ESTUDIO 4

Atypical antipsychotics attenuate MK801-induced social withdrawal in the RHA rat: a model of schizophrenia-relevant features

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ABSTRACT

Rationale: The administration of NMDA receptor (NMDAR) antagonists constitutes a widely used model that produce both positive (e.g., hyperactivity) and negative (e.g., social withdrawal) symptoms relevant for schizophrenia in rodents. These effects can be reversed with the administration of atypical (second and third generation) antipsychotics.

Objectives: In this study we combined the NMDAR-antagonist model with the Roman High-Avoidance (RHA) strain, a psychogenetically selected model of schizophrenia-relevant features. We also studied whether some atypical antipsychotics drugs (clozapine, ziprasidone, and aripiprazole) would be able to attenuate or reverse the behavioural alterations induced by MK801- and whether such effects might be dependent on the rat strain.

Methods: MK801 dose-response study was conducted in RHA and Roman Low-Avoidance (RLA) male rats. After that, the 0.15 mg/kg MK801 dose was selected to carry out pharmacological studies versus atypical antipsychotics.

Results: In the first experiment we establish that MK801 (dizocilpine), a NMDAR antagonist, produces dose-related hyperactivity and social withdrawal, which are more marked in RHA than RLA rats. The administration of the atypical antipsychotics clozapine (2.5. mg/kg) or ziprasidone (2.5 mg/kg) partially reversed or attenuated some of the social behaviour deficits and hyperactivity induced by the administration of MK801. Aripiprazole (3 mg/kg), a third-generation antipsychotic, reversed or attenuated the social preference deficit, the hyperactivity and the impairment of social latency induced by MK801.

Conclusions: These results seem to be in line with previous studies with the NMDAR-antagonist model and add face and predictive validity to the RHA rat strain as a model of schizophrenia-relevant features.

***Keywords**:* Schizophrenia; Negative symptoms; Social withdrawal; NMDAR antagonist; Atypical antipsychotics; RHA and RLA rats

1. INTRODUCTION

The glutamate model of schizophrenia is based on the action of the N-methyl-D-aspartate receptor (NMDAR) antagonists (Aghajanian & Marek, 2000; Krzystanek & Pałasz, 2019; Neill et al., 2010, 2014; Rung, et al., 2005). The administration of those NMDAR antagonists (e.g. ketamine, phencyclidine –PCP- or dizocilpine -MK801-) in rodents is widely used to produce cognitive deficits and social withdrawal relevant to cognitive and negative symptomatology of schizophrenia (Gururajan et al., 2012; Neill et al., 2010; Rung et al., 2005; Wilson & Koenig, 2014). It is well know that NMDAR antagonists also produce psychotic-like symptoms, such as hyperactivity, paranoia and hallucination, and the administration of NMDAR antagonists to patients with schizophrenia exacerbates their symptoms (Malhotra et al., 1997; and see review by Neill et al., 2010).

The relevance of pharmacological models based on the administration of NMDAR antagonists lies in the fact that their effects can be reversed by second-generation (but not first-generation) antipsychotics (APs, such as clozapine, risperidone, olanzapine or sertindole; e.g., Gururajan et al., 2012; Neill et al., 2010).

The Roman high- (RHA) and low-avoidance (RLA) rat strains were developed in Rome in the 1960's trough bidirectional selective breeding of Wistar rats for their rapid (RHA) or extremely poor acquisition (RLA) of the two-way active avoidance –TWAA- task (Bignami, 1965; Fernández-Teruel et al., 2021). Among many other phenotypic strain differences related to anxiety, vulnerability to stress and to drug abuse (see reviews by Fernández-Teruel et al., 2021; Giorgi et al., 2019), it is noteworthy that RHA rats display (compared with RLAs, and also with unselected outbred rats) a number of schizophrenia-relevant phenotypes. Thus, among other psychotic/schizophrenia-relevant traits (reviewed by Fernandez-Teruel et al. 2021), RHA rats present cognitive disfunction, as indicated by impairments of reference and working memory (Oliveras et al., 2015; Río-Álamos et al., 2019), attention-related deficits (e.g. latent inhibition and prepulse inhibition) and hyperactivity (Oliveras et al., 2017; reviewed by Fernandez-Teruel et al., 2021). We have recently reported that drug-free RHA rats also exhibit relative asociality (i.e. lowered preference for social interaction), compared with their RLA counterparts and outbred HS rats (Oliveras et al., 2022; Sampedro-Viana et al., 2021), which is considered to model social withdrawal, a negative symptom of schizophrenia.

At neurochemical and molecular levels, selection for extremely divergent rates of acquisition of TWAA, or comparison of RHA vs RLA rats, has been shown to be associated with differential expression of many genes at the cortical, hippocampal and amygdala levels (Díaz-Morán et al., 2013; Sabariego et al., 2011, 2013). Most interestingly, recent studies have shown that RHA rats present alterations of synaptic markers and trophic factors in the prefrontal cortex (PFC) and/or hippocampus (HPC), such as neuregulin1, homer1, synaptophysin, brain-derived neurotrophic factor (BDNF), and others, that have

been linked with glutamatergic disfunction, PFC maturation and schizophrenia (Elfving et al., 2019; Neill et al., 2010; reviewed by Fernandez-Teruel et al., 2021). Hence, the genetically-based RHA model appears to recapitulate a considerable number of neurobehavioral traits that are relevant for the disorder (Fernandez-Teruel et al., 2021; Giorgi et al., 2019).

In this context, the present study was aimed at expanding the schizophrenia-relevant phenotypic profiling of RHA vs RLA rats by performing a pharmacological characterization of their social behaviour. We first aimed at evaluating the effects of the administration of the NMDAR antagonist MK801 on social behaviour of RHA vs RLA rats. We also studied whether some atypical antipsychotic drugs (clozapine, ziprasidone, and aripiprazole) would be able to attenuate or reverse the behavioural alterations induced by MK801- and whether such effects might be dependent on the rat strain. We hypothesized that, (i) MK801 would lead to more profound social behaviour deficits and enhanced hyperlocomotion in RHA than RLA rats, and (ii) second- (clozapine, ziprasidone) and third-generation (aripiprazole) antipsychotics would reduce MK801-induced impairment of social behaviour and hyperactivity more markedly in RHA rats than in their RLA counterparts.

2. METHODS

2.1 Subjects

Animals used in the present study were naïve male rats from the inbred Roman high- (RHA, n=217) and low-avoidance (RLA, n=222) strains, from the permanent colonies maintained at the laboratory of the Medical Psychology Unit, Dept. Psychiatry and Forensic Medicine (School of Medicine, Autonomous University of Barcelona, Spain), since 1996. They were 4-5 months old at the beginning of the experiments with an average weight of 421,50g ±3,60g (mean ± SD).

Animals were housed in macrolon cages (standard size: 50 x 25 x 14cm) in same-sexed pairs. They were maintained under a 12:12h light-dark cycle (lights on at 8:00h) with controlled temperature (22ºC ± 2ºC) and humidity (50-70%). Food and water were available *ad libitum*. All testing was performed in the morning between 8:00 and 14:00h. All the experimental procedures agreed with the Spanish legislation on "Protection of Animal Used for Experimental and Others Scientific Purposes" (RD 53/2013) and the European Communities Council Directive (2010/63/EU).

2.2 Apparatus

The set-up used to test social interaction was based on the one used by Gururajan et al., (2012) which was a modified version of that initially designed by Panksepp et al., (1997). It consists of two acrylic boxes (65 x 23 x 20cm) placed facing one another. The cages were divided by lines, in three equal sectors: social, middle, and non-social. Both boxes had two 3cm-diameter holes on their right and left sides (social/non-social hole). To prevent physical contact, the social holes of both cages were separated by 12cm. Above the test set up, there was a video camera recording the session which was connected to a screen out (TV monitor) of the experimental room where experimenters observed and assessed behaviour *in situ*. Further analysis was performed by visualization of the video tapes on a computer.

