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## Differential effects of antipsychotic and propsychotic drugs on prepulse inhibition and locomotor activity in Roman high- (RHA) and low-avoidance (RLA) rats

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### Abstract

**Rationale**—Animal models with predictive and construct validity are necessary for developing novel and efficient therapeutics for psychiatric disorders.

**Objectives**—We have carried out a pharmacological characterization of the Roman high-(RHA-I) and low-avoidance (RLA-I) rat strains with different acutely administered propsychotic (DOI, MK-801) and antipsychotic drugs (haloperidol, clozapine), as well as apomorphine, on prepulse inhibition (PPI) of startle and locomotor activity (activity cages).

**Results**—RHA-I rats display a consistent deficit of PPI compared with RLA-I rats. The typical antipsychotic haloperidol (dopamine D2 receptor antagonist) reversed the PPI deficit characteristic of RHA-I rats (in particular at 65 and 70 dB prepulse intensities) and reduced locomotion in both strains. The atypical antipsychotic clozapine (serotonin/dopamine receptor antagonist) did not affect PPI in either strain, but decreased locomotion in a dose-dependent manner in both rat strains. The mixed dopamine D1/D2 agonist, apomorphine, at the dose of 0.05 mg/kg, decreased PPI in RHA-I, but not RLA-I rats. The hallucinogen drug DOI (5-HT2A agonist; 0.1–1.0 mg/kg) disrupted PPI in RLA-I rats in a dose-dependent manner at the 70 dB prepulse intensity, while in RHA-I rats, only the 0.5 mg/kg dose impaired PPI at the 80 dB prepulse intensity. DOI slightly decreased locomotion in both strains. Finally, clozapine attenuated the PPI impairment induced by the NMDA receptor antagonist MK-801 only in RLA-I rats.

**Conclusions**—These results add experimental evidence to the view that RHA-I rats represent a model with predictive and construct validity of some dopamine and 5-HT<sub>2A</sub> receptor-related features of schizophrenia.

### Keywords

Prepulse inhibition; Activity; Propsychotic drugs; Antipsychotic drugs; Roman; high- and low-avoidance rats; Genetically based rat model; Predictive validity; Schizophrenia

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### Introduction

Some very commonly used rodent models of schizophrenia symptoms tend to replicate aspects of the positive symptoms of the disorder, such as hyperactivity or prepulse inhibition (sensorimotor gating) deficits. Prepulse inhibition (PPI; reduction of the startle response elicited by a preceding non-startling stimulus that is shorter and less intense than the startling stimulus) of the acoustic startle response is a measure of sensorimotor gating that is thought to be relevant in some mental disorders like schizophrenia (Kohl et al. 2013), as schizophrenic patients have PPI deficits. Some of the most popular rodent models derive from the similarity of the human effects of psychotomimetic or psychostimulant drugs to the symptoms of schizophrenia. Thus, the administration of psychostimulant or psychotomimetic (hallucinogenic) drugs to rats or mice generally leads to PPI deficits and, very often, induces hyperactivity (Sawa and Snyder 2002; Powell and Miyakawa 2006; Jones et al. 2011; Del Río et al. 2014). These drugs may also produce some of the negative and/or cognitive deficits/symptoms of schizophrenia, although their specific profile of effects depends on the main neurochemical mechanisms influenced by each drug class. In general, these symptoms can be reversed either by typical or atypical antipsychotic drugs, which gives pharmacological (predictive) validity to these models (Sawa and Snyder 2002; Powell and Miyakawa 2006; Jones et al. 2011; Del Río et al. 2014). The findings that psychotic-like states or symptoms are produced by dopaminergic psychostimulants (dopamine receptor agonists) and that such symptoms are reversed by typical antipsychotic drugs (i.e., D<sub>2</sub> dopamine receptor antagonists; Geyer et al. 2001; Jones et al. 2011) support the dopaminergic hypothesis of schizophrenia. On the other hand, the finding that serotonergic psychotomimetic (serotonin receptor—5-HT<sub>2A</sub>—agonists) drugs, as well as NMDA (ionotropic glutamate receptor), antagonists also induce psychotic-like symptoms which are reversed by (mostly) atypical antipsychotic drugs (Halberstadt and Geyer 2013 and Geyer et al. 2001) has provided support to the more modern serotonin and glutamate hypotheses of schizophrenia (Sawa and Snyder 2002; Powell and Miyakawa 2006; Jones et al. 2011; Del Río et al. 2014, Halberstadt and Geyer 2013).

On the other hand, using schizophrenia models in which symptoms (or deficits) are spontaneously present in naive (untreated) animals is important in order to elucidate whether typical or atypical antipsychotics can ameliorate symptoms in vulnerable subjects (e.g., Geyer et al. 2001). We have recently proposed that one such model, the Roman rat strains, displays genetically determined deficits/impairments in several schizophrenia-related features (Del Río et al. 2014; Oliveras et al. 2015; Esnal et al. 2016). The Roman high- (RHA) and low-avoidance (RLA) rat lines/strains (*lines* refers to the outbred, and *strains*

refers to the inbred animals) have been selectively and bidirectionally bred for their rapid (RHA) vs extremely poor (RLA) ability to acquire the two-way active avoidance task (Bignami 1965; Driscoll and Battig 1982; Steimer and Driscoll 2003; Escorihuela et al. 1999, Rio-Alamos et al. 2015). There is extensive evidence showing that RHA/RLA rats display differential schizophrenia-relevant features. For instance, the RHA strain/line displays impaired performance in several learning/memory tasks (Aguilar et al. 2002; Driscoll et al. 1995; Escorihuela et al. 1995b; Nil and Bättig 1981; Oliveras et al. 2015) and enhanced impulsive behavior in the 5-CSRTT and DRL-20 operant tasks (Klein et al. 2014; Moreno et al. 2010; Zeier et al. 1978). RHA rats exhibit deficits in latent inhibition (Fernandez-Teruel et al. 2006; Esnal et al. 2016), augmented mesocortical dopaminergic response to stress (Giorgi et al. 2003), and enhanced locomotor as well as mesolimbic dopaminergic sensitization upon the repeated administration of dopaminergic psychostimulants (Corda et al. 2005; Giorgi et al. 2007; Guitart-Masip et al. 2008a, b), a more robust functional tone of nigrostriatal and mesolimbic dopamine neurons (Tournier et al. 2013) and neurochemical and neuromorphological evidence of decreased hippocampal function (Garcia-Falgueras et al. 2012; Meyza et al. 2009; Salles et al. 2001). With regard to other schizophrenia-linked neurotransmitter systems, like serotonin and glutamate, Klein et al. (2014) have shown that RHA-I rats exhibit a dramatically reduced density of mGluR2 receptors in the prefrontal cortex and hippocampus and increased binding density of 5HT2A receptors in the prefrontal cortex (Klein et al. 2014; Wood et al. 2016), similarly to what has been observed post mortem in the brains of schizophrenic patients. Gonzalez-Maeso et al. (2007, 2008) proposed that 5-HT2A and mGlu2 receptors engage in a functional interaction that plays a crucial role in pre-attentive processes (sensorimotor gating) and in psychotic states. Thus, RHA rats display a series of neurobehavioral traits that resemble some schizophrenia—relevant symptoms or associated neural processes.

On the basis of that evidence, we would expect that the typical antipsychotic haloperidol (a preferential dopamine D2 receptor antagonist) would reverse the PPI deficit of RHA-I rats whereas the dopamine (D1/D2) agonist apomorphine would further impair such a deficit. Moreover, given the above mentioned deficit of mGlu2 receptors in RHA-I rats, and because these receptors are functionally linked to 5-HT2A receptors (Gonzalez-Maeso et al. 2008), we would also predict that the propsychotic agent DOI (5-HT2A receptor agonist) and the atypical antipsychotic clozapine (5-HT2A receptor antagonist, but also antagonist at many other receptors, including D2 receptors) would be less effective on PPI and locomotion in RHA-I than RLA-I rats. Accordingly, it would also be expected that clozapine would show greater ability to reverse the MK-801-induced impairment of PPI in RLA-I than RHA-I rats. Most of these hypotheses were confirmed by our experimental results.

## Materials and methods

### Animals

Animals used for the present studies were male rats of the inbred Roman high-(RHA-I,  $N=283$ ) and low-avoidance (RLA-I,  $N=294$ ) rat strains from the permanent colonies maintained at our laboratory (Medical Psychology Unit, Dept. Psychiatry and Forensic Medicine, School of Medicine, Autonomous University of Barcelona) since 1996. They

were approximately 4 months old at the beginning of the experiments (weight range 320–420 g) and were housed in same-sexed pairs in standard (50 × 25 × 14 cm) macrolon cages. They were maintained under a 12:12 h light-dark cycle (lights on at 08:00 a.m.), with controlled temperature (22 ± 2 °C) and humidity (50–70%) and with free access to food and water. Naive rats were used for each of the ten experiments described below. Experimental procedures were approved by the Committee of Ethics of the Autonomous University of Barcelona and they were carried out in accordance with the European Council Directive (2010/63/EU) and Spanish legislation (RD 53/2013).

## Drugs

Haloperidol, clozapine, 2,5-dimethoxy-4-iodoamphetamine (DOI), MK-801 (dizocilpine), and apomorphine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Haloperidol and clozapine were dissolved in a small amount of glacial acetic acid and then diluted in distilled water. DOI, apomorphine, and MK-801 were dissolved in saline (0.9% NaCl). All the solutions were freshly prepared each day.

**Treatments**—All the drug doses and their respective vehicles were administered subcutaneously (s.c.) in a volume of 1 ml/kg body weight. Haloperidol (0.1, 0.25, 0.50, or 1 mg/kg) or its vehicle were administered 30 min before testing. Apomorphine (0.05 mg/kg) or its vehicle was administered 5 min before testing. DOI (0.1, 0.25, 0.50, or 1 mg/kg) or its vehicle was administered s.c. 15 min before testing. Clozapine (2.5, 5, or 10 mg/kg) or its vehicle was administered 60 min before testing, and MK-801 (0.1 mg/kg) or its vehicle was subcutaneously administered 20 min before testing.

To assess the effects on locomotor activity of haloperidol, DOI, and clozapine, the two lowest doses of each drug used in the PPI experiments were chosen and injected at the same time interval before starting the registration of locomotion as in the PPI studies. The effective dose of apomorphine was selected according to a previous report (Sanna et al. 2014a, b), but the effects of apomorphine on locomotor activity were not addressed here because they have been well characterized in our previous study (Giménez-Llort et al. 2005). For the experiment with clozapine and MK-801, the doses chosen were based on previous studies (Bast et al. 2000; Bubeníková et al. 2005; Cilia et al. 2010; Hadamitzky et al. 2007) and also on our pilot experiments showing that at a dose 0.1 mg/kg, MK-801 reduced significantly %PPI in both Roman strains (data not shown).

## Prepulse inhibition of the acoustic startle response

Four sound-attenuated boxes (SR-Lab Startle Response System, San Diego Inst., San Diego, USA) were used. Each box consists of a Plexiglas cylinder placed on top of a platform with a sensor that detects the intensity of the force made by the rat in each trial. Two speakers placed at 15 cm on each side of the cylinder deliver the acoustic stimuli, and a white noise generator provides the background noise (55 dB) throughout the whole session. Each box is lit by a 10 W lamp. The data are transduced by an accelerometer into a voltage which is saved into a computer for further analysis.

