Evaluating Early Preventive Antipsychotic and Antidepressant Drug Treatment in an Infection-Based Neurodevelopmental Mouse Model of Schizophrenia

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Current pharmacotherapy of schizophrenia remains unsatisfactory with little hope for complete functional restoration in patients once the disease has developed. A preventive approach based on intervention in the prodromal stage of the disease aiming to preserve functional integrity by halting the progress of the disease is therefore extremely attractive. Here, we investigated the effects of preventive antipsychotic or antidepressant drug treatment in a well-established neurodevelopmental mouse model of multiple schizophrenia-related abnormalities. Pregnant mice on gestation day 9 were exposed to the viral mimic polyribosin-polyribocytidylic acid (2 mg/kg, intravenously) or corresponding vehicle treatment, and the resulting offspring from both prenatal treatment conditions were subjected to chronic antipsychotic (haloperidol or clozapine), antidepressant (fluoxetine), or placebo treatment during the periadolescent stage of development. The effects of the preventive pharmacotherapy on behavioral and pharmacological functions were then investigated in adulthood using paradigms relevant to schizophrenia, namely prepulse inhibition, latent inhibition, and sensitivity to psychostimulant drugs. We show that periadolescent treatment with the reference antipsychotic and antidepressant drugs can successfully block the emergence of multiple psychosis-related behavioral and pharmacological abnormalities in subjects predisposed to adult brain pathology by exposure to prenatal immune challenge. At the same time, however, our study reveals numerous negative influences of the early pharmacological intervention on normal behavioral development in control subjects. Hence, even though preventive pharmacotherapy may be beneficial in individuals with predisposition to psychosis-related brain dysfunctions, chronic antipsychotic or antidepressant drug treatment in false-positive subjects is associated with substantial risk for long-term behavioral disturbances in adulthood.

Key words: clozapine/fluoxetine/haloperidol/prevention/ prodromal/psychosis

Introduction

There is a growing interest in the early detection and pharmacological intervention in the treatment of schizophrenia. This has led to the initiation of preventive intervention programs in recent years, which are based on early pharmacotherapy in individuals identified as being prodromally symptomatic of the disorder. The “prodromal” stage of schizophrenia refers to a muted form of psychosis-related behavior that precedes the onset of the full spectrum of psychotic pathology.1 It has been suggested that early pharmacological treatment during the prodromal phase may prevent the subsequent emergence of a full-blown psychotic episode by attenuating or even halting the progression of the underlying pathology.2–4 For this reason, chronic administration of antipsychotic and antidepressant drugs to periadolescent and/or adolescent subjects with prodromal symptoms has been introduced as preventive treatment of schizophrenia and other psychosis-related disorders in humans.5–9

In spite of the laudable rationale of the preventive approach, its implementation has provoked several ethical concerns and therefore still remains highly controversial.10–13 One relative unknown is the “conversion rate” among individuals with identified prodromal signs to full-blown psychosis. With an estimation as low as 50%,12,14,15 one immediate implication of such preventive practice is that a substantial number of “false-positive” subjects (who otherwise would not progress into full psychosis) would be exposed to unnecessary antipsychotic and/or antidepressant drug treatment,12,16,17 while the long-term side effects of such exposure in these individuals are unknown. Hence, the relative benefits (ie, successful prevention) and costs (ie, long-term sides effects in false-positive subjects) of preventive pharmacological interventions targeting periadolescent subjects, identified as being at high risk for schizophrenia in later life, must be comprehensively evaluated.
Considering the apparent lack of knowledge about the long-term consequences of early preventive pharmacotherapy, along with the ethical concerns and technical difficulties to address these issues in humans, the explorative investigation of early preventive strategies in preclinical animal models of schizophrenia is clearly warranted.\textsuperscript{18–20} Because a defined experimental manipulation allows the clear segregation of high-risk subjects from controls (CONs), the efficiency of preventive pharmacotherapy can be studied without potential confounds arising from treatment in false-positive subjects. Therefore, we evaluated in a well-established neurodevelopmental mouse model of schizophrenia-like disorder whether chronic antipsychotic or antidepressant drug treatment during periadolescence may prevent the subsequent emergence of psychosis-related behavioral and pharmacological abnormalities in adulthood. The experimental model used here is based on prenatal exposure to the viral mimic polyriboinosinic-polyribocytidylic acid (PolyI:C). The prenatal PolyI:C model is highly suitable for the experimental investigation of preventive pharmacological intervention in schizophrenia because it can mimic brain and behavioral abnormalities related to the full-blown schizophrenia phenotype in adult life and incorporates etiological significance and the neurodevelopmental perspective of the disorder.\textsuperscript{21–28} Importantly, the prenatal PolyI:C model captures the pathological progression from periadolescence to adulthood because the full spectrum of prenatal PolyI:C-induced behavioral, cognitive, and pharmacological abnormalities only emerges in adult but not periadolescent subjects.\textsuperscript{21,23,25,27} One exception is enhanced sensitivity to systemic amphetamine (AMPH) treatment, which can already emerge during the periadolescent stage of postnatal development, provided that the prenatal immunological manipulation is conducted during early fetal development (ie, on gestation day [GD] 9 in the mouse\textsuperscript{25}; see also Ozawa\textsuperscript{27}). Increased behavioral and neurochemical responses to acute AMPH treatment have been associated especially with the positive symptoms of schizophrenia.\textsuperscript{29,30} The appearance of attenuated positive symptoms is considered to be one of the critical prodromal symptoms preceding the onset of full-blown psychosis.\textsuperscript{13} Hence, in the present experimental model, we evaluated the efficacy of preventive antipsychotic or antidepressant drug treatment during a prodromal-like phase, which is characterized by periadolescent AMPH hypersensitivity but absence of other behavioral and pharmacological abnormalities related to the full-blown schizophrenia phenotypes.\textsuperscript{25}

Materials and Methods

Animals

Female and male C57BL/6 breeders were obtained from our in-house specific pathogen-free colony at the age of 10–14 weeks. Littermates of the same sex were kept in groups of 3–5 mice. Breeding began after 2 weeks of acclimatization to the new animal holding room, which was a temperature- and humidity-controlled (21 ± 1°C, 55% ± 5%) holding facility under a reversed light-dark cycle (lights off: 0800–2000). All animals had ad libitum access to food (Kliba 3430; Klímabühlen, Kaiseraugst, Switzerland) and water. All procedures described in the present study had been approved previously by the Zurich Cantonal Veterinary Office and are in agreement with the Principles of Laboratory Animal Care (National Institutes of Health publication number 86-23, revised 1985).

Timed Mating Procedure and Prenatal Immune Treatment

For the purpose of the prenatal immunological manipulation in mid-pregnancy, C57BL/6 breeders were subjected to a timed mating procedure as fully described before.\textsuperscript{22} Pregnant dams on GD 9 received either a single injection of PolyI:C or CON (saline [SAL]) solution via the intravenous route at the tail vein under mild physical constraint. PolyI:C (potassium salt) was obtained from Sigma-Aldrich (Buchs, St Gallen, Switzerland) and dissolved in isotonic 0.9% NaCl solution to obtain the desired dosage (2 mg/kg, calculated based on the pure form PolyI:C). The volume of injection was 5 ml/kg. All animals were returned to their home cages immediately after the injection procedures and left undisturbed until weaning of the offspring.

Periadolescent Drug Treatment

Offspring born to PolyI:C-treated and CON mothers were weaned and sexed at postnatal day (PND) 21. Littermates of the same sex were caged in groups of 2–3 mice and had ad libitum food (Kliba 3430; Klímabühlen) and water. They were kept in a temperature- and humidity-controlled (21 ± 1°C, 55% ± 5%) animal room under a 12:12 h reversed light-dark cycle (lights off at 0800) as described above.

For the periadolescent drug treatment, PolyI:C and CON offspring were randomly assigned to 1 of the 4 possible drug conditions: haloperidol (HAL), clozapine (CLZ), fluoxetine (FLX), or vehicle (VEH). The periadolescent drug regime started on PND 35 and lasted for 4 weeks, ie, until PND 65 (see figure 1). During this period, all animals were kept in groups of 2–3 mice in order to avoid the possible confounding factor of isolation housing. All drugs were administered via regular drinking bottles to avoid periadolescent stress exposure resulting from repeated injections. HAL, CLZ, and FLX were administered at a dose of 3, 15, and 20 mg/kg/day, respectively. The desired concentration for each drug was calculated based on the average liquid consumption and body weight per cage; this was adjusted every fourth day based on the liquid consumption and body weight assessed on the preceding days. The doses of HAL, CLZ, and FLX...
were selected based on previous drug administration protocols using regular drinking bottles in mice.\(^{31,32}\) HAL was obtained from Janssen-Cilag (Baar, Switzerland) in the form of ampoules consisting of 5 mg of HAL in 1 ml of solvent containing minimal amounts of lactic acid; it was further diluted in regular tap water. CLZ (Novartis, Basel, Switzerland) was first dissolved in 0.1 N hydrochloric acid (HCl) in 0.9% SAL solution, neutralized with Na\(_2\)CO\(_3\), and then further diluted in tap water. FLX hydrochloride was obtained from Sigma-Aldrich and dissolved in tap water at the desired concentration. Animals were not given an alternate source of water from the drinking bottles containing the antipsychotic or antidepressant drug solutions and, therefore, were motivated to drink the solutions by thirst. One-third of the animals assigned to periadolescent VEH treatment received tap water containing the appropriate amounts of 0.1 N HCl and Na\(_2\)CO\(_3\), and the remaining animals in the VEH group received tap water only.

