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ORIGINAL ARTICLE

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Reelin deficiency exacerbates cocaine-induced hyperlocomotion by enhancing neuronal activity in the dorsomedial striatum

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Abstract

The *Reln* gene encodes for the extracellular glycoprotein Reelin, which regulates several brain functions from development to adulthood, including neuronal migration, dendritic growth and branching and synapse formation and plasticity. Human studies have implicated Reelin signaling in several neurodevelopmental and psychiatric disorders. Mouse studies using the heterozygous Reeler (HR) mice have shown that reduced levels of *Reln* expression are associated with deficits in learning and memory and increased disinhibition. Although these traits are relevant to substance use disorders, the role of Reelin in cellular and behavioral responses to addictive drugs remains largely unknown. Here, we compared HR mice to wild-type (WT) littermate controls to investigate whether Reelin signaling contributes to the hyperlocomotor and rewarding effects of cocaine. After a single or repeated cocaine injections, HR mice showed enhanced cocaine-induced locomotor activity compared with WT controls. This effect persisted after withdrawal. In contrast, Reelin deficiency did not induce cocaine sensitization, and did not affect the rewarding effects of cocaine measured in the conditioned place preference assay. The elevated cocaine-induced hyperlocomotion in HR mice was associated with increased protein Fos expression in the dorsal medial striatum (DMS) compared with WT. Lastly, we performed an RNA fluorescent in situ hybridization experiment and found that *Reln* was highly co-expressed with the *Drd1* gene, which encodes for the dopamine receptor D1, in the DMS. These findings show that Reelin signaling contributes to the locomotor effects of cocaine and improve our understanding of the neurobiological mechanisms underlying the cellular and behavioral effects of cocaine.

KEYWORDS
cocaine, CPP, DMS, dopamine, locomotor activity, NAc shell, Reelin

1 | INTRODUCTION

Reelin is an extracellular glycoprotein expressed by Cajal Retzius cells during brain development and predominately by GABAergic interneurons in the postnatal brain.[1–3] The canonical Reelin signaling pathway includes the binding of Reelin to lipoprotein membrane receptors ApoER2 and VLDR, the phosphorylation of the intracellular adaptor protein Dab1 by two tyrosine kinases of the Src family, Src and Fyn, and the recruitment of signaling molecules of the Crk family.[4–7] During embryonic development, Reelin signaling is required for the proper migration of newborn neurons involved in the laminar formation of several brain structures, such as the cortex, hippocampus and cerebellum.[2,8] Mutant mice lacking *Reln* (Reeler mice) or other core components of the Reelin signaling show grossly inverted cortical layers due to defective neuronal positioning.[9] In humans, *Reln* gene mutations are associated with autosomal recessive lissencephaly with cerebellar hypoplasia, characterized by gross brain malformations similar to those observed in the Reeler mice.[10]

In the postnatal period, Reelin signaling is implicated in several brain functions linked to cognition, including dendritic spine formation, excitatory glutamatergic synaptic activity and activity-regulated transcription.[11–19] The heterozygous Reeler (HR) mice express reduced levels of Reelin and do not exhibit abnormal neuronal positioning during development.[20] In contrast, they show reduced dendritic spine density[21] and several behavioral abnormalities, including impaired learning and memory, decreased behavioral inhibition and increased impulsivity.[20,22–25] Therefore, HR mice have been proposed as a model for studying neurological disorders.[20] A link between Reelin signaling and psychiatric diseases is strongly supported by human genetic studies or postmortem brain studies showing that perturbation of Reelin signaling is associated with schizophrenia, bipolar disorder, autism, epilepsy and Alzheimer disease.[26–28]

Despite the evidence implicating Reelin signaling in several psychiatric disorders, including behavioral traits relevant to addiction, the influence of Reelin on the cellular and behavioral effects of addictive drugs remains largely unknown.

Here, we used the HR mice to assess the influence of reduced levels of Reelin on the hyperlocomotor and rewarding effects of cocaine, a psychostimulant that leads to robust cellular and behavioral changes in mice. We measured the effects of acute or repeated injections of cocaine on locomotor activity in HR and WT littermate controls. We used a conditioned place preference (CPP) assay to evaluate the effects of Reelin deficiency on the rewarding effects of cocaine. In addition to behavioral tests, we mapped the regional expression of the cocaine-induced Fos expression in the dorsomedial striatum (DMS) and the nucleus accumbens shell (NAc Shell) in HR and WT mice. Finally, we performed RNA fluorescent in situ hybridization (FISH) to examine *Reln* expression in specific cell types of the DMS.

