Reln haploinsufficiency alters fentanyl-induced striatal activity and adaptive responding without affecting opioid reinforcement

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Abstract

Reln gene encodes the extracellular glycoprotein Reelin that regulates synaptic plasticity and activity-dependent gene expression with implication in several neuropsychiatric disorders, including substance use disorder. Although reduction of *Reln* expression has been associated with altered responses to psychostimulants and cannabinoids, its role in opioid-evoked behaviors has not been investigated. Here, we tested whether *Reln* haploinsufficiency alters behavioral and molecular responses to the synthetic opioid fentanyl. Heterozygous Reeler (Reln+/) mice and wild-type littermates were assessed using multi-phase fentanyl intravenous self-administration, conditioned place preference, locomotor assays, and dorsal striatal immediate early gene expression. *Reln* haploinsufficiency did not alter acquisition, progressive ratio breakpoint, extinction, or cue-induced reinstatement during self-administration, indicating preservation of baseline fentanyl reinforcement and relapse-like responding. However, *Reln*+/− mice exhibited a genotype-by-dose interaction during dose-response testing, suggesting altered flexibility under changing reinforcement conditions. During cue-induced reinstatement, the expression of immediate early genes in the dorsal striatum did not show a difference between genotypes. Following acute, passive exposure to fentanyl, *Reln*+/− mice displayed increased fentanyl-induced locomotion and elevated Fos immunoreactivity in the dorsal striatum, while fentanyl conditioned place preference was not altered. Together, these findings indicate that *Reln* haploinsufficiency does not modify baseline opioid reinforcement or relapse-like behavior but is associated with altered opioid-evoked neuronal activation and behavioral adaptation to changing reinforcement conditions. These results position *Reln* as a modulatory factor in opioid-responsive neural circuits rather than a primary regulator of opioid reinforcement.

Introduction

Opioid use disorder (OUD) affects millions globally (Hser *et al.* 2001; Degenhardt *et al.* 2019) and is characterized by chronic drug intake, relapse vulnerability, and maladaptive learning processes involving the encoding of drug-associated cues and contexts (Everitt & Robbins 2005). These behaviors reflect molecular and cellular perturbations within corticostriatal circuits, particularly the dorsal striatum, which supports action-selection, habit-formation, and cost-benefit valuation of reward-seeking behaviors (Cucinello-Ragland *et al.* 2026). Opioids, including the highly potent synthetic agonist fentanyl, robustly engage striatal signaling pathways that regulate synaptic plasticity, neuronal excitability, and immediate early gene (IEG) expression (Bisagno & Cadet 2019). Although substantial progress has been made in defining opioid-induced behavioral adaptations, the molecular regulators that drive these plasticity mechanisms are not clearly understood.

Reelin (*Reln*) is a large extracellular glycoprotein (D'Arcangelo *et al.* 1997) expressed mainly in the striatum, cortex, and cerebellum (Ikeda & Terashima 1997). While best known for its developmental role in neuronal migration, Reelin is increasingly recognized for its functions in adult neuronal plasticity through modulation of IEG expression (Lee *et al.* 2014; Telese *et al.* 2015), dendritic spines regulation (Niu *et al.* 2008; Rogers *et al.* 2011; Bosch *et al.* 2016; Lee *et al.* 2023), and long-term potentiation (Beffert *et al.* 2005; Qiu *et al.* 2006) in the adult brain.

Altered RELN expression has been implicated in several neuropsychiatric conditions characterized by dysregulated plasticity (Fatemi *et al.* 2000), including autism (Zhang *et al.* 2002; Wang *et al.* 2014), schizophrenia (Costa *et al.* 2001; Grayson *et al.* 2005), Alzheimer's disease (Seripa *et al.* 2008; Cuchillo-Ibañez *et al.* 2016), and attention-deficit/hyperactivity disorder (Chen *et al.* 2017). Emerging evidence also links Reelin signaling to phenotypes associated with substances of abuse. Experimental reduction of *Reln* expression in mice modifies behavioral and cellular responses to cannabinoids (Iemolo *et al.* 2021; Silva-Hurtado *et al.* 2024; Zuo *et al.* 2025) and psychostimulants (Matsuzaki *et al.* 2007; de Guglielmo *et al.* 2022; Brida *et al.* 2025), including drug-induced locomotion, reward-related learning, and striatal activation patterns. In parallel, clinical studies report associations between altered RELN expression and phenotypes associated with alcohol use disorder (Escudero *et al.* 2023; Legaki *et al.* 2024). Together, these findings suggest that Reelin may influence how drug experiences are encoded within reward-related circuits.

Despite these advances, the role of Reelin in opioid-related behaviors has not been examined. Opioids differ from psychostimulants and cannabinoids in both receptor mechanisms and circuit recruitment, and they strongly recruit striatal neuronal pathways involved in reinforcement learning and adaptive responding to changing reward contingencies (Le Merrer *et al.* 2009). Given the established role of Reelin in regulating synaptic plasticity and activity-dependent transcription, alterations in Reelin signaling are well positioned to modulate opioid-evoked neuronal activation and behavioral adaptation. However, it remains unknown whether reduced *Reln* expression alters baseline opioid reinforcement, relapse-like behavior, or the neural activation associated with these processes.

