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Oxytocin attenuates schizophrenia-like reduced sensorimotor gating in outbred and inbred rats in line with strain differences in CD38 gene expression

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HIGHLIGHTS

- Oxytocin is proposed as an alternative natural antipsychotic drug for schizophrenia.
- Oxytocin improves PPI performance in HS rats stratified for low PPI.
- We tested oxytocin effects on PPI and mPFC gene expression of Roman rats.
- Oxytocin attenuates natural PPI deficits in RHA rats, but it does not affect RLAs.
- *CD38* expression is reduced in RHA rats, while oxytocin increases *OXTR* in both strains.
- Oxytocin shows potential antipsychotic effects that may depend on basal *CD38* differences.

ABSTRACT

Prepulse inhibition (PPI) of the startle response is a measure of sensorimotor gating that is impaired in many clinical conditions, including schizophrenia. The inbred Roman high-avoidance (RHA) rats, compared to their low-avoidance (RLA) counterparts, show distinct schizophrenia-like phenotypes, such as spontaneous deficits in PPI accompanied by decreased medial prefrontal cortex (mPFC) activity and volume. Schizophrenia-like deficits are usually attenuated by antipsychotic drugs, but these drugs often produce severe side effects. In order to reduce these side effects, the neuropeptide oxytocin has been proposed as an alternative natural antipsychotic for schizophrenia. Here, we examined the effects of peripheral oxytocin administration (saline, 0.04, and 0.2mg/kg) on PPI in the RHA vs. RLA rats, as well as in the outbred heterogeneous stock (HS) rats. Our results showed that oxytocin increased PPI in the HS rats and attenuated PPI deficits in the RHA rats, but it did not significantly affect PPI in the RLAs. To explore whether these divergent effects associated with differences in oxytocinergic mechanisms, we analyzed gene expression of the oxytocin receptor (*OXTR*) and the regulator of oxytocin release (*CD38*) in the mPFC of the Roman rats. Consistent with the differential oxytocin effects on PPI (RHA>RLA), constitutive *CD38* expression was reduced in the RHA rats compared to the RLAs, while oxytocin administration increased *OXTR* expression in both strains. Overall, the present work reveals that oxytocin administration shows antipsychotic-like effects on PPI in outbred and inbred rats, and it suggests that these effects may be related to basal differences in oxytocin-mediated mechanisms in the mPFC.

Keywords: schizophrenia, oxytocin, sensorimotor gating, qPCR, CD38, OXTR

INTRODUCTION

Prepulse inhibition (PPI) of the startle response is a measure of sensorimotor gating, in which the magnitude of a startle stimulus is attenuated by the presence of a prestimulus of lower intensity [1]. This attentional process is impaired in several neuropsychiatric conditions, such as schizophrenia, obsessive-compulsive disorder, and Tourette's syndrome [2–4]. Experimentally-induced PPI deficits in rodents are used as an endophenotype to model this basic schizophrenia-like deficiency [5–7]. PPI is an objective and useful measure to study the mechanisms underlying attentional/cognitive deficits, as it shows high test-retest stability [8,9], response to antipsychotic drugs [10,11], and genetic [12] and environmental [13] influences. Regarding its neural bases, PPI is modulated by the medial prefrontal cortex (mPFC) in rats [7,14–17] and the PFC in humans [18–20], among other brain regions [11,21,22].

The inbred Roman high-avoidance (RHA) rats display, compared to their low-avoidance counterparts (RLA), several schizophrenia-like behavioral phenotypes, such as (i) impaired PPI, working memory, and latent inhibition [23,24]; (ii) increased exploratory activity [25]; (iii) poorer maternal/nesting behavior [26]; (iv) decreased social behavior [27]; and various other schizophrenia-relevant behavioral traits [26]. Moreover, molecular, neuroanatomical, and neurochemical analyses have shown that the RHA rats exhibit several brain abnormalities that resemble schizophrenia [17,28–30]. For instance, the RHA rats show low PPI accompanied by reduced mPFC activity and volume [17]. Furthermore, pharmacological studies have shown that the administration of several antipsychotic drugs in the RHA rats can attenuate schizophrenia-like phenotypes, such as hyperactivity and PPI deficits [31]. In this regard, even though antipsychotic drugs usually attenuate schizophrenia-like impairments in rodents and humans [16,32,33], they produce severe side effects [34]. Importantly, the neuropeptide oxytocin has been proposed as an alternative natural antipsychotic for schizophrenia, which would have the advantage of presenting less side effects than antipsychotics [35–38]. Thus, oxytocin can become a candidate of interest to replace or be adjuvant of the current schizophrenia medication.

Oxytocin is a neuropeptide synthesized in the paraventricular and supraoptic nuclei of the hypothalamus [39,40]. It is known to promote uterine contractions and breastfeeding, but recent evidence has shown that oxytocin might be also critical for social attachments and cognitive-relevant behaviors [37,41]. Specifically, high oxytocin levels in blood plasma are associated with several positive events, such as trust, physical contact with

a partner, and reduced hormonal response to stressors or reduced anxiety. In contrast, low levels of oxytocin have been related to several psychiatric conditions, such as autism spectrum disorders, depression, and schizophrenia [35]. In this sense, recent findings have pinpointed that oxytocin administration induces several antipsychotic-like effects, as it increases trustworthiness in healthy subjects [42], reduces positive and negative symptoms in schizophrenia [43], increases eye gaze in schizophrenia [44], and improves several schizophrenia-like behaviors in rodents, such as social interaction [45] or PPI [46,47].