2.3 Drug treatment

Dizocilpine (MK801, M107), clozapine (CLZ, C6305), aripiprazole (ARI, SML0935) and ziprasidone (ZPR, Z2777) were purchased from Sigma-Aldrich (St. Louis, MO, USA). MK801 was dissolved in saline (0.9% NaCl). CLZ was dissolved in a small amount of glacial acetic acid and then diluted in distilled water with NaOH to neutralize the acidification. ZPR and ARI were dissolved in 2% of Tween 80 diluted in distilled water.

MK801 and CLZ and their respective vehicles were administered subcutaneously (s.c.) in a volume of 1 ml/kg body weight, 20 min and 60 min before testing, respectively. ARI and ZPR and their vehicles were administered intraperitoneally in a volume of 1 ml/kg body weight, 60 min and 30 min respectively before the test.

To assess the interactions between the propsychotic drug and the antipsychotics, the dose of 0.15 mg/kg of MK801 was selected according to its effects on "Non-social time", "Social time", "Social preference", "Social latency" and locomotion in Study 1. All the solutions were freshly prepared each day. The doses of each antipsychotic were selected according to pilot studies from our laboratory and/or the effective doses used in previous studies from our laboratory and others (Deiana et al., 2015; Oliveras et al., 2017; Ratajczak et al., 2016; Snigdha & Neill, 2008).

See experimental overview in Figure 1.

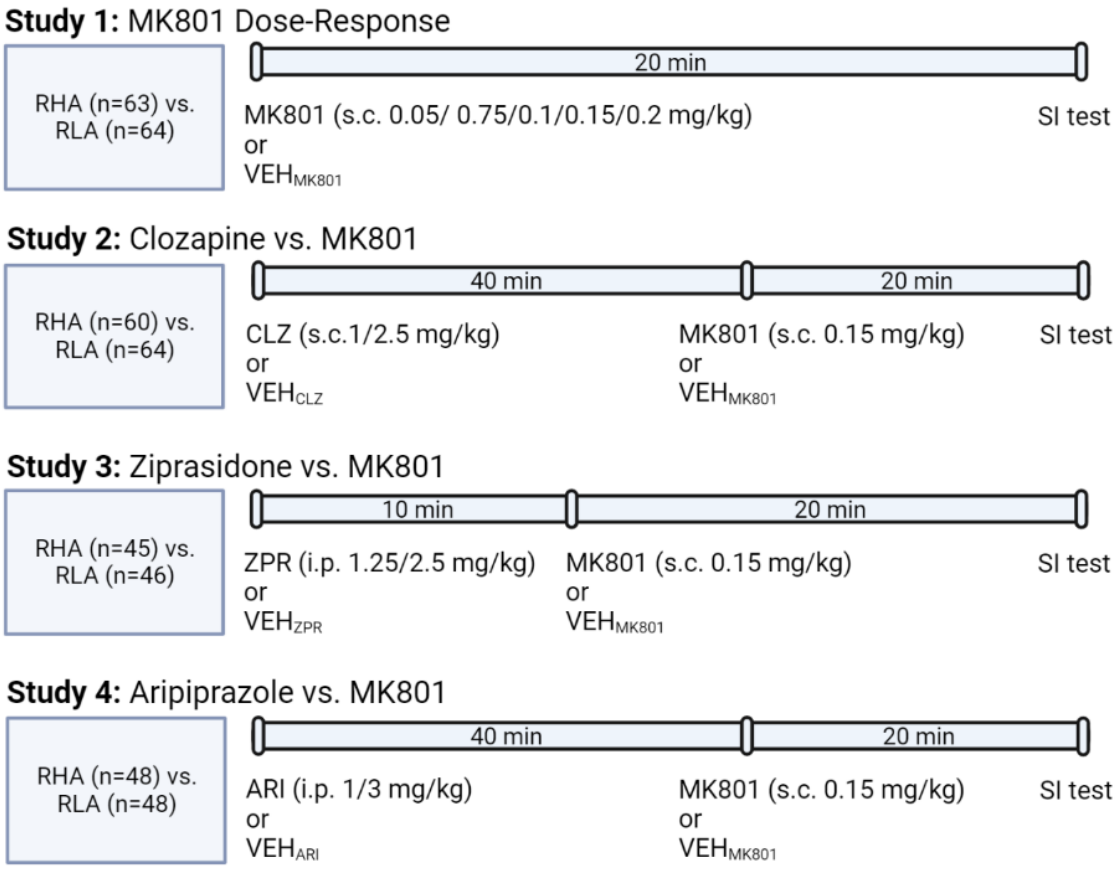

Figure 1. **Overview of the experimental schedules** (see "Materials and methods"). **Study 1:** different doses of MK801 were tested in the social interaction (SI) test. MK801 or its vehicle (VEH_{MK801}) were subcutaneously (s.c.) administered 20 minutes before the SI test. The 0.15 mg/kg MK801 dose was selected for the following studies. **Study 2**: Clozapine (CLZ; 1 or 2.5 mg/kg) or its vehicle (VEH_{CLZ}) were s.c. administered 40 min before the administration of MK801 or its vehicle (VEH_{MK801}); 20 min after MK801 administration the SI test was performed. **Study 3:** Ziprasidone (ZPR; 1.25/2.5 mg/kg) or its vehicle (VEH_{ZPR}) were administered i.p. 10 min before the administration of MK801 or its vehicle (VEH_{MK801}); 20 after the second administration the SI test was performed. **Study 4:** Aripiprazole (ARI; 1 or 3 mg/kg) or its vehicle (VEH_{ARI}) were intraperitoneally (i.p.) administered 40 min before the administration of MK801 or its vehicle (VEH_{MK801}); 20 min after the second administration the SI test was performed.

2.4 Experimental procedure

The experimental procedure was the same as the one used by Sampedro-Viana et al. (Sampedro-Viana et al., 2021). Pairs of unfamiliar weight-matched rats (±25gr.) were placed separately in each box for 30 minutes during the habituation sessions carried out 24h before testing, and then different pairs of unfamiliar weight-matched rats were placed one in each box for the 15-min test. The holding room was slightly illuminated with red light both during habituation and testing sessions. After testing each rat pair, the boxes were wiped clean with 70% ethanol to remove olfactory cues. The variables measured in each experiment were the same: "social latency", measured as the time elapsed until the first exploration of a social hole; "social time", measured by the time animals spend nose-poking at the social hole; "non-social time", measured by the time animals spent nose-poking at the non-social hole; "Social Preference", measured as the following equation: "Social Preference = (Social Time/ (Social Time + Non-social Time)) x 100"

"Locomotor activity" was measured as the number of crossings across the three equal sectors of the cage.

2.4.1 Study 1: MK801 Dose-Response

In study 1, we compared the effects of different MK801 (0.05, 0.075, 0.1, 0.15 and 0.2 mg/kg) doses. A total of 64 RLA rats and 64 RHA were tested in this study.

2.4.2 Antipsychotics vs. MK801

This study encompasses three experiments, in which the effects of MK801 on social behaviour and their possible reversal or attenuation by three different antipsychotics drugs, i.e., clozapine (CLZ), ziprasidone (ZPR) or (ARI), were evaluated.

Animal's matching, time of testing, room conditions and variables measures were the same as described above.