To address this gap, we evaluated heterozygous Reeler (*Reln*+/−) mice and wild-type (WT) littermates (D'Arcangelo *et al.* 1995) in complementary contingent and non-contingent models of fentanyl exposure. Using a multi-phase intravenous self-administration (IVSA) paradigm, we assessed acquisition, dose-response adaptation, progressive ratio performance, extinction, and cue-induced reinstatement to determine whether *Reln* haploinsufficiency alters opioid reinforcement or relapse-like responding. In parallel, we examined conditioned place preference (CPP), fentanyl-induced locomotion, and Fos immunoreactivity in the dorsal striatum to assess opioid-evoked neuronal activation under passive exposure to fentanyl. Altogether, this study dissociates the role of Reelin in reinforcement processes from adaptive responding associated with fentanyl exposure.

Methods

Mice

All animal care and experimental procedures were approved by the UCSD Institutional Animal Care and Use Committee. Male and female *Reln*+/- and WT littermate controls were maintained on a C57BL/6J background and genotypes were determined by PCR-based analysis of tail biopsies (de Guglielmo *et al.* 2022). Mice were group-housed (2-4 per cage) under a 12-h light/dark cycle with ad libitum food and water except during behavioral testing where mice were maintained on a reverse-like cycle. Mice were handled for a minimum of three days before the start of each experiment. For multi-phase IVSA, 8 = WT and n = 5 Reln+/− male mice, and n= 5 WT and n = 8 Reln+/- female mice were used across two cohorts. For CPP and locomotion, n = 4 WT and n = 4 Reln+/− male, n = 4 WT and n = 4 Reln+/− female mice were used. For Fos immunohistochemistry n = 4 WT and n = 4 Reln+/− male mice were used. Across all experiments, mice ranged from 12 to 16 weeks of age at the beginning of testing.

Drug Preparation

Powdered fentanyl citrate (Cayman Chemical Company) was dissolved in 0.9% saline to a stock concentration of 5 mg/ml. For IVSA, 5 mg/ml stock of fentanyl citrate and saline was further diluted to a working solution with saline for a final concentration of 0.025 mg/ml to allow a dynamic range of 15-1.5 ug/kg/infusion dependent on the duration of infusion. For acute exposure and CPP, 5 mg/ml stock of fentanyl citrate and saline was further diluted to a working solution with saline for a final concentration of 0.003 or 0.012 mg/ml to allow for 20 ug/kg and 80 ug/kg IP injections, respectively.

Fentanyl IVSA

For IVSA procedures, mice were individually housed and were food-restricted to 80% of their normal intake to increase motivation for operant conditioning.

Surgical procedures. Mice underwent intravenous jugular catheterization using standard procedure (Margiani *et al.* 2022; Valles *et al.* 2022). Following surgery, mice were given daily post-operative care with intravenous flushes of heparinized saline containing cefazolin antibiotic to prevent clogging and infection. Once mice recovered to pre-surgical body weight and had a dry wound area (3-5 days), the mice underwent fentanyl IVSA.

Acquisition (FR1, 15 µg/kg/infusion). Self-administration was conducted in standard operant conditioning chambers (Med Associates) equipped with two retractable levers positioned on one wall. an active lever that was reinforced with intravenous delivery of fentanyl as opposed to an inactive lever that featured no consequences. At the start of each IVSA session, the white house lights were illuminated, mice were given a passive infusion of fentanyl, and a yellow availability light cue above the active lever was illuminated. Upon interaction with the active lever, the yellow availability light cue extinguished illumination, fentanyl was delivered, and a pure tone was played for 10 seconds. Active lever presses within this 10 second tone did not allow another infusion delivery. Following the 10 second tone, the availability light was illuminated again to signal another fentanyl infusion is available. All active or inactive lever presses and the infusions were recorded. The house lights were illuminated with both active and inactive levers extended for the entire session. Each session was 2 hours in duration and conducted daily.

De-escalating Dose-Response Testing. Following acquisition, mice underwent dose-response testing in which fentanyl unit dose was sequentially reduced every three days (15, 10, 5, 1.5 µg/kg/infusion), with two consecutive 2-hour sessions at each dose under an FR1 schedule. Dose order was identical for all animals. Different IVSA doses were implemented by adjusting the duration of infusions (0.1

mg/sec) in the MedPC software while using the same concentration of fentanyl (0.25 mg/ml; Supplemental Table 1). Infusion number, active and inactive lever presses, and total intake were recorded.

Progressive Ratio Testing. Following dose–response testing and two dose-reset sessions at 15 µg/kg/infusion to re-stabilize responding, motivation was assessed under a linear PR schedule conducted at the lowest unit dose (1.5 µg/kg/infusion). Under the PR schedule, the response requirement increased incrementally for each subsequent infusion (1, 2, 3, 4, …). The breakpoint was defined as the highest completed ratio resulting in infusion delivery. Active and inactive lever presses, infusions, and breakpoints were recorded.