Oxytocin acts on oxytocin receptors (OXTR) and its secretion is regulated by the CD38 protein [48,49]. In this regard, studies in rodents lacking *OXTR* or *CD38* show impairments in oxytocin-related behaviors, such as maternal nurturing [48,50] and social behavior [48,50]. Interestingly, it has been recently found that mice lacking *CD38* show PFC abnormalities accompanied by alterations in several behaviors that depend on this region [51]. Of note, the *OXTR* and *CD38* genes have been implicated in complex human behaviors, such as the processing of anticipatory, appetitive, and aversive cognitive states [52]. Regarding the role of oxytocin in the brain, findings are generally consistent with the idea that oxytocin has an inhibitory profile, as it reduces glutamate and increases GABA release [53–55].

In order to test the oxytocin effects on PPI, based on previous works by [46,47,56], first we examined the effects of oxytocin on PPI in the outbred heterogeneous stock (HS) rats in the inbred RHA and RLA rat strains. Then, given the known behavioral differences between the Roman rats, we explored differences in oxytocinergic mechanisms (*OXTR* and *CD38*) by real-time quantitative polymerase chain reaction (qPCR) in the mPFC. Our major hypotheses were that oxytocin would improve PPI in HS rats and it would cause a more marked PPI improvement in the RHA than in the RLA rats and there would be between-strain differences in *OXTR* and *CD38* expression in the mPFC.

MATERIAL AND METHODS

Subjects

We used naïve male HS (the "National Institutes of Health genetically heterogeneous" rat stock; see [57,58]; n= 46), and inbred RHA (n=54) and RLA (n=45) rats from our breeding colonies (Dept. Psychiatry and Forensic Medicine, Universitat Autònoma de Barcelona). They were aged 3-4 months, weighting 320-390 g. They were housed in pairs in macrolon cages (50 x 25 x 14) and kept with food and water *ad libitum*, maintained under a 12:12h light-dark cycle (lights on at 08:00 a.m.) and with controlled temperature (22 ± 2°C) and humidity (50-70%).

The inbred RHA and RLA rat strains are Wistar-derived rats that were originally selected and bred in Rome for their rapid (RHA) vs. extremely poor (RLA) acquisition of the two-way active avoidance task [59]. Two inbred strains are maintained in our laboratory (since 1996) on the basis of breeding 20-30 pairs of each strain three times a year. The RHA and RLA rats used in the present study came from at least 12 different litters per strain.

The HS rat stock was derived from crossing 8 inbred rat strains, following an optimized rotational breeding schedule on the basis of 40 breeding pairs per generation and over 50 breeding generations, with the aim of obtaining the maximal possible genetic heterogeneity. Among the 8 parental strains, three were of Wistar origin [57,58]. The HS rats from the present study were randomly selected from 30 breeding pairs (i.e., 30 genetically different families). Extensive phenotyping work carried out by our group has shown that behavioral profiles of HS rats are usually different from RHA rats and closer to the profiles of RLAs (e.g., [23,25,27]).

Experimental procedures

Oxytocin administration and behavioral testing were conducted during the light cycle (9:00–14:00). Experiments were carried out in accordance with the Spanish legislation on "Protection of Animals Used for Experimental and Other Scientific Purposes" and the European Communities Directive (2010/63/EU) on this subject. Every effort was made to minimize any suffering of animals used in this study.

First, we carried out a study using the genetically heterogeneous (outbred) HS rats. As illustrated in Fig. 1, before the PPI test, the HS rats underwent a "screening" PPI test to stratify them according to relatively low PPI levels (compared to those usually found in the whole HS colony), because we expected that positive oxytocin effects on PPI could more easily appear in relatively low-PPI animals. This "screening" procedure have been used in previous studies and proved its reliability [60]**.** The aim of this experiment was to

test different oxytocin doses in the HS rats in order to select effective doses for the second study (i.e., the study with the Roman rat strains).

Second, we conducted the Roman rat study, in which we aimed to test the hypothesis that oxytocin would had improving effects on PPI particularly in the RHA rats. The Roman rats did not undergo the "screening" PPI test, since we already know from several previous studies their relative PPI performance (e.g. [17,23–25,28]).

As illustrated in Fig. 1, before the PPI test, the HS rats underwent a "screening" PPI test to stratify them according to relatively low PPI levels compared to those found in the HS colony, while the Roman rats did not undergo it.

Oxytocin administration

Following random assignment to group condition, HS groups and RHA and RLA rat strains received subcutaneous injections 30-min before the PPI test of either sterile 0.9% saline vehicle, 0.04mg/kg oxytocin or 0.2mg/kg oxytocin (VWR International Eurolab, S.L., Barcelona, Spain) dissolved in sterile saline, based on previous studies [46,47,56]. Pair-housed rats were injected and behaviorally tested at the same time.

Prepulse inhibition

PPI was conducted in four sound attenuated boxes (SR-Lab Startle Response System, San Diego Instruments, USA), as previously described by our laboratory with minor modifications [25]. Briefly, rats were located in a cylinder, which was situated in a dimly illuminated box and on the top of a platform with a sensor that detects the strength made by the rat in each trial. Noise bursts were presented via a speaker mounted 15cm above the cylinder. After 5 min of acclimation period, 10 "pulse-alone" trials (105dB(A), SPL, 40ms; Startle_Block1) were delivered in order to obtain a stable baseline of startle. After this pulse-alone period, six types of trials were randomly administered ten times (60 trials in total): (i) Pulse alone trials (105dB(A), SPL, 40ms; used to calculate the percentage of PPI; Startle_Block2); (ii) prepulses of 60/65/70/75dB(A), SPL, 20ms, followed by the pulse stimulus with an inter-stimulus interval of 100ms; or (iii) no-stimulus trials (background noise of 55dB). The interval between trials was 15± 5s. The percentage of PPI for each prepulse intensity was calculated by applying the following formula: %PPI = [100-(startle amplitude on prepulse trials/startle amplitude on pulse alone trials)x100]. %PPI is shown for each prepulse alone (i.e., from "PPI_60dB" to "PPI_75dB"), as well as for the average of all prepulse intensities (hereafter, "PPI_Total")

For selection of HS rats with relatively low PPI levels, based on a previous work [60], we used a shorter version of the PPI test. This session consisted of (i) a 2-min acclimation period; (ii) 5 pulse-alone trials; and (iii) randomly administered trials of 12 pulse-alone

trials and 3 pre-pulse 65dB + pulse trials. This baseline PPI ("PPI_screening" session) allowed us to select a sub-sample of HS rats by their similar and relatively low PPI levels to have room for improvement in the "final" PPI session upon oxytocin administration.