2 | MATERIALS AND METHODS

2.1 | Mice

All experimental procedures were approved by the institutional animal care and use committee at the University of California, San Diego. Mice were housed (3–4 per cage) under a 12 h light/12 h dark cycle and provided with food and water ad libitum. HR mice were bred in house using the B6C3Fe a/a-Relnrl/J line (The Jackson Laboratory, #000235).[9] The strain has been backcrossed to C57B6/J for more than 10 generations and it is an isogenic line. Littermate controls were used for each experiment.

2.2 | Genotyping

Genotyping was carried out by PCR using genomic DNA extracted from tail clips. PCR was performed using the DreamTaq Hot Start Green PCR Master Mix (Thermo Scientific, Cat. no. K1082), and the following primers: a forward primer common to both alleles 5′TAATCTGTCCTCACTCTGCC 3′, a reverse WT-specific primer 5′ACAGTTGACATACCTTAATC3′ and a reverse mutant-specific primer 5′TGCATTAATGTGCAGTGTTG3′. The expected sizes for the amplicons are 280 bp for the WT and 280 bp + 380 bp for the HET.

2.3 | Drugs

Cocaine HCl (National Institute on Drug Abuse, Bethesda, MD, USA and Sigma Aldrich, St. Louis, MO) was dissolved in 0.9% saline (Hospira, Lake Forest, IL, USA) at various doses (5, 10, 20 and 40 mg/kg) and injected intraperitoneally (IP). Cocaine solutions were prepared fresh daily.

2.4 | Locomotor activity chambers

Locomotor activity was measured in polycarbonate cages (42 × 22 × 20 cm) placed into frames (25.5 × 47 cm) mounted with two levels of photocell beams at 2 and 7 cm above the bottom of the cage (San Diego Instruments, San Diego, CA). These two sets of beams allowed for recording both horizontal (locomotion) and vertical (rearing) behavior. A thin layer of bedding material was applied to the bottom of the cage. Locomotor activity recording was carried out during the dark cycle, between 1 and 4 h after the lights went off.

2.5 | Cocaine-induced locomotor activity in HR and WT mice

The mice (20 HR and 21 WT, half males and half females) were tested immediately following IP administration of 0.9% saline solution (0.01 ml/g body weight) or 10 mg/kg cocaine HCl with locomotor

activity recorded for 15 min (in 1 min bins). Different groups of mice received saline or cocaine.

2.6 | Dose–response of cocaine-induced locomotor activity in HR and WT mice

The mice (26 HR and 23 WT, half males and half females) received 0.0 (saline), 5, 10, 20 and 40 mg/kg of cocaine HCl in a counterbalanced order, one dose every 5 days, and were tested in the locomotor activity cages for 15 min. In this way, a within-subjects dose–response curve was generated for each group.

2.7 | Cocaine-induced locomotor sensitization in HR and WT mice

All mice (10 HR and 8 WT) were first tested immediately following IP administration of 0.9% saline solution (0.01 ml/g body weight) with locomotor activity recorded for 15 min (in 1 min bins) on two consecutive days (days 1 and 2) to acclimate them to the test. Mice then received 10 mg/kg cocaine on days 3, 5, 7, 9 and 11 and were tested in the locomotor activity cages for 15 min. Intermittent cocaine exposure was used to examine the progression of the behavioral sensitization.[29–31] Finally, on day 12, all mice received saline before an identical locomotor test.

One week later, all mice received 10 mg/kg of cocaine to examine sensitivity to the same dose used in the sensitization test.

2.8 | Cocaine CPP in HR and WT mice

Place conditioning involves pairing a distinct environmental context (i.e., floor type) with a motivationally significant event (i.e., cocaine injection).[32] Rectangular Plexiglas black matte boxes (length 42 cm, width 22 cm, height 30 cm) divided by central partitions into two chambers of equal size (22 × 22 × 30 cm) were used. Distinctive tactile stimuli were provided in the two compartments of the apparatus. One chamber had no additional flooring (i.e., was smooth), and the other had lightly textured milky-colored flooring. During preconditioning and testing sessions, an aperture (4 × 4 cm) in the central partition allowed the animals to enter both sides of the apparatus. All testing occurred during the dark cycle under red light and was analyzed from video files using Noldus Ethovision software.

A new cohort of 42 mice, not previously exposed to any cocaine treatment or behavioral testing, was used for this experiment, 20 HR and 22 WT, half males and half females.