The startle session started with a 5-min habituation period in the startle chambers. Then, 10 *pulse-alone* trials (105 dB, 40 ms) were delivered in order to obtain a basal measure of the ASR (BASELINE 1). Next, each one of the 6 different types of trials was randomly administered 10 times (i.e., 60 trials in total):

1. Pulse-alone trials (105 dB 40 ms, BASELINE 2)
2. Prepulses of 65/70/75/80 dB (20 ms) followed by the startle stimulus (105 dB, 40 ms), with an inter-stimulus interval of 100 ms.
3. No stimulus trials (background noise 55 dB)

Finally, in order to measure the habituation to the startle stimulus, 5 *pulse-alone* trials were delivered (BASELINE 3).

The interval between trials was 10–20 s with a mean of 15 s. The startle magnitude was recorded for 200 ms after the onset of the pulse.

The degree of PPI (in percentage) was calculated according to the formula:

$$\%PPI = 100 - \left( \frac{\text{startle amplitude on prepulse trials}}{\text{startle amplitude on pulse - alone trials}} \right) \times 100$$

### Locomotor activity assessment

Locomotor activity of RHA-I and RLA-I rats was evaluated with 3 identical plexiglas activity cages (40 × 40 × 40 cm). These cages are equipped with photocell beams that are interfaced with a PC to record the locomotor activity of the rats using program Acti-track (Panlab, Spain).

The procedure used was identical in the four experiments except for the intervals between the injection of each drug and the beginning of the testing session, which were the same as in the respective PPI experiments (see above). There was no previous habituation to the apparatus or the testing room. Locomotor activity was measured as the total photocell beam breaks every 5 min for a total of 30 min.

### Statistical analysis

Statistical analysis was performed using the *Statistical Package for Social Science* (SPSS, version 17).

To assess the percentage PPI data as the dependent variable, we performed repeated measures ANOVAs, with the 4 prepulse intensities as a within-subject factor, and the 2 strains and the administered doses as between-subject factors. To assess the differences in the startle response during pulse-alone trials, we performed an ANOVA with baseline 2 as the dependent variable and the *doses* and *strain* as between-subject factors. For locomotor activity experiments, we assessed the distance traveled data as the dependent variable using repeated measures multifactor ANOVAs with *drugs doses* and *strains* as between-subject factors and 6 time intervals (5 min each) as a within-subject factor. Post hoc pair wise

contrasts with the LSD test were performed on PPI and activity measures after significant *Strain* × *Treatment* ANOVA effects.

To assess the pharmacological interaction between clozapine and MK-801, we conducted a repeated measures ANOVA with *strain*, *clozapine*, and *MK-801* as between-subject factors and the prepulse intensities as a within-subject factor. Post hoc pair wise contrasts with the LSD test were performed after significant *Strain* × *Treatment* ANOVA effects. The threshold for statistical significance was set at  $p = 0.05$ .

The number of animals in each experimental group is indicated in the legends to figures. Only the comparisons between the drug doses and the respective vehicle group are indicated in the figures except for the study with clozapine and MK-801 in which the significant comparisons with the veh/MK-801 group are also indicated.

## Results

### Effects of antipsychotics on PPI and locomotor activity

**Haloperidol (Exps. 1–2) effects on PPI**—In Exp. 1 (Fig. 1), the repeated measures factorial ANOVA (2 strains × 5 haloperidol doses × 4 prepulse intensities—as within-subject factor-) revealed significant *prepulse intensity* (Huynh-Feldt  $F(2.86, 182.86) = 373.91$ ,  $p = 0.001$ ), *prepulse intensity* × *strain*, and *prepulse intensity* × *treatment* effects (Huynh-Feldt  $F(6.11, 182.86) = 6.11$ ,  $p = 0.001$ , and  $F(11.43, 182.86) = 3.51$ ,  $p = 0.001$ , respectively). There were also *strain* and *treatment* effects ( $F(1, 64) = 13.00$ ,  $p = 0.001$ ;  $F(1, 64) = 3.64$ ,  $p = 0.010$ , respectively) (Fig. 1a). The *strain* effect is due to the overall better PPI performance of the RLA-I rats, and the *treatment* effect indicates a global increase of PPI with haloperidol.

A two-way ANOVA showed a significant *treatment* effect on baseline startle response ( $F(4, 64) = 5.21$ ,  $p = 0.001$ ), as haloperidol overall decreased this measure in both strains (Fig. 1c).

As a clear trend for an increase of PPI with haloperidol 1 mg/kg was observed in Exp. 1, Exp. 2 was carried out to test whether this dose had differential, strain-dependent effects. Indeed, the repeated measures ANOVA (2 strains × 2 haloperidol doses × 4 prepulse intensities—within subject factor-) showed that the second-order interaction (*prepulse intensity* × *strain* × *treatment*) was significant (Huynh-Feldt  $F(2.17, 78.23) = 4.91$ ,  $p = 0.008$ ) as well as the *strain* and *treatment* effects ( $F(1, 36) = 24.40$ ,  $p = 0.001$ , and  $F(1, 36) = 5.17$ ,  $p = 0.029$ , respectively, Fig. 2a). Further, post hoc pair wise comparisons, to determine the source of that interaction, revealed that %PPI was increased in RHA-I rats treated with 1 mg/kg haloperidol at 65 and 70 dB intensities whereas no effects were observed in RLA-I rats (Fig. 2a)

Two-way ANOVA on baseline startle response showed no significant differences between strains or treatments in Exp. 2 (Fig. 2c).

In summary, both Exps. 1 and 2 show that RHA-I rats display lower %PPI than RLA-I rats and that haloperidol globally increases %PPI, whereas Exp. 2 shows that haloperidol 1

mg/kg improves %PPI in RHA-I rats without significantly affecting %PPI in the RLA-I strain.

**Clozapine (Exp. 7) effects on PPI**—In Exp. 7, the repeated measures factorial ANOVA (2 strains  $\times$  4 clozapine doses  $\times$  4 prepulse intensities—as within-subject factor-) revealed a significant interaction between *strain* and *prepulse intensity* (Huynh-Feldt  $F(2.93, 161.37) = 13.19$ ,  $p = 0.001$ ). The only main effect found was the *strain* effect ( $F(1, 55) = 8.89$ ,  $p = 0.004$ ) (Fig. 7a), overall indicating the better performance of the RLA-I rats (Fig. 7a, b).

Assessment by two-way ANOVA of the baseline startle yielded significant *strain* and *treatment* effects ( $F(1, 55) = 8.70$ ,  $p = 0.005$ , and  $F(3, 55) = 12.19$ ,  $p = 0.001$ , respectively; Fig. 7c). The *strain* effect is due to the increased startle response of the RLA-I whereas the *treatment* effect reflects the overall reduction of startle response in clozapine-treated rats.

**Haloperidol (Exp. 3) and clozapine (Exp. 8) effects on locomotor activity**—In Exp. 3, the overall repeated measures ANOVA (2 strains  $\times$  3 haloperidol doses  $\times$  6 time intervals—within subject factor-) revealed a significant *interval*  $\times$  *strain*  $\times$  *treatment* interaction effect on locomotion (Huynh-Feldt  $F(8.85, 181.33) = 2.40$ ,  $p = 0.014$ , Fig. 3a, b). Moreover, there was a significant *treatment* effect ( $F(2, 41) = 14.07$ ,  $p = 0.001$ , Fig. 3a, b). *Post hoc* pair wise comparisons performed to dissect that interaction indicated that both haloperidol doses reduced locomotor activity in the first three 5-min intervals in RLA-I rats, whereas the 0.1 mg/kg dose was clearly less effective in RHA-I vs RLA-I rats (Fig. 3a, b). These results show that haloperidol reduced locomotion in both strains, but the effect elicited by 0.1 mg/kg haloperidol was more marked in RLA-I rats.

Repeated measures ANOVA (2 strains  $\times$  3 clozapine doses  $\times$  6 time intervals—within-subject factor-) was performed on activity (distance traveled). A significant *treatment*  $\times$  *interval* interaction was found (Huynh-Feldt  $F(8.98, 188.57) = 5.57$ ,  $p = 0.001$ ), as well as *strain* ( $F(1, 42) = 4.24$ ,  $p = 0.046$ ) and *treatment* ( $F(2, 42) = 12.87$ ,  $p = 0.001$ ) effects (Fig. 8a, b). The *strain* effect is due to the higher activity exhibited by the RHA-I rats, and the *treatment* effect is due to the global reduction of activity induced by clozapine.

Repeated measures ANOVA for the first 15 min of the sessions (3 intervals) for both drugs yielded similar results (data not shown).

### Apomorphine and DOI effects on PPI and locomotor activity

**Effects of apomorphine (Exp. 4) on PPI**—The repeated measures factorial ANOVA (2 strains  $\times$  2 apomorphine doses  $\times$  4 prepulse intensities—within subject factor-) revealed a significant *strain*  $\times$  *prepulse intensity* effect (Huynh-Feldt  $F(2.82, 118.22) = 5.19$ ,  $p = 0.003$ ). The interaction *strain*  $\times$  *treatment* was also significant ( $F(1, 42) = 4.46$ ,  $p = 0.042$ ), as well as the *strain* and *treatment* effects ( $F(1, 42) = 17.28$ ,  $p < 0.001$ , and  $F(1, 42) = 6.92$ ,  $p = 0.012$ , respectively) (Fig. 4a, b). *Post hoc* comparisons to determine the source of the *strain*  $\times$  *treatment* interaction revealed that RHA-I rats treated with apomorphine showed a reduction in %PPI at all prepulse intensities, whereas PPI performance of RLA-I rats was not disrupted by apomorphine at any prepulse intensity (Fig. 4a, b).

Assessment by means of two-way ANOVA baseline startle revealed a significant *strain* effect ( $F(1,42) = 15.99, p = 0.001$ ) and a significant *strain*  $\times$  *treatment* interaction ( $F(3,78) = 6.50, p = 0.015$ ) (Fig. 4c). Post hoc comparisons showed that baseline startle was reduced by apomorphine in RHA-I rats but not in their RLA-I counterparts (Fig. 4c).

**Effects of DOI (Exp. 5) on PPI**—Repeated measures ANOVA (2 strains  $\times$  5 DOI doses  $\times$  4 prepulse intensities—within subject factor-) revealed significant *strain* and *treatment* effects ( $F(1,78) = 5.70, p = 0.019$ , and  $F(4, 78) = 4.04, p = 0.005$ , respectively) and also a significant interaction between both factors ( $F(4,78) = 2.55, p = 0.046$ ) (Fig. 5a). The post hoc comparisons evidenced that 0.5 mg/kg DOI significantly reduced %PPI of RHA-I rats at 80 dB prepulse intensity (see post hoc tests in Fig. 5a, b). In RLA-I rats, all DOI doses significantly diminished %PPI at the 70 dB prepulse intensity in a dose-dependent manner (see post hoc comparisons in Fig. 5a, b).

Two-way ANOVA evaluation of the baseline startle revealed a significant *treatment* effect ( $F(4, 78) = 2.74, p = 0.034$ ), indicating an overall decrease of startle response magnitude induced by DOI in both strains (Fig. 5c).

**Effects of DOI (Exp. 6) on locomotor activity**—Repeated measures ANOVA (2 strains  $\times$  3 DOI doses  $\times$  6 time intervals—within-subject factor-) revealed a significant *interval*  $\times$  *treatment* interaction (Huynh-Feldt  $F(9.45,189.07) = 4.12, p = 0.001$ ). There were also significant *strain* ( $F(1, 40) = 7.52, p = 0.009$ ) and *treatment* effects ( $F(2, 40) = 3.33, p = 0.046$ ) (Figs 6a, b). The *strain* effect is due to more intense locomotion of the RHA-I rats, while the *treatment* effect is apparently due to the reduction of activity induced by 0.25 mg/kg DOI in both rat strains.