RNA extraction, Reverse Transcription, and qPCR

Immediately after the PPI test, a random and representative sample of RHA and RLA rats belonging to the saline or 0.2mg/kg oxytocin groups were euthanized to conduct qPCR analyses ($\geq$8 rats per group). Their mPFC was dissected out, immediately frozen in liquid nitrogen, and stored at -80°C.

RNA was extracted from the mPFC tissue as described earlier in [61] with minor modifications. Briefly, RNA from tissue samples (around 30µg) was extracted using the miRNeasy mini kit (Qiagen; cat. no. 217004) in a RNAase-free environment. RNA samples were subjected to DNAse treatment using the Turbo DNA-free kit (Ambion; cat. no. AM1907). RNA concentration was determined using Thermo ScientificTM NanoDrop 2000c spectrophotometer (ThermoFisher Scientific, USA). RNA integrity number (RIN) was determined using the 2100 Agilent Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) and only samples with RIN value $\geq$8 were and A260/A280 ratio ≥ 1.8 were included in the analyses. The purified RNA (200ng) was transcribed into complementary DNA (cDNA) using qScript cDNA SuperMix kit (Quanta Biosciences, cat. no. 95048), according to the manufacturer instructions. The cDNA products were diluted 1:5 with RNase/DNase-free water.

Each sample was run on a 96-well plate in duplicates and one reaction contained 3µL diluted cDNA, 0.6µL of each primer (final concentration 300nM), 0.8µL of RNAse-free water, and 5µL of Fast SYBR Green Master Mix (Applied Biosystems, cat. no. 438512). The following primers were used: *GAPDH* (*F*: CATCAAGAAGGTGGTGAAGCA, *R*: CTGTTGAAGTCACAGGAGACA); *RPL13A* (*F*: AGCAGCTCTTGAGGCTAAGG, *R:* GGGTTCACACCAAGAGTCCA); *CD38* (*F*: GAAAGGGAAGCCTACCACGAA, *R*: GCCGGAGGATTTGAGTATAGATCA); *OXTR* (F: TCGTACTGGCCTTCATCGTG, R: TGAAGGCAGAAGCTTCCTTGG). To compare the multiple samples between the assays, a positive control (a pool of cDNA from all samples used as a calibrator) and a negative control (RNase/DNase-free water) were included in each run. To check for gDNA contaminations, a negative control of RNA from which the reverse transcription had been omitted was included for each sample analysis.

All qPCR reactions were run on a QuantStudio3 qPCR system (Applied Biosystems) using a standardized 40-cycle Fast SYBR Green program with annealing/acquisition segments adjusted for each primer sets: *GAPDH*, *RPL13A*, and *CD38* at 61°C; *OXTR* at 58°C. Expression levels of housekeeping genes did not differ across groups. A

comparative cycle of threshold fluorescence (Ct) method was used and the relative transcription level of the target gene was normalized to that of average for housekeeping genes (*GAPDH* and *RPL13A*) and expressed as relative quantity to the calibrator sample using the Pfaffl method [62].

Statistics

All the analyses were performed using the "Statistics Package for Social Sciences" (SPSS). Significance level at $p < 0.05$.

3 x 4 ("3 treatments x 4 prepulse intensities") repeated measures ANOVA was applied followed by post hoc Duncan's test to determine differences among the three HS groups, as we had the *a priori* hypothesis that the drug treatment was expected to increase PPI levels [46,47]. 3 x 2 ("3 treatments x 2 startle Blocks – Startle_Block1 and Startle_Block2") repeated measures ANOVA was applied to determine differences among groups in baseline startle response (to pulse-alone trials).

2 x 3 x 4 ("2 strains x 3 treatments x 4 prepulse intensities") repeated measures ANOVA was applied to data from the Roman rat study, followed by post hoc Duncan's test, as we also had the *a priori* hypothesis that the drug treatment would increase PPI levels particularly in the PPI-impaired RHA strain. 2 x 3 x 2 ("2 strains x 3 treatments x 2 startle Blocks – Startle_Block1 and Startle_Block2") repeated measures ANOVA was applied to determine differences among groups.

2 x 2 ("2 strains x 2 treatments") ANOVAs were applied to data from the gene expression study, followed by post hoc Duncan's test to determine differences in gene expression between the RHA and RLA that underwent the two treatment conditions (saline vs. 0.2mg/kg oxytocin). Before the gene expression analysis, the Grubbs outlier test was run, and significant outliers removed (final sample was of 5-8 rats per group).

Spearman's rank correlations were performed between startle response, PPI scores, and gene expression values. Descriptive factor analysis, with oblique rotation (oblimin direct), was also applied to the same variables in the whole Roman rat sample.

RESULTS

In the HS rats, 3 x 4 repeated measures ANOVA revealed a "Prepulse" effect [$F(3,45)=24.52$; $p=<0.001$], as PPI was greater at higher prepulse intensities; while there was no "Treatment x Prepulse" effect [$F(6,45)=0.82$; $p=<0.560$]. However, there was a "Treatment" effect on PPI [$F(2,15)=4.20$; $p=0.036$]. Particularly, the HS group that received a dose of 0.04mg/kg showed higher PPI than the saline control group in "PPI_75dB" and "PPI_Total" measures, and it did not differ from the dose of 0.2mg/kg (Duncan's post hoc test, $p<0.05$; Fig. 2). Notably, there were no differences among the three groups in their levels of PPI in the "PPI_Screening" session [$F(2,15)=0.001$; $p=0.999$], indicating that the groups were matched correctly. With regard to baseline startle response, there was a "Block" effect [$F(1,15)=24.778$; $p=<0.001$], indicating startle habituation, while there was no significant "Block x Treatment" [$F(2,15)=0.035$; $p=0.966$] or "Treatment" [$F(1,15)=0.059$; $p=0.943$] effects (see Fig. 2). Thus, oxytocin had a specific effect on PPI in HS rats previously matched for their PPI levels in the "PPI_screening" session.