The experiment consisted of three phases: preconditioning, conditioning and testing in the following sequence. Day 1: Preconditioning phase with access to both compartments, days 2–7: Conditioning phase—drug or saline administration followed by immediate confinement in one compartment of the place conditioning apparatus, and day 8: test with access to both compartments in the drug-free state. For the preconditioning phase, each animal was placed in one compartment of the apparatus and allowed to explore the entire apparatus for 30 min. The mice were put in the apparatus at the doorway, randomly facing one of the two chambers. The time spent in each of the two compartments was measured. Mice showing unbiased exploration of the times sides of the apparatus (between 45% and 55% time spent on each side) were randomly assigned a chamber in which to receive cocaine. Mice showing biased exploration of either side were given cocaine in the least preferred compartment. Bias was observed in half of the mice tested and this was spread equally across each genotype and therefore did not confound the results. On the following 6 days, 30 min conditioning sessions were given in which animals were injected i.p. with either saline or 10 mg/kg cocaine and immediately confined to one side of the apparatus (alternating these treatments and sides each day). In this way, each mouse experienced three pairings of cocaine with one of the apparatus sides. On the day after the final conditioning trial, each mouse was allowed to explore the entire apparatus in a nondrugged state for 30 min. The time spent in each of the two compartments of the CPP apparatus was recorded. The time spent in the cocaine side was compared with the preconditioning session to examine preference development.

2.9 | Immunohistochemistry

To examine Fos expression by immunohistochemistry (IHC), we used 11 WT (7 females and 4 males) and 10 HET (7 females and 3 males) littermates. Mice were injected with 10 mg/kg of cocaine or saline. One hour after injection, mice were anesthetized with CO_2 and fixed via transcardial perfusion with 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS). Brain tissues were processed at a cryostat, as previously described.[33] Thirty micrometers thick coronal slices were stained with a primary antibody recognizing Fos (1:1000, ABE457, EMD Millipore, RRID:AB_2631318) and a secondary antirabbit antibody (donkey antirabbit Alexa Fluor 488; 1:1000, #A21206, Thermo Fisher Scientific). For vehicle-treated samples, DAB (Vector Labs) staining was used for chromogenic detection. Images were acquired with a fluorescent microscope (BZX800, Keyence Corporation, Osaka, Japan). The DMS and the NAc shell were identified using a mouse brain atlas as a reference.[34] For each region, we stained 4–5 sections from Bregma 0.5 to 1.94 mm, which span both areas. The number of Fos + cells for each mouse was normalized to the size of the chosen area (mm^2) and presented as mean Fos + per mm^2 ± SEM. Counts from all images from each mouse were averaged so that each mouse was an *n* of 1. One outlier (WT-DMS) was removed from the analysis (identified after the Grubbs' test with alpha set at 0.05).

2.10 | RNA fluorescent in situ hybridization

To examine the expression of various transcripts in specific cell types of the DMS, we performed RNA FISH using the RNAscope Multiplex Fluorescent Reagent Kit v2 (ACD, #323100) and following the

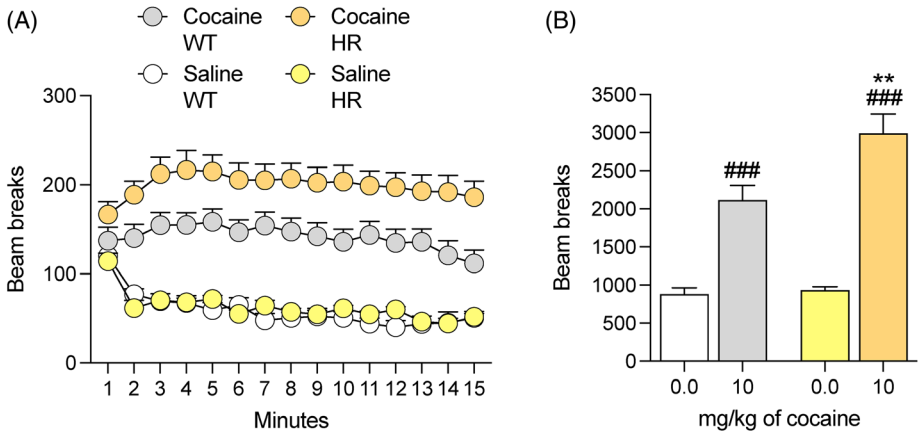

FIGURE 1 Locomotor activity in HR and WT mice after a single injection of cocaine. (A) Number of beam breaks per minute during the 15 minutes test. (B) Total number of beam breaks during the 15 minutes locomotor test. ** $p < 0.01$ versus WT; ### $p < 0.001$ versus dose 0.0 (saline)