**Behavioral Phenotyping**

Behavioral testing commenced 4 weeks after cessation of the periadolescent drug treatment, ie, when the animals reached PND 90 (see figure 1). Each experimental group consisted of subjects derived from multiple independent litters (18 PolyI:C litters and 23 CON litters). Animals of both sexes were included in all the behavioral and pharmacological tests described below. The number of subjects employed in each of the behavioral tests is listed in table 1.

**Prepulse Inhibition of the Acoustic Startle Reflex.** The prepulse inhibition (PPI) test was conducted using 4 startle chambers for mice (San Diego Instruments, San Diego, CA). The test apparatus has been fully described elsewhere.\(^{33}\) In the demonstration of PPI of the acoustic startle reflex, subjects were presented with a series of discrete trials comprising a mixture of 4 trial types. These included pulse-alone trials, prepulse-plus-pulse trials, prepulse-alone trials, and no-stimulus trials in which no discrete stimulus other than the constant background noise was presented. The pulse and prepulse stimuli employed were in the form of a sudden elevation in broadband white noise level (sustaining for 40 and 20 ms, respectively) from the background (65 dBA), with a rise time of 0.2–1.0 ms. In all, 3 different intensities of pulse (100, 110, and 120 dBA) and 3 intensities of prepulse (71, 77, and 83 dB A, which corresponded to 6, 12, and 18 dB above background, respectively) were employed. The stimulus-onset asynchrony of the prepulse and pulse stimuli on all prepulse-plus-pulse trials was 100 ms (onset-to-onset).

A session began with the animals being placed into the Plexiglas enclosure. They were acclimatized to the apparatus for 2 min before the first trial began. The first 6 trials consisted of 6 startle-alone trials, comprising 2 trials of each of the 3 possible pulse intensities. These trials served to habituate and stabilize the animals’ startle response and were not included in the analysis. Subsequently, the animals were presented with 10 blocks of discrete test trials. Each block consisted of the following: 3 pulse-alone trials (100, 110, or 120 dBA), 3 prepulse-alone trials (+6, +12, or +18 dB above background), 9 possible combinations of prepulse-plus-pulse trials (3 levels of prepulse × 3 levels of prepulse), and 1 no stimulus. The 16 discrete trials within each block were presented in a pseudorandom order, with a variable intertrial interval of a mean of 15 s (ranging from 10 to 20 s).

For each of the 3 pulse intensities (100, 110, or 120 dBA), PPI was indexed by percent inhibition of the startle response obtained in the pulse-alone trials by the following expression: 100% \(\times (1 - \frac{\text{mean reactivity on prepulse-plus-pulse trials}}{\text{mean reactivity on pulse-alone trials}})\), for each subject, and at each of the 3 possible prepulse

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**Fig. 1.** Experimental Design Used to Study Early Preventive Antipsychotic and Antidepressant Drug Treatment in the Present Infection-Based Neurodevelopmental Mouse Model of Schizophrenia-Like Disorder. Pregnant mice on GD 9 were exposed to PolyI:C (2 mg/kg, intravenously) or corresponding control (CON, saline) treatment. The resulting offspring from both treatment conditions (ie, PolyI:C offspring and CON offspring) were then subjected to chronic antipsychotic (haloperidol or clozapine), antidepressant (fluoxetine), or vehicle treatment during the periadolescent stage of development, ie, between postnatal days (PNDs) 35 and 65. On PND 65, the periadolescent drug regime was stopped. Behavioral and pharmacological testing of the offspring was conducted in adulthood (ie, between PNDs 90 and 120) in a drug-free state.
Table 1. Summary of the Number of Subjects Used in the Behavioral and Pharmacological Assays at Adult Age

|                  | CON Offspring | PolyI:C Offspring |
|------------------|---------------|-------------------|
|                  | VEH | HAL | CLZ | FLX | VEH | HAL | CLZ | FLX |
| PPI NPE          | 13  | 14  | 6   | 8   | 6   | 5   | 6   | 6   |
| PPI PE           | 11  | 12  | 6   | 4   | 5   | 5   | 6   | 5   |
| AMPH sensitivity | 8   | 7   | 6   | 5   | 4   | 4   | 5   | 5   |
| MK-801 sensitivity | 10 | 9   | 4   | 5   | 5   | 5   | 5   | 4   |

Note: CON, control; PolyI:C, polyriboinosinic-polyribocytidylic acid; VEH, vehicle; HAL, haloperidol; CLZ, clozapine; FLX, fluoxetine; PPI, prepulse inhibition; LI, latent inhibition; NPE, non-preexposure; PE, preexposure; AMPH, amphetamine; PND, postnatal day. Male and female offspring born to PolyI:C-exposed or CON mothers were treated with VEH, HAL, CLZ, or FLX during periadolescence, ie, between PNDs 35 and 65. They were then subjected to repeated behavioral and pharmacological phenotyping at adult age (PNDs 90–120) in drug-free state. Phenotyping of the offspring started with the paradigm of PPI, followed by LI and sensitivity to the psychostimulant drugs AMPH and dizocilpine (MK-801). All subjects were behaviorally naive at the beginning of testing (PPI). In order to increase the number of subjects necessary for the LI test, behaviorally naive animals were also included in this test in addition to the ones tested previously in the PPI paradigm. The AMPH and MK-801 sensitivity tests were conducted in 2 separate cohorts of drug-naive animals to avoid repeated psychostimulant drug exposure.

cent PPI was analyzed as a function of prepulse and pulse intensities with the between-subjects factors of prenatal treatment (PolyI:C or CON), periadolescent drug treatment (VEH, HAL, CLZ, or FLX), and sex (male or female). In addition, the startle reactivity to prepulse-alone trials and to pulse-alone and prepulse-plus-pulse trials was subjected to statistical analysis.

Latent Inhibition. Latent inhibition (LI) was assessed in a conditioned 2-way active avoidance procedure using 4 identical 2-way shuttle boxes (model H10–11M-SC; Coulbourn Instruments, Whitehall, PA) as fully described before.23 The test procedures consisted of 2 phases: preexposure (PE) and conditioning, conducted 24 h apart. Animals from each of the 4 treatment groups were allocated to 1 of 2 PE conditions: conditioned stimulus (CS)-PE or non-preexposure (NPE).

In the PE phase, the PE subjects were placed in the appropriate test chambers and presented with 100 discrete exposures to a 5-s burst of white noise (80 dB) in the conditioned stimulus (CS)—according to a random interstimulus interval schedule of 40 ± 15 s. The NPE subjects were confined to the chamber for an equivalent period of time without any stimulus presentation. The total number of shuttle responses performed during the PE session was recorded and analyzed with the between-subjects factors of PE (NPE or PE), prenatal treatment (PolyI:C or CON), periadolescent drug treatment (VEH, HAL, CLZ, or FLX), and sex (male or female).

On the conditioning day, the subjects were returned to the same shuttle boxes and received a total of 100 avoidance trials presented with an intertrial interval (ITI) of 40 ± 15 s. A trial began with the onset of the noise CS. If the animal shuttled within 5 s of CS onset, the CS was terminated and the animal avoided the electric shock (unconditioned stimulus [US]) on that trial. Avoidance failure led immediately to an electric foot shock presented in conjunction to the CS. This could last for a maximum of 2 s but could be terminated by a shuttle response during this period (ie, an escape response).

To index conditioned avoidance learning, the mean number of avoidance responses recorded on successive blocks of 10 trials were analyzed with the between-subjects factors of PE (NPE or PE), prenatal treatment (PolyI:C or CON), periadolescent drug treatment (VEH, HAL, CLZ, or FLX), and sex (male or female). In order to account for general locomotor activity, the spontaneous shuttles performed during the interstimulus intervals were also subjected to statistical analysis.

Spontaneous and Drug-Induced Locomotor Activity. Spontaneous and drug-induced locomotor activity was assessed in 4 identical open-field arenas (40 × 40 × 35-cm high) made of wood and painted white as described before.22 They were located in a testing room under dim diffused lighting (approximately 35 lux as measured in the center of the arenas). A digital camera was mounted directly above the 4 arenas. Images were captured at a rate of 5 Hz and transmitted to a PC running the Ethovision (Noldus, Wageningen, The Netherlands) tracking system.