Study 2: Clozapine vs. MK801

In study 2, the effects of the pro-psychotics MK801 (0.15 mg/kg) and the antipsychotic CLZ (1 and 2.5 mg/kg), were assessed on both rat strains in a 2 x 2 x 3 factorial design (i.e., "2 strains" x 2 "MK801 levels" x "3 CLZ levels"). A total of n=64 RLA and n=60 RHA were used.

Study 3: Ziprasidone vs. MK801

In study 3, the effects of MK801 and ZPR (1.25 and 2.5 mg/kg) were tested in a 2 x 2 x 3 factorial design (i.e., "2 strains" x "2 MK801 levels" x "3 ZPR levels"). A total of n=46 RLA and n=45 RHA were tested in this experiment.

Study 4: Aripiprazole vs. MK801

In study 4, the effects of MK801 and ARI (1 and 3 mg/kg), were evaluated on both strains in a 2 x 2 x 3 factorial design (i.e., “2 strains” x “2 MK801 levels” x “3 ARI levels”). A total of n=48 RLA and n=48 RHA were used

2.5 Statistical analysis

Statistical analysis was performed using the *Statistical Package for Social Science 17 (*SPSS, Inc., Chicago, IL, USA). The data from Study1 were evaluated with two-way ANOVAs (2 “Strain” x 6 “Treatment”). If the two-way ANOVA revealed “Strain”, “Treatment” or “Strain x Treatment”, post hoc contrasts with Duncan’s multiple range tests to explore differences between groups were performed. The data from studies 2, 3 and 4 were evaluated with a three-way ANOVA (2 “Strain” x 2 “MK801” x 3 “APs” levels). If the ANOVA revealed “Strain”, “MK801”, “Clozapine”/ “Ziprasidone”/ “Aripiprazole”, or interactions between these factors post hoc Duncan’s multiple range tests to explore differences between groups were performed. All the results are expressed as mean ± SEM. The threshold for statistical significance was set at $p< 0.05$ for all measures.

3. RESULTS

Study 1: MK801 Dose-Response

The results of study 1 are presented in Figure 2. Factorial ANOVA (2 "strains" x 6 "Treatment") revealed a "Treatment" ($F_{(5,116)}$=15.641, p<0.001) effect on "Non-social Time", indicating a global decrease in this measure by MK801 administration. It also revealed a "Strain x Treatment" ($F_{(5,116)}$=2.943, p<0.05) effect, as the MK801-induced reduction of non-social time in RHA was higher than in their RLA counterparts (Figure 2a, and Duncan's test).

Regarding "Social time", there were "Strain" ($F_{(1,116)}$=6.503, p<0.05), "Treatment" ($F_{(5,116)}$=17.558, p<0.001) and "Strain x Treatment" ($F_{(5,116)}$=3.280, p<0.01) effects, the latter being due to the stronger effect of MK801 on social time in RHA rats compared with their RLA counterparts (Figure 2b, and Duncan's test).

ANOVA also revealed "Strain" ($F_{(1,116)}$=44.669, p<0.001) and "Strain x Treatment" ($F_{(5,116)}$=4.201, p<0.001) effects on the "Social preference" measure. Duncan's post hoc comparison revealed a MK801-induced deficit in social preference by the highest doses of MK801 (0.15 and 0.2 mg/kg) in RHA rats (Figure 2c, and Duncan's test).

As for "Social Latency", there were "Strain" ($F_{(1,116)}$=43.257, p<0.001), "Treatment" ($F_{(5,116)}$=23.864, p<0.001) and "Strain x Treatment" ($F_{(5,116)}$=7.941, p<0.001) effects, reflecting the increase of social latency due to MK801 treatment and the stronger effect on the RHAs compared with RLA rats (Figure 2d, and Duncan's test).

With regard to "Locomotor Activity", there were "Strain" ($F_{(1,116)}$=65.284, p<0.001) and "Treatment" ($F_{(5,116)}$=65.284, p<0.001) effects (Figure 2e).

Study 2: Clozapine vs. MK801

The results of study 2 are presented in Figure 3 and Figure 4. Since in this study there were no significant effects of MK801 (0.15 mg/kg; see comparison of VEH-VEH and VEH-MK groups in RHA rats, Figure 3e) on "Social preference" considering the whole 15-min SI test, the first 5-min period of the test was also statistically analysed.

Factorial ANOVA (2 "Strain" x 2 "MK801 dose" x 3 "CLZ dose") revealed a "Clozapine" ($F_{(2,112)}$=7.85, p<0.001) and "MK801" ($F_{(1,112)}$=36.987, p<0.001) effects on "Non-social time" (Figure 3a, and Duncan's test). Similar "Clozapine" ($F_{(2,112)}$=11.834, p<0.001) and "MK801" ($F_{(1,112)}$=72.423, p<0.001) effects on that variable were found during the first five minutes of the test (Figure 3b).

As for "Social time", there were "Clozapine" ($F_{(2,112)}$=6.931, p<0.001) and "MK801" ($F_{(1,112)}$=37.041, p<0.001) effects (Figure 3c, and Duncan's test), which were also present during the first five minutes of the test ("Clozapine" effect $F_{(2,112)}$=5.148, p<0.05,; "MK801"effect $F_{(1,112)}$=50.511, p<0.001) (Figure 3d).

ANOVA also revealed "Strain" ($F_{(1,112)}$=16.108, p<0.001), "Clozapine" ($F_{(2,112)}$=4.822, p<0.001), "Strain x MK801" ($F_{(1,112)}$=13.209, p<0.001) and "Strain x MK801 x Clozapine" ($F_{(2,112)}$=3.779, p<0.05) effects on "Social preference" (Figure 3e, and Duncan's test). Moreover, "Strain" ($F_{(1,112)}$=30.167, p<0.001), "Clozapine" ($F_{(2,112)}$=5.593, p<0.001), "MK801" ($F_{(1,112)}$=7.212, p<0.01) and "Strain x MK801" ($F_{(1,112)}$=15.804, p<0.001) effects were observed during the first five minutes of SI testing. The above interaction effects indicate, first, that MK801 effects are more marked in RHA than RLA rats, and second, the highest dose of clozapine (2.5 mg/kg) reversed the relative deficit in social preference showed by MK801-treated RHA rats (see comparisons between the RHA CLZ2.5-MK vs VEH-MK and CLZ1-MK groups, in Figure 3e and 3f, and Duncan's tests). Remarkably also, the RHA VEH-MK801 group was different (i.e. decreased social preference) from RHA VEH-VEH group during the first five minutes of testing (Figure 3f, and Duncan's test).

With respect to "Locomotor Activity", there were "Strain" ($F_{(1,112)}$=30.562, p<0.001), "Clozapine" ($F_{(2,112)}$=9.666, p<0.001), "MK801" ($F_{(1,112)}$=77.073, p<0.001) and "Clozapine x MK801" ($F_{(2,112)}$=4.383, p<0.05) effects. This interaction indicates that only the highest dose of clozapine (2.5 mg/kg) reversed the MK801-induced hyperactivity in both rat strains (Figure 4a, and Duncan's test). Similarly, "Strain" ($F_{(1,112)}$=45.807, p<0.001), "Clozapine" ($F_{(2,112)}$=9.148, p<0.001), "MK801" ($F_{(1,112)}$=70.155, p<0.05) and "Strain x MK801" ($F_{(1,112)}$=21.596, p<0.001) effects were observed during the first five minutes of SI testing, reflecting that MK801-induced hyperactivity was higher in RHA rats than in their RLA counterparts (Figure 4b and Duncan's test).

As for "Social latency", "Strain" ($F_{(1,112)}$=29.888, p<0.001), "MK801" ($F_{(1,112)}$=51.931, p<0.001) and "Strain x MK801" ($F_{(1,112)}$=22.431, p<0.001) effects were observed, indicating that MK801 increased social latency more markedly in RHAs than in RLA rats (Figure 4c, and Duncan's test).

