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Sex Differences in Aripiprazole Sensitization from Adolescence to Adulthood

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Abstract

The present study investigated the potential sex differences in repeated aripiprazole (ARI) treatment-induced behavioral sensitization from adolescence to adulthood, and to determine whether ARI sensitization can be transferred to olanzapine (OLZ) and/or clozapine (CLZ) using the conditioned avoidance response (CAR) and phencyclidine-induced (PCP) hyperlocomotion tests of antipsychotic activity. Male and female Sprague-Dawley adolescence rats (P46) were first treated with ARI (10 mg/kg) for 5 consecutive days (P46–50) and tested for avoidance response and ARI-induced inhibition of PCP-induced hyperlocomotion. After they became adults (>P68), rats were challenged with ARI (1.5 mg/kg, sc) (P70), OLZ (0.5 mg/kg, sc; P73), CLZ (5 mg/kg, sc; P76) and again with ARI (1.5 mg/kg, sc; P84) and tested for avoidance response and ARI-induced inhibition of PCP-induced hyperlocomotion again. During the drug treatment period in adolescence, repeated ARI treatment suppressed avoidance response, inhibited the PCP-induced hyperlocomotion, and these effects were progressively increased across the 5-day period in both males and females, confirming the induction of ARI sensitization. On the challenge days, rats previously treated with ARI in adolescence also had significantly lower avoidance and lower PCP-induced hyperlocomotion than the previous vehicle rats, confirming the expression of ARI sensitization and its persistence into adulthood. More importantly, female rats made significantly more avoidances than males in both ARI and vehicle groups, indicating higher sensitivity to the acute and long-term effects of ARI. Further, on the OLZ and CLZ challenge days, prior ARI treatment seemed to increase sensitivity to OLZ exposure, however, this increase was not significant. Similarly, rats also showed an ARI sensitization to OLZ and CLZ on challenge days. Collectively, results from this experiment demonstrated a sex difference in response to ARI and enhanced inhibition of PCP-induced hyperlocomotion in animals that were pretreated with ARI as compared to controls.

Keywords

Aripiprazole; sex difference; conditioned avoidance response; phencyclidine; sensitization; development

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1. Introduction

In recent years, there has been a significant increase in prescription rates for antipsychotics in adult males and females. Yet, most clinical studies have precluded females, thus much of the information available on the side effects and effectiveness of antipsychotics has been inferred from the effects found in males (Smith, 2010). Regardless, there have been some studies that have shown that sex differences in response to antipsychotic treatment exist, although not well understood. For example, it has been shown that females show increased sensitivity to the effects of antipsychotics (e.g. weight gain, type 2 diabetes, dyslipidemia, digestive, neurological/sensory symptoms and increased rates of side effects) as compared to men (Covell, Weissman, & Essock, 2004; Bigos et al., 2008). These differences are thought to be influenced by the bioavailability, distribution, metabolism, and/or excretion in the pharmacokinetics of drug response (Waxman, 2009). In fact, studies have demonstrated that sex differences in metabolism are thought to be the primary influence in response to antipsychotic treatment (Bigos et al, 2008; Seeman, 2004). For example, the main metabolizing enzyme (CYP1A2) of olanzapine is less active in females than males and it is thought to contribute to higher olanzapine and clozapine blood concentrations shown in females. This could help explain the incidence of increased severity of side effects as seen in females.

Another important factor when considering differences in antipsychotic response is the developmental period in which treatment begins. There has been a dramatic increase of antipsychotic prescription rates in children and adolescents in recent years to treat various mental disorders (e.g. schizophrenia, disruptive behavior disorder, autism, mood disorder) (Correl, 2008; Vitiello et al., 2009; Rani et al., 2008). Most (90%) of these children and adolescents are treated with atypical antipsychotic medication (e.g. risperidone, olanzapine and aripiprazole) for the management of these disorders (Olfson et al., 2006). Surprisingly, clinical research generally only focuses on the efficacy, tolerability, and side effect profiles of these drugs. However, there have been some preclinical studies that have strongly suggested that antipsychotic exposure during adolescence could alter brain and behavioral functions. For example, animal receptor binding studies show that antipsychotic exposure during adolescence increases or decreases various neuroreceptors, including various dopamine receptors (Qiao et al., 2014; Vinish et al., 2013), serotonin 5-HT1A/ 5-HT2A receptors (Choi et al., 2010), and ionotropic NMDA and AMPA glutamatergic receptors (Choi et al., 2009). Further, behavioral studies have demonstrated that early adolescent antipsychotic exposure enhances animals' sensitivity to reward stimuli (Vinish et al., 2013), impairs working memory, and delays the extinction process of fear memory in adulthood (Milstein et al., 2013). Consequently, due to the lack of research in this area, it is not well understood the long-term consequences that antipsychotic treatment will have on an immature developing brain.

Importantly, the long-term consequences should be of concern as most individuals regardless of sex or developmental age typically continue antipsychotic treatment throughout their lifetime (Harrow et al., 2012). Studies have shown that neurotransmitter release, changes in neuroreceptor levels, receptor-mediated second messenger activities, cell electrophysiology, and behaviors can be affected by antipsychotic treatment (Gao, Qin, & Li, 2015). These

changes can result in either an augmentation (sensitization) or decrease (tolerance) of the effects of the drug. For example, low doses of risperidone and olanzapine have been shown to be effective in the treatment of acute psychotic symptoms (Arango et al., 2004; Sikich et al., 2004), while haloperidol-induced sensitization has been associated with the development of extrapyramidal motor effects (Turrone et al., 2005), and increased dopamine sensitivity (Samaha et al., 2007). A critical issue associated with chronic long- term administration of antipsychotic drugs is the potential for changes in the acute effects over time. Moreover, it is likely that these changes are biological and developmentally mediated and thus will impact behavioral and neurochemical response to antipsychotics. For example, previous work in our laboratory has shown that repeated aripiprazole treatment disrupted avoidance responding and inhibition of PCP-induced hyperlocomotion, demonstrating induced sensitization behavioral effects (Gao & Li, 2015). Undoubtedly, these results emphasize the need for more research designed to examine the impact of chronic administration of antipsychotic drugs and likely sex and developmental differences that would impact overall efficacy of treatment. In addition, efficacy of treatment is directly affected by compliance of treatment that is most often mediated by severity of side effects reported. Consequently, the noted increases in prescription rate in adolescents and adults have been attributed to the availability of new antipsychotics with fewer extrapyramidal side effects (Cooper et al, 2006) and greater efficacy for broader target symptoms (Buckley, 2001), ultimately improving the potential for compliance (Dolder et al., 2002; Menzin et al., 2003).