Repeated measures ANOVA for the first 15 min of the sessions (3 intervals) yielded similar results (data not shown).

### Clozapine effects on MK-801-induced deficits on PPI (Exp. 9)

Repeated measures ANOVA (2 strains  $\times$  2 clozapine doses  $\times$  2 MK-801 doses  $\times$  4 prepulse intensities—within-subject factor-) revealed a significant *prepulse intensity*  $\times$  *strain* interaction ( $F(3, 216) = 2.82, p = 0.040$ ), as well as significant effects of the three main factors (*strain*  $F(1,72) = 13.21, p = 0.001$ ; *clozapine*  $F(1, 72) = 4.60, p = 0.035$ ; *MK-801*  $F(1, 72) = 41.93, p = 0.001$ ). There was also a significant *strain*  $\times$  *clozapine*  $\times$  *MK-801* interaction ( $F(1,72) = 4.39, p = 0.040$ ) (Fig. 9a, b).

Post hoc tests applied to clarify the source of this second-order interaction showed that MK-801 impaired PPI in RHA-I rats only at the 80 dB prepulse intensity and this effect was not attenuated by clozapine (see Fig. 9a), while the impairment in RLA-I rats was observed at all prepulse intensities (Fig. 9a) and it was attenuated by clozapine at the 75 dB intensity (Fig. 9a). Likewise, post hoc comparisons on the *total % PPI* measure (Fig. 9b) showed that MK-801 did not significantly impair total %PPI in RHA-I rats, whereas this measure was impaired in RLA-I rats and clozapine significantly (though partially) attenuated such an impairment (Fig. 9b).

Three-way ANOVA on baseline startle revealed significant *strain* ( $F(1,72) = 4.13, p = 0.046$ ), *clozapine* ( $F(1,72) = 11.98, p = 0.001$ ), and *MK-801* ( $F(1,72) = 15.90, p = 0.001$ ) effects, as baseline startle was overall higher in RLA-I vs RHA-I rats and was decreased by clozapine but enhanced by MK-801 (Fig. 9c).

### Clozapine effects on MK-801-induced hyperactivity (Exp. 10)

Repeated measures ANOVA on activity results (the whole 30-min session) revealed a significant *time interval*  $\times$  *clozapine* interaction (Huynh-Feldt  $F(3.50, 161.06) = 6.53, p = 0.001$ ). Significant effects of *MK-801* and *clozapine* ( $F(1,46) = 21.38, p = 0.001$  and  $F(1, 46) = 20.25, p = 0.001$ , respectively) were also found (Fig. 10a, b), indicating that in both strains, MK-801 induced a statistically significant hyperactivity while clozapine reduced locomotor activity.

We also conducted a repeated measures ANOVA with the first three intervals (i.e., first 15 min) of the session as within-subject factor. Main effects of *clozapine* and *MK-801* were observed ( $F(1,46) = 28.04, p = 0.001$ , and  $F(1, 46) = 24.22, p = 0.001$ , respectively), as well as a *clozapine*  $\times$  *interval* ( $F(1.80, 82.63) = 14.39, p = 0.001$ ) and a *strain*  $\times$  *clozapine*  $\times$  *interval* interaction ( $F(1.80, 82.63) = 3.27, p = 0.048$ ). Post hoc comparisons to dissect this second-order (i.e., *strain*  $\times$  *clozapine*  $\times$  *interval*) interaction on locomotor activity during the first three 5-min (0–5, 6–10, 11–15) intervals showed that MK-801 increased locomotor activity of RHA-I rats during the three 5-min intervals (Fig. 10a), but only during the first 5-min interval in RLA-I rats (Fig. 10b). Moreover, the clozapine + MK-801 treatment produced a more marked attenuation of MK-801-induced hyperactivity in RHA-I (see intervals 0–5 and 6–10 in Fig. 10a) than in RLA-I rats (see interval 0–5 in Fig. 10b).

## Discussion

The present report provides the first pharmacological characterization of the effects of antipsychotics (haloperidol, a typical antipsychotic, and clozapine, an atypical antipsychotic), propsychotic drugs (DOI and MK-801), and the mixed dopamine D1/D2 receptor agonist apomorphine on PPI and locomotor activity in the Roman rat strains. The main findings are as follows: (1) haloperidol normalized the deficit in PPI showed by RHA-I rats, without affecting PPI in RLA-I rats; moreover, haloperidol reduced locomotor activity more markedly in the RLA-I strain; (2) clozapine (a 5-HT<sub>2A</sub> and dopamine D2 receptor antagonist) failed to affect PPI in either strain, but reduced locomotor activity in both strains; (3) apomorphine impaired PPI only in RHA-I rats; (4) the propsychotic drug DOI (5-HT<sub>2A</sub> receptor agonist) disrupted PPI in RLA-I rats in a dose-dependent manner while only the 0.5 mg/kg dose impaired PPI in RHA-I rats; (5) DOI moderately decreased locomotor activity in both strains; (6) clozapine significantly antagonized the MK-801-induced PPI deficit only in RLA-I rats; and (7) clozapine appeared to antagonize MK-801-induced hyperactivity more effectively in RHA-I vs RLA-I rats.

Remarkably, a general finding of all PPI experiments was that the total averaged %PPI was consistently lower in RHA-I than in RLA-I rats, which is in agreement with previous studies showing that the RHA-I strain displays a relative deficit in PPI (Oliveras et al. 2015; Esnal et al. 2016; Del Río et al. 2014).

## Haloperidol and clozapine effects on PPI and locomotor activity

In order to assess the predictive validity of genetically based rat models of schizophrenia-related symptoms, it is crucial that propsychotic and antipsychotic drugs are tested alone in naive animals, to evaluate whether they are able to further impair or reverse PPI deficits, respectively (e.g., Geyer et al. 2001). For example, using this approach, Hadamitzky et al. (2007) found that the deficits displayed by rats bred for their low scores of PPI could be restored by haloperidol, but not by clozapine. Moreover, Cilia et al. (2010) reported that Brattleboro rats (which have a mutant gene for vasopressin and display impaired PPI) show a significant improvement of PPI upon treatment with haloperidol and atypical antipsychotics like clozapine and risperidone. In the present study, we have used this approach in order to gather evidence on the pharmacological validity of the Roman rat strains (which markedly differ in their basal PPI performance) as a model of differential schizophrenia-related features.

We report that haloperidol 1 mg/kg reverses the PPI deficit of RHA-I rats, without affecting RLA-I rats, whereas the atypical antipsychotic clozapine does not affect PPI in either strain. Moreover, haloperidol reduced baseline startle of both strains in Exp. 1 but did not affect it in Exp. 2, while clozapine (Exp. 7) also decreased startle in both strains. The fact that in Exp. 2, haloperidol increased PPI in RHA-I rats in the absence of changes of baseline startle, strengthens the idea that the drug genuinely improves sensorimotor gating in this strain (Swerdlow et al. 2000). Conversely, the lack of effects of clozapine might be related to its startle-decreasing effects (in pulse-alone trials) in both strains. Similar effects were observed by Hadamitzky et al. (2007) in their selectively bred low-PPI rat line, as they also found that haloperidol but not clozapine improved PPI. The authors attributed the lack of effects of clozapine to its (baseline) startle-inhibiting effects, although they also noted that when this drug has an effect, it is often in a narrow dose range (i.e., it is likely to miss an effective dose in a particular study) and that its effects (both on startle and on PPI) vary between different rat strains, with Wistar rats (which are the original strain from which the RHA/RLA lines were derived) being a particularly resistant strain (Hadamitzky et al. 2007). These results are in contrast with previous studies usually showing that typical antipsychotics do not ameliorate PPI deficits while atypical antipsychotics revert PPI deficits (among other negative and cognitive symptoms) in naive rats (see Geyer et al. 2001; but also see Cilia et al. 2010; Feifel et al. 2001; Hadamitzky et al. 2007).

Finally, haloperidol more markedly reduced locomotor activity in RLA-Is than RHA-I rats, while clozapine reduced activity of both rat strains to a similar extent. While there have been no previous studies with clozapine in these strains, the effects of haloperidol on locomotor activity are consistent with previous studies with the Roman rats. For example, Sanna et al. (2014b) reported that haloperidol impaired copulatory behavior in RLA, but not RHA rats. The authors interpreted that these effects may be due to the lowered dopaminergic tone and increased D2 receptor density (Tournier et al. 2013) in the brain of RLA rats, which might make this strain more sensitive than RHA rats to the inhibition of locomotion induced by haloperidol, thereby interfering with copulatory behavior. Moreover, in keeping with this hypothesis and with our present results, Eilam and Szechtman (1989) reported that RHA rats are less sensitive than their RLA counterparts to the inhibitory effect on locomotion of

quinpirole, which is thought to be mediated by the activation of presynaptic inhibitory D2 autoreceptors.

### **Apomorphine and DOI effects on PPI and locomotor activity**

The mixed D1/D2 receptor agonist apomorphine and the propsychotic (5-HT<sub>2A</sub> agonist) DOI globally impaired PPI, in a strain-dependent manner. Thus, the apomorphine-induced impairment was evident only in RHA-I rats, whereas the PPI-disrupting effects of DOI were more robust in RLA-I rats. Moreover, apomorphine decreased startle responses to pulse-alone stimuli in RHA-I rats, and a similar trend was observed with DOI. It might be argued that the disruption of PPI induced by apomorphine in RHA-I rats might be related to the observed reduction of startle responses in that strain; however, the PPI-disrupting apomorphine effect is a well-established phenomenon (e.g., Swerdlow et al. 2000), which is sometimes paralleled by a drug-induced reduction of startle responses to pulse-alone trials (e.g., Breier et al. 2010; Swerdlow et al. 2000). As the phenomenon has been replicated in several rat strains and has been shown to be heritable, regardless of whether or not it is sometimes associated to a reduction of startle responses, it is generally considered as solid evidence that apomorphine induces an impairment of sensorimotor gating (e.g., Breier et al. 2010; Swerdlow et al. 2000). Similarly, the PPI-disruptive effect of DOI, also a well-characterized phenomenon (e.g., Farid et al. 2000; Wischhof et al. 2012, see below), is present in RLA-I rats at doses devoid of effects on baseline startle response (see Fig. 5b, c). A moderate decrease of locomotor activity was also observed with DOI (as said in “Materials and methods” section, the effects of apomorphine on locomotor activity in both strains were not addressed here because they have been well characterized in our previous study; Gimenez-Llort et al. 2005).

The above results give support to our hypotheses as, based on neurochemical and neuropharmacological evidence of differential central dopaminergic function between both Roman rat lines/strains (see “Introduction”), we predicted that RHA-I rats would be more sensitive to the haloperidol-improving and apomorphine-impairing effects on PPI. In fact, there is mounting evidence supporting the view that the functional tone of nigrostriatal and mesolimbic dopamine neurons is more robust in RHA than RLA rats, and it has been suggested that the lower availability of inhibitory D2 autoreceptors in the cell bodies of nigrostriatal and mesolimbic dopaminergic neurons of RHA rats (Tournier et al. 2013) may account (at least in part) for the behavioral differences between the two rat lines/strains (e.g., Driscoll et al. 2009; Giorgi et al. 2007; Guitart-Masip et al. 2008a, b; Sanna et al. 2013, 2014a, b; Tournier et al. 2013). Together with the finding that RHA rats show increased sensitivity to dopaminergic psychostimulants (Corda et al. 2005; Giorgi et al. 2007; Gimenez-Llort et al. 2005) and to the behavioral and neurochemical sensitization effects of these drugs (Corda et al. 2005; Giorgi et al. 2007; Guitart-Masip et al. 2008a), the results of the present PPI experiments using haloperidol and apomorphine lend further support to the validity and usefulness of RHA-I vs RLA-I rats as a model of some dopamine-related schizophrenia-relevant symptoms.