instructions for the "fixed-frozen tissue sample" of the user manual (ACD, USM-323100). Thirty micrometers thick coronal slices were obtained from 5 WT mice, as described above. Tissue sections corresponding to DMS (Bregma 0.86 mm) from 5 WT mice (2–3 section per mouse) were hybridized with a mix of three probes; Reelin (ACD, # 405981) + Drd1 (ACD, 406491-C2) + Drd2 (ACD, 406501-C3). We used DAPI as a nuclear stain. To assess both tissue RNA integrity and assay procedure, a separate group of sections was incubated with negative probes (data not shown). Images were acquired with the Keyence fluorescent microscope. We used 13 sections across five mice to calculate the number of Reln/Drd1- or Reln/Drd2-positive cells. The number of nuclei was counted manually and normalized to the size of the area selected (mm^2). Counts from all images from each mouse were averaged so that each mouse was an *n* of 1.

2.11 | Statistical analysis

The data are expressed as mean ± SEM. For comparisons between only two groups, *p* values were calculated using unpaired *t*-tests as described in the Section 3. Comparisons across more than two groups were made using one-way analysis of variance (ANOVA), and two- or three-way ANOVA was used when there was more than one independent variable. ANOVA was followed by Bonferroni's multiple comparison test when appropriate. Differences were considered significant at $p \leq 0.05$. The standard error of the mean is indicated by error bars for each data group. All of these data were analyzed using Statistica 7 software.

3 | RESULTS

3.1 | HR mice exhibit enhanced hyperlocomotor effects of cocaine compared with WT

We injected HR and WT mice with 10 mg/kg of cocaine or saline and measured their locomotor activity for 15 min after injection. The three-way ANOVA with genotype (HR and WT) and dose (0.0 and 10 mg/kg) as between factors and the time as within factor indicated a significant genotype * dose interaction ($F_{1,4} = 49.15$; $p < 0.001$). We did not detect a genotype × dose × time interaction ($F_{14,555} = 0.16$; $p = 0.16$). This analysis indicates that the effect of cocaine on locomotion depends on genotype, and this effect was stronger in HR mice independently of the time points (Figure 1A). This result was corroborated by the analysis of the total locomotor activity during the 15 min test recording (Figure 1B). The two-way ANOVA of the mean locomotor activity with genotype (HR and WT) and dose (0.0 and 10 mg/kg) as between factors showed a significant genotype × dose interaction ($F_{1,37} = 4.09$; $p = 0.05$). Post-hoc comparisons with Bonferroni's test corrections showed that cocaine significantly increased the mean locomotor activity compared with saline in both genotype groups ($p < 0.001$ vs. saline). This effect was significantly higher in the HR mice compared with WT ($p < 0.01$, Figure 1B).

3.2 | Dose–response of cocaine-induced locomotor activation in HR and WT mice

We compared the locomotor activity of WT and HR mice after the injection of different doses of cocaine. The two-way ANOVA of the mean locomotor activity with genotype (HR and WT) and dose (0.0, 5, 10, 20 and 40 mg/kg) as between factors showed a significant effect of the genotype ($F_{1,46} = 9.82$; $p < 0.01$), of the dose ($F_{4,46} = 27.64$; $p < 0.0001$), but no genotype × dose interaction. The lack of genotype × dose interaction might reflect the slightly higher locomotor activity of the HR compared with WT in the basal condition in this specific experiment (Figure 2A). We then examined the influence of sex on the increased stimulatory effects of cocaine observed in HR mice. The three-way ANOVA with genotype (HR and WT), cocaine dose and sex as factors showed a significant effect of sex ($F_{1,220} = 13.39$; $p < 0.001$), dose ($F_{4,220} = 22.45$; $p < 0.0001$) and genotype ($F_{1,220} = 18.14$; $p < 0.0001$), demonstrating that female mice are overall more active than males. However, the statistical analysis did not show any sex × dose ($F_{4,220} = 22.45$; $p = 0.92$), sex × genotype ($F_{1,220} = 2.827$; $p = 0.09$) or sex × genotype × dose