One such new antipsychotic drug available is Aripiprazole (ARI), a third-generation antipsychotic drug, with demonstrated improved extrapyramidal side-effects compared with first generation drugs such as haloperidol and lessened metabolic effects compared with second generation drugs such as olanzapine (Khanna et al, 2014). The reduction in harmful side effects may be due in part to the mechanisms of aripiprazole, although the exact mechanisms remain unclear (Pan et al., 2015). For example, aripiprazole is a partial dopamine D2 receptor agonist, which in part may work to normalize dopamine activity. This may be accomplished by the drugs unique high affinity for dopamine D2 receptors but only as a partial agonist and not a full antagonist. Consequently, at D2 receptor sites where dopaminergic transmission is decreased aripiprazole acts as an agonist. However, at dopaminergic sites of normal or increased transmission, it functions as a stabilizer (Aihara et al., 2004; Shapiro et al., 2003; Burris et al., 2002). In addition, chronic administration of ARI has been shown to be brain region dependent (Pan et al., 2016). Clearly, this may help delineate ARI unique clinical profile and effects. Regardless, these findings suggest drug specificity in antipsychotic drug sensitization and tolerance and demonstrate a clear need to further examine this phenomenon.

The present study investigated this phenomenon by examining the long-term consequences of ARI sensitization in male and female adolescent rats, sex differences in ARI sensitization, and whether ARI sensitization can be transferred to OLZ and/or CLZ, using the conditioned avoidance (CAR) model and the PCP-induced hyperlocomotion model. This paradigm has been validated in previously conditioned place avoidance (CAR) and PCP-induced hyperlocomotion work. For example, repeated administration of ARI produced a sensitization effect in normal adult male rats in the CAR model (Gao et al., 2015). Additionally, it has been shown that repeated OLZ treatment causes sensitization, whereas

repeated CLZ treatment causes tolerance (a decreased disruption of avoidance response) in both adolescent and adult rats (Shu et al., 2014). However, it is unclear whether long-term sensitization can be induced in adolescent rats in both sexes.

2. Methods

2.1 Animals

Adolescent male and female Sprague-Dawley rats (51–75 g upon arrival, Charles River, Portage, Michigan, USA) were housed two per cage, in transparent polycarbonate cages (48.3×26.7×20.3) with food and water available ad libitum, and all animals were maintained on a 12:12 on/off/light/dark cycle. All behavioral testing occurred during the light cycle. All procedures were approved by the University of Nebraska-Lincoln Committee on Animal Care which is consistent with the NIH Guide on Care and Use of Animals.

2.2 Drugs and choice of doses

Aripiprazole (gift from the National Institute of Mental Health drug supply program) was dissolved in a mixed double-distilled water solution containing 30% (v/v) dimethylformamide and 1% (v/v) glacial acetic acid. The dose of aripiprazole (10 mg/kg) was determined based on previous studies in our lab (Gao et al., 2015; Li et al., 2005) and reports in the literature (Carli et al., 2011; Cosi et al., 2006; Li et al., 2004). This dose of aripiprazole was chosen because it results in 85% occupancy, respectively, at one hour post-injection (Natesan et al., 2006), but does not cause catalepsy (Hirose et al., 2004). Importantly, this chosen dose provides animals receptor occupancies that are comparable to observed levels (65–70% occupancy) seen in the clinical population (Kapur et al., 2003). The dose of PCP has been shown in previous studies (Gleason & Shannon, 1997; Kalinichev et al., 2008) to induce a robust hyperlocomotion effect without causing extreme stereotypical behavior. All drugs were administered subcutaneously (sc) at 1.0 ml/kg. OLZ and CLZ (gifts from the National Institute of Mental Health and drug supply program) was dissolved in distilled sterile water with 1% glacial acetic acid. One dose of OLZ (.5 mg.kg) and one dose of CLZ (5 mg/kg) were tested. It has been demonstrated that repeated OLZ treatment causes sensitization, whereas repeated CLZ causes tolerance in both adolescent and adult rats (Gao, Qin & Li, 2015). These doses were tested to determine how ARI sensitization would affect OLZ and CLZ exposure.

2.3 Two-way Avoidance Conditioning Apparatus

Eight identical two-way shuttle boxes custom-designed and manufactured by Med Associates (St Albans, VT) were used. Each box was housed in a ventilated, sound-insulated isolation cubicle (96.52 cm W x 35.56 cm D x 63.5 cm H). Each box was 64 cm long, 30 cm high (from grid floor), and 24 cm wide, and was divided into two equal-sized compartments by partition with an arch style doorway (15 cm high x 9 cm wide at base). A barrier (4 cm high) was placed between two compartments, which allowed the rats to jump from one compartment to the other. The grid floor consisted of 40 stainless-steel rods with a diameter of 0.48 cm, spaced 1.6 cm apart center to center. A scrambled footshock (unconditioned stimulus, US, 0.8 mA, maximum duration: 5 s) was delivered by a constant current shock generator (Model ENV-410B) and scrambler (Model ENV-412) through the grid floor. All

rats location and crossings within the boxes was monitored via a set of 16 photobeams (ENV-256-8P) affixed at the bottom of each box (3.5 cm above the grid floor). Illumination was provided by two houselights mounted at the top of each compartment. The conditioned stimulus (CS; 76 dB white noise) was produced by a speaker (ENV 224 AMX) mounted on the ceiling of the cubicle, centered above the shuttle box. Background noise (74 dB) was provided by a ventilation fan affixed at the top corner of each isolation cubicle. All training and testing procedures were controlled by Med Associates programs running on a computer.