On the other hand, in keeping with previous reports, DOI significantly disrupted PPI in both strains (e.g., Sipes and Geyer 1997; Johansson et al. 1995; Padich et al. 1996; Sipes and

Geyer 1995b), and consistent with our hypothesis, this effect was more pronounced in RLA-I than in RHA-I rats (see Fig. 5). A differential strain-related sensitivity to the PPI-disrupting effect of DOI has also been reported by Wischhof et al. (2012), who observed DOI-induced PPI deficits in Wistar, but not Lister Hooded, rats. Our results also show that RLA-I rats are more sensitive to DOI than their RHA-I counterparts. Several mechanisms may explain these strain-related differences in the effects of DOI on PPI, including drug sensitivity, receptor densities or affinities, as well as the coupling to second messengers (Wischhof et al. 2012). In this context, the recently discovered relationship between 5-HT<sub>2A</sub> (which are the molecular target of DOI) and mGlu<sub>2</sub> receptors (Delille et al. 2012; Gonzalez-Maeso et al. 2008) may also be involved in the contrasting effects mentioned above. Thus, Gonzalez-Maeso et al. (2008) and Delille et al. (2012), among others, have proposed that a receptor complex containing 5-HT<sub>2A</sub> and mGlu<sub>2</sub> integrates serotonergic and glutamatergic signaling in order to convey sensorimotor information in a proper manner. Furthermore, the finding that these receptors are dysregulated in schizophrenic patients supports the idea that the 5-HT<sub>2A</sub>/mGlu<sub>2</sub> receptor complex plays a major role in the neurochemistry of schizophrenia. Accordingly, the expression of psychotic states by 5-HT receptor agonists and antipsychotic effects by atypical antipsychotic drugs (i.e., 5-HT receptor antagonists) requires the presence of both 5-HT<sub>2A</sub> and mGlu<sub>2</sub> receptors (Fribourg et al. 2011; Gonzalez-Maeso et al. 2008; Klein et al. 2014). Remarkably, as reported by Klein et al. (2014), RHA-I rats showed no detectable protein levels of mGlu<sub>2</sub> receptor protein in the frontal cortex, hippocampus, and the striatum, a deficit that is apparently due to a mutation in the *grm2* gene (Wood et al. 2016). Thus, in line with the above evidence, it seems plausible that the reduced PPI-impairing effects of DOI in RHA-I rats (relative to RLA-Is) may be due to their low cerebral density of mGlu<sub>2</sub> receptors. On the other hand, although there were overall decreasing effects of DOI on baseline startle, taking both rat strains together, the impairment of PPI in RLA-I rats was independent of DOI effects on the basal startle responses (see Fig. 5b, c).

Finally, it is noteworthy that both DOI (i.e., 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> agonist) and clozapine (5-HT<sub>2A</sub> antagonist and antagonist of various 5-HT, DA, and other receptors), despite having partially opposite neurochemical mechanisms of action (i.e., agonist and antagonist of 5-HT<sub>2A</sub> receptors, respectively), induced decreases of locomotor activity that were similar in both rat strains. At least three possibilities may be proposed to explain this discrepancy: (i) the locomotor activity test is not specific enough to distinguish between opposite drug-receptor interactions, (ii) the neural mechanism(s) of action that lead to the decrease of locomotion may be different from those involved in PPI effects, and (iii) given that RHA-I and RLA-I rats present the above mentioned differences in central 5-HT<sub>2A</sub> receptor levels, the mechanisms underlying clozapine- and DOI-induced reduction of activity may not involve (at least not preferentially) 5-HT<sub>2A</sub> receptors.

### **Impairment of PPI and hyperactivity induced by MK-801: reversal by clozapine**

It is well established that NMDA receptor antagonists (PCP, MK-801, ketamine) impair PPI in rodents (for review, see Geyer et al. 2001). Most studies indicate that the disruption of PPI induced by PCP can be reversed/attenuated by clozapine in different species (Andreasen et al. 2006; Ballmaier et al. 2001; Linn et al. 2003; Lipina et al. 2005; Swerdlow et al. 1996),

but there is comparatively more controversial evidence on the effects of atypical antipsychotics on MK-801-impaired PPI (Bakshi et al. 1994; Bast et al. 2000; Bubenikova et al. 2005; Fijal et al. 2014; Levin et al. 2005, 2007; Zangrando et al. 2013). However, the experimental evidence seems to suggest that the PPI deficits induced by the administration of NMDA antagonists in rodents can be reversed/attenuated by some atypical antipsychotics but not by the typical antipsychotic haloperidol (e.g., Adell et al. 2012; Bubenikova et al. 2005; Jentsch and Roth 1999). Thus, following the recent finding that RHA-I rats exhibit increased 5-HT<sub>2A</sub> receptor density (related to their mGluR2 deficit) and to assess whether this characteristic may lead to different pharmacological-behavioral profiles as compared to RLA-I rats, we chose to antagonize MK-801 effects on PPI and locomotion with clozapine.

The results show that MK-801 produced PPI deficits in both rat strains. Interestingly, the MK-801-induced deficit in PPI was more clearly attenuated by clozapine in RLA-I rats. Also, in agreement with previous reports, MK-801 induced a global increase of startle responses in both rat strains (Bakshi et al. 1994; Bast et al. 2000; Bubenikova et al. 2005; Fijal et al. 2014; Levin et al. 2005). As discussed for the effects of DOI on PPI (see above), the finding that clozapine antagonizes the PPI deficit elicited by MK-801 more effectively in RLA-I than RHA-I rats is consistent with the putative important role of the 5-HT<sub>2A</sub>/mGluR2 receptor complex in sensorimotor gating and preattentive processes (Gonzalez-Maeso et al. 2008). Thus, because of their dramatic deficit of mGluR2 receptors (Klein et al. 2014), RHA-I rats would be expected to display weaker effects of 5-HT<sub>2A</sub>-receptor ligands on PPI as compared with their RLA-I counterparts (Gonzalez-Maeso et al. 2008; Fribourg et al. 2011). This prediction is confirmed by the effects on PPI of DOI (Exp. 4) and clozapine + MK-801 (Exp. 9) reported herein.

The finding that MK-801 increases the baseline startle response suggests that this effect may have contributed to the impairment of PPI induced by MK-801 observed in the present study. However, several lines of evidence argue against this hypothesis. First, the PPI-disrupting effect of MK-801 in rats is a well-established phenomenon that has been observed either in the presence (Bakshi et al. 1994; Bast et al. 2000; Bubenikova et al. 2005; Fijal et al. 2014; Levin et al. 2005) or in the absence of increases of the baseline startle response (Bast et al. 2000; Levin et al. 2005, 2007; Zangrando et al. 2013). Second, it is generally assumed that calculating individual PPI as a percentage of each animal's startle response diminishes the influence that changes in startle reactivity have on PPI (e.g., Feifel et al. 2001; Swerdlow et al. 1994; but see also Swerdlow et al. 2000). Third, baseline startle response and %PPI appear to be dissociable, as suggested by manipulations that change baseline startle but not %PPI (e.g., Feifel et al. 2001; Rigdon 1990; Swerdlow et al. 1986), treatments that alter %PPI but not startle (e.g., Feifel et al. 2001; Furuya et al. 1999; Rigdon 1990; Swerdlow et al. 1990b), or interventions that lead to changes of baseline startle and %PPI either in the same or opposite directions (e.g., Acri et al. 1995; al-Amin and Schwarzkopf 1996). Fourth, according to several studies, individual startle amplitude and %PPI are not necessarily correlated (Feifel 1999; Feifel et al. 2001; Oliveras et al. 2015; Paylor and Crawley 1997), although sometimes, they show low positive associations (Logue et al. 1997; Oliveras et al. 2015; Sanchez-Gonzalez et al. 2016). Fifth, although it has been reported that baseline startle tends to be negatively related with %PPI in some studies with rodents and humans (Csomor et al. 2008; Ellenbroek et al. 1995), other studies, using the

Roman rat strains as well as the genetically heterogeneous NIH-HS rat stock, have demonstrated just the opposite, i.e., a positive association between baseline startle and %PPI (Del Rio et al. 2014; Esnal et al. 2016; Oliveras et al. 2015; Sanchez-Gonzalez et al. 2016). Sixth, evidence supporting the dissociation of baseline startle and %PPI comes also from the present study, as haloperidol increased %PPI and did not alter startle in RHA-I rats (Exp. 2), apomorphine and DOI reduced %PPI and also baseline startle in RHA-I rats, MK-801 reduced %PPI but increased the baseline startle, while DOI reduced %PPI at doses not affecting startle in RLA-I rats.

Finally, we observed that MK-801 induced a more robust increment in locomotion in RHA-I vs RLA-I rats, especially during the first 15 min of the activity test, which was also more effectively attenuated by clozapine in the RHA-I strain. One possible explanation for these result is that the larger increment in locomotion elicited by MK-801 in RHA-I vs RLA-I rats could depend on the stimulatory effect of MK-801 on mesolimbic dopaminergic neurons which, under basal conditions, display a more robust physiological tone in RHA vs RLA rats (see Giorgi et al. 2007, and references therein). In fact, there is evidence suggesting that NMDA antagonists mediate some symptoms, such as hyperactivity and sensorimotor deficits, through their actions on limbic dopaminergic pathways (Meltzer et al. 2011). Specifically, the NMDA antagonist-induced stimulation of DA release in the nucleus accumbens has been suggested to result from decreased mesocortical dopaminergic function, which in turn enhances (i.e., disinhibits) mesolimbic dopaminergic activity (Del Arco et al. 2007; Del Arco and Mora 2008, 2009; Lannes et al. 1991; Jentsch et al. 1998; Zangrando et al. 2013). The different physiological tone of the mesolimbic dopaminergic projections of RHA and RLA rats may also be involved in the more effective antagonism of the MK-801-elicited locomotor activation by clozapine in RHA-I vs RLA-I rats.

## Conclusions

In conclusion, regarding sensorimotor gating, the main novel findings of the present study are that (see Table 1), compared to RLA-I, RHA-I rats show higher sensitivity to the improving effects of haloperidol and to the impairing effects of apomorphine on PPI. Conversely, RLA-I rats are more sensitive than their RHA-I counterparts to the PPI-impairing effects of DOI and MK-801, as well to the reversal of MK-801 effect by clozapine.

With regard to locomotor activity, its reduction by haloperidol is more pronounced in RLA-I rats, whereas MK-801-induced hyperactivity and its reversal by clozapine appear to be more marked in RHA-I rats (see Table 1).

The well-known between-strain differences in central dopaminergic function (RHA > RLA) may underlie the observed strain-dependent effects of haloperidol and apomorphine on PPI and locomotor activity. As discussed above, these dopaminergic differences may also be involved in the more robust hyperactivity induced by MK-801 (and its reversal by clozapine) in the RHA-I strain.