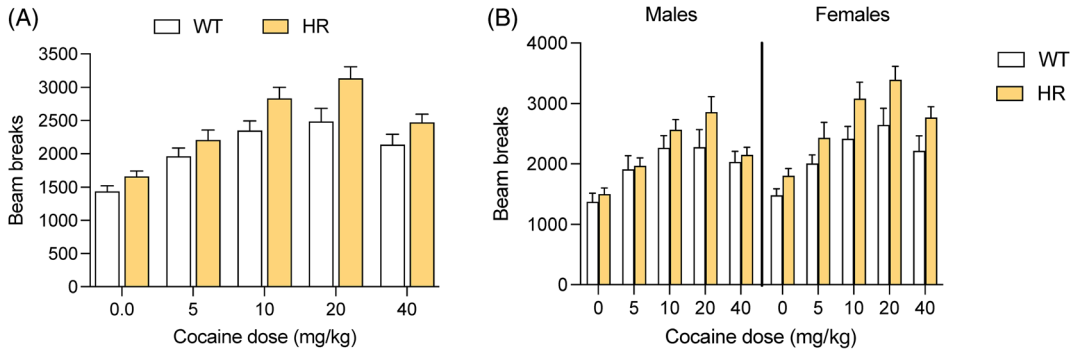

FIGURE 2 Dose–response for the effects of a single cocaine injection on locomotor activity in HR and WT mice. (A) Dose–response for the total population. (B) Dose–response in male and female mice

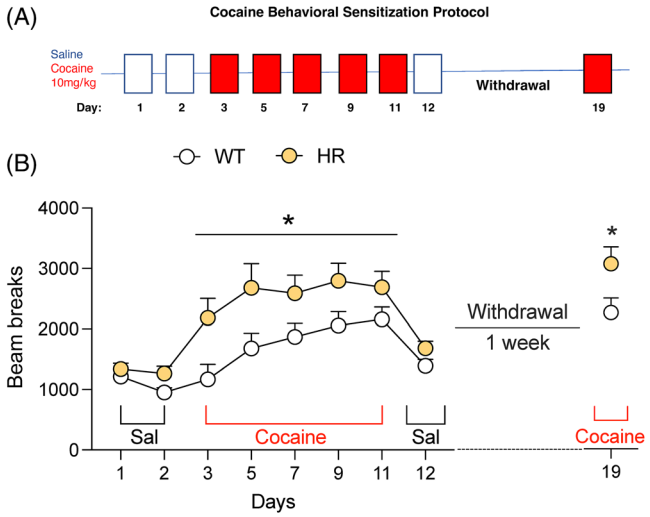

FIGURE 3 Reelin downregulation increases cocaine sensitization in HR mice. (A) Schematic timeline of the experiment. (B) Development of locomotor sensitization in HR and WT mice. * $p < 0.05$ versus WT

interactions ($F_{4,220} = 0.084$; $p = 0.98$), demonstrating that sex does not influence the response to cocaine in either genotype (Figure 2B).

3.3 | HR mice show increased cocaine-induced hyperlocomotion after repeated cocaine injections compared with WT

We explored the development of behavioral sensitization in WT and HR mice following repeated exposures to 10 mg/kg of cocaine (Figure 3A). No significant differences in locomotion were observed between the HR and WT mice in the first 2 days while receiving saline injections (Figure 3B, $p = 0.99$ for day 1 and $p = 0.34$ for day 2). This observation indicates that Reelin deficiency does not impact novelty-induced hyperlocomotion or habituation to the novel environment. Over the course of the experiment, HR mice injected with cocaine showed a dramatic increase in locomotor activity compared with WT (Figure 3B). The two-way ANOVA of the cocaine injection "day" (day 3–6 and 19) showed a significant effect of the genotype ($F_{1,16} = 4.72$; $p < 0.05$) and a significant effect of day ($F_{4,16} = 10.14$; $p < 0.001$), but not of the genotype × day interaction ($F_{4,64} = 0.37$; $p = 0.82$). The lack of interaction indicates that the genotype differences in the locomotor effects induced by repeated cocaine injections are independent of the day of injection. There were no detectable differences in locomotion on day 12 when the animals were re-exposed to saline ($p = 0.63$).

3.4 | Reelin deficiency does not influence the rewarding effects of cocaine

To investigate the impact of Reelin deficiency on the rewarding effects of cocaine, we compared WT and HR mice in the CPP assay to measure the preference of mice for a context associated with the cocaine reward. Out of the 42 animals used in this experiment, 12 WT (half males and half females) and 11 HR (5 males and 6 females) mice showed a biased preference for one of the two compartments. Data from the preference for these animals are shown in Figure 4A. The two-way ANOVA showed no effect of the genotype ($F_{1,10} = 0.018$; $p = 0.89$), sex ($F_{1,9} = 1.152$; $p = 0.31$) or the genotype × sex interaction ($F_{1,9} = 1.669$; $p = 0.22$) in the bias for the preferred side in the baseline test, demonstrating that there were no genotype or sex effects in meeting the bias criteria.