2.4 Locomotor Apparatus

Sixteen identical motor activity monitoring boxes (48.3 x 26.7 x 20.3 cm transparent polycarbonate cages) equipped with a row of six photocell beams (7.8 cm between two adjacent photobeams) placed 3.2 cm above the floor of the cage. A computer with recording software (Aero Apparatus Six-beam Locomotor System v1.4, Toronto, Canada) was used to detect the disruption of the photocell beams and recorded the number of beam breaks.

2.5 ARI sensitization induced in adolescence and assessed in adolescence

We examined the long-term consequences of ARI sensitization in male and female adolescent rats, and whether ARI sensitization can be transferred to OLZ and/or CLZ, using the conditioned avoidance (CAR) model (Table 1 for the experimental design) and PCP-induced hyperlocomotion model (Table 2 for experimental design). The CAR model consisted of three phases: avoidance training, induction of ARI sensitization, and sensitization assessment. The PCP model consisted of two phases: induction and expression.

2.5.1 Avoidance training—Thirty-four rats (P46) were habituated (P46–47) to the CAR boxes for 2 days (30 min/day) and then trained (30 trials) for conditioned avoidance responding for 8 consecutive days. A white noise (CS) was presented at the beginning of every trial for a period of 10 s, followed by a continuous scrambled footshock (0.8 mA, US, maximum duration = 5 s) on the grid floor. If the rat traveled from one compartment to the other within 10 s of CS presentation, the rat avoided shock, and this shuttling response was recorded as ‘avoidance’. If the rat remained in the same compartment for more than 10 s and then traveled into the other compartment upon receiving the footshock, this response was recorded as ‘escape’. If the rat did not respond during the entire (5 s) presentation of the shock, the trial was then terminated and recorded as ‘escape failure’. The total number of avoidance responses was recorded for each trial.

2.5.2 Induction of ARI sensitization—At the end of the training trial (P45), rats were first matched based on avoidance performance on the last training day (ie, predrug) in order to create blocks of rats (n = 3 rats/block) that were approximately equal in performance. Within each block, they were then randomly assigned to one of four groups: male, vehicle (n=7), male, ARI (ARI 10 mg/kg; n = 9), female, vehicle (n = 9), female, ARI (ARI 10 mg/kg; n = 9), and tested daily (5 days) for avoidance response. The CS-only (no shock, 30 trials) condition was used to eliminate any possible relearning effect that would be caused by the presence of the US. Before each trial began, all rats were injected with either ARI or vehicle and placed in the CAR boxes one hour after injection. The total number of avoidance responses was recorded for each trial.

2.5.3 Sensitization assessment—All rats were retrained drug-free for 1 day under the CS-only (no shock, 30 trials; P68), and for 1 day under the CS-US (shock, 30 trials; P69) condition to ensure all groups had a comparable levels of avoidance responding before the sensitization assessment. After two retraining sessions, all rats were challenged with ARI (1.5 mg/kg) on P70, OLZ (0.5 mg/kg) on P73, CLZ (5 mg/kg) on P76 and again with ARI (1.5 mg/kg) on P84, placed in CAR boxes 1 hour after injection, and tested for avoidance performance in the CS-only (no shock, 30 trials) condition. This procedure of using a lower challenge dose of drug has been successfully used in previous studies in our lab (Li et al, 2012; Sparkman & Li, 2012; Swalve & Li, 2012; Zhang & Li, 2012). Further, a lower challenge dose avoids the floor effect because a high dose may interrupt avoidance response by causing a maximal avoidance disruption, preventing demonstration of a sensitization or tolerance effect.

2.5.4 Induction phase—Forty-eight male and female adolescent rats were randomly assigned to one of four groups: VEH + VEH (vehicle + saline, n=12, 6M & 6F); VEH + PCP (vehicle + PCP 3.20 mg/kg, n=12, 6M & 6F); ARI +PCP (ARI 10.00 mg/kg + PCP 3.20 mg/kg, n=12, 6M & 6F); ARI + VEH (ARI 10.00 mg/kg +saline, n=12, 6M & 6F). All rats were first handled (P38–40) and then habituated (P41–42) to the locomotor activity apparatus for two days (30 min/day). On the second day of habituation, all animals were first injected with saline and immediately placed in the boxes for 30 min. Locomotor activity (number of photobeam breaks) was measured at 5 min intervals throughout the entire 30-min testing session. On each of the next five consecutive days (P43–47), all animals were first injected with either vehicle (30% (v/v) dimethylformamide and 1% (v/v) glacial acetic acid in water), or ARI 10 mg/kg and then immediately placed into the locomotor boxes for 30 min. At the end of the 30-minute period, all animals were taken out and injected with vehicle (saline) or PCP (3.20 mg/kg, sc) and placed back into the boxes for a period of 60 min. Locomotor activity (number of photobeam breaks) was measured at 5 min intervals throughout the entire 90-min testing session.