To acclimatize the animals to the open field, they were placed in the center of the arena and allowed to explore freely for 20 min. At the end of this time period, the animals were removed from the apparatus and injected with SAL solution. They were then immediately returned to the same arenas and allowed to explore for another 20 min. Subsequently, the animals were briefly removed from the apparatus once more, administered with...
AMPH or dizocilpine (MK-801), and returned to the same arenas again. The locomotor responses to the acute drug challenges were then monitored for a period of 80 min. d-Amphetamine sulfate (Sigma-Aldrich) and MK-801 (Merck Sharp & Dohme, Hoddesdon, UK) were dissolved in isotonic 0.9% NaCl solution to achieve the desired concentration for injection. AMPH and MK-801 were administered via the intraperitoneal route at a dose of 2.5 and 0.15 mg/kg, respectively. The volume of injection was 5 ml/kg for both drugs. All solutions were freshly prepared on the day of testing.

Locomotor activity was indexed by the distance traversed in the entire open-field arena as a function of 5-min bins and analyzed with the between-subjects factors of prenatal treatment (PolyI:C or CON), periadolescent drug treatment (VEH, HAL, CLZ, or FLX), and sex (male or female). The data collected on the 3 phases of the experiment (ie, no treatment, SAL treatment, and AMPH or MK-801 treatment) were analyzed separately.

**Serum Concentrations of Antipsychotic and Antidepressant Drugs**

The efficacy of the periadolescent drug regime to elevate peripheral antipsychotic and antidepressant drug levels was ascertained by measuring the serum concentrations of HAL, CLZ, and FLX, as well as their major metabolites (reduced HAL, desmethylclozapine, and norfluoxetine, respectively) after subchronic (5 days) and chronic (15 days) oral drug treatment. The antipsychotic and antidepressant drugs were administered to a separate cohort of male and female C57BL/6 mice via home cage drinking bottles from PND 35 onward as described above. Blood was taken from the orbital sinus 5 or 15 days following the commencement of antipsychotic and antidepressant drug administration under methoxyflurane (2,2-dichloro-1,1-difloroethymethyl ether; Pitman-Moore, Washington, NJ) anesthesia. The collected blood was allowed to clot at room temperature for 1 h before centrifugation at 12 000 rpm for 4 min at 4°C, and the resulting serum from each animal was stored at −80°C.

Serum levels of HAL, CLZ, FLX, and their metabolites were measured simultaneously using a reversed-phase high-performance liquid chromatography (HPLC) separation method with ultraviolet detection following a solid phase extraction. The HPLC system consisted of a WATERS 2697 Alliance separations module and a Waters 2487 dual wavelength UV detector, set at 214 and 254 nm (WATERS, Milford, MA). The chromatographic separation was achieved by using an end-capped C18 reversed-phase HPLC column (MERCK, Darmstadt, Germany). The mobile phase consisted of a mixture of octanesulfonic, acetonitrile, and 50 mM sodium dihydrogen phosphate buffer (pH 2.29, adjusted with H₃PO₄) and was run using an isocratic elution.

Two-hundred microliters of serum and 20 μl of internal standard in 0.1 M H₃PO₄ were applied onto a preconditioned 3cc Oasis MAX (HAL; internal standard: cyclobenzaprine) or MCX (CLZ, desmethylclozapine, FLX, desmethylfluoxetine; internal standard: taktin) cartridge. After flushing, the eluent was evaporated to dryness, and the residue was reconstituted with 40 μl of 2.0 mM H₃PO₄. An aliquot of 35 μl was injected onto the HPLC column. Stock solutions of the analytes (1 mg/ml) were prepared in methanol and added to drug-free serum to establish 5 different calibration concentrations. The 5-point calibration curves were plotted by the peak high ratios of each analyte/internal standard vs concentrations of the respective analyte in serum.

**Statistical Analysis**

All data (except serum concentrations of antipsychotic and antidepressant drugs) were analyzed using parametric ANOVA, followed by Fisher’s least significant difference (LSD) post hoc comparisons or restricted ANOVA whenever appropriate. Fisher LSD post hoc test was chosen in order to avoid possible type II errors. Serum concentrations of antipsychotic and antidepressant drugs were analyzed using independent Student t tests. Statistical significance was set at P < .05. Analyses were conducted using the statistical software StatView (version 5.0) implemented on a personal computer running the Windows XP operating system.

**Results**

**Serum Levels of Antipsychotic and Antidepressant Drugs Following Subchronic or Chronic Oral Administration During Periadolescence**

We measured the serum concentrations of the reference typical and atypical neuroleptic drugs HAL and CLZ, respectively, and of the selective serotonin reuptake inhibitor (SSRI) FLX after subchronic (5 days) and chronic (15 days) oral administration in order to ascertain the efficacy of the periadolescent drug regime to elevate peripheral antipsychotic and antidepressant drug levels. In addition, we assessed the major metabolites of HAL, CLZ, and FLX (reduced HAL, desmethylclozapine, and norfluoxetine, respectively). All drugs were readily detectable in the serum following subchronic (5 days) or chronic (15 days) treatment and so were the major metabolites of CLZ (desmethylclozapine) and FLX (norfluoxetine). The levels of reduced HAL were below detection limit at both sampling intervals. The mean ± standard error of the mean (SEM) serum levels of the antipsychotic and antidepressant drugs and their metabolites are summarized in table 2.

In order to assess possible effects of subchronic and chronic antipsychotic or antidepressant drug exposure, we also analyzed liquid consumption of the animals every fourth day. These analyses provided no evidence for
altered liquid intake as a consequence of the subchronic or chronic drug treatment (data not shown).

**Periadolescent Antipsychotic or Antidepressant Drug Treatment in the Prevention of Adult PPI Deficiency**

We tested whether periadolescent antipsychotic or antidepressant drug exposure would prevent the emergence of adult schizophrenia-like behavioral abnormalities induced by prenatal immune challenge. First, we evaluated the effects of periadolescent treatment with HAL, CLZ, or FLX, in comparison to corresponding VEH treatment, on prenatal infection-induced sensorimotor gating deficiency. Sensorimotor gating was assessed using the paradigm of PPI of the acoustic startle response, which refers to the reduction of startle reaction to a startle-elicitng stimulus (pulse) when it is shortly preceded by a weak stimulus (prepulse). PPI deficiency has been linked to several neuropsychiatric disorders with a presumed neurodevelopmental origin, including schizophrenia.34

Consistent with previous reports,22,26–28,35 prenatal PolyI:C-induced immune challenge impaired PPI (indexed as percent inhibition) regardless of sex relative to CON offspring (figure 2a). The PPI deficit was most pronounced in conditions of 120-dBA pulse stimulus but not so with pulse stimulus of lower intensities (100 or 110 dBA; data not shown). The prenatal PolyI:C-induced attenuation of PPI in the 120-dB pulse stimulus condition was consistently seen across all prepulse intensities, leading to a significant reduction in the mean percent PPI in VEH-treated PolyI:C offspring relative to VEH-treated CON offspring (figure 2a). Periadolescent CLZ and FLX treatment normalized the prenatal PolyI:C-induced PPI deficits in the 120-dBA pulse condition (figure 2a). In contrast, chronic HAL exposure during peradolescence was ineffective in restoring the PPI impairments in prenatally immune-challenged offspring (figure 2a). Instead, peradolescent HAL exposure tended to exacerbate the PPI attenuation in PolyI:C offspring and led to a significant PPI deficit in adult offspring born to CON mothers (figure 2a). Chronic CLZ and FLX administration during peradolescence did not significantly alter PPI in adult CON offspring (figure 2a). Statistical support for these interpretations was obtained by the 2 × 4 × 2 × 3 (prenatal treatment × peradolescent treatment × sex × prepulse intensity) ANOVA of percent PPI in 120-dBA pulse condition, which yielded a significant main effect of peradolescent treatment ($F_{3,173} = 9.65; P < .001$) and its interaction with prenatal treatment ($F_{3,173} = 3.82; P < .001$). Subsequent Fisher post hoc comparisons further verified the significant differences between VEH-treated CON and PolyI:C offspring ($P < .01$), between VEH-treated and HAL-exposed offspring born to CON mothers ($P < .001$), between VEH-treated and CLZ-exposed offspring born to PolyI:C-treated mothers ($P < .05$), and between VEH-treated and FLX-exposed PolyI:C offspring ($P < .001$).