The preference is expressed as the time spent in the compartment associated with cocaine. The two-way ANOVA with genotype as between factor and preference (BSL vs. Test) as within factor showed a significant effect of preference ($F_{1,40} = 129.2$; $p < 0.001$, Figure 4B), but not detectable genotype effect, demonstrating that cocaine induced a robust place preference in both HR and WT mice.

When sex was added as a factor in the analysis, the three-way ANOVA showed a main effect of the preference ($F_{1,38} = 150.1$; $p < 0.0001$, Figure 4C) without detectable sex effects, demonstrating that cocaine induced a robust place preference in both HR and WT mice, independently of the sex.

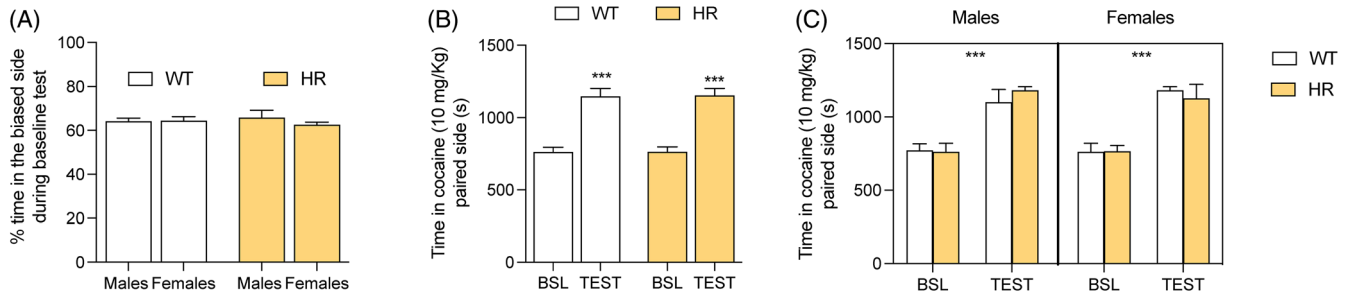

FIGURE 4 Reelin downregulation does not affect the rewarding properties of cocaine in a place conditioning paradigm. (A) Percentage of time spent in the preferred side during pretest in animals showing preference bias. (B) Time spent in the cocaine-paired side in HR and WT mice. (C) Place conditioning results in male and female mice. ** $p < 0.01$ versus baseline (BSL)

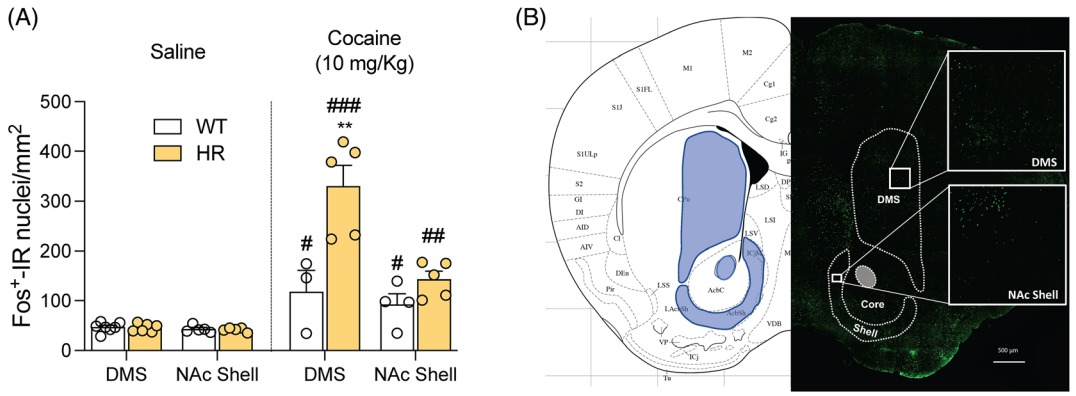

FIGURE 5 HR mice showed higher levels of Fos + cells in the DMS compared with WT after a single injection of cocaine. (A) Fos + cells in the DMS and the NAc shell. (B) Representative images of the immunostaining. ** $p < 0.01$ versus WT; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ versus Saline

3.5 | Cocaine-induced hyperlocomotion in HR mice is associated with increased *Fos* expression in the dorsal medial striatum

To gain deeper insights into the neuronal ensembles underlying the increased locomotor activity enhanced by cocaine in HR mice, we mapped the regional expression of Fos protein by IHC in DMS and NAc shell, two regions implicated in the acute behavioral responses to cocaine.