2.5.5 Expression phase (Challenge Tests)—On P75, all rats were rehabituated drug-free to the locomotor activity apparatus. One day later (P76), all rats were injected (sc) with ARI (3.0 mg/kg) and then immediately placed in the locomotor boxes for 30 min. At the end of the 30-min period, all animals were removed from boxes and injected (sc) with PCP (3.20 mg/kg) and placed back in the boxes for another 60 min. All animals rested for one day (P77), and on days P78–79 were re-habituated to the locomotor boxes. On P80, all animals were injected (sc) with OLZ (.5 mg/kg) and then immediately placed in the locomotor boxes for 30 min. At the end of the 30-min period, all animals were taken out and injected (sc) with PCP (3.20 mg/kg) and placed in the boxes for 60 min. All animals rested for one day (P81), and on days P82–83 were re-habituated to the locomotor boxes. On P84, all animals were injected (sc) with CLZ (5 mg/kg) and immediately placed in the locomotor boxes for 30 min. At the end of the 30-min period, all animals were removed from boxes and injected (sc) with PCP (3.20 mg/kg) and placed back in the boxes for a period of 60 min. All animals rested for one day (P85), and on days P86–87 were re-habituated to the locomotor boxed for 30 min. On P88, all animals were injected (sc) with ARI (3.0 mg/kg) and immediately placed in the locomotor boxes for 30 min. At the end of the 30-min period, all animals were

removed from boxes and injected with PCP (3.20 mg/kg) and placed back in the boxes for a period of 60 min.

2.6 Statistical Analysis

All data were expressed as mean + SEM. Avoidance data from the five drug test sessions were analyzed using a factorial repeated measures analysis of variance (ANOVA) with the between-subjects factor being drug group and the within-subjects factor being test day, followed by *post hoc* LSD tests. Data from the retraining/predrug days and from the challenge test days were analyzed by a one-way ANOVA. Motor activity data from the five drug test days were analyzed using a factorial ANOVA with the between-subjects factor being the drug group and the within-subjects factor being the test day, followed by *post hoc* LSD test to examine group difference. Data from the challenge tests were analyzed by one-way ANOVA. All analysis were conducted using IBM SPSS Statistics 22, and $p<.05$ was considered statistically significant.

3. Results

3.1 Avoidance response

Figure 1(a) shows the number of avoidance responses on the last day of training (predrug) and five drug test days. There was no group difference on the last training day. ARI disrupted avoidance responding consistently throughout the training days. Repeated measures ANOVA revealed a main effect of sex, $F(1,30)=13.350$, $p<.001$, a main effect of drug, $F(1,30)=113.29$, $p<.001$, and a main effect of days, $F(1,30)=12.22$, $p<.001$. *Post hoc* LSD tests revealed that the two ARI groups differed significantly from the VEH groups, all $p < .001$, with females demonstrating reduced sensitivity to ARI as compared to males.

To analyze avoidance response a repeated measures day * number of avoidances ANOVA was used. On the ARI challenge day, there was a main effect of drug, $F(1,30)=16.94$, $p<.001$ and a main effect of sex, $F(1,30)=23.90$, $p<.01$. Both male and female rats previously treated with ARI had significantly lower avoidance than the vehicle group, but females made significantly more avoidances than males in both ARI and vehicle groups.

3.1.2 ARI Sensitization in CAR—All rats were challenged with OLZ and CLZ in adulthood. Prior ARI treatment seemed to increase sensitivity to OLZ exposure; however, the increase was not significantly significant. $F(1,30)=2.79$, $p=.107$. There was a main effect for sex, $F(1,30)=5.79$, $p=.023$. Prior ARI treatment did not alter CLZ exposure. Both CLZ and vehicle groups exhibited a similar number of avoidance in each sex. $F(1,30)=.242$, $p=.626$. There was a main effect of sex, $F(1,30)=13.57$, $p=.001$. Collectively, the above results demonstrate that both male and female rats previously treated with ARI had significantly lower avoidance than the vehicle group, but females made significantly more avoidances than males in both ARI and vehicle groups. However, ARI sensitization induced in adolescence persists into adulthood but this effect is not transferrable to OLZ and CLZ.

3.1.3 ARI sensitization in the PCP-induced hyperlocomotion test—Figure 2(a,c) shows the mean motor activity of the four groups of rats for the first 30-minute test before

PCP or vehicle injection throughout the five days of drug testing. During the first 30 min time block repeated measures ANOVA revealed a significant main effect of sex ($F(1,40)=7.139$, $p < .05$)), ARI ($F(1, 40)=19.637$, $p < .01$) and PCP ($F(1, 40)=12.335$, $p < .01$). *Post hoc* LSD test revealed there was significantly lower motor activity in the female and male ARI + PCP (***) and ARI + SAL (**) groups as compared to the VEH + SAL and VEH + PCP groups (groups (all p*s* <.05).

Figure 2(b,d) shows the mean motor activity during the 60-minute test period after PCP or vehicle injection. A two-way repeated measures ANOVA revealed a significant main effect of sex ($F(1,40)=4.492$, *$p < .05$), ARI ($F(1, 40)=60.705$, *$p < .01$) and PCP ($F(1, 40)=75.849$, $p < .001$). There was a significant interaction effect of ARI * PCP ($F(1, 40)=31.039$, *$p < .001$). During the drug treatment period, repeated ARI treatment inhibited the PCP-induced hyperlocomotion, and this inhibition was progressively increased across the 5-day period in both males and females, suggesting a sensitization effect.

On the re-habituation day (Figure 3(a)), a one-way ANOVA revealed a main effect of sex $F(1,40)=4.49$, $p < .05$. *Post hoc* LSD revealed there was significantly higher motor activity in the female VEH + SAL group as compared to all other groups. There were no interaction effects.