In addition to its effects on PPI, peradolescent antipsychotic or antidepressant drug treatment also significantly affected startle reactivity to prepulse-alone trials. CLZ-treated PolyI:C offspring displayed increased startle reactivity to prepulse-alone trials compared with all other peradolescent drug regimes (figure 2b). This effect emerged regardless of sex and was most consistently seen in trials, in which the low (71 dBA) or middle (77 dBA) prepuces were presented. Chronic CLZ treatment also significantly increased startle reactivity to prepulse-alone trials in offspring born to CON mothers (see figure 2c). On the other hand, peradolescent HAL exposure in PolyI:C offspring led to a reduction in the reactivity to prepulse-alone trials relative to VEH treatment. Again, this was independent of sex and was significant at the middle (77 dBA) or high (83 dBA) prepulse-alone trials were presented (figure 2b). HAL treatment during peradolescence exerted no significant effects on prepulse-alone reactivity in offspring born to

### Table 2. Serum Levels (ng/ml) of the Antipsychotic Drugs HAL and CLZ (and Their Major Metabolites Reduced HAL and Desmethylclozapine, Respectively) and of the Antidepressant Drug FLX and Its Metabolite Norfluoxetine After Subchronic (5 d) and Chronic (15 d) Treatment

|                  | HAL     | Reduced HAL | CLZ     | Desmethylclozapine | FLX     | Norfluoxetine |
|------------------|---------|-------------|---------|--------------------|---------|--------------|
| 5-d treatment    | 110.4 ± 24.3 | ND         | 25.6 ± 7.0 | 73.7 ± 10.1   | 576.3 ± 59.2 | 823.8 ± 67.6 |
| 15-d treatment   | 110.3 ± 33.4 | ND         | 33.8 ± 5.2 | 147.9 ± 21.3*  | 1035.1 ± 72.6  | 1126.9 ± 58.4 |

*Note:* HAL, haloperidol; CLZ, clozapine; FLX, fluoxetine; ND, not detected. The antipsychotic and antidepressant drugs were administered to male and female C57BL/6 mice via home cage drinking bottles at doses of 3 mg/kg/day (HAL), 15 mg/kg/day (CLZ), and 20 mg/kg/day (FLX). The drug regime was conducted during the peradolescent stage of development, ie, from postnatal day 35 onward. Preliminary statistical analyses indicated that there were no significant differences between the serum drug levels in male and female mice. The antipsychotic and antidepressant drugs and their major metabolites were no longer detectable in the serum following a washout period of 3 wk (data not shown). *$P < .01$ between desmethylclozapine levels after 5- and 15-d treatment, #$P < .01$ between FLX levels after 5- and 15-d treatment, and ‡$P < .001$ between norfluoxetine levels after 5- and 15-d treatment based on independent Student t tests. $N$(HAL) = 10 (4 ♀, 6 ♂), $N$(CLZ) = 10 (5 ♀, 5 ♂), and $N$(FLX) = 10 (6 ♀, 4 ♂). The serum levels of reduced HAL were below detection limit (ND). All values are means ± standard error of the mean.
CON mothers (figure 2b). Statistical support for these impressions was obtained by the 2\( \times \)3\( \times \)4 (prenatal treatment \times periadolescent treatment \times prepulse intensity) ANOVA of startle reactivity to prepulse-alone trials, which revealed a significant main effect of periadolescent treatment (\( F_{3,173} = 11.83; P < .001 \)) and its interaction with prepulse intensity (\( F_{9,519} = 3.89; P < .001 \)). The prenatal treatment \times periadolescent treatment \times prepulse intensity also reached statistical significance (\( F_{9,519} = 2.25; P < .05 \)). Subsequent Fisher post hoc comparisons at each of the 3 prepulse levels were then conducted. These analyses confirmed the significance difference between VEH-treated and CLZ-exposed CON offspring at the middle (77 dBA) and high (83 dBA) prepulse intensities (all \( P < .05 \)), between VEH-treated and CLZ-exposed PolyI:C offspring at the low (71 dBA) and middle (77 dBA) prepulse intensities (all \( P < .05 \)), and between VEH-treated and HAL-exposed PolyI:C offspring at the middle (77 dBA) and high (83 dBA) prepulse intensities (all \( P < .05 \)).
Prenatal PolyI:C exposure did not significantly affect startle reactivity to the 120 dB pulse-alone trials in comparison with prenatal CON treatment (figure 2c). However, periadolescent FLX treatment significantly reduced the reactivity to pulse-alone trials compared with all other periadolescent drug conditions (figure 2c). This specific effect of FLX exposure emerged regardless of the prenatal treatment histories and sex. The $2 \times 4 \times 2$ (prenatal treatment $\times$ periadolescent treatment $\times$ sex) ANOVA of startle reactivity to pulse-alone trials revealed a significant main effect of periadolescent treatment ($F_{3,173} = 2.74; P < .05$). Subsequent Fisher post hoc comparisons confirmed the significant difference between VEH-treated and FLX-exposed animals ($P < .05$). The startle reactivity to pulse-alone trials was generally lower in female offspring compared with male offspring, leading to a significant main effect of sex ($F_{3,173} = 13.98; P < .001$) in the ANOVA of startle reactivity to pulse-alone trials. Importantly, this effect of sex was independent of the prenatal and periadolescent manipulations: The between-subjects factor of sex did not significantly interact with any of the other between-subjects factors. The mean $\pm$ SEM startle reactivity to the 120 dB pulses was $113.4 \pm 6.1$ and $154.1 \pm 9.2$ (in arbitrary units) in female and male mice, respectively.

**Periadolescent Antipsychotic or Antidepressant Drug Treatment in the Prevention of Adult LI Deficiency**

Next, we explored whether periadolescent antipsychotic or antidepressant drug exposure may prevent the emergence of deficient selective associative learning following prenatal immune challenge by PolyI:C.$^{21-23,28}$ Selective learning was studied by assessing the LI effect, in which nonreinforced PEs to a to-be-CS retard subsequent conditioning between the same CS and US. This form of selective learning is known to be impaired in acutely psychotic patients, especially in patients with marked positive symptoms.$^{36,37}$ Here, we used a 2-way active avoidance procedure to demonstrate LI, which is expressed as a retardation in avoidance learning in the PE subjects relative to the NPE subjects.

A robust LI effect was observed in adult CON offspring treated with VEH during periadolescence regardless of sex (figure 3a). Consistent with previous studies,$^{21-23,28}$ prenatal PolyI:C-induced immune activation led to a complete abolition of LI (figure 3b). The prenatal PolyI:C-induced LI disruption was reversed by periadolescent HAL or CLZ administration, ie, HAL- or CLZ-treated PolyI:C offspring displayed significant LI (figure 3b). In contrast, periadolescent FLX treatment not only failed to prevent the emergence of adult LI disruption induced by prenatal immune challenge (figure 3b) but also abolished the LI effect in offspring born to CON mothers (figure 3a). On the other hand, neither HAL nor CLZ administration during periadolescence interfered with the development of LI in CON offspring—the presence of a statistically significant LI effect was evident in HAL- or CLZ-treated offspring born to CON mothers (figure 3a).

Statistical support for these interpretations was yielded by the $2 \times 4 \times 2 \times 2 \times 10$ (prenatal treatment $\times$ periadolescent treatment $\times$ stimulus PE $\times$ sex $\times$ 10-trial blocks) ANOVA for repeated measures of avoidance shuttles, which yielded significance for the stimulus PE $\times$ blocks ($F_{9,1665} = 4.61; P < .001$), prenatal treatment $\times$ periadolescent treatment $\times$ blocks ($F_{27,1665} = 1.72; P < .01$), and prenatal treatment $\times$ periadolescent treatment $\times$ stimulus PE $\times$ blocks ($F_{27,1665} = 1.54; P < .05$) interactions. Additional $2 \times 10$ (stimulus PE $\times$ 10-trial blocks) ANOVAs for repeated measures restricted to each of the 8 experimental groups were then conducted in order to further verify the presence or absence of LI. These analyses revealed a significant main effect of stimulus PE in CON offspring treated with VEH ($F_{1,48} = 11.51; P < .001$), HAL ($F_{1,26} = 8.00; P < .01$), or CLZ ($F_{1,20} = 5.10; P < .05$) and in PolyI:C offspring treated with HAL ($F_{1,14} = 4.48; P < .05$). A significant $2 \times 10$ (stimulus PE $\times$ blocks) interaction was obtained in PolyI:C offspring exposed to chronic CLZ treatment ($F_{9,153} = 2.25; P < .05$), and additional ANOVAs restricted to each 10-trial block revealed a significant main effect of stimulus PE in blocks 5–7 as well as in blocks 9 and 10 (all $P < .05$). The between-subjects factor of sex never interacted significantly with any other between-subjects factor.

In order to account for general locomotor activity in the test of LI, we also analyzed the spontaneous shuttles performed during the PE phase as well as the ITI shuttles during conditioning. HAL treatment during periadolescence led to a significant increase in spontaneous shuttles during the PE phase of the test. This effect was independent of prenatal treatment, sex, and stimulus PE (table 3). The $2 \times 4 \times 2 \times 2$ (prenatal treatment $\times$ periadolescent treatment $\times$ sex $\times$ PE) ANOVA of total shuttles revealed a significant main effect of periadolescent treatment ($F_{1,188} = 20.19; P < .001$), and the subsequent Fisher post hoc comparison verified the significant difference between animals treated with HAL compared with all other periadolescent drug conditions (all $P < .001$). Likewise, periadolescent HAL administration also increased spontaneous locomotor activity as indexed by the spontaneous ITI shuttles performed during conditioning (table 3). This was supported by the $2 \times 4 \times 2 \times 2$ (prenatal treatment $\times$ periadolescent treatment $\times$ sex $\times$ PE) ANOVA of total ITI shuttles, which yielded a significant main effect of periadolescent treatment ($F_{3,188} = 6.45, P < .001$). Subsequent post hoc comparisons confirmed the significant increase in the mean number of ITI crosses in HAL-treated subjects relative to all other drug conditions (all $P < .001$).
Periadolescent Antipsychotic or Antidepressant Drug Treatment in the Prevention of Increased Sensitivity to the Locomotor-Enhancing Effects of Psychostimulant Drugs in Adulthood

One of the critical pharmacological abnormalities in schizophrenia and other psychosis-related disorders is enhanced sensitivity to psychostimulant drugs, including dopamine receptor agonists and N-methyl-D-aspartate (NMDA) receptor antagonists. Prenatal immune activation by PolyI:C in rodents can successfully mimic these schizophrenia-related pharmacological abnormalities by increasing the sensitivity to the indirect dopamine receptor agonist AMPH and the non-competitive NMDA receptor antagonist dizocilpine (MK-801). Here, we evaluated whether chronic antipsychotic or antidepressant drug exposure during periadolescence prevents the emergence of prenatal PolyI:C-induced enhancement in the locomotor response to acute AMPH and MK-801 challenge in adulthood. The sensitivity to the psychostimulant drugs was assessed by measuring locomotor reaction to the acute drug exposure in the open field as described previously.