Moreover, the results obtained with DOI and clozapine + MK-801 on PPI are consistent with the finding that the RHA-I strain has an alteration of the 5-HT<sub>2A</sub>/mGluR2 complex (i.e., a deficit of mGlu2 receptors and a relative increase of 5-HT<sub>2A</sub> receptors; Klein et al. 2014; Wood et al. 2016), similar to that observed in schizophrenic patients (Gonzalez-Maeso et al. 2008). Thus, RHA-I rats may represent a model of dopamine- and 5-HT<sub>2A</sub>/mGluR2-related schizophrenia-relevant features.

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## Abbreviations

<b>PPI</b>	Prepulse inhibition
<b>RLA</b>	Roman high-avoidance
<b>RHA</b>	Roman low-avoidance

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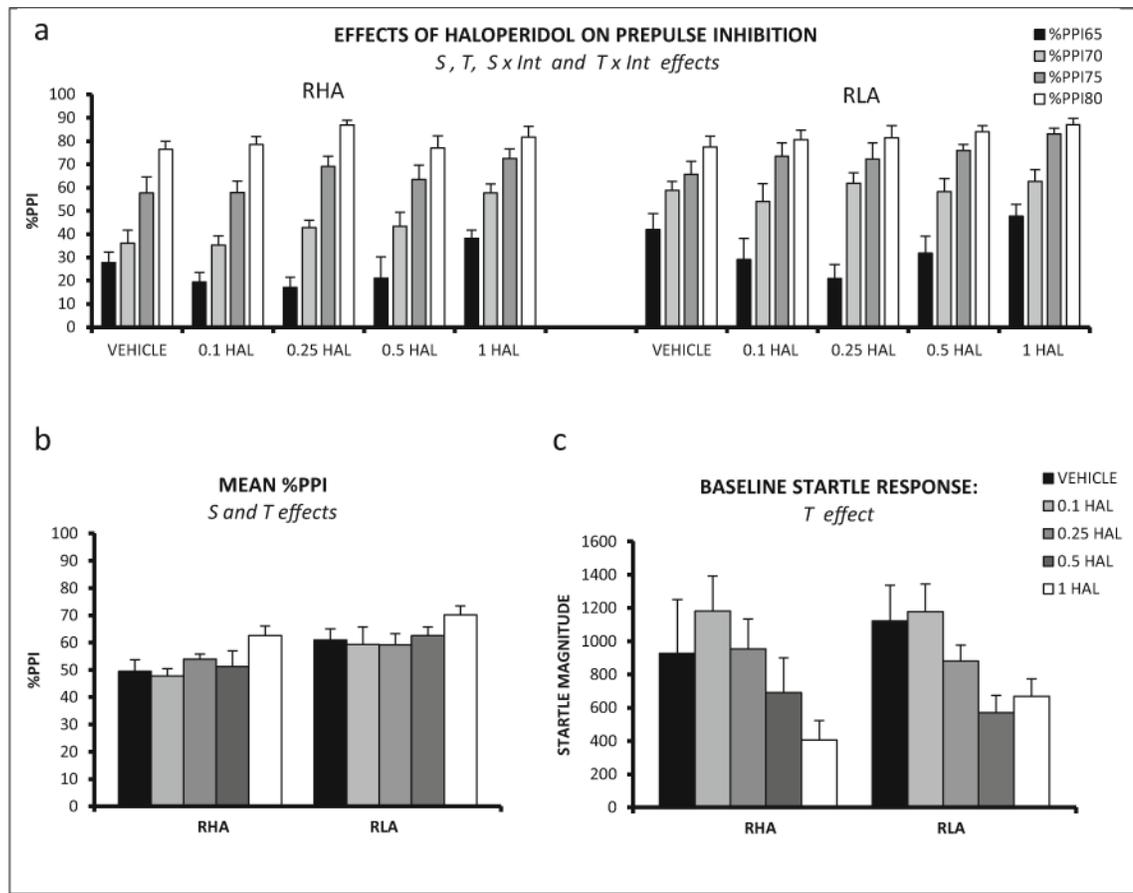
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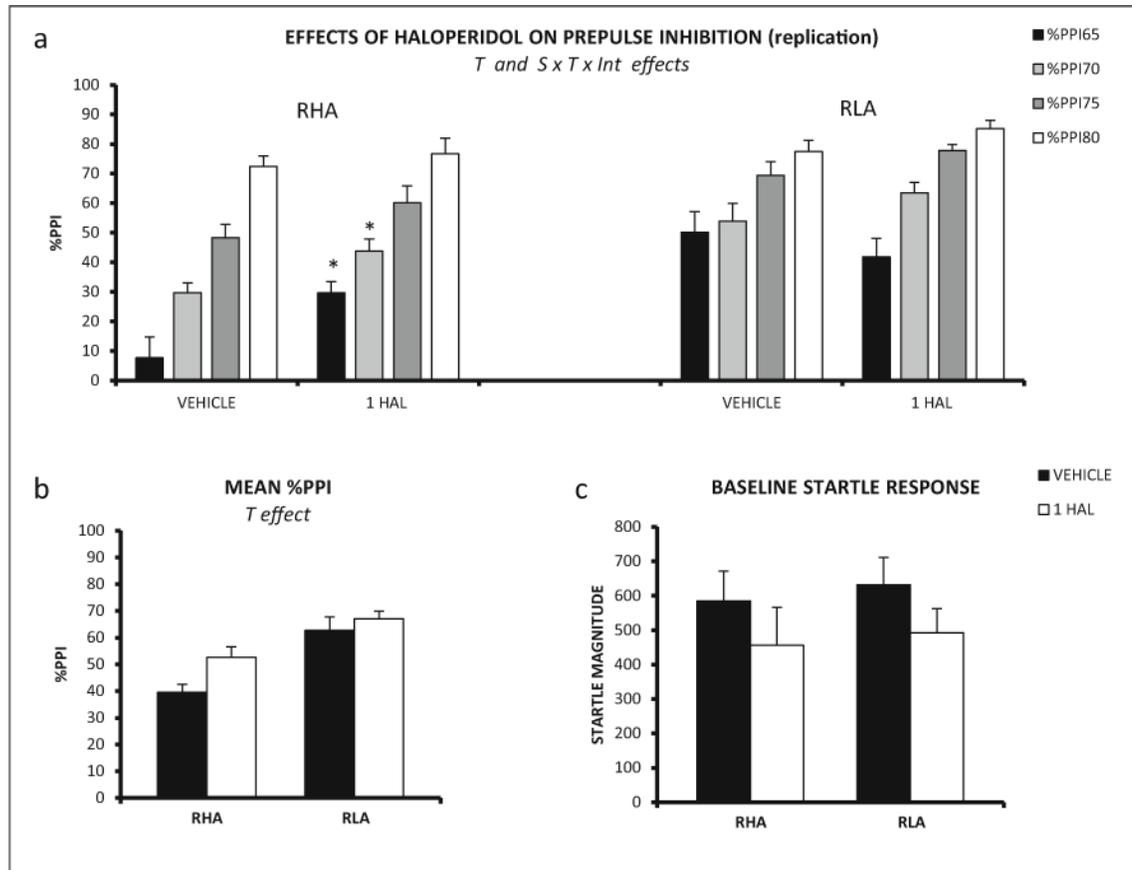
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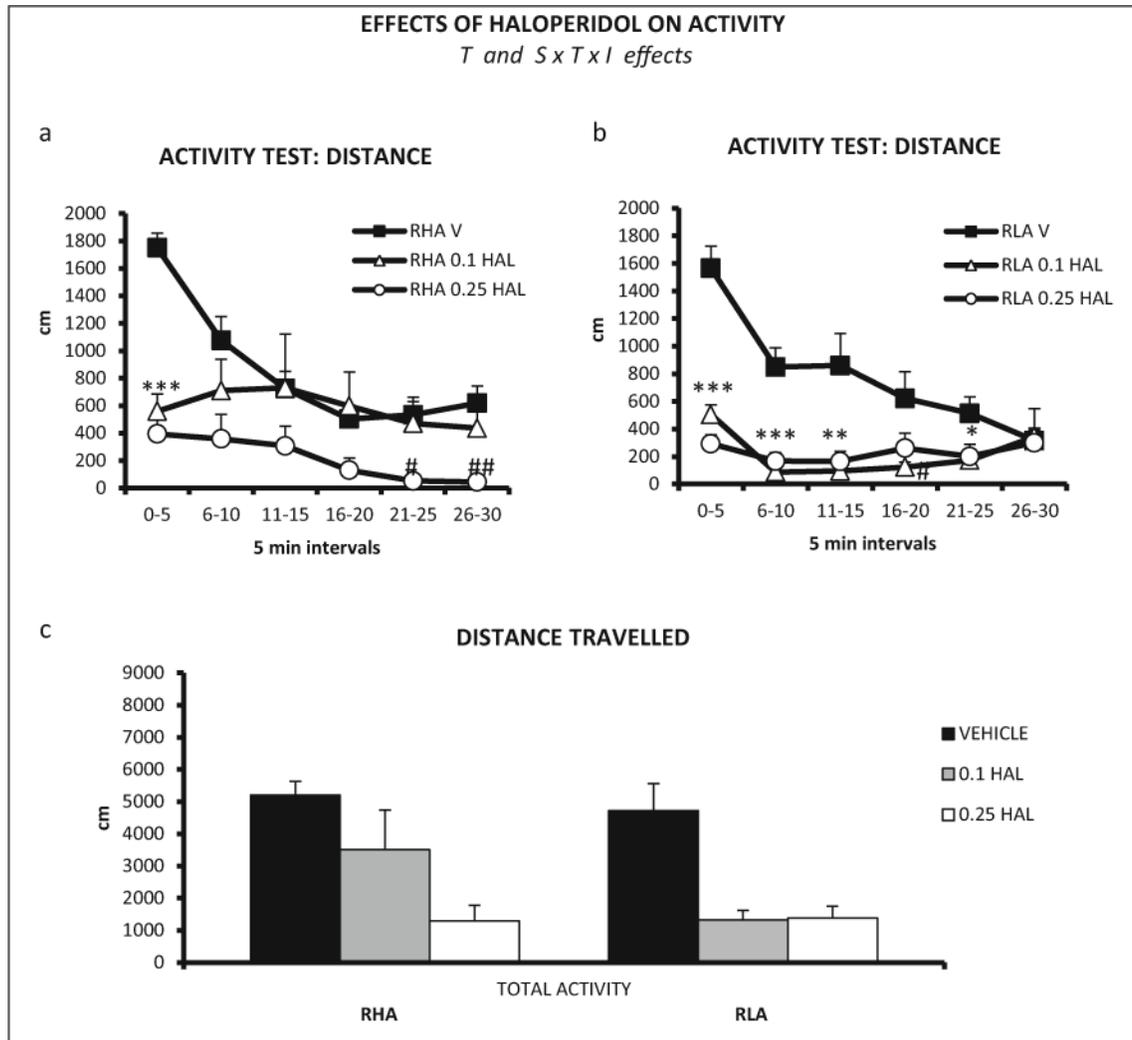
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**Fig. 1.**