Study 3: Ziprasidone vs. MK801

The results of study 3 are presented in Figure 5. Factorial ANOVA (2 "strain" x 2 "MK801 dose" x 3 "ZPR dose") revealed "Strain" ($F_{(1.79)}$=5.105, p<0.05), "MK801" ($F_{(1,79)}$=47.151, p<0.001) and "Strain x MK801" ($F_{(1,79)}$=9.591, p<0.05) effects, reflecting a reduction of "Non-social time" in RHA rats compared with their RLA counterparts (Figure 5a, and Duncan's test).

As for "Social Time", there were "MK801" ($F_{(1,79)}$=110.649, p<0.001) and "Strain x MK801" ($F_{(1.79)}$=17.143, p<0.001) effects, the interaction being due to the stronger effect of MK801 on RHA than RLA rats (Figure 5b, and Duncan's test).

Regarding "Social preference", there were "Strain" ($F_{(1,79)}$=20.528, p<0.001), "Strain x MK801" ($F_{(1,79)}$=9.646, p<0.05) and "Strain x MK801 x Ziprasidone" ($F_{(1,79)}$=3.345, p<0.05) effects, the latter being due to the specific effect of ZPR (2.5mg/kg) to reverse the

MK801-induced deficit in social preference in RHA, but not in RLA rats (Figure 5c, and Duncan's test).

Related to the "Social latency" variable, "Strain" ($F_{(1,79)}$=15.406, p<0.001), "MK801" ($F_{(1,79)}$=43.695, p<0.001) and "Strain x MK801" ($F_{(1,79)}$=17.730, p<0.001) were observed, reflecting that the MK801-induced increase of social latency was more marked in RHA rats compared with their RLA counterparts (Figure 5d, and Duncan's test).

ANOVA also revealed "Strain" ($F_{(1,79)}$=9.336, p<0.05), "Ziprasidone" ($F_{(2,79)}$=3.451, p<0.05) and "MK801" ($F_{(1,79)}$=98.090, p<0.001) effects on "Locomotor activity" (Figure 5e, and Duncan's test).

Study 4: Aripiprazole vs. MK-801

The results of study 4 are presented in Figure 6. Factorial ANOVA (2 "strain" x 2 "MK801 dose" x 3 "ARI dose") revealed an "MK801" effect on "Non-social time" ($F_{1,84)}$= 75.194, p<0.001), indicating a global decrease in this measure in all groups. It also revealed a "Strain x MK801" effect ($F_{(1,84)}$=11.110, p<0.001), as the reduction of non-social time in RLA rats was lower than in their RHA counterparts (Figure 6a, and Duncan's test).

Regarding "Social time", there was an "MK801" effect ($F_{(1,84)}$= 73.952, p<0.001), reflecting that the drug decreased social behaviour in all groups, and a "Strain x MK801" ($F_{(1,84)}$=13.315, p<0.001) effect, as the reduction of social time was more marked in RHA rats (Figure 6b, and Duncan's test).

As for "Social Preference", there were "Strain" ($F_{(1,84)}$=10.534, p<0.05), "MK801" ($F_{(1,84)}$=7.747, p<0.05) and "Strain x MK801" ($F_{(1,84)}$=6.331, p<0.05) effects, the latter being due to the reversal of the MK801-induced reduction of social preference in the RHA rats by the highest ARI dose (Figure 6c, and Duncan's test).

ANOVA also revealed "Strain" (F(1,84)=29.991, p<0.001), "MK801" (F(1,84)=79.711, p<0.001) and "Strain x MK801" (F(1,84)=10.716, p<0.05) effects on "Social latency". Duncan's post hoc comparison revealed a difference between the highest dose of ARI (3 mg/kg) and "VEH-MK" group in RHA rats, meaning that the MK801-increased social latency was partially reversed by ARI (Figure 6d, and Duncan's test).

Related to the "Locomotor activity" variable, results showed a "Strain" effect ($F_{(1,84)}$=29.991, p<0.001), as RHA rats present global higher levels of activity than RLA rats. Moreover, an "MK801" effect ($F_{(1,84)}$=79.711, p<0.001) was found, reflecting an overall increase in locomotion in all groups administered with MK801 (Figure 6e and Duncan's test). A significant "Strain x MK801" interaction ($F_{(1,84)}$=10.716, p<0.01) was also observed, indicating that a more marked effect of MK801 in RHA rats (Figure 6e, and Duncan's test).

4. DISCUSSION

The main findings of the present study may be summarized as follows: (1) The dose-response experiment (study 1) shows that MK801 0.15 and 0.2mg/kg doses induced a similar significant decrease of non-social behaviour ("Non-social time") in both Roman rat strains, but the drug reduced "Social time" and especially "Social preference" specifically in RHA rats. (2) Moreover, MK801 was more effective in increasing "Social latency" and hyperlocomotion in RHA rats. (3) The higher doses of ARI and ZPR attenuated MK801-induced hyperlocomotion in the RHA but not in RLA rats. This effect was also observed with CLZ, although this drug reduced activity globally and in both rat strains in parallel to its reversal of MK801-induced hyperactivity. (4) ARI 3 mg/kg and ZPR 2.5 mg/kg reversed the MK801-induced impairment of social preference of RHAs, being devoid of effects in RLA rats. (5) We analysed the first 5 min in the CLZ study (Study 2) because MK801 detrimental effects on social behaviour (specifically on "social preference") were more clear during that initial interval than during the whole 15-min test. Thus, only during the first 5 min of the SI test CLZ 2.5 mg/kg reversed the MK801-impaired social preference in RHA rats. (6) Among the three APs tested, ARI appears to be the most potent in reducing MK801-induced deficits, since apart from reversing MK801 effects on "Social Preference", ARI was the only drug that attenuated the effect of MK801 on "Social latency" (see Figure 6d) and produced a net increase of "Social time" in RHA rats (see Figure 6b).

It is worth to highlight here that measuring "Social preference", a parameter that has not been used in previous pharmacological studies carried out with the present SI procedure (e.g. Gururajan et al., 2010, 2012), enables the detection of some specific drug effects that do not arise when measuring just "Social time" and "Non-social time". The effects observed with MK801, and with the combination of this drug and the antipsychotics, suggest that social preference may better reflect, at least in some instances (e.g. following some particular drug treatments), the motivation of the animals for social interaction with relative independency of the absolute levels of social or non-social time. On the other hand, it is also worth mentioning that vehicle-treated (RLA vs. RHA) rats did not show differences in social preference in any of the present studies. This contrasts with our earlier (and replicated) findings indicating that treatment-naïve RLA rats consistently exhibit higher social preference than their RHA counterparts (Oliveras et al., 2022; Sampedro-Viana et al., 2021). These apparently contrasting findings open the reasonable possibility that the mild stress involved in handling and vehicle injection a few minutes before SI testing may lead to (slight) changes in the behavioural SI profiles of the rats. This possibility will certainly deserve further study.

The relevance of this study lies on the fact that this is the first time that: (i) Strain-related NMDA-antagonist (MK801)-induced impairments in social preference are reported in RHA vs. RLA rats and, (ii) attenuation of MK801-induced deficits in social preference (and locomotion) by atypical antipsychotics is shown specifically in RHA rats.

We have previously reported that RHA rats exhibit, compared with RLAs and other rat strain/stocks, several schizophrenia-relevant phenotypic traits related with positive and attentional/cognitive deficits of the disorder (Esnal et al., 2016; Fernández-Teruel et al., 2006; Giorgi et al., 2019; Oliveras et al., 2015; Río-Álamos et al., 2017, 2019; Tapias-Espinosa et al., 2018, 2019). Our present results go along with these findings and give further support to the schizophrenia-like profile of RHA rats by adding NMDA-antagonist detrimental effects on social behaviour (which is considered to model negative symptoms –asociality- of the disorder) as a new phenotype differing in the Roman rats.