Fig. 3. Modulation of the Latent Inhibition (LI) Effect by Periadolescent Antipsychotic and Antidepressant Drug Treatment in Offspring Born to PolyI:C-Treated and Control (CON) Mothers. LI was assessed in a 2-way active avoidance procedure, in which a white noise stimulus served as the to-be-conditioned stimulus (CS) and electric footshock as the unconditioned stimulus. To index conditioned avoidance learning, the mean number of avoidance shuttles performed on successive 10-trial blocks was analyzed and depicted in the figure. LI is manifested when subjects pre-exposed to the CS (PE) display retarded active avoidance learning relative to non-preexposed (NPE) subjects. (a) A clear LI effect was observed in adult CON offspring treated with vehicle (VEH) during periadolescence, as well as in CON offspring exposed to periadolescent haloperidol (HAL) or clozapine (CLZ) treatment. Chronic treatment with fluoxetine (FLX) led to the complete abolition of LI in adult offspring born to CON mothers. (b) Prenatal immune challenge by PolyI:C led to the loss of LI in VEH-treated offspring. Chronic HAL or CLZ treatment, but not FLX treatment during periadolescence, prevented from the emergence of PolyI:C-induced LI disruption in adulthood. *P < .05, **P < .01, ***P < .001, based on restricted ANOVAs. The number of subjects in each group is listed in table 1. All values are means ± standard error of the mean.
Table 3. Effects of Periadolescent Antipsychotic or Antidepressant Drug Exposure on Locomotor Activity as Indexed by the Spontaneous Shuttles Performed During the Latent Inhibition Test

| Periadolescent Drug Treatment | VEH | HAL | CLZ | FLX |
|------------------------------|-----|-----|-----|-----|
| PE day (total spontaneous shuttles) | 131 ± 6 | 212 ± 11* | 132 ± 10 | 1431 ± 8 |
| Conditioning day (total ITI shuttles) | 13 ± 1 | 27 ± 4* | 11 ± 2 | 14 ± 1 |

Note: VEH, vehicle; HAL, haloperidol; CLZ, clozapine; FLX, fluoxetine; PE, preexposure; ITI, intertrial interval; in the PE phase of the test, the total number of spontaneous shuttles between the 2 compartments of the shuttle box was taken to index locomotor activity; on the day of conditioning, the mean number of ITI shuttles per 10-trial block served as an index for locomotor activity. Periadolescent exposure to HAL significantly increased the number of spontaneous shuttles during the PE phase in comparison to periadolescent VEH, CLZ, or FLX treatment. This effect of the HAL regime emerged regardless of the prenatal treatment histories, stimulus PE conditions, and sex. Periadolescent HAL administration also increased spontaneous locomotor activity as indexed by the spontaneous ITI shuttles performed during conditioning. *P < .001 between animals treated with HAL compared with all other peridolescent drug conditions, based on Fisher post hoc comparisons. All values are means ± standard error of the mean.

Consistent with previous results,21,22,25,26 prenatal PolyI:C exposure did not significantly affect basal locomotor activity as indexed by the distance traveled during the initial acclimatization period and after SAL treatment (see figures 4 and 5). However, offspring treated with HAL during peridolescence displayed a significant increase in basal locomotor activity (ie, during the acclimatization phase and after SAL treatment) compared with offspring treated with VEH during peridolescence (see figures 4 and 5). These effects of HAL are in agreement with the results obtained in the LI procedure (see table 3) and emerged regardless of the prenatal treatment histories and sex. Statistical support for these interpretations was yielded by the 2 × 4 × 2 × 4 (preadolescent treatment × sex × 5-min bins) ANOVAs for repeated measures of distance traveled after AMPH challenge, which yielded a significant main effect of bins (F15,1110 = 79.16; P < .001) and a significant prenatal treatment × peradolescent treatment × sex × bins interaction (F45,1110 = 1.39; P < .05). Subsequent post hoc comparisons between the 8 experimental groups were then conducted for each of the 5-min bins after AMPH challenge. These analyses confirmed the significant difference between VEH-treated CON offspring and VEH-treated PolyI:C offspring in bins 14–19 (all P < .05), between HAL-treated and VEH-treated CON offspring in bins 13–17 (all P < .05), and between VEH-treated PolyI:C offspring and HAL- or FLX-treated PolyI:C offspring in bins 16–19 (all P < .05) (see figure 4).

Prenatal PolyI:C-induced immune activation also markedly potentiated the locomotor reaction to systemic MK-801 in comparison to prenatal CON treatment—an effect that was observed regardless of sex (figures 5a,b). The prenatal PolyI:C-induced potentiation of MK-801 sensitivity was completely blocked by peradolescent treatment with HAL but not by peradolescent CLZ or FLX treatment (figure 5a). Again, this specific effect of HAL treatment was seen in both male and female PolyI:C offspring. Similar to its effects on AMH sensitivity in CON animals (see figure 4a), chronic HAL exposure during peradolescence increased the sensitivity to acute MK-801 challenge in adult offspring born to CON mothers (figure 5a). A comparable effect was
Fig. 4. Modulation of the Sensitivity to Systemic Amphetamine (2.5 mg/kg, Intraperitoneally) Challenge by Periadolescent Antipsychotic and Antidepressant Drug Treatment in Offspring Born to PolyI:C-Treated and Control (CON) Mothers. The graph illustrates the distance traveled in the open field after nontreatment (bins 1–4), saline (SAL) treatment (bins 5–8), and AMPH treatment (bins 9–24) for CON (a) and PolyI:C offspring (b); the mean distance traveled after AMPH challenge is shown in (c). Periadolescent exposure to haloperidol (HAL) significantly increased basal locomotor activity (ie, after nontreatment and SAL treatment) compared with periadolescent vehicle (VEH) treatment regardless of the prenatal treatment histories. $P < .01$ between HAL-exposed and VEH-treated subjects, based on Fisher post hoc tests. Prenatal PolyI:C exposure significantly enhanced the locomotor reaction to acute AMPH challenge compared with prenatal CON treatment, ie, VEH-treated PolyI:C offspring displayed an increase in distance traveled in the open field compared with VEH-treated CON offspring following AMPH administration. This effect was attenuated by periadolescent HAL and fluoxetine (FLX) treatment but not by clozapine (CLZ) exposure. In addition, periadolescent HAL treatment (but not CLZ or FLX treatment) potentiated the locomotor reaction to AMPH in CON offspring. *$P < .05$ between VEH-treated CON offspring and VEH-treated PolyI:C offspring; $^{\#}P < .05$ between HAL-treated and VEH-treated CON offspring; and $^{\£}P < .05$ between VEH-treated PolyI:C offspring and HAL- or FLX-treated PolyI:C offspring. The number of subjects in each group is listed in table 1. SED in (a) and (b) refers to twice the standard error of difference derived from the error variance associated with the prenatal treatment × periadolescent treatment × 5-min bins interaction term from the overall ANOVA. All values in (c) are means ± standard error of the mean.
Fig. 5. Modulation of the Sensitivity to Systemic Dizocilpine (MK-801; 0.15 mg/kg, Intraperitoneally) Challenge by Periadolescent Antipsychotic and Antidepressant Drug Treatment in Offspring Born to PolyI:C-Treated and Control (CON) Mothers. The graph illustrates the distance traveled in the open field after nontreatment (bins 1–4), saline (SAL) treatment (bins 5–8), and MK-801 treatment (bins 9–24) for CON (a) and PolyI:C offspring (b); the mean distance traveled after MK-801 challenge is shown in (c). Periadolescent exposure to haloperidol (HAL) significantly increased basal locomotor activity (ie, after nontreatment and SAL treatment) compared with periadolescent vehicle (VEH) treatment regardless of the prenatal treatment histories. # $P < .05$ between HAL-exposed and VEH-treated subjects, based on Fisher post hoc tests. Prenatal PolyI:C exposure led to a significant increase in the locomotor reaction to acute MK-801 challenge compared with prenatal CON treatment, ie, VEH-treated PolyI:C offspring displayed a significant increase in mean distance traveled in the open field compared with VEH-treated CON offspring following MK-801 administration. The prenatal PolyI:C-induced potentiation of MK-801 sensitivity was blocked by chronic HAL treatment during periadolescence but not by periadolescent exposure to CLZ or FLX. On the other hand, HAL and FLX (but not CLZ) treatment significantly increased the sensitivity to acute MK-801 challenge in CON offspring. * $P < .05$ between VEH-treated CON offspring and VEH- or CLZ-treated PolyI:C offspring; $^\dagger$ $P < .05$ between VEH-treated CON offspring and HAL- or FLX-treated CON offspring; and $^\ddagger$ $P < .05$ between HAL-treated PolyI:C offspring and VEH- or CLZ-treated PolyI:C offspring. The number of subjects in each group is listed in Table 1. SED in (a) and (b) refers to twice the standard error of difference derived from the error variance associated with the prenatal treatment × periadolescent treatment × 5-min bins interaction term from the overall ANOVA. All values in (c) are means ± standard error of the mean.
also noticeable in CON offspring treated with FLX during periadolescence, which displayed a significant increase in the locomotor reaction to systemic MK-801 compared with VEH-treated CON subjects (figure 5a).