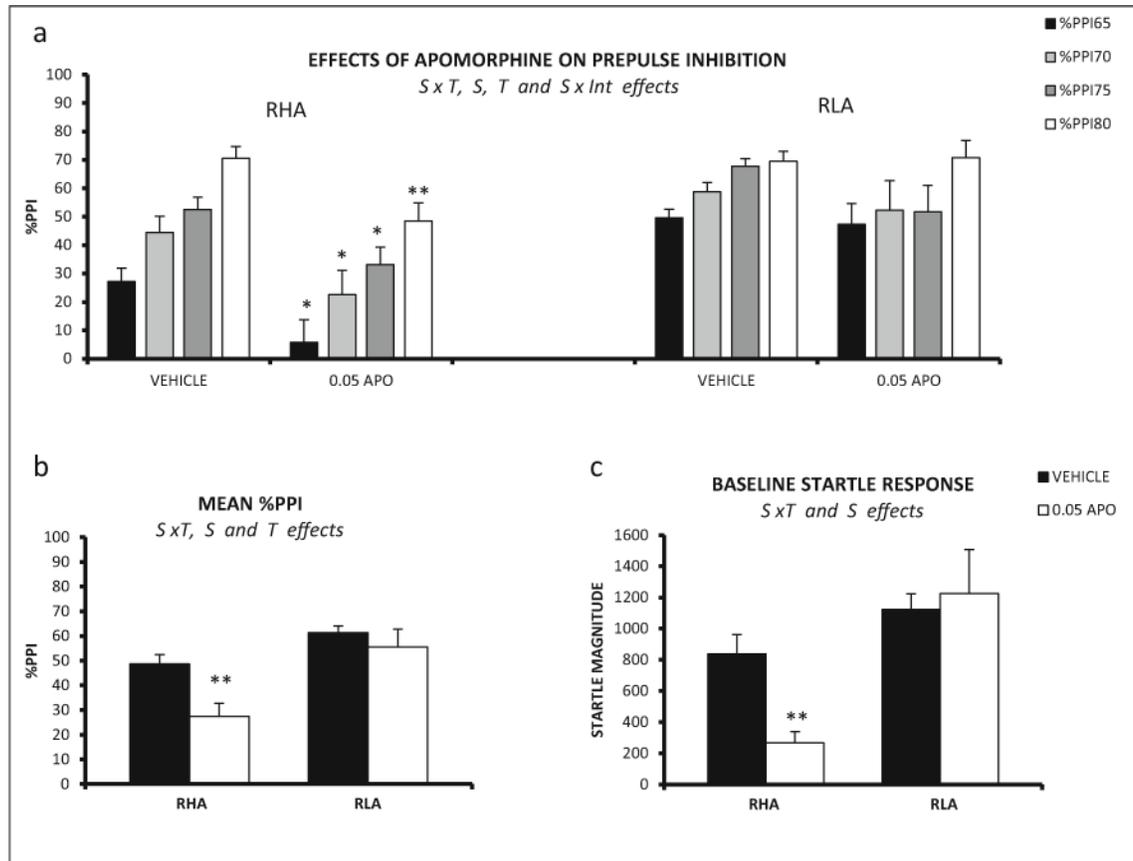
**a** Mean prepulse inhibition ( $\pm$ SEM) of RHA-I and RLA-I rats is shown for each prepulse intensity and haloperidol doses. **b** Mean prepulse inhibition (averaged for the four intensities  $\pm$  SEM) is shown for all the treatments in RHA-I and RLA-I. **c** Mean  $\pm$  SEM of the startle response magnitude in BASELINE 2 for each treatment administered to the RHA-Is and RLA-Is. *S* strain effect (ANOVA), *T* treatment effect (ANOVA), *S*  $\times$  *Int* strain  $\times$  prepulse intensity interaction (ANOVA), *T*  $\times$  *Int* treatment  $\times$  prepulse intensity interaction (ANOVA). The number of rats in each experimental group was as follows: RHA-I, vehicle,  $n = 6$ ; 0.1 mg/kg,  $n = 6$ ; 0.25 mg/kg,  $n = 6$ ; 0.5 mg/kg,  $n = 6$ ; 1 mg/kg,  $n = 10$ ; RLA-I, vehicle,  $n = 8$ ; 0.1 mg/kg,  $n = 7$ ; 0.25 mg/kg,  $n = 7$ ; 0.5 mg/kg,  $n = 8$ ; 1 mg/kg,  $n = 10$

**Fig. 2.**

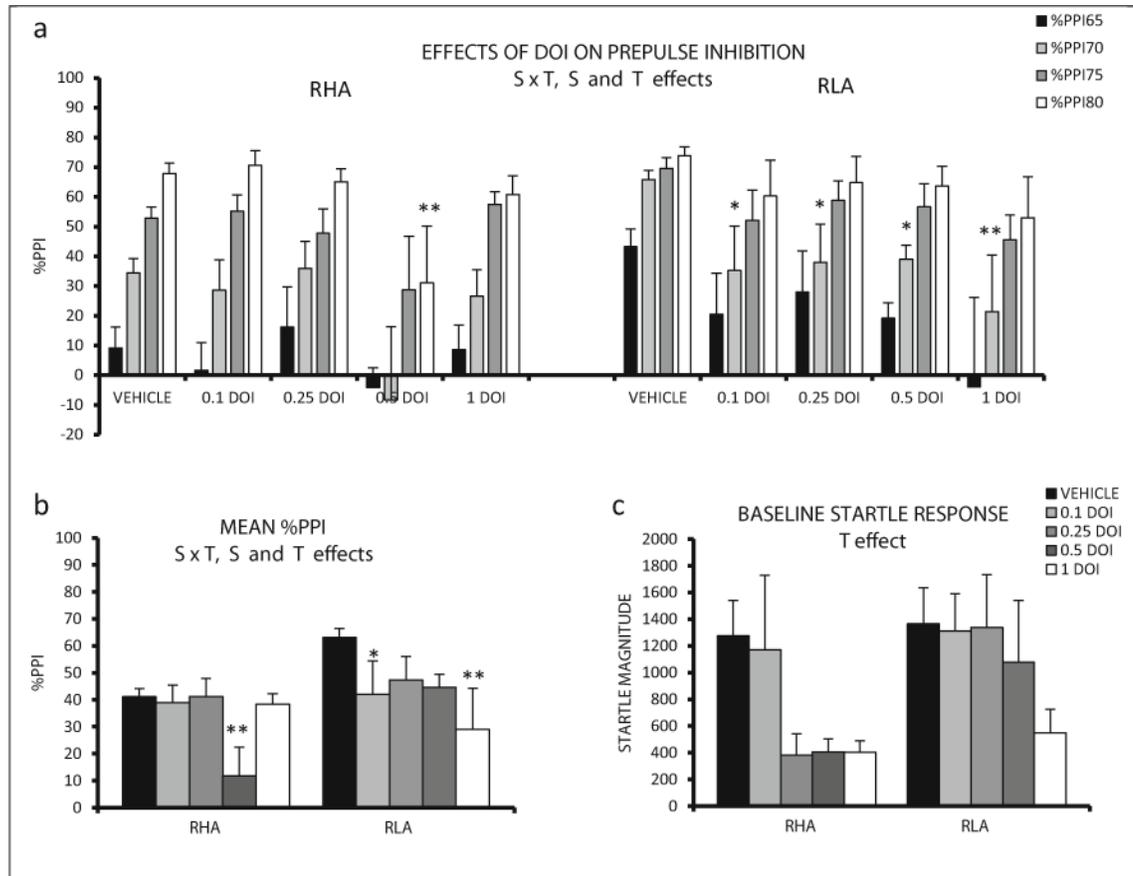
**a** Mean prepulse inhibition ( $\pm$ SEM) of RHA-I and RLA-I rats is shown for each prepulse intensity and haloperidol doses ( $n = 10$ /group in both strains). **b** Mean prepulse inhibition (averaged for the four intensities  $\pm$  SEM) is shown for all the treatments of RHA-I and RLA-I rats. **c** Mean  $\pm$  SEM of the baseline startle response for each treatment administered to the RHA-I and RLA-I rats. *T* treatment effect (ANOVA), *S*  $\times$  *T*  $\times$  *Int* strain  $\times$  treatment  $\times$  prepulse intensity second-order interaction (ANOVA). \* $p < 0.05$  vs the prepulse intensity-matched and vehicle-treated RHA-I group (LSD test after significant *S*  $\times$  *T*  $\times$  *Int* effect in ANOVA)

**Fig. 3.**

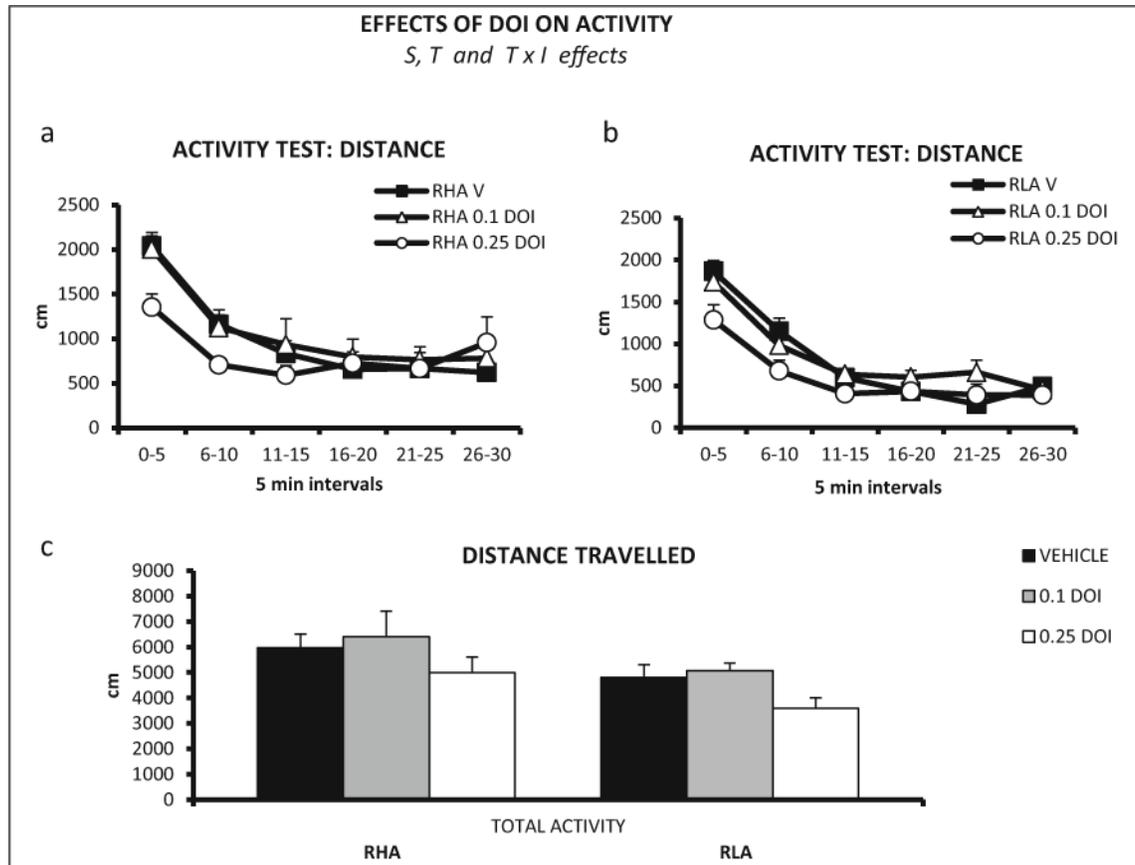
**a** Distance ( $\pm$ SEM) traveled in each 5-min interval of the locomotor activity test, by RHA-I rats and haloperidol doses (vehicle,  $n = 8$ ; 0.1 mg/kg,  $n = 8$ ; 0.25 mg/kg,  $n = 7$ ). **b** Distance ( $\pm$ SEM) traveled in each 5-min interval of the locomotor activity test, by RLA-I rats and haloperidol doses ( $n = 8$ /group). **c** Total distance ( $\pm$ SEM) traveled by RHA-I and RLA-I rats in the 30-min activity session. **a, b** \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$  both doses vs the vehicle-treated group (V); ## $p < 0.01$ , # $p < 0.05$  vs the vehicle-treated group (dose indicated). *T* treatment effect (ANOVA), *S*  $\times$  *T*  $\times$  *I* strain  $\times$  treatment  $\times$  time interval second-order interaction (ANOVA). **c** \*\*\* $p < 0.001$ , \*\* $p < 0.01$  vs vehicle-treated group (post hoc LSD test following significant *S*  $\times$  *T*  $\times$  *I* effect in ANOVA)