In the Roman rats, 2 x 3 x 4 repeated measures ANOVA showed a "Prepulse" effect on PPI [$F(3,279)=110.80$; $p=<0.001$], as PPI was higher at higher prepulse intensities; and a "Strain x Prepulse" effect on PPI [$F(3,279)=4.91$; $p=0.009$], as PPI was higher in the RLA rats than their RHA counterparts. No significant "Strain x Treatment x Prepulse" effect on PPI was found [$F(6,279)=1.28$; $p=0.265$]. Importantly, however, there was a "Treatment x Prepulse" effect on PPI [$F(6,279)=2.98$; $p=0.008$], as RHA rats treated with the dose of 0.2mg/kg significantly improved PPI compared to the RHA saline control, and did not statistically differ from the RLA rats at the "PPI_75dB" (Duncan's post hoc test, $p<0.05$; Fig. 3). According to the above "Strain x Prepulse" interaction we applied separate repeated measures ANOVA ("3 treatment x 4 prepulse" levels) to %PPI data from each strain, which yielded a significant "treatment x prepulse" interaction effect only in RHA rats (RHA, $F(6,153)=3.02$; $p=0.008$; RLA, $F(6,126)=1.12$; $p=0.357$). Post-hoc Duncan tests revealed that such an interaction in RHA rats is due to the fact that the oxytocin 0.2 mg/kg dose significantly improved %PPI at the 75 dB prepulse intensity (Fig. 3).

Regarding baseline startle, there was a "Block" effect [$F(1,93)=67.77$; $p=<0.001$], indicating startle habituation from Startle_Block1 to Startle_Block2, and a "Strain" effect [$F(1,93)=21.10$; $p=<0.001$], as startle was overall higher in the RLA rats than the RHA rats. However, there was no "Block x Treatment" [$F(2,93)=0.842$; $p=0.434$] or "Block x

Strain x Treatment" [$F(2,93)=0.347$; $p=0.708$] effects (see Fig. 3). Thus, oxytocin had a specific improving effect on PPI in RHA rats that was absent in RLA rats.

In line with the behavioral data, as shown in Fig. 4, ANOVA revealed a "Strain" effect on *CD38* gene expression in the mPFC [$F(1,21)=11.61$; $p=0.003$], which was higher in the RLA rats than the RHA rats. This finding coheres with the fact that oxytocin was effective to improve PPI in the RHAs, while being devoid of effects in the RLA rats. Moreover, there was a "Treatment" effect on *OXTR* expression [$F(1,21)=5.50$; $p=0.029$], as it was globally higher in subjects treated with oxytocin than in saline groups (Fig. 4).

Finally, as shown in Fig. 5a-b, factor analysis of gene expression (*CD38* and *OXTR*), PPI (PPI_Total) and Startle (BL1 and BL2_Startle) values in Roman rats (including all the rats from both strains) revealed a 2-factor solution, which grouped PPI, BL1_Startle, and CD38 expression in the first factor. Moreover, in line with the factor analysis, Spearman's rank correlations yielded significant positive correlations between *CD38* expression, PPI and BL1_Startle (Fig. 5c-e). This finding indicates that higher *CD38* expression is associated with better PPI performance.

DISCUSSION

The main aim of the current study was to test the potential antipsychotic-like effects of oxytocin on PPI in our rat model of schizophrenia-relevant symptoms, i.e., the RHA rats. Our results showed that oxytocin was effective to improve PPI performance in inbred RHA rats compared to the saline group, while oxytocin had no significant effect on the RLAs. Importantly, the RHA rats treated with oxytocin did not significantly differ from their RLA counterparts, suggesting that oxytocin may have antipsychotic-like effects. Moreover, a novel outstanding finding is that gene expression analysis revealed differences in oxytocinergic mechanisms in the mPFC between the Roman rats, as RHA rats showed lowered expression of *CD38* compared with their RLA counterparts. Additionally, we also showed that oxytocin improves PPI in the outbred HS rats.

Findings in the HS and RHA rats are in line with previous studies showing that oxytocin administration reverses the PPI-disrupting effects of amphetamine and the NMDA antagonist MK-801 [46], attenuates the natural PPI deficits in Brown-Norway rats [47], and increases PPI in C57BL/6N mice [63]. In this sense, it has been reported that the sensitivity to the PPI disrupting effects of the NMDA antagonist PCP are increased in oxytocin knock-out mice [36]. Apart from PPI, oxytocin has shown a potential antipsychotic profile in other behaviors and cognitive functions, such as hyperactivity [64], social withdrawal [45,65], aggressive behavior [66], and impaired latent inhibition [56].

At the prepulse intensities and oxytocin doses tested here, we found a more powerful oxytocin effect on the HS rats that on the RHA rats. The HS rats in the 0.04mg/kg oxytocin group showed significant differences in both “PPI_75dB” and “PPI_Total”, while the improving effect of oxytocin 0.2mg/kg in the RHA was only observed at “PPI_75dB”. In this sense, higher prepulse intensities (i.e. PPI_75dB) are known to elicit higher PPI levels [15,67], which might be more sensitive to drug effects [68–71]. On the other hand, albeit not significant, we observed a trend in the RLA in the 0.04mg/kg group. These findings, i.e., the different potency of oxytocin on HS and RHA rats, may also be related to the fact that the genetic background of the animals is very different. In relative terms, there is higher genetic variability in HS rats (derived from crossing of 8 inbred strains [57,58]) than the inbred Roman rat strains [72,73]. Thus, genetic background is likely an important influencing factor that might account for both differences in PPI performance and oxytocin response among the rat strains used here.