Pharmacological models have already shown effectiveness of NMDAR antagonists to mimic not only positive and cognitive but also negative symptoms of schizophrenia on standard laboratory rat strains. Specifically, the most used NMDAR antagonists which have shown to induce social withdrawal are PCP, ketamine and MK801, being the latter more potent than the former ones (Gururajan et al., 2010; Neill et al., 2010). Hence, our results with MK801 administration confirm what has already been shown in other rat strains (not genetically selected). Importantly, using both Roman rat strains allowed us to add the novel finding that RHA rats are clearly more affected by MK801 than their RLA counterparts, in line with what we could expect from a rat strain presenting a schizophrenic-relevant profile. In line with the fact that negative symptoms of schizophrenia are thought to be related to frontal cortex and hippocampal dysfunction, RHA rats also present decreased prefrontal cortex and hippocampus (HPC) activity and reduced volume of both regions compared with their RLA counterparts (Meyza et al., 2009; Río-Álamos et al., 2017, 2019; Tapias-Espinosa et al., 2019). Administration of NMDA receptor antagonists is thought to reproduce these cortical and hippocampal dysfunctions, as the reduction of glutamatergic activity produces a disinhibition of mesolimbic dopaminergic function and an inhibition of mesocortical dopaminergic activity (e.g. Fernández-Teruel et al., 2021; Neill et al. 2014). RHA and RLA rats are known to differ in dopaminergic function in several brain regions, so that these divergences could underlie the between-strain differences in MK801 effects observed in the present work (e.g. Fernández-Teruel et al., 2021; Giorgi et al., 2007, 2019).

Differences in glutamatergic NMDA-receptor-mediated transmission might also be involved in the strain-related (RHA vs RLA) effects of MK801 on social interaction (e.g., Elfving et al. 2019). Recent studies have been devoted to elucidate whether RHA and RLA rats differ in parameters related to neurotransmitter receptor alterations in PFC and HPC, as well as pre- and post-synaptic markers of neural activity. Thus, RHA rats exhibit an increase of *Grin2b* (glutamate receptor NMDA2B) mRNA expression in PFC and enhanced NMDA2B in the HPC (Elfving et al., 2019; Klein et al., 2014). In addition, RHA rats exhibit enhanced 5-HT2A mRNA expression in the HPC and increased 5-HT2A receptor binding in the PFC (Elfving et al., 2019; Klein et al., 2014). Related to that, in particular in the context of the involvement of the 5-HT2A/mGlu2 receptor complex in schizophrenia (e.g. González-Maeso et al. 2008), it is worth to highlight that RHA rats exhibit a dramatic

deficit of mGlu2 receptors (mGlu2R) and *Grm2* expression (the gene for mGlu2R) in PFC, HPC and striatum (Elfving et al., 2019; Fomsgaard et al., 2018; Klein et al., 2014), due to a stop codon mutation in that receptor that makes RHA rats a naturally-occurring knock-out for it (Wood et al., 2017).

Comparing the effects of the three APs on MK801-induced alterations of social behaviour, it is worth highlighting that both CLZ and ZPR produced global significant effects on activity (see ANOVA main drug effects). In particular, CLZ dose-dependently reversed MK801-induced hyperactivity in both rat strains (see Figure 4a,b), which coheres with our previous findings using a specific activity test procedure (Oliveras et al., 2017). Conversely, ARI did not produce a global effect on locomotion (i.e., as also shown by the absence of significant main factor "ARI" effects on that measure), but its effects on activity were restricted to the attenuation of MK801-induced hyperlocomotion in RHA rats, but not in their RLA counterparts. Thus, it seems that CLZ and ZPR produce stronger and more general effects on activity than ARI. MK801 hyperlocomotor effects have been related to the increase of mesolimbic dopaminergic activity induced by NMDA receptor antagonism (e.g. Meltzer et al., 2011a, 2011b; Oliveras et al., 2017). Since RHA rats have a higher functional tone of the mesolimbic DA system than RLA rats, this could be the reason for the stronger hyperlocomotor effects of MK801 in the former strain (Giorgi et al., 2019). D2 receptor antagonism, which reduces mesolimbic dopaminergic activity, has in turn been related to the reduction of locomotor activity produced by antipsychotic drugs (e.g. Oliveras et al., 2017, and references therein). Hence, the global effects on activity observed with CLZ and ZPR might be related to their D2 antagonist properties. Conversely, since ARI acts as a partial D2 receptor agonist (Mauri et al., 2014) this could be the reason for the absence of global effects of ARI on activity while it retains a specific capacity to attenuate MK801-induced hyperactivity in RHA rats.

Moreover, the three antipsychotics have in common that they act as a antagonists of 5-HT2A receptors, and they also have partial agonist activity at 5-HT1A receptors, with CLZ showing the weakest and ARI showing the most potent partial agonist activity of the three drugs (e.g., see Odagaki and Toyoshima 2007). 5-HT1A partial agonist activity has been proposed as a key neurochemical mechanism in the attenuation or reversal of PCP-induced deficits on social behaviour (Snigdha & Neill, 2008). In fact, the three APs attenuate the deficits of "Social Preference" induced by MK801. It is nevertheless noteworthy that CLZ and ZPR appear to produce that effect by a trend to a relative reduction of "Non-Social Time" (relative to "Social Time"), whereas ARI is the only drug able to specifically and dose-dependently increase the "Social Time" (see Figure 6b).

Importantly, in addition, ARI is the only of the three APs that significantly reduces the "Social Latency" (Figure 6d) of MK801-treated RHA rats (i.e., a reduction of >200s in "ARI3+MK" group vs. "VEH-MK" group). Collectively, these findings of ARI attenuation of MK801 effects on social time, hyperlocomotion and social latency, besides its effects on social preference, confirm that ARI is the most potent of all three APs drugs under the

present conditions. This differential profile of effects of ARI (vs. CLZ and ZPR) might suggest that its more potent partial agonist effect at 5-HT1A receptors, relative to CLZ and ZPR (Odagaki & Toyoshima, 2007), and perhaps its D2 partial agonist activity, might underlie those effects on social behaviour. Since it has been shown that antagonism of 5-HT1A receptor prevents the ARI attenuation of PCP-induced deficits on social behaviour in rats (Snigdha & Neill, 2008), the observed ARI specific effects give support to the notion that they might be predominantly mediated by its potent partial agonism at 5-HT1A receptors.

5-HT1A receptors have been implicated in the cognitive/affective anomalies present in schizophrenia (e.g. Aznar and Hervig 2016; Ohno 2011) and, in line with that, previous reports have shown enhanced 5-HT1A receptor levels in the PFC of schizophrenic patients (Abi-Dargham, 2007; Burnet et al., 1996; Joyce et al., 1993; Meltzer & Sumiyoshi, 2008; Selvaraj et al., 2014). It has been proposed that postsynaptic 5-HT1A receptors tonically inhibit cholinergic and glutamatergic neuronal activity in septo-hippocampal/cortical areas, so that 5-HT1A-receptor partial agonists (such as atypical antipsychotics) might ameliorate some of the above symptoms due to a disinhibition of these cholinergic/glutamatergic neurons or systems (e.g. Ohno 2011). Consistent with the above findings from patients, rats showing deficits of prepulse inhibition, such as the Low-PPI-stratified outbred HS rats and RHA rats, which are known to also display correlated cognitive deficits (Oliveras et al., 2015), exhibit increased mRNA expression or binding of 5-HT1A receptors in the PFC (Elfving et al., 2019; Klein et al., 2014; Oliveras et al., 2017; Østerbøg et al., 2020). In addition, we have shown that prepulse inhibition of the startle response (which is impaired in patients with schizophrenia) is negatively correlated with mRNA expression of 5-HT1A receptors in PFC in a mixed sample of RHA, RLA and HS rats (Oliveras, 2017). Hence, the aforementioned profile of 5-HT1A receptors in RHA rats might also underlie the specific effects of ARI in this rat strain observed in the present study.