**Fig. 4.**

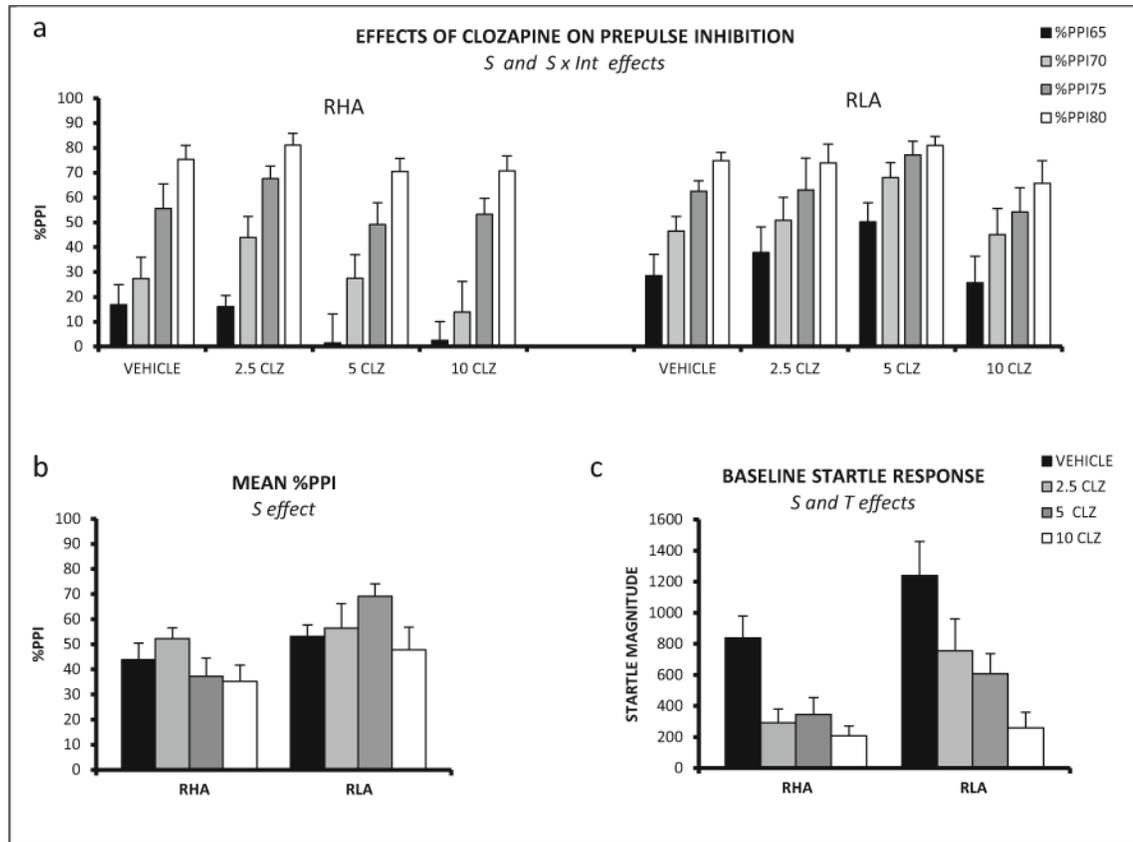
**a** Mean prepulse inhibition ( $\pm$ SEM) of RHA-I and RLA-I rats is shown for each prepulse intensity and apomorphine dose (RHA-I: vehicle  $n = 15$ ; 0.05 mg/kg,  $n = 9$ ; RLA-I: vehicle,  $n = 15$ ; 0.05 mg/kg,  $n = 7$ ). **b** Mean prepulse inhibition (averaged for the four intensities  $\pm$  SEM) is shown for both treatments and both strains. **c** Mean  $\pm$  SEM of the baseline startle response for each treatment administered to the RHA-I and RLA-I rats. *S* strain effect (ANOVA), *T* treatment effect (ANOVA), *S*  $\times$  *T* strain  $\times$  treatment interaction (ANOVA), *S*  $\times$  *Int* strain  $\times$  prepulse intensity interaction (ANOVA). \*\* $p < 0.01$ , \* $p < 0.05$  (0.05 mg vs the respective vehicle group (post hoc pair wise contrasts with the LSD test following significant *S*  $\times$  *T* effect from ANOVA)

**Fig. 5.**

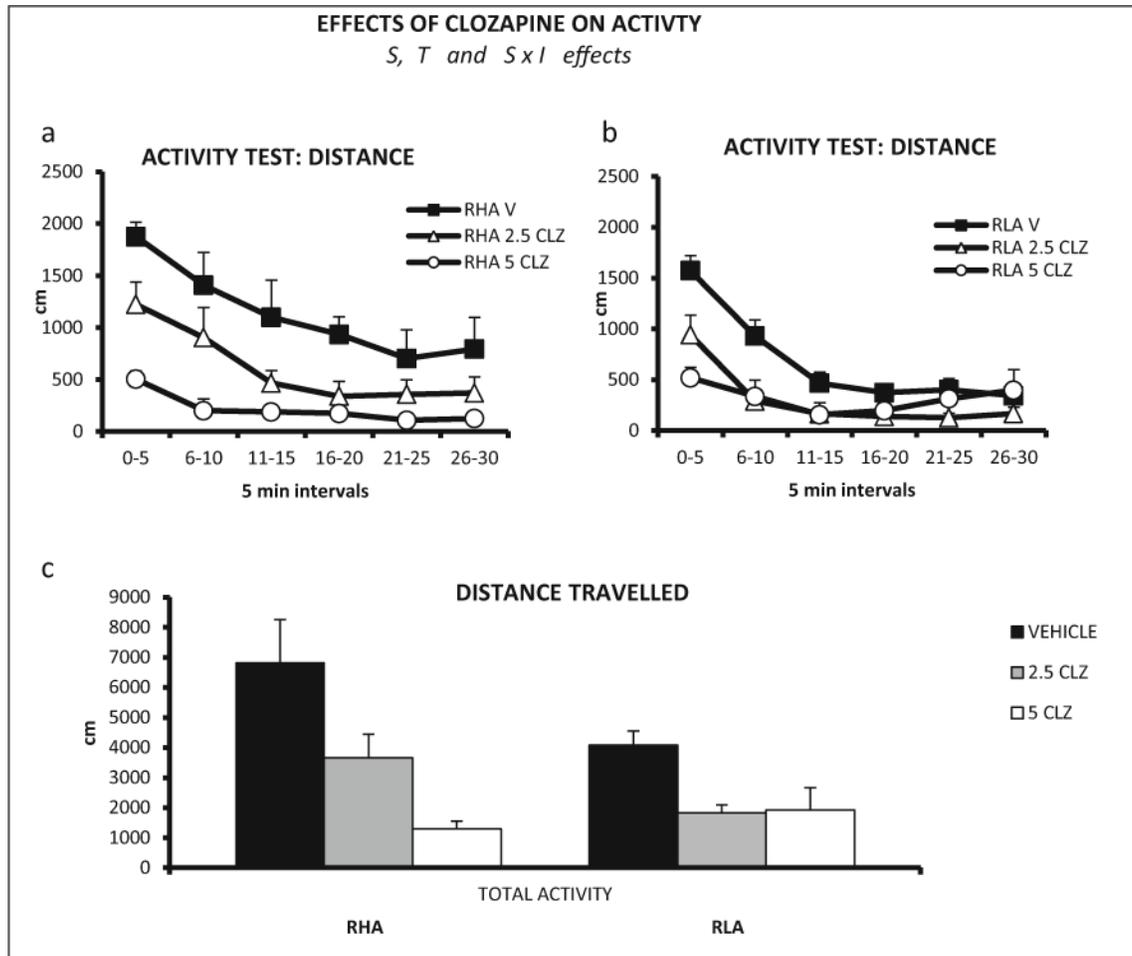
**a** Mean prepulse inhibition ( $\pm$ SEM) of RHA-I and RLA-I rats is shown for each prepulse intensity and DOI doses. **b** Mean prepulse inhibition (averaged for the four intensities  $\pm$  SEM) is shown for all the treatments in RHA-I and RLA-I rats. **c** Mean  $\pm$  SEM of baseline startle for each treatment administered to the RHA-Is and RLA-Is. *S* strain effect (ANOVA), *T* treatment effect (ANOVA), *S*  $\times$  *T* strain  $\times$  treatment interaction (ANOVA).  $**p < 0.01$ ,  $*p < 0.05$  vs the respective vehicle group (post hoc pair wise contrasts with the LSD test following significant *S*  $\times$  *T* effect from ANOVA). The number of rats in each experimental group was as follows: RHA, vehicle,  $n = 14$ ; 0.1 mg/kg,  $n = 8$ ; 0.25 mg/kg,  $n = 8$ ; 0.50 mg/kg,  $n = 6$ ; 1 mg/kg,  $n = 8$ ; RLA-I, vehicle,  $n = 16$ ; 0.1 mg/kg,  $n = 8$ ; 0.25 mg/kg,  $n = 6$ ; 0.50 mg/kg,  $n = 8$ ; 1 mg/kg,  $n = 6$



