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# Differential effects of intermittent versus continuous haloperidol treatment throughout adolescence on haloperidol sensitization and social behavior in adulthood

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ABSTRACT

Animal work on the behavioral effects of antipsychotic treatment suggests that different dosing regimens could affect drug sensitivity differently, with an intermittent treatment regimen tending to cause a sensitization effect, while a continuous treatment causing a tolerance. In this study, we explored how haloperidol (HAL) sensitization induced throughout adolescence and tested in adulthood was differentially impacted by these two dosing regimens in the conditioned avoidance response (CAR) test. We also examined how these two dosing regiments affected social interaction and social memory in adulthood. Male adolescent Sprague-Dawley rats were treated with HAL via either osmotic minipump (HAL-0.25 CONT; 0.25 mg $kg^{-1}$ $day^{-1}$, n = 14) or daily injection (HAL-0.05 INT; 0.05 mg $kg^{-1}$ $day^{-1}$ injection, sc, n = 14), or sterile water (n = 14) from postnatal days (PND) 44 to 71. HAL sensitization was assessed in a challenge test in which all rats were injected with HAL (0.025 and 0.05 mg/kg, sc) on PND 80 and PND 82. Two days later, half of the rats from each group (n = 7/group) were assayed in two 4-trial social interaction tests in which a subject rat was given four 5-min social encounters with a familiar or novel juvenile rat (PND 35-40) at 10 min intervals. Another half were tested in a quinpirole-induced hyperlocomotion assay to assess the potential HAL-induced change in $D_2$-mediated function. Results show that only the intermittent dosing group under the HAL 0.05 mg/kg challenge showed a robust sensitization effect as rats in this group made significantly fewer avoidance responses than those in the vehicle and HAL-0.25 CONT groups. Adolescent HAL treatment did not affect social behavior and social memory, as rats from all 3 groups exhibited a similar level of social interaction and showed a similar level of sensitivity to the change of social stimuli. Similarly, adolescent HAL treatment also did not produce a long-lasting change in $D_2$ function, as all 3 groups exhibited a similar level of increase in motor activity under quinpirole challenge. These findings suggest that HAL sensitization is a dosing-specific phenomenon. It is more likely to be seen under an intermittent dosing regimen than under a continuous dosing one. The findings that the intermittent HAL treatment did not impair social functioning and did not alter $D_2$ function suggest a dissociation between drug-induced alterations in drug sensitivity and those in social function and neuroreceptors.



## 1. Introduction

Recent work suggests that adolescent antipsychotic exposure could alter antipsychotic response in adulthood (Qiao et al., 2013, 2014a; Shu et al., 2014). Two patterns of such alterations have been identified and named *antipsychotic sensitization and tolerance from adolescence to adulthood*. Antipsychotic sensitization from adolescence to adulthood refers to the increased effectiveness of a given antipsychotic drug to alter behavioral and brain functions assessed in adulthood due to past drug experience in adolescence, whereas antipsychotic tolerance refers to the opposite response pattern (i.e. decreased effectiveness due to past drug exposure). In the clinic, supersensitivity psychosis, tardive dyskinesia, and time-dependent increase in antipsychotic efficacy are several well-known examples of antipsychotic sensitization and tolerance (Agid et al., 2003; Fallon and Dursun, 2011; Kapur et al., 2006). However, clinical examples reflecting the sensitization and tolerance across the developmental period are less understood and currently understudied.

Many researchers use a rat conditioned avoidance response (CAR) test to measure antipsychotic efficacy (Arnt, 1982; Wadenberg and Hicks, 1999). In a typical CAR paradigm, a rat is placed in a two-compartment

*Abbreviations:* ANOVA, analysis of variance; CAR, conditioned avoidance response; CS, conditioned stimulus; CONT, continuous; HAL, haloperidol; HAL-0.05 INT, intermittent haloperidol 0.05 mg/kg; HAL-0.25 CONT, continuous HAL 0.25 mg/kg/day; INT, intermittent; PND, postnatal day; PolyI:C, polyinosinic-polycytidylic acid sodium salt, US, unconditioned stimulus; VEH, vehicle.

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box and trained daily to make a motor response (e.g. shuttling between two compartments) to avoid a footshock when it hears a sound. The sound typically precedes the shock for a few seconds (10 s). Antipsychotic drugs can be administered before the acquisition of avoidance responding or after. Studies over the past 60 years have consistently shown that at clinically relevant doses, all clinically approved antipsychotic drugs acutely suppress the acquisition and expression of avoidance response (Arnt, 1982; Wadenberg et al., 2001). Our CAR work has been focusing on the repeated effects of drug administration. We show that adult rats that had been treated with olanzapine or risperidone in adolescence responded to these drugs in a more sensitive way than those treated with vehicle (Qiao et al., 2013, 2014a), whereas adult rats that had been treated with clozapine in adolescence responded to this drug in a less sensitive way than those treated with vehicle (Qiao et al., 2013). Specifically, in a single avoidance challenge test in which all rats were injected with a low dose of olanzapine or risperidone, adult rats that had been treated with these drugs for only 5 days in adolescence showed much lower avoidance responding than those previously treated with vehicle, whereas those treated with clozapine tended to have higher avoidance. We further show that adolescent risperidone treatment could even enhance sensitivity to olanzapine treatment in adulthood (Qiao et al., 2014b). From these studies, it becomes quite clear that antipsychotic treatment in adolescence can induce a long-term change in drug responsiveness that persists into adulthood.