Chronic CLZ treatment did not significantly affect the locomotor reaction to systemic MK-801 treatment in PolyI:C or CON offspring (figures 5a,b). Statistical support for these interpretations was yielded by the $2 \times 4 \times 2 \times 16$ (prenatal treatment $\times$ periadolescent treatment $\times$ sex $\times$ 5-min bins) ANOVA for repeated measures of distance traveled after MK-801 challenge, which revealed significant prenatal treatment $\times$ periadolescent treatment ($F_{3,75} = 4.00; P < .05$) and prenatal treatment $\times$ periadolescent treatment $\times$ bins ($F_{45,1125} = 1.42; P < .05$) interactions. Subsequent post hoc comparisons further verified the significant differences between VEH-treated CON offspring and VEH- or CLZ-treated PolyI:C offspring (both $P < .05$), between VEH-treated CON offspring and HAL- or FLX-treated CON offspring (both $P < .05$), between CLZ-treated CON offspring and HAL- or FLX-treated CON offspring (both $P < .05$), and between HAL-treated PolyI:C offspring and VEH- or CLZ-treated PolyI:C offspring (both $P < .05$) (see figure 5).

Discussion

By evaluating preventive antipsychotic and antidepressant drug treatment in an infection-based neurodevelopmental mouse model of schizophrenia-like disorder, the present study provides experimental evidence for both beneficial and detrimental effects of these early pharmacological interventions on adult behavioral functions. In subjects predisposed to adult psychopathology as a result of prenatal immune challenge, preventive treatment with the reference antipsychotic and antidepressant drugs HAL, CLZ, and FLX during periadolescence attenuated the emergence of multiple psychosis-related behavioral and pharmacological abnormalities in adulthood (for a summary, see table 4). Hence, in the absence of preventive pharmacotherapy, offspring born to PolyI:C-challenged mothers developed multiple adult disturbances known to be implicated in schizophrenia, namely PPI disruption, LI deficiency, and hypersensitivity to dopaminergic and glutamatergic psychostimulant drugs. This indicates that periadolescent pharmacotherapy was effective in halting the pathological progression into a full-blown schizophrenia-related phenotype after infection-mediated neurodevelopmental disturbances. At the same time, our results show that chronic periadolescent pharmacotherapy as such is sufficient to induce adult pathological behavior when initiated in CON subjects that were not primed to develop schizophrenia-related abnormalities (table 4). Indeed, we revealed clear bidirectional effects for some of the long-term consequences of periadolescent pharmacotherapy in CON subjects (ie, offspring born to CON mothers) and subjects with predisposition for adult schizophrenia-related disturbances (ie, offspring born to PolyI:C-treated mothers). One particular example is periadolescent HAL treatment, which completely abolished the development of increased AMPH sensitivity in PolyI:C offspring but as such induced an enhancement of AMPH responsiveness in CON offspring (see figure 4).

It is important to emphasize that the beneficial effects of periadolescent drug exposure in prenatally immune-challenged animals cannot be simply accounted for by “masking effects” of the preventive pharmacotherapy. For example, we show that periadolescent CLZ treatment blocks the prenatal PolyI:C-induced PPI and LI deficits without concomitantly enhancing PPI and LI in CLZ-treated animals born to CON mothers (see figures 2 and 3). Hence, the periadolescent pharmacotherapy exerted corrective but not additive effects on behavioral and pharmacological functions in adulthood. It is well known that offspring born to gestationally immune-challenged mothers display several neuronal behavioral and pharmacological dysfunctions during the periadolescent period. Importantly, these early neuronal abnormalities can exist in the absence of multiple behavioral and pharmacological dysfunctions at this stage of development and may be readily associated with the drugs’ effects on developmental and maturational processes occurring during periadolescent development. Likewise, the identified long-term detrimental effects of chronic periadolescent pharmacotherapy in offspring born to CON mothers are likely to be accounted for by drug-induced interference with normal periadolescent brain development and/or maturation. Further studies are clearly warranted in order to identify the critical neuronal mechanisms underlying these associations in prenatally immune-challenged animals as well as CON subjects.

Both the beneficial and detrimental long-term effects of the periadolescent pharmacotherapy were critically dependent on the pharmacological specificity of the compound administered as well as on the precise psychopathological trait being evaluated (for a summary, see table 4). Specifically, periadolescent HAL exposure in

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Table 4. Summary of the Identified Beneficial and Detrimental Effects of Preventive Pharmacotherapy During Periadolescence on Adult Behavioral and Pharmacological Functions in Prenatally Immune-Challenged Mice (PolyI:C Offspring) and CON Mice (CON Offspring)

|                           | CON Offspring | PolyI:C Offspring |
|---------------------------|---------------|-------------------|
|                           | HAL CLZ FLX   | HAL CLZ FLX       |
| Prepulse inhibition       | – 0 0         | 0 + +             |
| Latent inhibition         | 0 0 –         | + + 0             |
| AMPH sensitivity          | – 0 0         | + 0 +             |
| MK-801 sensitivity        | – 0 –         | + 0 0             |

Note: PolyI:C, polyriboinosinic-polyribocytidylic acid; CON, control; HAL, haloperidol; CLZ, clozapine; FLX, fluoxetine; AMPH, amphetamine. Symbols (–, +, and (0) denote detrimental, beneficial, and no significant effects of the periadolescent drug treatment, respectively, with respect to corresponding placebo treatment in CON and PolyI:C offspring.

PolyI:C offspring was effective for the prevention of 3 of 4 schizophrenia-related abnormalities, namely LI deficiency (figure 3) and increased sensitivity to acute AMPH (figure 4) as well as MK-801 (figure 5) challenge. On the other hand, chronic CLZ treatment successfully blocked the emergence of PPI (figure 2) and LI (figure 3) deficits induced by prenatal PolyI:C treatment, while it did not exert any corrective effects against psychostimulant hypersensitivity (figures 4 and 5). Interestingly, the beneficial effects of periadolescent CLZ treatment on PolyI:C-induced PPI disruption seems to be, at least in part, linked to the drug's effect on enhancing the reactivity to prepulse-alone stimuli (figure 2b). This is consistent with the recent findings that normalization of sensorimotor gating deficiency in medicated schizophrenic patients may be related to the effects of antipsychotic medication on enhancing prepulse-elicited reactivity. Finally, chronic treatment with the SSRI FLX attenuated PPI deficits (figure 2) and enhanced AMPH reaction (figure 4) induced by prenatal PolyI:C exposure, which however was ineffective against the disruption of LI (figure 3) and the enhanced reaction to MK-801 (figure 5). In offspring born to CON mothers, periadolescent HAL treatment induced marked PPI impairments (figure 2) and increased basal and psychostimulant-induced locomotor activity (figures 4 and 5), whereas chronic FLX administration disrupted LI (figure 3) and potentiated MK-801 sensitivity (figure 5) in adult life. In contrast, chronic CLZ treatment during periadolescent development was devoid of any negative behavioral effects examined here in adult CON subjects. At the present stage, we can only speculate about the possible mechanisms underlying these differential brain and behavioral effects of periadolescent treatment with distinct classes of pharmacological compounds. One clear possibility would be that chronic antagonism and/or activation of distinct receptor classes and neurotransmitter systems during periadolescent development may be involved in the distinct long-term effects of the early pharmacological intervention, given that the pharmacology of HAL, CLZ, and FLX can be readily distinguished, at least in part, by their specific neurochemical activities and receptor affinities.