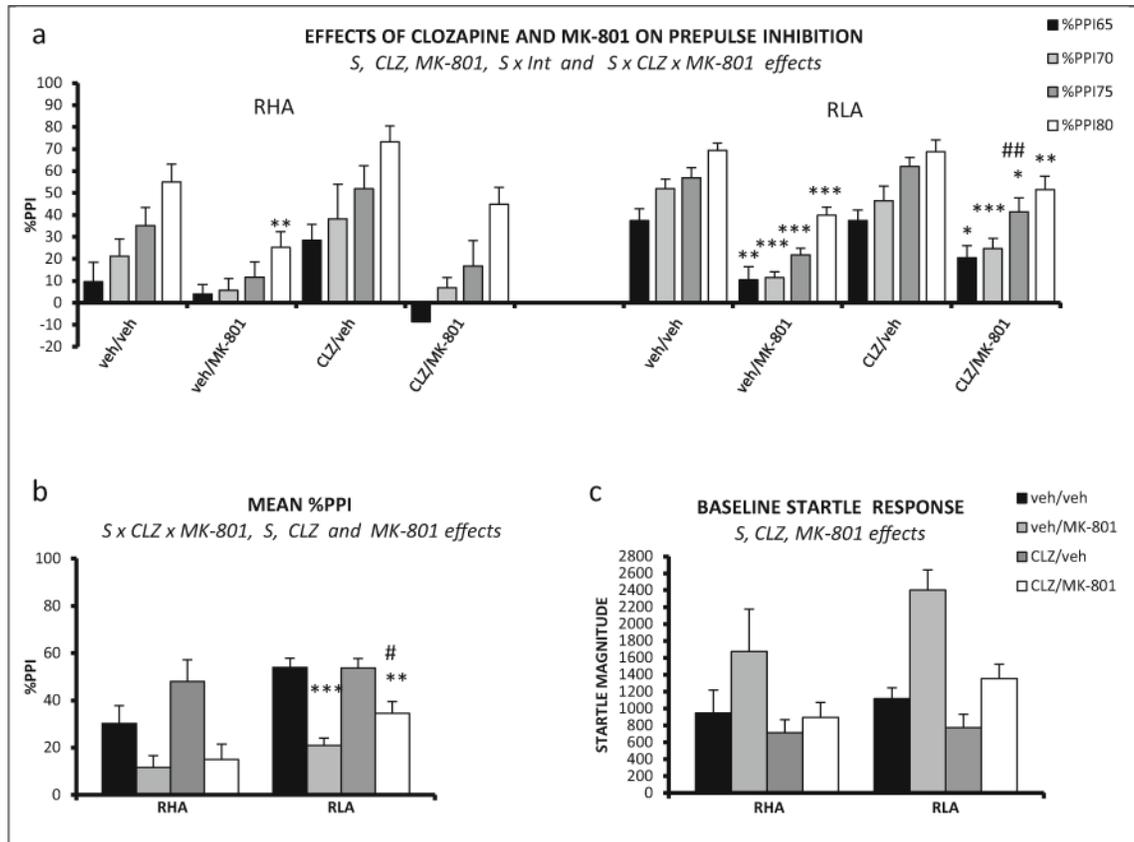
**Fig. 6.** **a** Distance ( $\pm$ SEM) traveled in each 5-min interval of the activity test, by RHA-I rats and DOI doses (vehicle,  $n = 8$ ; 0.1 mg/kg,  $n = 7$ ; 0.25 mg/kg,  $n = 7$ ). **b** Distance ( $\pm$ SEM) traveled in each 5-min interval of the activity test, by RLA-I rats and DOI doses ( $n = 8$  rats/group). **c** Total distance ( $\pm$ SEM) traveled by RHA-I and RLA-I rats in the 30-min activity session. *S* strain effect (ANOVA), *T* treatment effect (ANOVA), *T*  $\times$  *I* treatment  $\times$  time interval interaction (ANOVA)

**Fig. 7.**

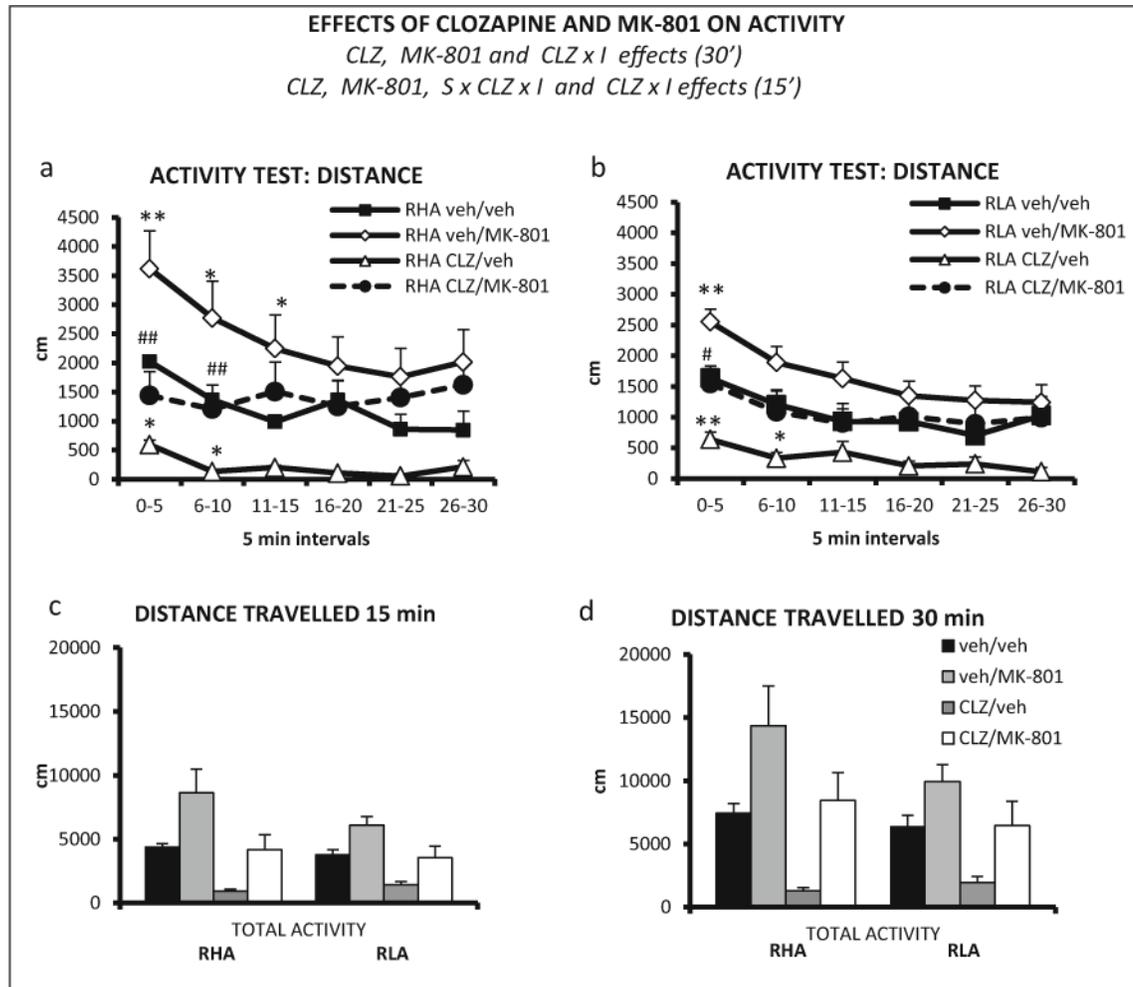
**a** Mean prepulse inhibition ( $\pm$ SEM) of RHA-I and RLA-I rats is shown for each prepulse intensity and clozapine dose. **b** Mean prepulse inhibition (averaged for the four intensities  $\pm$  SEM) is shown for all the treatments in RHA-I and RLA-I rats. **c** Mean  $\pm$  SEM baseline startle response for each treatment administered to the RHA-I and RLA-I rats. *S* strain effect (ANOVA), *T* treatment effect (ANOVA), *S*  $\times$  *Int* strain  $\times$  prepulse intensity interaction (ANOVA). The number of rats in each experimental group was as follows: RHA, vehicle,  $n = 9$ ; 2.5 mg/kg,  $n = 7$ ; 5 mg/kg,  $n = 8$ ; 10 mg/kg,  $n = 8$ ; RLA-I, vehicle,  $n = 8$ ; 2.5 mg/kg,  $n = 7$ ; 5 mg/kg,  $n = 8$ ; 10 mg/kg,  $n = 8$

**Fig. 8.**

**a** Distance ( $\pm$ SEM) traveled in each 5-min interval of the locomotor activity test, by RHA-I rats and clozapine doses ( $n = 8$  rats/group). **b** Distance ( $\pm$ SEM) traveled in each 5-min interval of the activity test, by RLA-I rats and clozapine doses ( $n = 8$  rats/group). **c** Total distance ( $\pm$ SEM) traveled by RHA-I and RLA-I rats in the 30-min activity session. *S* strain effect (ANOVA), *T* treatment effect (ANOVA), *S* × *I* strain × time interval interaction (ANOVA)



**Fig. 9.** **a** Mean prepulse inhibition ( $\pm$ SEM) of RHA-I and RLA-I rats is shown for each prepulse intensity and each treatment combination. **b** Mean prepulse inhibition (averaged for the four intensities  $\pm$  SEM) is shown for all the treatments in RHA-I and RLAI rats. **c** Mean  $\pm$  SEM of baseline startle response for each treatment administered to the RHA-Is and RLA-Is. *S* strain effect (ANOVA), *CLZ* clozapine effect (ANOVA), *MK-801* MK-801 effect (ANOVA), *S*  $\times$  *CLZ*  $\times$  *MK-801* strain  $\times$  clozapine  $\times$  MK-801 (ANOVA). \*\*\* $p$  < 0.001, \*\* $p$  < 0.01, \* $p$  < 0.05 vs vehicle-treated group; ## $p$  < 0.01 vs veh/MK-801 (post hoc LSD test, following significant *S*  $\times$  *CLZ*  $\times$  *MK-801* effect in ANOVA). The number of rats in each experimental group was as follows: RHA-I, veh/veh,  $n$  = 11; veh/MK-801,  $n$  = 9; CLZ/veh,  $n$  = 8; CLZ/MK-801,  $n$  = 8; RLA-I, veh/veh,  $n$  = 14; veh/MK-801,  $n$  = 10; CLZ/veh,  $n$  = 10; CLZ/MK-801,  $n$  = 10

**Fig. 10.**

**a** Distance ( $\pm$ SEM) traveled in each 5-min interval of the activity test, by RHA-I rats and treatment combinations ( $n = 6$  rats/group). **b** Distance ( $\pm$ SEM) traveled in each 5-min interval of the activity test, by RLA-I rats and treatment combinations (veh/veh,  $n = 7$ ; veh/MK-801,  $n = 7$ ; CLZ/veh,  $n = 7$ ; CLZ/MK-801,  $n = 8$ ). **c** Total distance ( $\pm$ SEM) traveled by RHA-I and RLA-I rats in the first 15 min of the activity session. Total distance ( $\pm$ SEM) traveled by RHA-I and RLA-I rats in the 30 min of the activity session. **d** *CLZ* clozapine effect (ANOVA), *MK-801* MK-801 effect (ANOVA), *CLZ*  $\times$  *I* clozapine  $\times$  time interval interaction (ANOVA), *S*  $\times$  *CLZ*  $\times$  *I* strain  $\times$  clozapine  $\times$  time interval interaction.  $**p < 0.01$ ,  $*p < 0.05$  vs veh/veh group;  $##p < 0.01$ ,  $#p < 0.05$  between CLZ/MK-801 and veh/MK-801 groups (post hoc pair wise contrasts with the LSD test following significant *S*  $\times$  *CLZ*  $\times$  *I* effect from ANOVA)

**Table 1**

Qualitative summary of the main effects on %PPI and locomotor activity observed with the tested drugs

	<u>RHA</u>		<u>RLA</u>	
	<u>%PPI</u>	<u>Locomotor activity</u>	<u>%PPI</u>	<u>Locomotor activity</u>
<i>Exps. 1, 2-3</i>				
Haloperidol	↑	↓	=	↓↓
<i>Exps. 7-8</i>				
Clozapine	=	↓	=	↓
<i>Exp. 4</i>				
Apomorphine	↓	NT	=	NT
<i>Exps. 5-6</i>				
DOI	↓	↓	↓↓	↓
<i>Exps. 9-10 (clozapine + MK801)</i>				
Clozapine	=	↓	=	↓
MK-801	↓	↑↑	↓↓	↑
Clozapine + MK-801	=	↑↑	↑	↑

(antagonism of MK-801 effects by CLZ) \*

Symbols: = no effect; ↑, ↑↑ increase;

\* antagonist effect; ↓, ↓↓ decrease or impairment *NT* not tested, *CLZ* clozapine

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