; Salgado *et al*, 2000; Swerdlow *et al*, 2003; Thornton *et al*, 1996) and in acutely psychotic schizophrenia patients (Baruch *et al*, 1988; Gray *et al*, 1992a, 1995b; Rascle *et al*, 2001; but also see Swerdlow *et al*, 2005). A failure to inhibit attention to irrelevant stimuli is likely to give rise to aberrantly increased salience perception and distractibility that are associated with psychotic symptoms (Kapur *et al*, 2005; Weiner and Arad, 2009). These results show that unlike the reduced efficacy of APDs in OVX rats, the efficacy of amphetamine is not compromised by OVX. The latter suggests that the pro-psychotic action of amphetamine, and by extension, of increased dopaminergic function does not require estrogen. Similar results were reported with PPI. OVX spared PPI, which was disrupted by the DA agonist apomorphine (Gogos *et al*, 2009; Van den Buuse and Eikelis, 2001). PPI in OVX rats was disrupted also by the pro-psychotic NMDA antagonist MK-801. We have also found that MK-801 exerts the same effect on LI in OVX and sham rats (M Arad and I Weiner, unpublished data). Taken together, these results indicate that pro-psychotic drugs from different classes remain effective in OVX rats.

As shown repeatedly in the past using males (for review see Weiner, 2003; Weiner and Arad, 2009), amphetamine-

induced LI disruption in sham female rats was reversed here by both clozapine and haloperidol, at both doses used. However, both drugs were less effective in OVX rats. Clozapine was effective only at the higher 10 mg/kg dose, and haloperidol failed to restore LI at both the 0.1 and 0.3 mg/kg doses. These outcomes extend our previous demonstration of reduced APD efficacy in reversing LI disruption in pharmacologically nontreated OVX rats (Arad and Weiner, 2009). Moreover, LI disruption in amphetamine-treated OVX rats is more resistant to APDs because in the previous study, clozapine was effective at the 5 mg/kg dose. These results support our previous conclusion that OVX-induced loss of estrogen reduces the potency of APDs, and further indicate that loss of estrogen coupled with hyperdopaminergia aggravates the loss of APDs efficacy so that not only typical but also atypical APDs, which are considered more effective antipsychotics in general (Horacek et al, 2006; Stone and Pilowsky, 2006) and in women in particular (de Leon et al, 2004), lose their efficacy.

Failure of APDs to restore disrupted LI, and in particular the different efficacies of haloperidol and clozapine, in OVX rats, are of particular interest given that in male rats, reversal by both classes of APDs has characterized to date all known instances of LI disruption, be they induced by pharmacological (Barak and Weiner, 2007; Russig et al, 2003), brain lesion (Coutureau et al, 1999; Weiner et al, 1996a), neurodevelopmental (Zuckerman et al, 2003), or parametric (Killcross et al, 1994b; Shadach et al, 1999; Weiner et al, 1996b) manipulations. Thus, loss of estrogen in female rats appears to exert a unique and powerful interference with APD action.

Following our previous demonstration that concurrent 17β-estradiol treatment potentiated the action of APDs (Arad and Weiner, 2009), here we postulated that ineffective APD doses would also regain their capacity to block amphetamine-induced disrupted LI in the presence of estrogen. Indeed we found that coadministration of low haloperidol and low clozapine doses with an ineffective dose of 17β-estradiol restored the efficacy of haloperidol and clozapine, although each of the drugs alone (in these doses) did not restore LI. These results indicate that 17β-estradiol restores specifically the anti-psychotic (anti-amphetamine) action of APDs, both typical and atypical. The latter outcome is consistent with other reports that the behavioral effects of D2 antagonists in OVX rats are potentiated by coadministration of 17β-estradiol (Bedard et al, 1982; Daniel, 2006; De Ryck et al, 1982; Di Paolo et al, 1979, 1984; Nicoletti et al, 1983; Palermo-Neto and Dorce, 1990).

17β-Estradiol not only potentiated the anti-amphetamine action of APDs but also prevented amphetamine from disrupting LI when given on its own. Gogos et al (2009) recently found that chronic estradiol blocked disruption of PPI induced by apomorphine. Importantly, here 17β-estradiol blocked amphetamine effects in both OVX and sham rats. Furthermore, although the anti-amphetamine action was exerted in sham rats by both 50 and 150 µg/kg 17β-estradiol, in OVX rats only the highest dose of 150 µg/kg exerted such action. The fact that a higher 17β-estradiol dose was needed to counteract amphetamine action in OVX rats suggests that low level of hormones and hyperdopaminergia are synergistic, supporting the notion that OVX-induced hormonal reduction is pro-psychotic.