The dissociation of the long-term effects of preventive pharmacotherapy in subjects predisposed to adult schizophrenia-related pathology and CON subjects highlights some of the major difficulties and concerns associated with the recent attempts in humans to prevent the onset or to attenuate the severity of psychosis by early pharmacological interventions during the prodromal stage. First, the relatively low conversion rates from the initial prodromal stage to the eventual psychotic phase implies the exposure of a substantial number of false-positive individuals to unnecessary and possibly harmful drug treatment. Reports about long-term detrimental effects of early pharmacological intervention on subsequent brain and behavioral development in false-positive subjects are still lacking because attempts to prevent psychotic behavior by drugs is a relatively recent event. However, this possibility is not at all surprising because prodromal-based pharmacological intervention in humans is often initiated during periadolescence or early adolescence, which represents a time window of significant ongoing brain development and maturation. The present study provides direct experimental evidence for this possibility by identifying numerous detrimental effects of the chronic exposures to antipsychotic or antidepressant medication during periadolescence on subsequent adult brain and behavioral functions in false-positive subjects (ie, offspring born to CON mothers).

Second, the low conversion rates from the initial prodromal to the full-blown psychotic stage may also undermine the evaluation and interpretation of possible beneficial effects of early pharmacotherapy in the preventive treatment of schizophrenia. Hence, even though a low conversion rate to psychosis may truly reflect positive benefits of the early pharmacological intervention, such seemingly beneficial effects may be occluded by presence of false-positive subjects whose prognosis would have been satisfactory even in the absence of any medication. Our experimental design is expected to yield results devoid of such interpretative
problems because it allows an evaluation of such early pharmacological intervention within an explicit comparison between 2 distinct groups of subjects that can be prospectively identified as being predisposed to multiple schizophrenia-like adult behavioral abnormalities (ie, adult PolyI:C offspring) or not (ie, CON offspring). Against this background, our findings of beneficial effects of preventive medication readily support the hypothesis that early pharmacological intervention in psychosis-prone subjects can successfully block the conversion into a full-blown psychotic disorder.\(^5\)\(^-\)\(^9\) Interestingly, the data thus far available in humans suggest that such early pharmacological intervention is not effective for the prevention of the whole spectrum of psychosis-related abnormalities after treatment with one specific class of pharmacological compounds. For example, Cornblatt and colleagues\(^8\) recently reported that exposure to antidepressant drugs during the initial prodromal phase was only effective in preventing the emergence of 3 out of 5 positive symptoms. This is in agreement with the experimental data presented here, which suggest that early pharmacological intervention with distinct antipsychotic or antidepressant compounds leads only to a partial but not full blockade of further progression into the full-blown schizophrenia-related phenotype.

In a recent study, Tenn et al\(^{19}\) have explored a model of progressive behavioral/psychomotor sensitization in rats as a putative animal model of the prodromal state of schizophrenia. In this model, rats were treated with different regimes of AMPH to produce full behavioral sensitization (analogous to the full psychotic phenotype) or partial sensitization (analogous to the prodromal phenotype), and they were then treated with the typical and atypical antipsychotic drug HAL and CLZ, respectively, in order to mimic preventive pharmacological intervention. The authors showed that animals having experienced full sensitization displayed significant behavioral deficits implicated in schizophrenia, including reduced PPI, deficient LI, and enhanced sensitivity to acute AMPH treatment.\(^{19}\) On the other hand, animals subjected to a partial sensitization regimen showed only a muted phenotype, ie, they displayed potentiated AMPH sensitivity but normal PPI and LI.\(^{19}\) Most importantly, fully sensitized animals that received early preventive HAL or CLZ treatment did not progress from the “prodromal” to the full-blown phenotype when tested in a drug-free state.\(^{19}\)

The outcomes of early preventive antipsychotic drug treatment as evaluated in the present study share some striking similarities with those yielded in the experimental model of partial AMPH sensitization in rats.\(^{19}\) However, the 2 models also critically differ in several aspects. Consistent with the present results, preventive HAL and CLZ treatment both restored LI abnormalities induced by the experimental manipulation (ie, AMPH sensitization or prenatal PolyI:C exposure) without affecting LI in CON subjects. In the AMPH-based model of the prodromal state of schizophrenia,\(^{19}\) both HAL and CLZ were similarly effective in blocking the emergence of PPI deficiency and AMPH hypersensitivity. Here, only periadolescent HAL (and FLX) but not CLZ exposure successfully prevented the prenatal PolyI:C-induced AMPH potentiation in adulthood (figure 4); and only CLZ and FLX but not HAL treatment during peradolescence normalized the prenatal PolyI:C-induced PPI deficits (figure 2). Another critical distinction between the 2 experimental models is that chronic antipsychotic drug exposure in CON animals (ie, SAL-treated subjects) did not exert any negative influences on PPI or sensitivity to acute AMPH challenge when tested in a drug-free state.\(^{19}\) Here, we clearly revealed such detrimental long-term effects of chronic antipsychotic or antidepressant drug exposure when given to CON animals (ie, offspring born to CON mothers). It is conceivable to explain these differences by the precise timing of the preventive pharmacological manipulation. Indeed, in our model, subjects were exposed to the preventive pharmacotherapy during peradolescent development, whereas all experimental manipulations were conducted during adulthood in the AMPH-based experimental model of the psychosis prodrome.\(^{19}\) Because preventive pharmacological intervention during the initial prodromal phase in humans is most often initiated in peradolescence or early adolescence, our model described here more closely mimics the human condition. This is therefore unlike the approach adopted by Tenn et al,\(^{19}\) in which all manipulations were conducted in adult subjects. Hence, the sensitivity of the prenatal PolyI:C model to detrimental behavioral effects of chronic pharmacotherapy in CON subjects represents an important improvement over previous models of prodromal-based preventive intervention in adulthood because it highlights some of the possible risk factors associated with unnecessary preventive pharmacotherapy in false-positive subjects.

One limitation of the present experimental evaluation of the effects of chronic pharmacotherapy for preventive reasons is that we only evaluated one specific dose for each drug. Periadolescent CLZ treatment at the selected dose here (15 mg/kg/day) led to a serum concentration of approximately 30 ng/ml after subchronic or chronic administration (table 2). This is about 10 times lower compared with clinically effective serum concentrations after subchronic or chronic CLZ treatment in adult human patients.\(^{14}\)\(^-\)\(^{16}\) On the other hand, the daily administration of HAL (3 mg/kg/day) and FLX (20 mg/kg/day) led to serum concentrations of ~110 and 570 ng/ml, respectively, after subchronic treatment and to ~110 and 1030 ng/ml after chronic treatment (table 2). For both drugs, these concentrations are 2–6 times higher than the usual concentrations targeted in humans.\(^{41}\)\(^-\)\(^{46}\) It is important to emphasize that the half-life times of many antipsychotic and antidepressant drugs in rodents are 4–6 times faster than in humans.\(^{41}\)\(^-\)\(^{45}\) Higher drug regimes
are thus often needed in rodents relative to humans in order to obtain receptor occupancies that are comparable with those present during clinically effective pharmacotherapy in humans.\(^{45}\) Undoubtedly, a dose-response approach would be warranted in order to further confirm the present findings of both beneficial and detrimental effects of the early pharmacological interventions. Specifically, it would be of great interest to evaluate whether HAL and FLX treatment at lower doses may still exert preventive effects against the emergence of schizophrenia-related behavioral and pharmacological abnormalities without inducing significant brain and behavioral deficits when given to CON subjects and whether perinatal administration of CLZ at higher doses may also induce negative behavioral effects in CONs.

Taken together, our results provide experimental evidence that early pharmacological intervention may provide a successful strategy for the prevention of multiple psychosis-related abnormalities in subjects predisposed to adult dysfunctions. Because the experiments reported here are based on prenatal infection-induced interference with normal brain development, the present findings may be especially relevant for subjects at high risk for schizophrenia following prenatal exposure to infection.\(^{48–50}\) Of equal importance are the findings that chronic antipsychotic or antidepressant drug treatment in false-positive subjects is associated with substantial risk for long-lasting brain and behavioral disturbances in adulthood. The present study thus also highlights the critical importance of targeting early preventive pharmacotherapy only to psychosis-prone individuals. This readily suggests the necessity for a careful examination of possible risks and benefits before initiating any early pharmacological intervention programs designed to reduce the incidence of schizophrenia and related disorders among high-risk individuals.