3.1.4 ARI Challenge—Figure 3(b) shows the mean motor activity of the four groups of rats for the first 30-minute test before PCP or vehicle injection. A one-way ANOVA revealed a main effect of sex $F(1,40)=5.490$, $p < .05$) and ARI ($F(1, 40)=28.312$, $p < .001$). The ARI + PCP (**$p < .001$) and ARI + SAL (**$p < .001$) demonstrated significantly less motor activity than the VEH + SAL and VEH + PCP groups. Figure 3(c) shows the mean motor activity during the 60-minute test period after PCP or vehicle injection. In the 60-minute test period after PCP injection, a one-way ANOVA revealed main effect of ARI $F(1,40)=36.597$, $p < .001$) and PCP $F(1, 40)=43.609$, $p < .001$). There was a significant interaction effect of ARI* PCP ($F(1, 40)=13.780$, $p=.001$). *Post-hoc* LSD tests revealed that the ARI + PCP (**$p < .001$) and the ARI + SAL (**$p < .001$) had significantly lower motor activity than controls. These results suggest that rats previously treated with ARI showed a stronger inhibition of PCP-induced hyperlocomotion (i.e. sensitization) than those previously treated with vehicle.

3.1.5 OLZ Challenge—On the rehabituation day (Figure 4(a)), a one-way ANOVA revealed a main effect of sex $F(1,40)=5.25$, $p < .05$ and a main effect of ARI $F(1,40)=5.10$, $p < .05$). *Post hoc* LSD revealed that females in the ARI + PCP group (**$p < .001$) had significantly higher motor activity than all other groups, and males in the VEH + PCP group (* $p < .05$) had significantly lower motor activity than all other groups. There were no interaction effects.

Figure 4(b) shows the mean motor activity of the four groups of rats for the first 30-minute test before PCP or vehicle injection. In the first 30 min, a one-way ANOVA revealed a main effect of ARI ($F(1, 40)=6.762$, $p < .05$). The ARI + PCP (**$p < .001$) and ARI + SAL (**$p < .001$) demonstrated significantly less motor activity than the VEH + PCP (*) group. Figure 5(c) shows the mean motor activity of the four groups of rats for the 60-minute test after

PCP or vehicle injection. A one-way ANOVA revealed main effect of sex (F(1, 40)=4.12, **p < .05), ARI (F(1, 40)=37.510, **p < .001), and PCP (F(1, 40)=20.017, **p < .001). There was a significant interaction effect of ARI * PCP (F(1, 40)=20.017, p < .01). *Post-hoc* LSD tests show that females in the VEH + SAL group (*p < .001) had significantly higher motor activity as compared to males and females in all other groups.

3.1.6 CLZ Challenge—On the rehabituation day (Figure 5(a)), a one-way ANOVA revealed a main effect of PCP F(1, 40)=5.26, p < .05. *Post-hoc* LSD tests revealed that the VEH + PCP and ARI + PCP groups demonstrated significantly overall motor activity than all other groups.

Figure 5(b) shows the mean motor activity of the four groups of rats for the first 30-minute test before PCP or vehicle injection. In the first 30 min, a one-way ANOVA revealed a significant main effect of sex F(1, 40)=5.252, *p < .05), ARI (F(1, 40)= 4.364, *p < .05), and PCP F(1, 40)=4.476, *p < .05). *Post-hoc* LSD tests revealed that females in the ARI + PCP group (*p*s* < .001) demonstrated significantly higher motor activity than males. Figure 6(c) shows the mean motor activity of the four groups of rats for the 60-minute test after PCP or vehicle injection. A one-way ANOVA revealed a main effect for ARI (F(1,40)=29.573. **p < .004), PCP (F(1, 40)=65.895, **p < .001, and significant interaction effects of Sex*ARI (F(1,40)= *p < .05), ARI*PCP (F(1,40)=21.746, p < .001), and Sex*ARI*PCP F(1,40)=8.729, p < .005). *Post-hoc* LSD tests revealed that females in both the ARI + PCP (p < .001) and ARI + SAL (p < .001) groups had significantly higher motor activity counts than did males.

3.1.7 ARI challenge (second challenge)—On the rehabituation day (Figure 6(a)), a one-way ANOVA revealed a main effect of sex F(1,40)=11.65, p = .001. *Post-hoc* LSD tests revealed males in the VEH + SAL group *p < .001) had significantly higher motor activity accounts than did females. There were no significant interaction effects.

Figure 6(b) shows the mean motor activity of the four groups of rats for the first 30-minute test before PCP or vehicle injection. In the first 30 min, a one-way ANOVA revealed a significant main effect of ARI F(1,40)=24.28, p < .001. *Post hoc* LSD tests revealed that the ARI + SAL and ARI + PCP groups had significantly less overall motor activity compared to all other groups. Figure 7(c) shows the mean motor activity of the four groups of rats for the 60-minute test after PCP or vehicle injection. A one-way ANOVA revealed a main effect for ARI F(1,40)=26.5, **p < .001, PCP F(1,40)=62.03, ***p < .001 and a significant interaction effect of ARI * PCP F(1, 40), p = .003. *Post-hoc* LSD tests revealed that both males and females in the ARI + SAL and ARI + PCP groups had significantly lower motor activity than all other groups.

Collectively, results from this experiment demonstrated a sex difference in response to ARI and enhanced inhibition of PCP-induced hyperlocomotion in the animals that were pretreated with ARI as compared to controls. Specifically, it appears that ARI reduces PCP-induced increases in locomotor activity and this effect appears to be more robust in males.

4. Discussion

ARI is a fairly new antipsychotic drug with a pharmacological profile that is not shared among more conventional and atypical antipsychotics (Mamo et al., 2007). While the effectiveness of ARI on psychosis has been demonstrated in both humans (Takahata et al., 2012) and animal models (Carli et al., 2011), there has been very little research to access the long-term effect (i.e. sensitization or tolerance). Further, while there have been some studies examining the sex differences in pharmacokinetics, pharmacodynamics, receptors and transporters, it remains largely uncharacterized.