The low dose of 17β-estradiol (10 µg/kg), although having no effect on amphetamine-induced disruption in both sham and OVX rats, disrupted LI in both sham and OVX rats. Nofrey et al (2008) have also reported that under conditions in which OVX spared LI, 10 µg/kg of 17β-estradiol disrupted LI. Taken together, results by Nofrey et al (2008) and by us suggest that high doses of estradiol exert an antipsychotic action whereas low doses exert a pro-psychotic action.

Amphetamine-induced LI disruption and its reversal by APDs are mediated by increased DA release and blockade of DA transmission, respectively, within the nucleus accumbens (Gray et al, 1995a; Warburton et al, 1996; Weiner, 2003). Consequently, our results imply that acute high dose of estradiol, which blocked the effects of amphetamine and potentiated the effects of haloperidol on LI, reduced mesolimbic DA function, whereas low dose, which disrupted LI, increased DA release within the nucleus accumbens. Results consistent with both reduction and increase of striatal dopaminergic function by estradiol have been reported for all indices of dopaminergic activity, including receptor levels/binding, membrane dopamine transporter levels, and release, depending on dose and treatment paradigm (Arvin et al, 2000; Bazzett and Becker, 1994; Becker and Beer, 1986; Becker and Rudick, 1999; Di Paolo, 1994, 1982, 1984, 1985; Disshon et al, 1998; Disshon and Dluzen, 2000; Dluzen, 1997; Landry et al, 2002; McDermott, 1993; McDermott et al, 1994; Morissette et al, 2008; Morissette and Di Paolo, 1993; Peris et al, 1991; Shieh and Yang, 2008; Thompson and Moss, 1994; Zhou et al, 2002). It has been suggested that antidopaminergic effects are primarily exerted by high doses of estrogen or chronic administration, whereas pro-dopaminergic actions are more associated with lower physiological levels of estrogen (Barber et al, 1976; Becker, 1999; Bedard et al, 1977; Cyr et al, 2002; Di Paolo, 1994; Di Paolo et al, 1981; Hruska and Silbergeld, 1980; McEwen and Alves, 1999; Riddoch et al, 1971). The specific mechanisms by which estradiol exerts the effects observed here remain to be elucidated.

OVX had been proposed to model gonadal hormone withdrawal occurring at menopause (Bosse and Di Paolo, 1995; Le Saux and Di Paolo, 2006; Vaillancourt et al, 2002). In support of this notion, we showed previously that OVX disrupts LI and impairs the efficacy of APDs to restore LI in OVX rats, in line with reduced APD efficacy reported in menopausal women with schizophrenia (Arad and Weiner, 2009). In this study, we used a combination of reduced hormonal level induced by OVX and increased dopaminergic activity induced by amphetamine to more closely model what may be occurring during menopause in women vulnerable to psychosis. Using disrupted LI and its restoration by 17β-estradiol and APDs as a behavioral readout for this biological constellation, our results have extended and strengthened our previous findings and their implications for the often-debated relationship between hormonal level and vulnerability to psychosis.

First, cessation of hormones interferes with the anti-psychotic action of APDs as reflected in lowered efficacy to reverse amphetamine-induced LI disruption. Because the behavioral effects of amphetamine in general and amphetamine-induced LI disruption in particular are well-established models of psychosis, fortified by the capacity of amphetamine to induce and exacerbate psychosis in

healthy and schizophrenic humans as well as enhance striatal DA release in schizophrenia patients (Laruelle *et al*, 1996, 1999), reduced efficacy of APDs in blocking amphetamine-induced behavioral abnormality allows a strong conclusion that loss of estrogen reduces specifically the antipsychotic potency of APDs. This reduced anti-amphetamine efficacy of APDs on the background of low hormonal level provides a close parallel to reduced efficacy of APD treatment in women with schizophrenia during periods associated with low levels of hormones (Kulkarni *et al*, 1996; Saugstad, 1989; Seeman, 1989; Seeman and Lang, 1990).