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**References**

1. Klosterkötter J, Hellmich M, Steinmeyer EM, Schultz-Lutter F. Diagnosing schizophrenia in the initial prodromal phase. Arch Gen Psychiatry. 2001;58:158–164.
2. McGlashan TH. Early detection and intervention in schizophrenia: research. Schizophr Bull. 1996;22:327–345.
3. Cornblatt B, Lencz T, Obuchowski M. The schizophrenia prodrome: treatment and high-risk perspectives. Schizophr Res. 2002;54:177–186.
4. Ruhrmann S, Schultze-Lutter F, Maier W, Klosterkötter J. Pharmacological intervention in the initial prodromal phase of psychosis. Eur Psychiatry. 2005;20:1–6.
5. Woods SW, Breier A, Zipursky RB, et al. Randomized trial of olanzapine versus placebo in the symptomatic acute treatment of the schizophrenic prodrome. Biol Psychiatry. 2003;54:453–464.
6. Woods SW, Tully EM, Walsh BC, et al. Aripiprazole in the treatment of the psychosis prodrome: an open-label pilot study. Br J Psychiatry Suppl. 2007;51:s96–s101.
7. McGlashan TH, Zipursky RB, Perkins D, et al. The PRIME North America randomized double-blind clinical trial of olanzapine versus placebo in patients at risk of being prodromally symptomatic for psychosis. I. Study rationale and design. Schizophr Res. 2003;61:7–18.
8. McGlashan TH, Zipursky RB, Perkins D, et al. Randomized, double-blind trial of olanzapine versus placebo in patients prodromally symptomatic for psychosis. Am J Psychiatry. 2006;163:790–799.
9. Cornblatt BA, Lencz T, Smith CW, et al. Can antidepressants be used to treat the schizophrenia prodrome? Results of a prospective, naturalistic treatment study of adolescents. J Clin Psychiatry. 2007;68:546–557.
10. Cornblatt BA, Lencz T, Kane JM. Treatment of the schizophrenia prodrome: is it presently ethical? Schizophr Res. 2001;51:31–38.
11. McGlashan TH. Psychosis treatment prior to psychosis onset: ethical issues. Schizophr Res. 2001;51:47–54.
12. Corcoran C, Malaspina D, Hercher L. Prodromal interventions for schizophrenia vulnerability: the risks of being “at risk”. Schizophr Res. 2005;73:173–184.
13. Haroun N, Dunn L, Haroun A, Cadenhead KS. Risk and protection in prodromal schizophrenia: ethical implications for clinical practice and future research. Schizophr Bull. 2006;32:166–178.
14. Yung AR, Phillips LJ, McGorry PD, et al. Prediction of psychosis. A step towards indicated prevention of schizophrenia. Br J Psychiatry Suppl. 1998;172:14–20.
15. Yung AR, Phillips LJ, Yuen HP, et al. Psychosis prediction: 12-month follow up of a high-risk ("prodromal") group. Schizophr Res. 2003;60:21–32.
16. Block JJ. Ethical concerns regarding olanzapine versus placebo in patients prodromally symptomatic for psychosis. Am J Psychiatry. 2006;163:1838.
17. Fusar-Poli P, Valmaggia L, McGuire P. Can antidepressants prevent psychosis? Lancet. 2007;370:1746–1748.
18. Lee C, McGlashan TH, Woods SW. Prevention of schizophrenia: can it be achieved? CNS Drugs. 2005;19:193–206.
19. Tenn CC, Fletcher PJ, Kapur S. A putative animal model of the “prodromal” state of schizophrenia. Biol Psychiatry. 2005;57:586–593.
20. Powell SB, Risbrough VB, Geyer MA. Potential use of animal models to examine antipsychotic prophylaxis for schizophrenia. Clin Neurosci Res. 2003;3:289–296.
21. Zuckerman L, Rehavi M, Nachman R, Weiner I. Immune activation during pregnancy in rats leads to a postpubertal emergence of disrupted latent inhibition, dopaminergic hyperfunction, and altered limbic morphology in the offspring: a novel neurodevelopmental model of schizophrenia. Neuropsychopharmacology. 2003;28:1778–1789.
22. Meyer U, Feldon J, Schedlowski M, Yee BK. Towards an immuno-precipitated neurodevelopmental animal model of schizophrenia. *Neurosci Biobehav Rev*. 2005;29:913–947.

23. Meyer U, Schwendener S, Feldon J, Yee BK. Prenatal and postnatal maternal contributions in the infection model of schizophrenia. *Exp Brain Res*. 2006;173:243–257.

24. Meyer U, Yee BK, Feldon J. The neurodevelopmental impact of prenatal infections at different times of pregnancy: the earlier the worse? *Neuroscientist*. 2007;13:241–256.

25. Meyer U, Nyffeler M, Schwendener S, Knuesel I, Yee BK, Feldon J. Relative prenatal and postnatal maternal contributions to schizophrenia-related neurochemical dysfunction after in utero immune challenge. *Neuropsychopharmacology*. 2008;33:441–456.

26. Meyer U, Nyffeler M, Yee BK, Knuesel I, Feldon J. Adult brain and behavioral pathological markers of prenatal immune challenge during early/middle and late fetal development in mice. *Brain Behav Immun*. 2008;22:469–486.

27. Ozawa K, Hashimoto K, Kishimoto T, Shimizu E, Ishikura H, Iyo M. Immune activation during pregnancy in mice leads to dopaminergic hyperfunction and cognitive impairment in the offspring: a neurodevelopmental animal model of schizophrenia. *Biol Psychiatry*. 2006;59:546–554.

28. Smith SE, Li J, Garbett K, Mirnics K, Patterson PH. Maternal immune activation alters fetal brain development through interleukin-6. *J Neurosci*. 2007;27:10695–10702.

29. Laruelle M, Abi-Dargham A, van Dyck CH, et al. Single photon emission computerized tomography imaging of amphetamine-induced dopamine release in drug-free schizophrenic subjects. *Proc Natl Acad Sci USA*. 1996;93:9235–9240.

30. Laruelle M, Abi-Dargham A, Gil R, Kegeles L, Innis R. Increased dopamine transmission in schizophrenia: relationship to illness phases. *Biol Psychiatry*. 1999;46:56–72.

31. Dunstan R, Jackson DM. Long-term haloperidol-treatment of mice: a change in beta-adrenergic receptor responsiveness. *J Neural Transm*. 1979;44:187–195.

32. Dulawa SC, Holick KA, Gundersen B, Hen R. Effects of chronic fluoxetine in animal models of anxiety and depression. *Neuropsychopharmacology*. 2004;29:1321–1330.

33. Yee BK, Chang T, Pietropaolo S, Feldon J. The expression of prepulse inhibition of the acoustic startle reflex as a function of three pulse stimulus intensities, three prepulse stimulus intensities, and three levels of startle responsiveness in C57BL6/J mice. *Behav Brain Res*. 2005;163:265–276.

34. Braff DL, Geyer MA, Swerdlow NR. Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. *Psychopharmacology*. 2001;156:234–258.

35. Shi L, Fatemi SH, Sidwell RW, Patterson PH. Maternal influenza infection causes marked behavioral and pharmacological changes in the offspring. *J Neurosci*. 2003;23:297–302.

36. Weiner I. The “two-headed” latent inhibition model of schizophrenia: modeling positive and negative symptoms and their treatment. *Psychopharmacology*. 2003;169:257–297.

37. Lubow RE. Construct validity of the animal latent inhibition model of selective attention deficits in schizophrenia. *Schizophr Bull*. 2005;31:139–153.

38. Lieberman JA, Kane JM, Alvir J. Provocative tests with psychostimulant drugs in schizophrenia. *Psychopharmacology*. 1987;91:415–433.

39. Lahti AC, Koffel B, LaPorte D, Tamminga CA. Subanesthetic doses of ketamine stimulate psychosis in schizophrenia. *Neuropsychopharmacology*. 1995;13:9–19.

40. Csomor PA, Yee BK, Feldon J, Theodoridou A, Studerus E, Vollenweider FX. Impaired prepulse inhibition and prepulse-elicted reactivity but intact reflex circuit excitability in unmedicated schizophrenia patients: a comparison with healthy subjects and medicated schizophrenia patients. *Schizophr Bull*. In press [Epub ahead of print [PMID: 18245063]]

41. DeVane CL. Metabolism and pharmacokinetics of selective serotonin reuptake inhibitors. *Cell Mol Neurobiol*. 1999;19:443–466.

42. Miyamoto S, Duncan GE, Marx CE, Lieberman JA. Treatments for schizophrenia: a critical review of pharmacology and mechanisms of action of antipsychotic drugs. *Mol Psychiatry*. 2005;10:79–104.

43. Spear LP. The adolescent brain and age-related behavioral manifestations. *Neurosci Biobehav Rev*. 2000;24:417–463.

44. Spina E, Avenoso A, Faciolì G, et al. Relationship between plasma concentrations of clozapine and norclozapine and therapeutic response in patients with schizophrenia resistant to conventional neuroleptics. *Psychopharmacology*. 2000;148:83–89.

45. Kapur S, VanderSpek SC, Brownlee BA, Nobrega JN. Antipsychotic dosing in preclinical models is often unrepresentative of the clinical condition: a suggested solution based on in vivo occupancy. *J Pharmacol Exp Ther*. 2003;305:625–631.

46. Perez-Costas E, Guidetti P, Melendez-Ferro M, Kelley JJ, Roberts RC. Neuroleptics and animal models: feasibility of oral treatment monitored by plasma levels and receptor occupancy assays. *J Neural Transm*. 2008;115:745–753.

47. de Oliveira IR, de Sena EP, Pereira EL, et al. Haloperidol blood levels and clinical outcome: a meta-analysis of studies relevant to testing the therapeutic window hypothesis. *J Clin Pharm Ther*. 1996;21:229–236.

48. Brown AS, Susser ES. In utero infection and adult schizophrenia. *Ment Retard Dev Disabil Res Rev*. 2002;8:51–57.

49. Brown AS. Prenatal infection as a risk factor for schizophrenia. *Schizophr Bull*. 2006;32:200–202.

50. Patterson PH. Neuroscience. Maternal effects on schizophrenia risk. *Science*. 2007;318:576–577.