To conclude, the main finding of the present study is that, in line with the literature, MK801 induces social withdrawal in the Roman rats, but this is clearly more marked in RHA rats than in their RLA counterparts. The results also show that the three atypical antipsychotics used here can reverse the MK801-induced impairment in "Social preference" in the RHA rats, whereas ARI presents the most potent "therapeutic" profile of the three APs. These results are consistent with previous human and rodent studies showing that NMDAR antagonists induce some negative symptomatology of schizophrenia and hyperactivity, and these effects could be reversed by the administration of second and third generation antipsychotics (Abel et al., 2003; Deakin et al., 2008; Neill et al., 2014). The present findings add further evidence that RHA rats may be a valid model of schizophrenia-relevant symptoms. Further studies, using sub-chronic or chronic administration of MK801 and/or other NMDAR antagonists, in combination

with atypical antipsychotics and different cognitive/attentional tests/tasks, are warranted to extend the present findings to other symptom-related phenotypes.

Author contributions

D.S-V., T.C., C.R-A., A.T. and A.F-T. conceived and designed the experiments. D.S-V., T.C. and I.O. conducted the behavioural experiments. F.S., V.L., P.T., S.C., A.S-G., C.T-E. and L.M. assisted in the behavioural experiments. A.F-T. and D.S-V. analyzed the data and wrote the original manuscript. A.T., T.C., I.O., C.R-A., C.T-E and A.S-G. provided critical review of the original draft. All authors read and approved the manuscript.

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Conflict of interest

The authors declare that they have no conflict of interest.

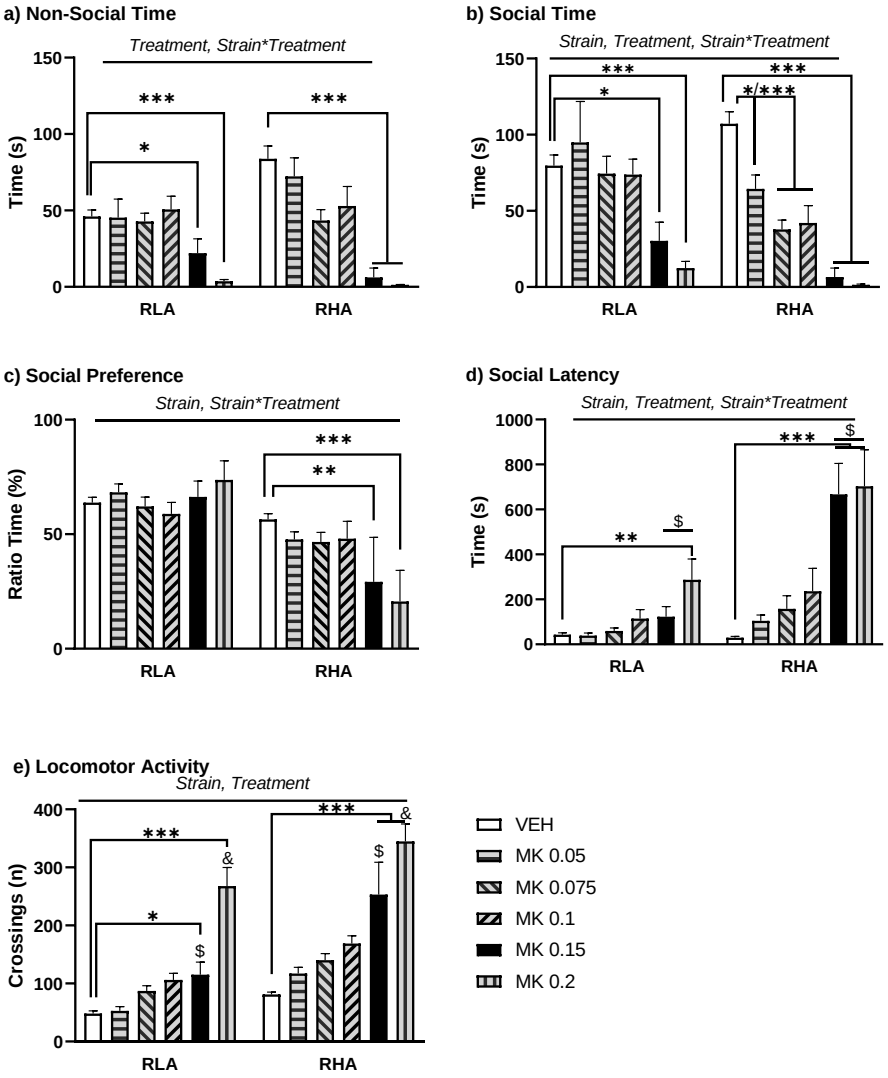

Figure 2. Dose-response effects of MK801 in the social interaction test in the Roman rat strains. a) Mean non-social time (±SEM) of RHA and RLA rats is shown for each MK801 dose. b) Mean social time (±SEM) of RHA and RLA rats is shown for each MK801 dose. c) Mean social preference (±SEM) of RHA and RLA rats is shown for all MK801 doses. d) Mean social latency (±SEM) of RHA and RLA rats is shown for each MK801 dose. d) Social latency time (±SEM) of RHA and RLA rats is shown for each MK801 dose. e) Mean number of crossings (±SEM) of RHA and RLA rats is shown for all the MK801 doses. RLA groups: VEH n=19; MK 0.05 n=10; MK 0.075 n=10; MK 0.1 n=10; MK 0.15 n=9; MK 0.2 n=6. RHA groups: VEH n=20; MK 0.05 n=10; MK 0.075 n=10; MK 0.1 n=10; MK 0.15 n=5; MK 0.2 n=8. "Strain", "Treatment" and "Strain*Treatment" effects (ANOVA). * p<0.05; ***p<0.001, between the groups indicated (Duncan's multiple range test). $, p<0.001 between the groups with the same symbol; &, p<0.01 between the groups with the same symbol (all posthoc comparisons with Duncan's multiple range test).