In the present study, we demonstrated significant long-term behavioral changes and a sex difference response induced by repeated ARI drug treatment during adolescence, across both the CAR model and PCP-induced hyperlocomotor model. In the CAR model, previous research has shown that when ARI (10 and 30 mg/kg) is administered in acute dosing, significant suppression of conditioned avoidance response in rats occurs (Natesan et al., 2006). The present study extended these findings to show that repeated administration of ARI progressively increased the disruption of avoidance response in both males and females, suggesting a sensitization effect. Specifically, rats that had prior ARI exposure had a significant lower avoidance than vehicle rats. Interestingly, it appears that despite its novel mechanisms, ARI shares an induced sensitization effect similar to other atypical antipsychotics (e.g. olanzapine, risperdone). Therefore, it was important to examine whether ARI induced sensitization in the CAR model could be generalized and whether sex differences observed in the CAR model would be observed in the PCP model as this model is commonly used to detect antipsychotic activity.

In the PCP model, we showed that ARI treatment during adolescence induced a sensitization effect that remained into adulthood, a full 41 days after the last drug treatment, suggesting a long-lasting effect. This was expressed as an enhanced inhibition of PCP-induced hyperlocomotion (a validated measure of antipsychotic activity) and a sex difference response to ARI in animals that were pretreated with ARI as compared to control animals. During the drug treatment period, repeated ARI treatment inhibited the PCP-induced hyperlocomotion, and this inhibition was progressively increased across the 5-day period in both males and females, suggesting a sensitization effect. In regards to sex differences, females demonstrated significant increased motor activity as compared to males. On the challenge day, rats previously treated with ARI showed a stronger inhibition of PCP-induced hyperlocomotion (i.e. sensitization) than those previously treated with vehicle. Similarly, rats also demonstrated a sensitization to OLZ and CLZ on challenge days.

Sex differences were seen in the sensitization effect of ARI that manifested as the progressively enhanced disruption of avoidance in the CAR model and enhanced inhibition of PCP-induced hyperlocomotion during the induction phase and an enhanced sensitivity to ARI challenge in the expression phase (Shu, Hu, & Li, 2014; Qiao, Li, & Li, 2012; Swalve & Li, 2012). This effect was demonstrated in both male and female rats previously treated with ARI in the CAR and PCP models. Specifically, in CAR female rats made significantly more avoidances than male rats in both ARI and vehicle groups. Prior ARI treatment seemed to increase sensitivity to OLZ and CLZ exposure, however, this increase was not significant.

Therefore, it would appear that the effect of ARI sensitization induced in adolescence that persisted into adulthood was not transferrable to OLZ or CLZ in the CAR model. However, this was not the case in the PCP model; rats that had been previously treated with ARI did show a stronger inhibition of PCP-induced hyperlocomotor (i.e. sensitization).

We demonstrated that antipsychotic exposure during adolescence can engender long-lasting changes in the behavioral development of animals. These changes manifest in alterations of the behavioral response of antipsychotics in either an increase or decrease in sensitization response to antipsychotics in adulthood. In addition, a while it remains unclear the mechanisms underlying sex differences in response to antipsychotic treatment, there have been a few studies examining the pharmacodynamic difference in these effects associated with developmental age. For example, adult rats have shown age related functional changes in dopamine receptors which can attenuate brain serum levels (Pizzolato et al., 1985). Taken in contrast with adolescence which represents a developmental time of pruning and re-organization of the dopamine system, it is possible that a similar process could play a role in the sex differences observed here. For example, both atypical and typical antipsychotics block dopamine D2 receptors resulting in positively correlated clinical potency (Aihara et al., 2004). Therefore, it could be concluded that ongoing developmental changes in dopamine receptors result in age and sex difference response to antipsychotic treatment. Clearly, more research is needed to examine the underlying mechanisms involved.

5. Conclusions

Most adolescents and children who have been diagnosed with schizophrenia and/or other disorders will typically require treatment with antipsychotics throughout their lifetime regardless of sex (Alanen, Finne-Soveri, & Leinonen, 2008). Long-term antipsychotic treatment induces drug sensitization, enhancing the behavioral effects of the drug. While this in itself is considered a behavioral mechanism underlying the therapeutic effects of antipsychotic treatment (Kapur et al., 2006), it has also been demonstrated to underlie the drug-induced extrapyramidal motor syndrome and tardive dyskinesia observed in long-term antipsychotic treatment (Turrone et al., 2005). ARI is rapidly becoming more readily prescribed to treat schizophrenia, schizoaffective disorder and many other disorders in adolescents and children due to the effectiveness and reported less severe drug side effects (Docherty et al., 2010; Samaha et al., 2007; Takashi et al., 2009; Corell et al., 2011). While this leads to increased medication compliance, ARI does appear to share a similar behavioral profile with other antipsychotic drugs (e.g. OLZ, risperidone, asenapine). For example, there have been many studies that have shown repeated treatment with antipsychotics induced a sensitization and tolerance effect across several behavior domains (Shu, Hu, & Li, 2014). One important mechanism that is potentially involved in the sensitization effect is possible functional changes in the dopamine D2/D3 system. This should be of a concern because adolescence is a developmental period in which the dopamine system has not yet reached full maturity, yet most treatment with antipsychotics for adolescents and children begin during this developmental period. We do not understand fully how chronic antipsychotic treatment affects the immature dopamine system. Moreover, it is likely that biological differences in males and females will impact their behavioral and neurochemical response to antipsychotics, thereby effectively having a direct consequence of the efficacy of treatment.

From this clinical perspective, it is important that more research is conducted to examine aripiprazole sensitization, associated sex differences and the underlying neurobiological mechanisms that may be involved in antipsychotic treatment response.