The tree-way ANOVA with genotype, treatment and brain region as factors showed a significant genotype × treatment × brain region interaction ($F_{1,32} = 22.59$; $p < 0.01$, Figure 5). The post-hoc comparisons with the Bonferroni's correction showed that there were no basal differences in Fos expression between HR and WT mice. A single injection of cocaine increased Fos expression in the DMS and the NAc Shell in HR and WT mice compared with the vehicle. However, HR mice showed increased levels of Fos-positive cells in the DMS, but not in the NAc shell, compared with WT mice (adj $p < 0.01$, Figure 5).

3.6 | Reelin is highly coexpressed with Drd1 in the dorsal medial striatum

Acute responses to cocaine lead to increased dopamine levels in the ventral tegmental area and are mediated by neuronal populations expressing the dopamine receptors, Drd1 and Drd2, which are highly expressed in the medium spiny neurons (MSNs) of DMS. We analyzed *Reln* expression in the Drd1 and Drd2 subpopulations of the DMS by RNA FISH. While similar expression of *Drd1* and *Drd2* transcripts was detected in the DMS (Figure 6A), we found increased co-expression of *Reln* transcript in Drd1+ compared with Drd2+ neurons (63% of the Drd1+ cells and 48% of the Drd2+ cells co-expressed *Reln*), as showed by the *t*-test ($t_8 = 3.848$; $p < 0.01$, Figure 6B).

4 | DISCUSSION

Our results showed that reduced levels of Reelin influence the locomotor effects of cocaine, but not the cocaine rewarding effects. In our experiments, HR mice showed similar baseline locomotor activity compared with WT, indicating that Reelin deficiency does not influence novelty-induced hyperlocomotion or habituation to the novel environment (Figures 1 and 3). However, when injected with a single dose of cocaine, HR mice showed increased cocaine-induced hyperlocomotion compared with WT. This effect was independent of the cocaine dose, as showed by the dose–response curve for cocaine-induced locomotor activation shown in Figure 2. A possible explanation for these differences in cocaine sensitivity might be related to differences in cocaine pharmacokinetics between HR and WT mice.

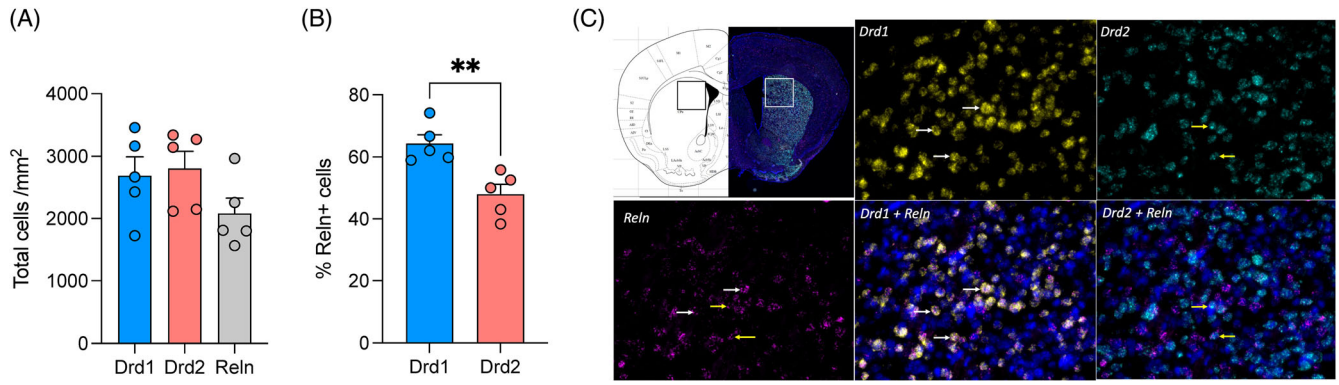

FIGURE 6 Reelin expression in Drd1 and Drd2 DMS neurons. (A) Total number of Drd1+, Drd2+ and Reln+ cells in the DMS. (B) Percent of colocalization Drd1+/Reln+ and Drd2+/Reln+ in the DMS. * $p < 0.05$ and *** $p < 0.01$ versus Drd2. (C) Representative images for the RNA fluorescent in situ hybridization (FISH)

Future studies aimed at determining blood levels after a single injection of cocaine in HR and WT mice help explain the differences in cocaine sensitivity and cocaine-induced hyperlocomotion. A limitation of the dose–response experiment is related to the within-subject design, which does not account for the possible sensitizing effects of repeated cocaine administration. However, this limitation is balanced by the observation that HR mice showed increased locomotor response to cocaine in the experiments described in Figures 1 and 3. Previous literature showed sex differences in basal[35,36] and cocaine-induced locomotor activity in rodents, with females showing increased activity.[36,37] Our data confirmed these results with female mice showing an overall increased locomotor activity at basal conditions and in response to cocaine. However, sex did not play a role in the genotype differences. The effects on locomotion were confirmed in the cocaine sensitization model. While we cannot conclude that sensitization is enhanced, HR mice showed higher locomotion after repeated cocaine injections compared with their WT littermates (Figure 3). This increased response to the locomotor effects of cocaine was also observed after a week of withdrawal (Figure 3).