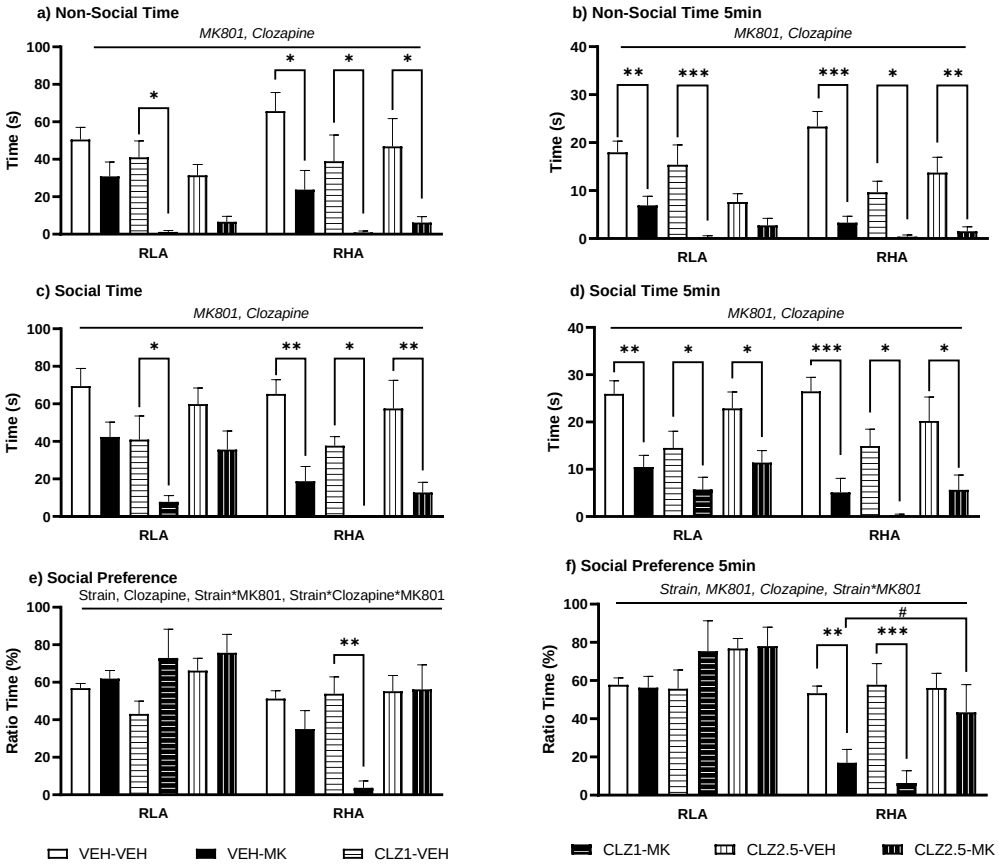

Figure 3. Clozapine (CLZ) vs. MK801 (0.15mg/kg) results for the social interaction test in the Roman rat strains. a) Mean non-social time (±SEM) of RHA and RLA rats is shown for each MK801 and CLZ dose. b) Mean non-social time (±SEM) of the first five minutes of RHA and RLA rats is shown for each MK801 and CLZ dose. c) Mean social time (±SEM) of RHA and RLA rats is shown for each MK801 and CLZ dose. d) Mean social time (±SEM) of the first five minutes of RHA and RLA is shown for each MK801 and CLZ dose. e) Mean social preference (±SEM) of RHA and RLA rats is shown for all MK801 and CLZ doses. f) Mean social preference (±SEM) of the first five minutes of RHA and RLA rats is shown for each MK801 and CLZ dose. RLA groups: VEH-VEH n=16; VEH-MK n=16; CLZ1-VEH n=6; CLZ1-MK n=6; CLZ2.5-VEH n=10; CLZ2.5-MK n=10. RHA groups: VEH-VEH n=12; VEH-MK n=16; CLZ1-VEH n=6; CLZ1-MK n=6; CLZ2.5-VEH n=10; CLZ2.5-MK n=10. "Strain", "MK801", "Clozapine", "Strain*MK801", "Clozapine*MK801" and "Strain*Clozapine*MK801" effects (ANOVA). * p<0.05; ** p<0.01; ***p<0.001; # p<0.05 between the groups indicated (Duncan's multiple range test).

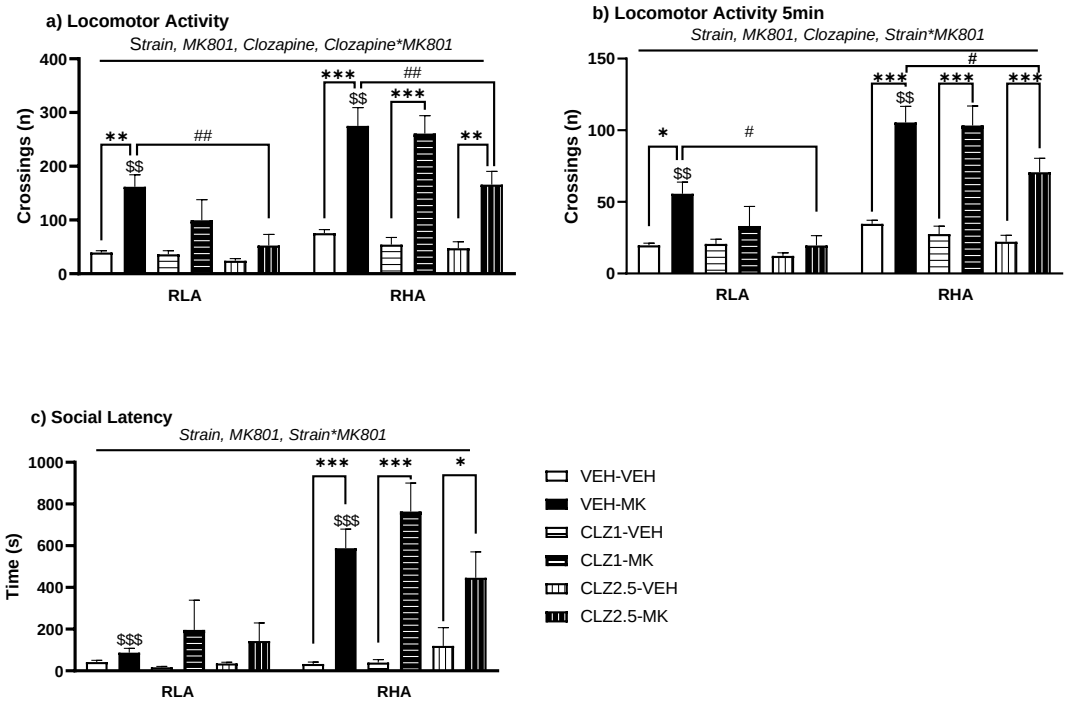

Figure 4. Clozapine (CLZ) vs. MK801 (0.15mg/kg) results for the social interaction test in the Roman rat strains. a) Mean number of crossings (±SEM) of RHA and RLA rats is shown for each MK801 and CLZ dose. b) Mean number of crossings (±SEM) of the first five minutes of RHA and RLA rats is shown for each MK801 and CLZ dose. c) Mean social latency time (±SEM) of RHA and RLA rats is shown for each MK801 and CLZ dose. RLA groups: VEH-VEH n=16; VEH-MK n=16; CLZ1-VEH n=6; CLZ1-MK n=6; CLZ2.5-VEH n=10; CLZ2.5-MK n=10. RHA groups: VEH-VEH n=12; VEH-MK n=16; CLZ1-VEH n=6; CLZ1-MK n=6; CLZ2.5-VEH n=10; CLZ2.5-MK n=10. "Strain", "MK801", "Clozapine", "Strain*MK801", "Clozapine*MK801" and "Strain*Clozapine*MK801" effects (ANOVA). * p<0.05; ** p<0.01; ***p<0.001; # p<0.05; $$ p<0.01; $$$ p<0.001 between the groups indicated. $$, $$$, p<0.01, p<0.001 respectively, between the groups with the same symbol (all posthoc comparisons with Duncan's multiple range test)