Second, cessation of hormones may be synergistic with hyperdopaminergia induced by amphetamine. Although our data do not show directly that amphetamine is more efficacious in disrupting LI in OVX rats, indirectly the data indicate that the effects of amphetamine on LI in OVX rats were more potent than in sham rats. Thus, a three times higher dose of 17β-estradiol was needed to block the LI disruptive effect of amphetamine in OVX than the dose needed in sham rats; in addition, both low and high doses of haloperidol and clozapine reversed disrupted LI in sham and OVX rats, but only high dose of clozapine was effective in OVX rats injected with amphetamine. It remains to be shown directly that OVX rats are more sensitive to LI disruptive effects of amphetamine. Such increased sensitivity could be shown by proving that amphetamine is effective in disrupting LI in OVX rats under conditions at which it loses capacity to disrupt LI in normal rats (De la Casa *et al*, 1993; Killcross *et al*, 1994a), or that LI disruption in OVX rats can be achieved by lower amphetamine doses than in control rats. However, be it normal or higher compared to sham, the potency of amphetamine in OVX rats sharply contrasts with the loss of potency of APDs after OVX. Reduced sensitivity to dopaminergic blockade coupled with intact/increased sensitivity to increased DA transmission in OVX rats may provide clues as to how loss of estrogen exacerbates or triggers psychosis in vulnerable women.

Finally, our results show that 17β-estradiol can exert antipsychotic activity as reflected in reversal of amphetamine-induced LI disruption in OVX and sham rats. As detailed in the Introduction, it has been suggested that exogenous estrogen may have antipsychotic properties or increase response to APDs in women with schizophrenia. Although the latter has been supported in several studies (Agius *et al*, 2009; Akhondzadeh *et al*, 2003; Cyr *et al*, 2002; Korhonen *et al*, 1995; Kulkarni, 2009; Kulkarni *et al*, 1996, 2001, 2008a,; Lindamer *et al*, 2001; Mortimer, 2007; Rao and Kolsch, 2003), other studies reported that estrogen treatment failed to improve or even worsened symptoms (Bergemann *et al*, 2005; Chua *et al*, 2005; Gattaz *et al*, 1994; Lindamer *et al*, 2001; for review see Mortimer, 2007). We previously provided support for antipsychotic capacity of 17β-estradiol by showing a synergistic effect of ineffective doses of 17β-estradiol and APDs in reversing OVX-induced disrupted LI (Arad and Weiner, 2009), and have replicated such a synergistic effect here for amphetamine-induced disrupted LI. However, although a synergistic action between APDs and 17β-estradiol implies that APDs require a certain level of estrogen to be effective, such dependence does not confer 17β-estradiol a direct antipsychotic action. In contrast, the capacity of 17β-estradiol given on its own to reverse amphetamine-induced disrupted LI in OVX rats as well as in sham rats strongly supports a direct antipsychotic action of 17β-estradiol. We have recently found that 17β-estradiol also reverses amphetamine-induced disrupted LI in male rats (M Arad and I Weiner, unpublished data).

In summary, the differential sensitivity to blockade and enhancement of dopaminergic transmission after OVX as seen here, with reduced sensitivity to DA blockade and spared or potentiated sensitivity to DA stimulation, may provide a novel model of menopausal psychosis that is associated with low levels of estrogen along with hyperfunction of the dopaminergic system. This constellation appears to rather accurately model the combination of increased vulnerability to psychosis with reduced response to APD treatment in female patients during menopause (Hafner, 2003; Seeman, 1989). Such a model may have important implications for the clinical progression and treatment of schizophrenia in women. Regarding the latter, our data are clear in showing that estrogen exerts antipsychotic activity. Unfortunately, in OVX rats and by extension in menopausal women, very high doses of 17β-estradiol would be needed. However, our data do suggest that using physiological 17β-estradiol doses as an add-on treatment may augment APD efficacy, and in fact may be more effective than raising the dose of APD.

A gender focus on mental disorders has been rapidly emerging in the last few years kindled by the acknowledgment of salient gender differences in all major psychiatric disorders. Much effort still needs to be invested by both clinical and basic research to study the biological and psychological causes and impacts of psychiatric disorders in the female gender. The present model is a step in this direction.

DISCLOSURE

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