The present work adds further evidence on the potential antipsychotic value of oxytocin for two main reasons. First, we show that oxytocin improved PPI in a sub-sample of genetically heterogeneous rats (representing the whole colony of 30 families we maintain at our laboratory), stratified by their relatively low PPI scores in relation to the HS population. The HS rats are known to have higher genetic heterogeneity than other laboratory rats, which confers these results an enhanced translational value in view of the heterogeneity of the human population [25,57]. Second, we report divergent strain-related effects of oxytocin in the Roman rats, as the treatment improved PPI in the PPI-deficient RHA strain but not in the RLA strain. This suggests possible differences in endogenous oxytocin related to PPI, which highlights the importance of taking into account strain-linked differences when dealing with the effects of oxytocin on sensorimotor gating processes (e.g., PPI).

To see whether these divergent effects in the Roman rats were related to differences in the oxytocinergic mechanisms, we analyzed gene expression of the oxytocin receptor (*OXTR*) and the oxytocin regulator (*CD38*) in the mPFC. Consistent with the above suggestion and the differential oxytocin 0.02mg/kg effects on PPI (RHA>RLA), our data revealed that *CD38* expression in the mPFC was reduced in RHA rats compared to their RLA counterparts. Interestingly, loss of *CD38*, which is associated with low oxytocin levels [48], causes abnormalities in PFC-dependent behaviors [51]. Together with the fact that RHA rats display attentional and sensorimotor gating deficits, reduced social behavior, and impaired working memory, the reduced *CD38* expression in the PFC of RHA rats may be consistent with alterations in the oxytocinergic mechanisms reported from animal models of schizophrenia-relevant features [36,51,74–76] and human schizophrenia studies [35,49,77,78]. For example, the vasopressin-deficient Brattleboro rats show abnormal oxytocin release in response to stress [79], as well as several natural schizophrenia-like deficits, including impairments in PPI, social discrimination, and memory [80]. Thus, it seems reasonable that the RHA rats would benefit more from oxytocin administration than the RLAs. Conversely, oxytocin in the RLA showed a non-significant tendency to improve PPI at 0.04mg/kg, but not at 0.2mg/kg, that may be explained by these basal oxytocin differences. On the other hand, *OXTR* expression was increased in both RHA and RLA rats that underwent oxytocin administration. In agreement with our data, a recent finding indicates that oxytocin administration reverses the reduced *OXTR* expression in the mPFC caused by prolonged stress [81]. In addition, the schizophrenia-like Wisket rats show reduced *OXTR* expression related to decreased acute pain sensitivity [76]. Moreover, it has been reported that *OXTR* expression is reduced in post-mortem brains of schizophrenic patients [78]. Regarding oxytocin

administration, the entry of exogenously administered oxytocin into the brain is still a matter of controversy [63,82]. However, our data indicate that oxytocin administration increased *OXTR* in the mPFC of the Roman rats. Thus, oxytocin increased *OXTR* in the mPFC in both rat strains, but not *CD38*. Since the Roman rats were euthanized around 1 hour after the administration of oxytocin (when they finished the PPI test), the possibility remains that the *CD38* gene in the mPFC, if altered by oxytocin administration, had already returned to its basal levels (RHA < RLA), while oxytocin would have more long-lasting effects on the *OXTR*. Also, even though we did not observe "treatment" effect in *CD38* levels in the PFC, we cannot rule out that exogenous oxytocin administration increases *CD38* in the hypothalamus [40]. Moreover, correlation and factor analyses indicate a positive association between PPI and *CD38* expression in the mPFC. Our findings showing higher PPI and *CD38* expression in RLA rats and a positive PPI-*CD38* association in the whole sample of Roman rats are consistent with previous results indicating that oxytocin improves PPI and is associated with higher sensorimotor gating [36,46,47,63]. These analyses also show a positive association between BL1_Startle (i.e., initial –or baseline– startle reactivity) and both PPI and *CD38* expression. In spite of these associations, our previous findings clearly indicate that PPI in the Roman strains and in the HS stock is independent from the initial baseline startle reactivity (BL1_Startle) displayed by the animals ([17] and unpublished results). Moreover, the present correlation and factor analyses also indicate that BL2_Startle (i.e., habituated startle responses; those that are used to calculate the %PPI) are not associated with PPI or *CD38* expression (Fig. 5).

Here, we focused on the mPFC, as previous studies have indicated differences in volume, activity, and gene expression between the Roman rats strains in this region [17,28,30,83]. However, focusing only on the mPFC is a limitation of the present study, as for instance the hippocampus or the nucleus accumbens have also been involved in PPI modulation [4,15,17] and the hypothalamus is critical for oxytocin synthesis [39,40]. Future studies will be addressed to a wider range of brain areas.

Regarding the neural mechanisms through which oxytocin improves PPI, one could speculate that it does it through the inhibition of dopamine transmission, as oxytocin attenuates the PPI disrupting effects of amphetamine [46] and the cocaine-induced mesolimbic dopamine release and hyperactivity [64]. Accordingly, impaired dopamine transmission has been described as one of the fundamental factors in the etiopathology of psychotic symptoms of schizophrenia [84]. However, recent findings have highlighted that dopamine transmission is regulated by the balance between neocortical excitatory

glutamatergic and inhibitory GABAergic neurons, and this balance is primarily affected in schizophrenia [84,85]. Specifically, schizophrenic patients would have excessive glutamate release and reduced GABA inhibition. In this sense, previous findings indicate that, compared to RLAs, the RHA rats could have an excessive glutamatergic and dopaminergic tone in the PFC and striatum that could drive an imbalance between excitation and inhibition [30,59]. Consequently, as oxytocin administration has been associated with a reduction in glutamate and an increase in GABA release [53–55,63], and *OXTR* is expressed in GABAergic interneurons [86], it is possible that oxytocin reduces dopamine transmission by increasing inhibition in mPFC neurons.