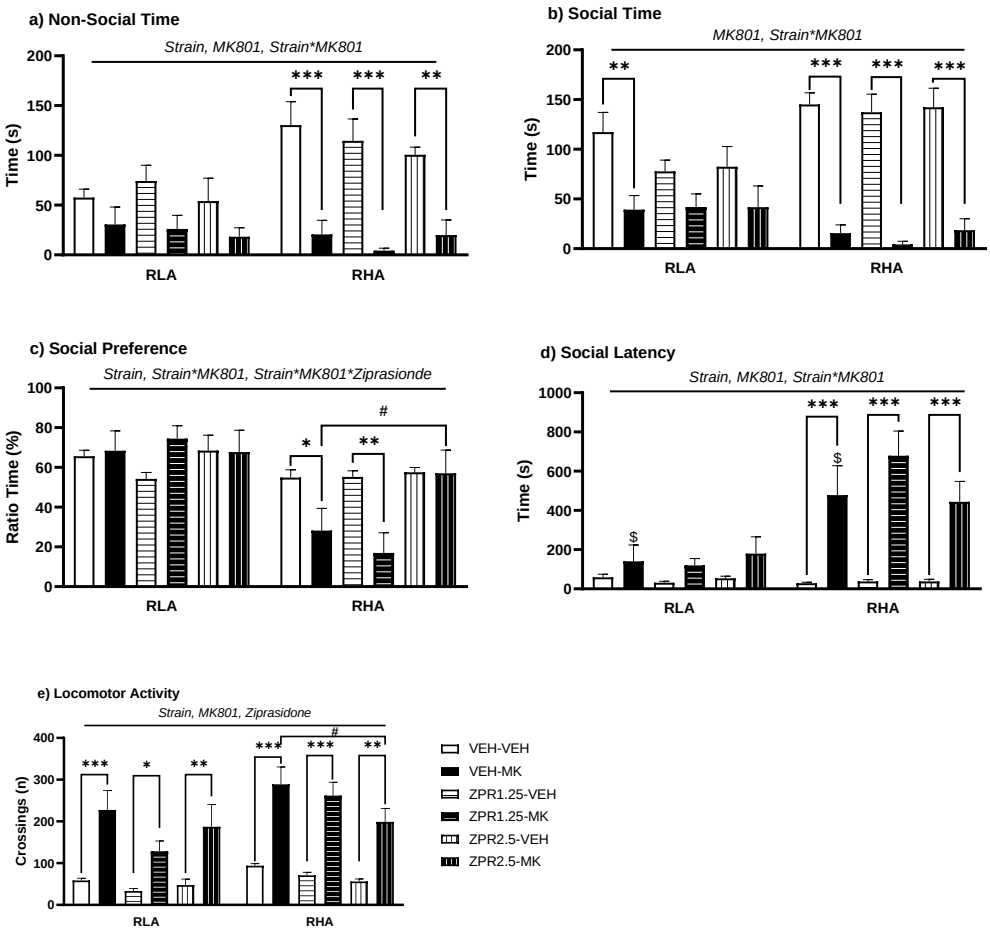

Figure 5. Ziprasidone (ZPR) vs. MK801 (0.15mg/kg) results for the social interaction test in the Roman rat strains. a) Mean non-social time (±SEM) of RHA and RLA rats is shown for each MK801 and ZPR dose. b) Mean social time (±SEM) of RHA and RLA rats is shown for each MK801 and ZPR dose. c) Mean social preference (±SEM) of RHA and RLA rats is shown for each MK801 and ZPR dose. d) Mean Social latency (±SEM) of RHA and RLA is shown for each MK801 and ZPR dose. e) Mean number of crossings (±SEM) of RHA and RLA rats is shown for all MK801 and ZPR doses. RLA groups: VEH-VEH n=8; VEH-MK n=6; ZPR1.25-VEH n=10; ZPR1.25-MK n=8; ZPR2.5-VEH n=8; ZPR2.5-MK n=6. RHA groups: VEH-VEH n=8; VEH-MK n=7; CLZ1-VEH n=6; CLZ1-MK n=8; CLZ2.5-VEH n=6; CLZ2.5-MK n=10. "Strain", "MK801", "Ziprasidone", "Strain*MK801", and "Strain*Ziprasidone*MK801" effects (ANOVA). * p<0.05; ** p<0.01; ***p<0.001; # p<0.05 between the groups indicated. $, p<0.05 between the groups with the same symbol (all posthoc comparisons with Duncan's multiple range test).

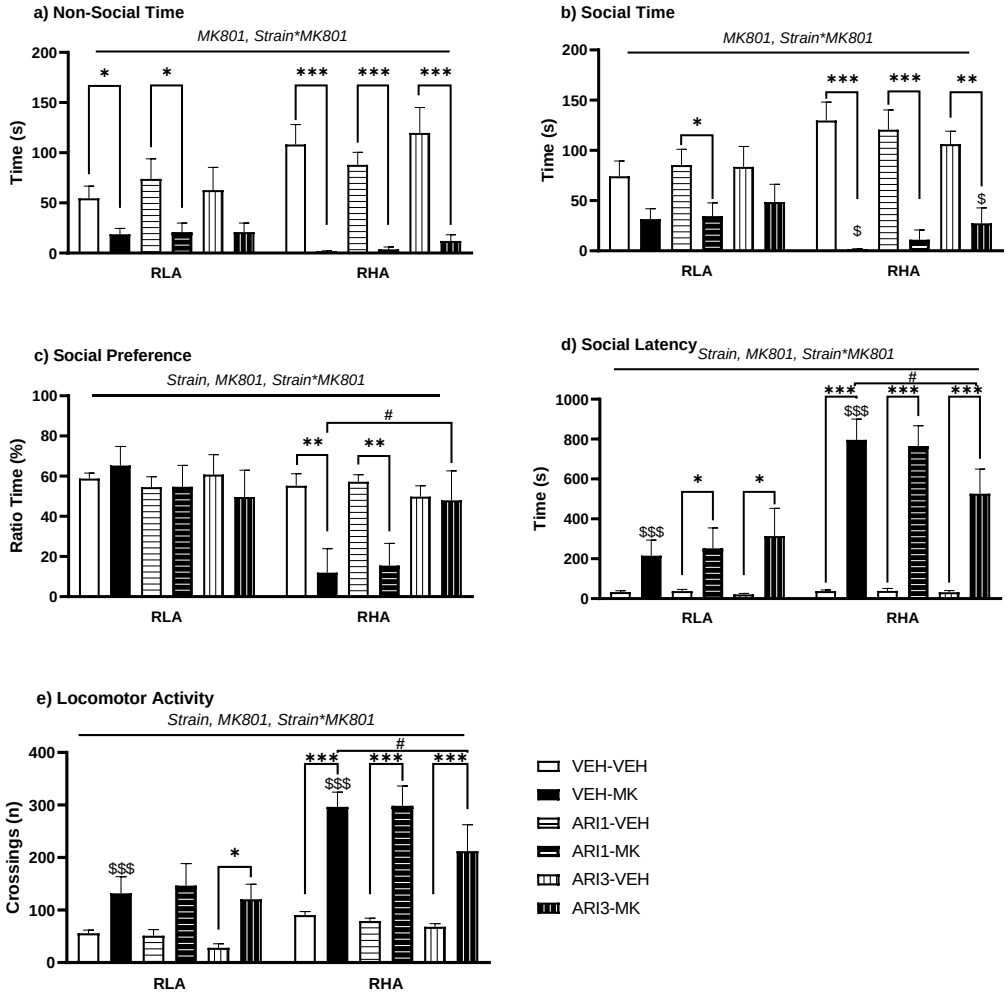

Figure 6. Aripiprazole (ARI) vs. MK801 results for the social interaction test in the Roman rat strains. a) Mean non-social time (±SEM) of RHA and RLA rats is shown for each MK801 and ARI dose. b) Mean social time (±SEM) of RHA and RLA rats is shown for each MK801 and ARI dose. c) Mean social preference (±SEM) of RHA and RLA rats is shown for each MK801 and ARI dose. d) Mean social latency time (±SEM) of RHA and RLA is shown for each MK801 and ARI dose. e) Mean number of crossings (±SEM) of RHA and RLA rats is shown for all MK801 and ARI doses. RLA n=8/group. RHA n=8/group. "Strain", "MK801" and "Strain*MK801" effects (ANOVA). * p<0.05; ** p<0.01; ***p<0.001; # p<0.05 between the groups indicated; $, $$$, p<0.05, p<0.001 respectively, between the groups with the same symbol (all posthoc comparisons with Duncan's multiple range test).

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