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Highlights

- Sex differences exist in response to adolescent ARI treatment into adulthood.
- Adolescent ARI treatment enhances inhibition of PCP-induced hyperlocomotion.
- ARI reduces PCP-induced increases in motor activity with more robust effects in males as compared to females

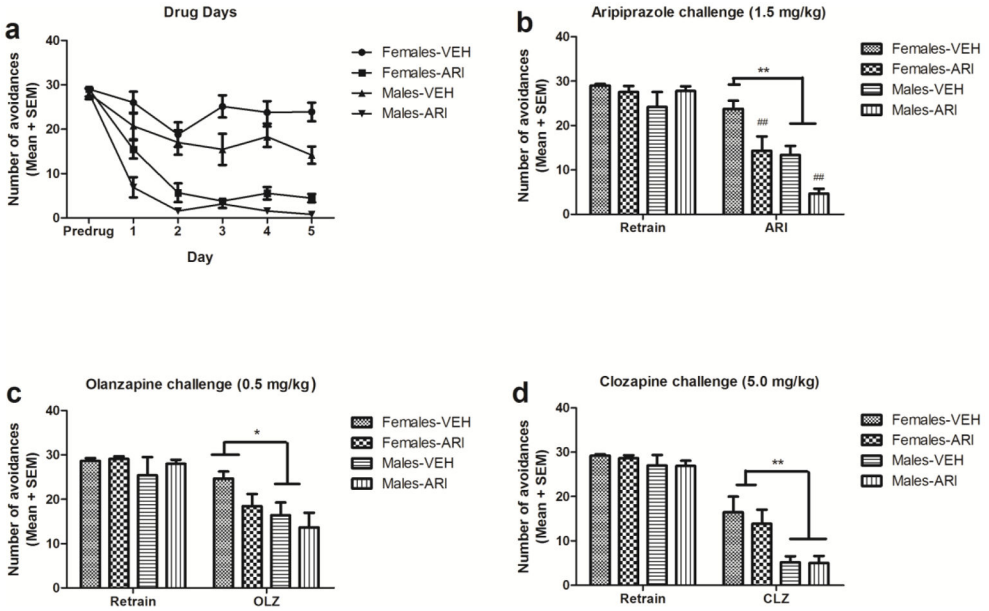

Fig. 1.

ARI sensitization induced in adolescence and assessed in adolescence. Number of avoidance responses (a) made by the rats from ARI (10 mg/kg) and vehicle (30% (v/v) dimethylformamide and 1% (v/v) glacial acetic acid in water) groups on the last training (predrug) day and the 5 test days. Avoidance responses are expressed as mean + SEM. The same measure on the second retraining (predrug) day and the ARI (1.5 mg/kg), OLZ (.5 mg/kg) and CLZ (5 mg/kg) sensitization assessment day are also expressed as mean + SEM and depicted in b, c, and d with $p < .05$ relative for all groups. Astericks (*) indicates $p < .05$, double astericks (**) indicates $p < 0.01$, and hashtag (#) indicates significant one-way ANOVA for sex.

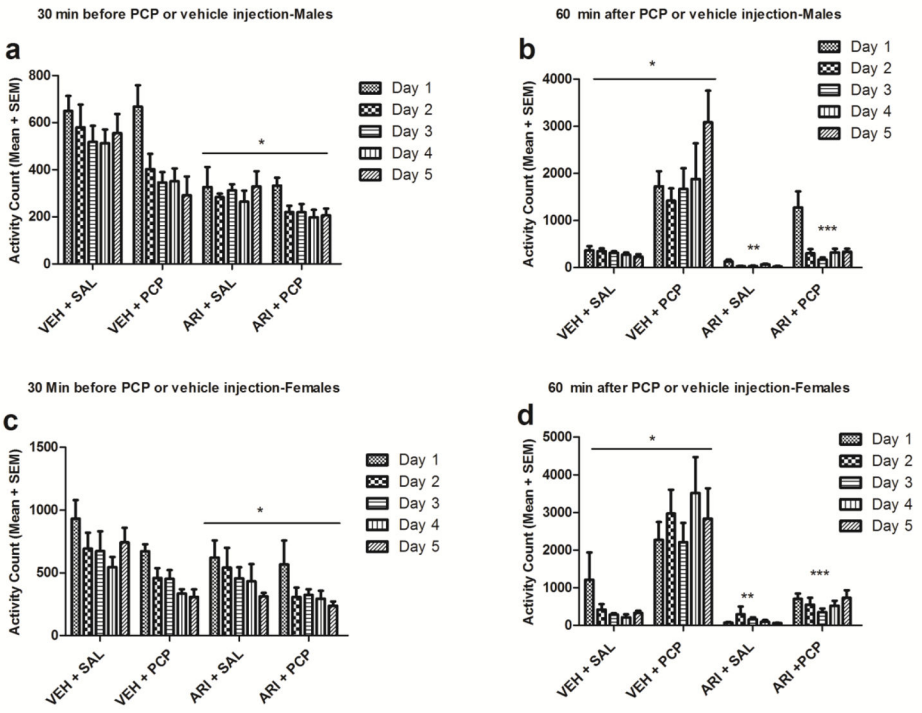

Fig. 2.

Effect of repeated ARI (3 mg/kg) treatment on phencyclidine (PCP)-induced hyperlocomotion across the initial five days of testing. Locomotor activity (a, c) in the 30 min before PCP or VEH injection and locomotor activity (b, d) 60 min after PCP or VEH injection is expressed as mean + SEM for each group. *p<.05 relative to the ARI + SAL, ARI + VEH, VEH + SAL and VEH + PCP groups. Astericks (*) indicates $p < .05$, double astericks (**) indicates $p < 0.01$, and triple astericks (***) indicates significant one-way ANOVA for drug.

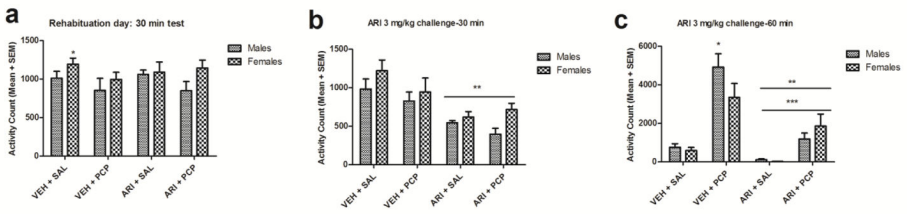

Fig. 3.