These data align with a previous report showing an inhibitory effect of Reelin overexpression on cocaine-induced hyperlocomotion and behavioral sensitization,[38] confirming a possible role of Reelin signaling in the modulation of the psychostimulant effects of cocaine. However, these results are in contrast with a recent report that showed no differences in methamphetamine-induced increased locomotion between HR and WT mice.[39] This might be related to the different pharmacological properties of methamphetamine compared with cocaine (prolonged stimulant effect[40]). Thus, this discrepancy may be explained by the different experimental conditions (drugs and doses), and further studies will be needed to clarify these contrasting findings.

Importantly, we report that the differences observed in cocaine-induced hyperlocomotion and cocaine sensitization did not correlate with differences in the rewarding effects of cocaine. HR and WT rats developed a similar preference for the cocaine-paired side when subjected to a cocaine place conditioning paradigm. Without a saline control group in the CPP experiment, we cannot exclude that changes in cocaine preference could be non-specific for one or both genotypes. This limitation is reduced because our results are in line with previous data on Reelin overexpressing mice showing no differences in cocaine self-administration rates[38] compared with WT mice, further demonstrating that manipulation of Reelin does not affect cocaine reward.

Considerable evidence suggests that in humans there are sex differences in the pharmacological effects of cocaine and the prevalence of cocaine use disorders. Research in humans and rodents suggests that women may be more vulnerable to the reinforcing (rewarding) effects of stimulants, with females acquiring cocaine abuse faster and at higher levels than males.[41–44]

Notably, the HR mice exhibit sex differences in neurotransmitter signaling and receptor function.[45,46] Similarly, HR mice showed sex differences in behavioral disinhibition and stress reactivity following adolescent exposure to THC.[22] However, our study did not find specific sex-biased responses to cocaine in HR mice, demonstrating that sex does not play a role in the effects of enhanced locomotor activation after cocaine injection in HR mice.

The HR mice showed an increased number of Fos+ cells in the DMS after a single cocaine injection compared with WT mice (Figure 5). Previous work has shown that cocaine increased Fos expression in the DMS compared with saline, but not in the dorsolateral and ventral striatum.[47] The increase of Fos-positive cells is probably a result of the cocaine-induced increase of dopamine levels in the striatum,[48] which is hypothesized to increase the activity of MSNs through the activation of dopamine 1 (D1R) and D2 receptors.[49,50] In particular, MSNs expressing D1R appear responsible for the psychomotor effects of cocaine because mice lacking D1R fail to show the motor-stimulating effects of cocaine.[51] We found increased expression of Reelin in the DRD1 cells compared with the DRD2 cells in the DMS (Figure 6). We speculate that Reelin acts as a modulator of DR1 receptor function, which could explain the increased locomotor and DMS neural activations observed after acute injection of cocaine in mice with Reelin deficiency. This hypothesis is reinforced by previous

findings demonstrating that both D1 and D2 classes of dopamine receptors are reduced in the striatum of Reeler mice.[52] Future studies investigating the effects of the manipulation of Reelin expression, specifically in DRD1 cells of the DMS, will be necessary to test this hypothesis.

The rewarding effects of cocaine are driven by its ability to increase dopamine levels in the nucleus accumbens.[53,54] This is reinforced by the fact that blockade of dopamine transmission reduces the rewarding effects of psychostimulants.[55] In particular, all drugs of abuse are thought to activate the shell subregion of the NAc.[56,57] In line with the behavioral data that showed no differences in the rewarding effects of cocaine, we did not find any differences in Fos activation in the nucleus accumbens shell of HR and WT mice (Figure 5) after acute cocaine treatment.

5 | CONCLUSIONS

In conclusion, our study indicates that Reelin might have a role in modulating the hyperlocomotor effects of cocaine, but not cocaine reward. Future studies are needed to elucidate the neurobiological mechanisms underlying these findings, such as the relationship between Reelin and the dopaminergic system in the DMS.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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