CONCLUSIONS

Overall, the present work reveals that oxytocin administration shows antipsychotic-like effects on PPI in both outbred HS and inbred RHA rats, and it suggests that these effects may depend on basal differences in oxytocinergic mechanisms. Moreover, we found positive associations between PPI and *CD38* expression. To the best of our knowledge, this is the first study that combines the oxytocin effects on PPI and gene expression of oxytocin-related genes in the mPFC in a genetic rat model of schizophrenia-like features, i.e., the RHA rats. Our present results support the notion that oxytocin administration regulates sensorimotor gating in a strain-dependent manner, while highlighting differences in *CD38* expression in the mPFC between the Roman rats that may be relevant for neurobiological research on schizophrenia.

FUNDING AND DISCLOSURE

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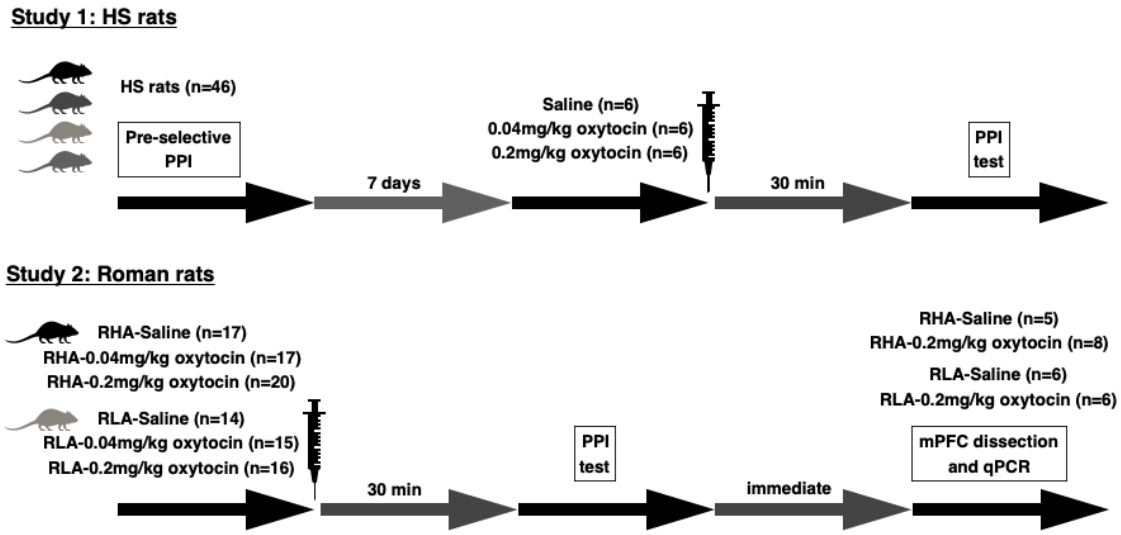


Figure 1. Experimental timeline. Study 1: HS rats underwent a short pre-selective version of the PPI test that was used to conform three similar random groups with relative low PPI. After a 7-day rest period, animals were injected with saline solution, 0.04mg/kg oxytocin, or 0.2mg/kg oxytocin 30-min before a PPI test. Study 2: RHA and RLA rats were randomly distributed in three groups that received saline solution, 0.04mg/kg oxytocin, or 0.2mg/kg oxytocin 30-min before a PPI test. Immediately after ending the PPI session, random and representative samples of the saline and 0.2mg/kg oxytocin from both strains were euthanized and the mPFC was dissected out to conduct gene expression analyses by quantitative polymerase chain reaction (qPCR).