Locomotor activity (a) on rehabituation day, locomotor activity (b) on ARI challenge day during the first 30-min test period before PCP or VEH injection and locomotor (c) activity during the 60-min test after PCP or VEH injection. *p<.05 relative to all groups. Astericks (*) indicates p < .05, double astericks (**) indicates p < 0.01, and triple astericks (***) indicates significant one-way ANOVA for sex.

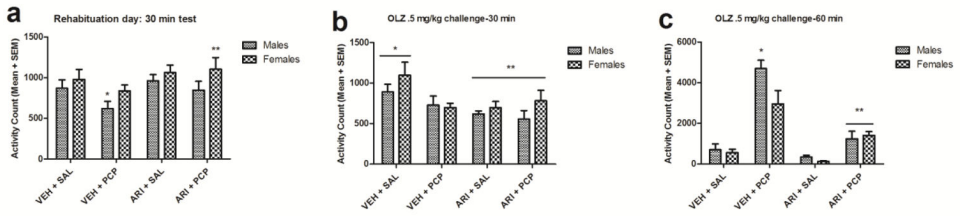

Fig. 4.

Locomotor activity (a) on rehabituation day, locomotor activity (b) on OLZ challenge day during the first 30-min test period before PCP or VEH injection and locomotor (c) activity during the 60-min test after PCP or VEH injection. *p<.05 relative to all groups. Astericks (*) indicates p < .05, double astericks (**) indicates p < 0.01.

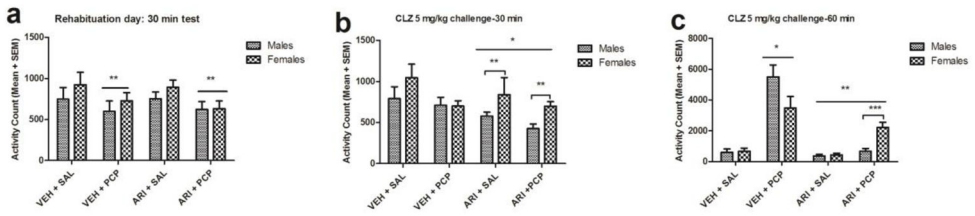

Fig. 5.

Locomotor activity (a) on rehabituation day, locomotor activity (b) on ARI challenge day during the first 30-min test period before PCP or VEH injection and locomotor (c) activity during the 60-min test after PCP or VEH injection. *p<.05 relative to all groups. Astericks (*) indicates p < .05, double astericks (**) indicates p < 0.01, and triple astericks (***) indicates significant one-way ANOVA for sex.

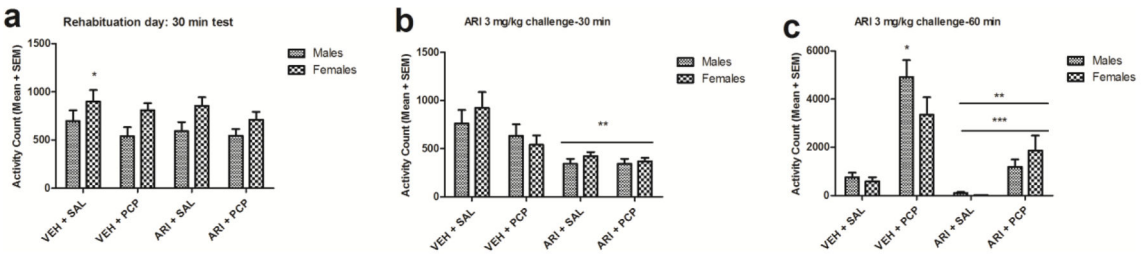

Fig. 6.

Locomotor activity (a) on rehabituation day, locomotor activity (b) on ARI challenge day during the first 30-min test period before PCP or VEH injection and locomotor (c) activity during the 60-min test after PCP or VEH injection. *p<.05 relative to all groups. Astericks (*) indicates p < .05, double astericks (**) indicates p < 0.01, and triple astericks (***) indicates significant one-way ANOVA for sex

Table 1

Timeline of events in CAR model.

Days of study	Approximate age	Manipulation
1–2	P34–35	Habituate
1–2	P36–45	Train
1–5	P46–50	Five days of ARI (10 mg/kg) treatment
1–26	P51–66	Rest to adulthood
1	P67–69	Retrain
1	P70	ARI (1.5mg/kg) challenge
1	P71–72	Retrain
1–2	P73	OLZ (.5 mg/kg) challenge
1	P74–75	Retrain
1	P76	CLZ (5 mg/kg) challenge
1–2	P77–81	Rest
1	P82–83	Retrain
1	P84	ARI (1.5 mg/kg) challenge

ARI: aripiprazole; OLZ: olanzapine; CLZ: clozapine.

Table 2

Timeline of events in PCP model.

Days of study	Approximate age	Manipulation
1–2	P39–40	Handle
1–2	P41–42	Habituation to locomotor boxes
1–5	P43–47	Five days of ARI (10 mg/kg) treatment
1–26	P48–74	Rest to adulthood
1	P75	Rehabituate to locomotor boxes
1	P76	ARI (3mg/kg) challenge test
1	P77	Rest
1–2	P78–79	Rehabituate to locomotor boxes
1	P80	OLZ (.5 mg/kg) challenge test
1	P81	Rest
1–2	P82–83	Rehabituate to locomotor boxes
1	P84	CLZ (5 mg/kg) challenge test
1	P85	Rest
1–2	P86–87	Rehabituate to locomotor boxes
1	P88	ARI (3 mg/kg) challenge test

ARI: aripiprazole; OLZ: olanzapine; CLZ: clozapine.