Much of our adolescent antipsychotic sensitization and tolerance work has relied on a daily intermittent drug injection schedule for a short period of time (e.g. 5 days). Whether this effect is subject to change in magnitude has never been examined. Given that drug dosing regimens determine many features of long-term treatment outcomes, with an intermittent treatment tending to cause a sensitization effect while a continuous treatment causing a tolerance (Remington and Kapur, 2010), it is quite possible that antipsychotic sensitization would be different under the two dosing regimens. This notion is consistent with evidence in the literature. For example, it has been shown that continuous haloperidol or olanzapine exposure to rats via osmotic minipump caused a greater increase in vacuous chewing movements (VCMs, a proxy for tardive dyskinesia in humans) than transient subcutaneous injections (Turrone et al., 2005). Similarly, continuous haloperidol treatment resulted in increased amphetamine-induced locomotor activity following antipsychotic discontinuation (a measure of supersensitivity psychosis) and caused an attenuated disruption (tolerance) of avoidance responding (a measure of antipsychotic activity). In contrast, intermittent haloperidol treatment did not cause an increased sensitivity to amphetamine challenge and it actually potentiated avoidance disruption (sensitization) (Samaha et al., 2007, 2008). Thus, the primary goal of the present study was to investigate whether continuous haloperidol treatment in adolescence could induce a sensitization that differs from the one induced by intermittent haloperidol treatment, and whether this sensitization effect was mediated by drug-induced dopamine $D_2$ supersensitivity, as previously shown in the risperidone-induced sensitization (Gao and Li, 2013).

Adolescent antipsychotic treatment is known to exert long-term impacts of basic behavioral and brain functions. Several studies demonstrate that early adolescent antipsychotic exposure causes an impairment of animals' working memory and delays the extinction process of fear memory in adulthood (Milstein et al., 2013). It is effective in preventing the development of various psychosis-like behaviors (e.g. prepulse inhibition deficit, latent inhibition deficit, etc.) induced by maternal immune activation (PolyI:C injection during pregnancy), while impairing certain behavioral functions of normal animals (Meyer et al., 2010; Piontkewitz et al., 2009, 2011, 2012). However, no studies have examined whether adolescent antipsychotic treatment would affect social functioning in adulthood, one of seven primary cognitive domains that are affected in schizophrenia (Floresco et al., 2005; Green et al., 2004). Therefore, the secondary goal of this study was to examine how intermittent and continuous haloperidol treatment may potentially impact social interaction and social memory used a paradigm that we validated in amphetamine and phencyclidine-based animal models of schizophrenia (Li et al., 2012a). Findings from this study could help determine the clinical significance of adolescent haloperidol sensitization and enhance our understanding of the positive and negative impacts of adolescent antipsychotic treatment on drug response and behavioral functions.

## 2. Materials and methods

### 2.1. Animals

Adolescent male Sprague-Dawley rats (n = 72, postnatal days, PND 25-27 upon arrival, averaged age were assumed to be PND 26) were purchased from Charles River Inc. (Portage, MI). They were housed two per cage, in 39.5 cm × 34.6 cm × 21.3 cm transparent polycarbonate cages under 12-h light/dark conditions (light on between 6:30 am and 6:30 pm). Room temperature was maintained at 22 ± 1°C with a relative humidity of 45-60%. Food and water was available *ad libitum*. Animals were allowed at least 5 days of acclimation to the animal facility before being used in experiments. All behavioral tests took place between 9 am and 4 pm in the light cycle. All experimental treatment and procedures were approved by the Institutional Animal Care and Use Committee at the University of Nebraska-Lincoln.

### 2.2. Drugs and choice of doses

Haloperidol (HAL) is dopamine $D_2$ receptor antagonist (Strange, 2001). HAL (5.0 mg/ml Ampoules, Shanghai Xudong Haipu Pharmaceutical Co. Ltd, Shanghai, China) was dissolved in distilled sterile water. Quinpirole hydrochloride (Tocris Bioscience, Bristol, UK) was dissolved in 0.9% saline. All drugs were administered subcutaneously in a volume of 1.0 ml/kg body weight. The continuous and intermittent treatment doses of HAL were based on the rat striatal $D_2$ receptor occupancy data (65-80%) that corresponds to clinically relevant conditions (Kapur et al., 2003) and prior studies using the conditioned avoidance response task (Samaha et al., 2007, 2008) and social behavior (Li et al., 2005). The doses of quinpirole (a selective dopamine $D_{2/3}$ receptor agonist) were chosen based on previous work showing that this dose was effectively induced hyperactivity, and to assess $D_2$ receptor-mediated motor activity (Gao and Li, 2013; Luque-Rojas et al., 2013; Nakamura et al., 1994; Qiao et al., 2014a).

### 2.3. Two-way avoidance conditioning apparatus

Ten 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 × 35.56 cm D × 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 a partition with an arch style doorway (15 cm high × 9 cm wide at base). A barrier (4 cm high) was placed between the two compartments, so the rats had 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, through which 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). The rat location and crossings between compartments were monitored by a set of 16 photobeams (ENV-256-8P) affixed at the bottom of the 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; i.e. 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 (approximately 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.