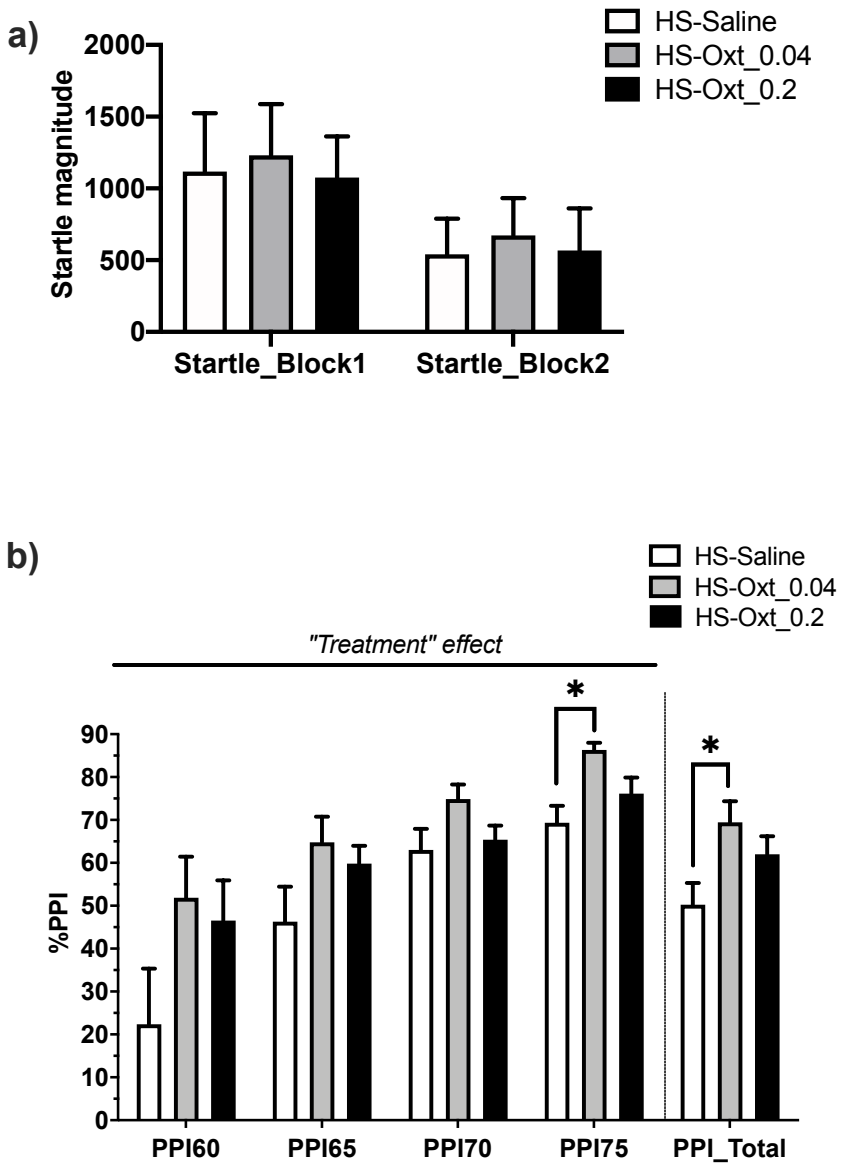


Figure 2. Oxytocin improves PPI in the HS rats treated with oxytocin compared to saline group and has no effects on startle response. **a)** No "treatment" effects were observed among the three groups of HS rats treated with saline solution, oxytocin 0.04mg/kg, or 0.2mg/kg in the baseline startle blocks (1 and 2). **b)** A significant "treatment" effect was observed among the three groups of HS rats across the different prepulse intensities (PPI60, 65, 70, and 75dB), as PPI was higher in the HS rat group treated with oxytocin 0.04mg/kg compared to the saline group. Bars and error bars show Mean±SEM. *, $p<0.05$ (Duncan's multiple range test).

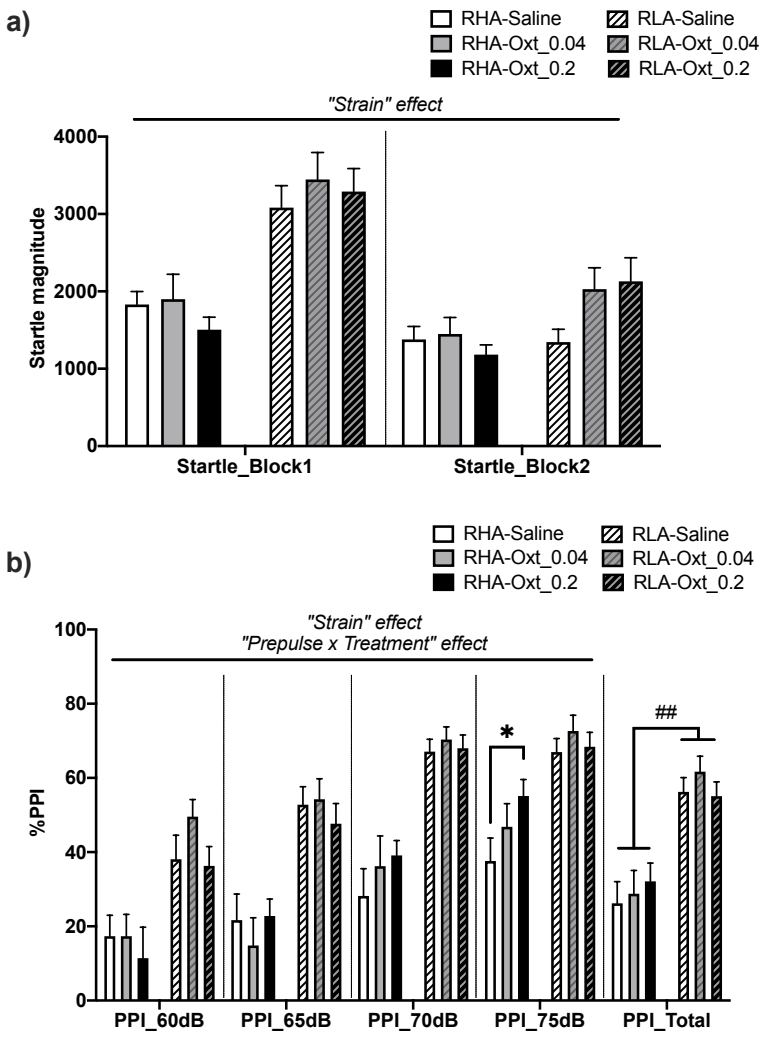


Figure 3. Oxytocin attenuates PPI deficits in the RHA rats at the 75dB prepulse intensity and has no effects on startle response. **a)** No “treatment” or “strain x treatment” effects were observed among the six groups of RHA and RLA rats treated with saline solution, oxytocin 0.04mg/kg, or 0.2mg/kg in the baseline startle blocks (1 and 2). However, there was a “strain” effect, as startle was globally higher in the RLA rats than in the RHAs. **b)** A significant “prepulse x treatment” effect was observed among groups across the different prepulse intensities (PPI_60, 65, 70, and 75dB), as oxytocin improved PPI in the RHA rats treated with oxytocin 0.2mg/kg compared to saline group at the 75dB prepulse intensity. Bars and error bars show Mean±SEM. *, $p<0.05$; ##, $p<0.01$ (Duncan’s multiple range test).

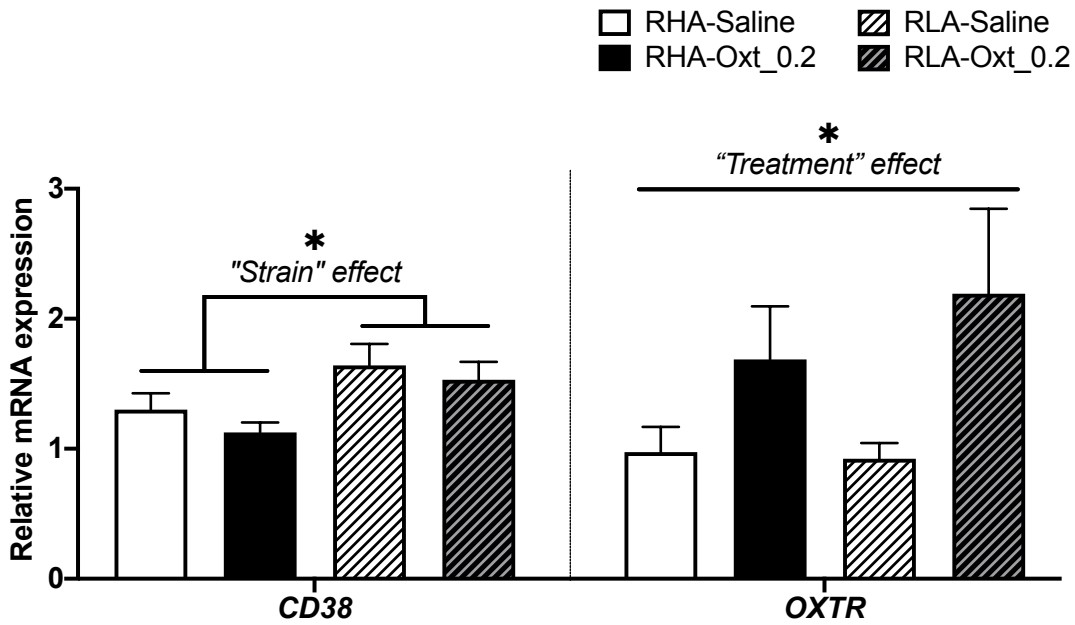
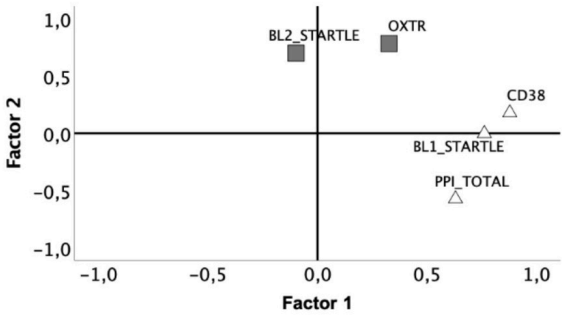


Figure 4. The RHA rats show lower *CD38* expression than the RLA rats, while oxytocin increases *OXTR* expression in the medial prefrontal cortex (mPFC). A significant "strain" effect was observed in *CD38* relative mRNA expression in the mPFC, as the RLA rats globally showed higher expression than the RHAs. On the other hand, there was a significant "treatment" effect in *OXTR* expression in the mPFC, as both RHA and RLA rats treated with oxytocin 0.2mg/kg showed higher expression than RHA and RLA rats treated with saline solution. Bars and error bars show Mean±SEM. Vertical axis shows relative mRNA expression with respect to the housekeeping genes *GAPDH* and *RLP13A* (see "Material and Methods" section). *, $p<0.05$.

a)

	Factors	
	1	2
OXTR	-	0.772
CD38	0.873	-
PPI_TOTAL	0.652	-
BL1_STARTLE	0.763	-
BL2_STARTLE	-	0.703
% Cumulative variance	37.7%	66.7%
Factor correlations	1	
	-0.36	1

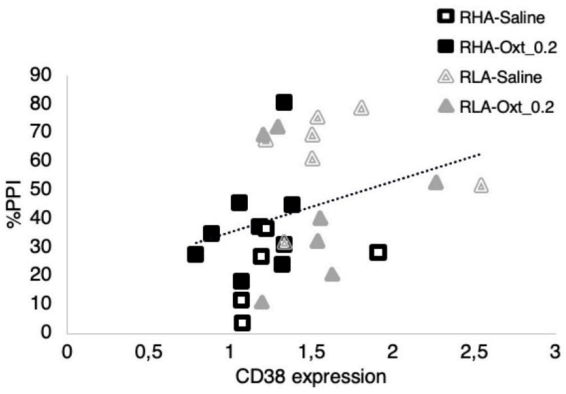
b)



c)

Variables	*1*	*2*	*3*	*4*	*5*
OXTR	1				
CD38	0.246	1			
PPI_TOTAL	-0.216	**0.439***	1		
BL1_STARTLE	0.048	**0.538****	0.350	1	
BL2_STARLE	-0.072	-0.340	-0.326	-0.272	1

d)



e)

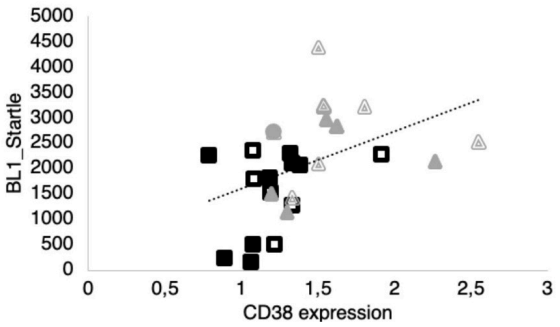


Figure 5. Factor and correlation analyses of PPI, Startle response, and *OXTR* and *CD38* gene expression in the Roman rats (n=25). a-b) Two-fold solution of factor analysis (oblique rotation) with the first factor grouping *CD38* expression, PPI_TOTAL (average of all prepulse intensities) and BL1_STARTLE (baseline startle 1, first 10 pulse-alone trials of the session) and the second factor grouping *OXTR* expression and BL2_STARTLE (baseline startle 2, pulse-alone trials used for the PPI calculation). Only loadings > 0.60 are shown. c-e) Spearman's correlations (c), which show positive correlations between CD38 expression and PPI_TOTAL (d) and BL1_STARTLE (e). *, p<0.05; **p<0.01 (2-tailed).

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