Extinction and Cue-Induced Reinstatement. Following PR testing, mice underwent extinction training in which lever presses had no programmed consequences (no fentanyl delivery, no cues). Extinction sessions were conducted daily for 10 sessions. Cue-induced reinstatement was conducted by reintroducing the previously fentanyl-paired cues without drug delivery. Lever presses during reinstatement were compared to the final extinction session.

RT-qPCR

Following cue-induced reinstatement, mice were euthanized 90 minutes after session termination. Dorsal striatal tissue punches were collected and sectioned using a vibratome (Leica), flash frozen and stored at -80C. Total RNA was extracted from frozen tissues using Direct-Zol RNA Miniprep (Zymo Research, #R2050). cDNA was synthesized from 100 ng RNA using the Maxima H Minus First Strand cDNA Synthesis Kit with dsDNase (Thermo Fisher Scientific, #K1682). PCR was performed using the following primer sequences (Supplemental Table 2). RT-PCR was performed using SsoAdvanced Universal SYBR Green Supermix (Bio-Rad, #1725270) Gene expression was normalized to the housekeeping gene using the 2^-ΔCt method (Livak & Schmittgen 2001).

Fentanyl CPP

CPP was conducted in a two-compartment apparatus with distinct visual and tactile cues. On the pre-test day, mice were allowed free access to both chambers for 30 minutes to assess baseline contextual preference. A biased design was used. If a mouse displayed over 55% preference for one chamber during the pre-test session, the least preferred chamber was assigned as the fentanyl paired context. If no strong baseline preference was observed (≤55%), pairing assignment was counterbalanced across genotypes. Fentanyl conditioning consisted of eight total treatment days where treatments alternated daily (four fentanyl, four saline in opposite chambers). Furthermore mice were treated with fentanyl in two separate cohorts, a high dose (80µg/kg) and a low dose (20µg/kg). On saline-pairing days, mice received an equivalent volume of saline and were confined to the alternate chamber. On the test day, mice were allowed free access to both compartments for 30 minutes in a drug-free state.

All sessions were 30 minutes in length and recorded using red lighting and a camera positioned above the apparatus. CPP videos were analyzed using DeepLabCut v3.0 to track the mouse midpoint across frames. The mouse was scored as occupying a chamber when its centroid fell within that chamber's boundary. Time spent in each chamber was calculated as the number of frames assigned to each chamber divided by the video frame rate (30 frames per second). Videos were visually inspected to confirm accurate chamber assignments. Preference score was calculated as the change in percentage time spent in the fentanyl-paired chamber between pre-test and the test sessions.

Locomotion Analysis

Locomotor activity during fentanyl exposure was quantified using DeepLabCut v3 to track the mouse location across frames. The total distance traveled was calculated as the total sum of the difference

between the animal's centroid position in consecutive frames. All analyses were performed using custom Python and Matlab (Mathworks) scripts and were conducted blind to genotype.

Acute Fentanyl Exposure and Fos Immunostaining

In a separate cohort, mice received an acute fentanyl injection (20 µg/kg, i.p.) or saline, and were perfused in 1x PBS followed by 4% PFA 90 minutes later. Brains were post-fixed (overnight in 4 °C, 4% PFA), cryoprotected (30% sucrose solution, until sinking), and sectioned coronally using a microtome (Microm HM440E). Three mounted sections (20um) comprising the dorsal striatum (bregma 0.74 mm) per each genotype were used. Immunohistochemistry (IHC) for Fos protein was performed as follows: 1-hour incubation in blocking buffer [1x PBS (Millipore Sigma; # P4244-100ML), 10% normal goat serum (Millipore Sigma; # S26-M), 0.3% TritonX-100 (Millipore Sigma; #X100-5ML)] at room temperature followed by overnight incubation in Fos primary antibody dilution solution [1x PBS, 5% normal goat serum, 0.1% TritonX-100, and 1:2500 anti-Fos rabbit (Synaptic Systems; # 226 008)] at 4C, three 15-minute washes in washing buffer (1x PBS, 0.3% TritonX-100), 2-hour incubation in secondary antibody solution [1x PBS, 5% normal goat serum, 0.1% TritonX-100, and 1:500 Donkey anti-Rabbit (ThermoFisher; # A10043)] at room temperature, three 15-minute washes in washing buffer at room temperature, 30-minute incubation in 1x PBS with 600 nM DAPI at room temperature, two 15-minute washes in washing buffer at room temperature. Sections were coverslipped with mounting medium (Millipore Sigma, # 10981). Images of the dorsal striatum were acquired using a fluorescence microscope (Keyence; BZ-X810) under identical exposure settings across animals. Cropped region-of-interest (ROI) images corresponding to the dorsal striatum were generated in ImageJ (Schneider *et al.* 2012) using standardized anatomical boundaries. Automated cell quantification was performed using CellProfiler (Carpenter *et al.* 2006) to identify DAPI-positive nuclei and Fos-positive nuclei. For each mouse, three coronal slices (six total sections) were analyzed. The average number of Fos+/DAPI+ cells per section was calculated and used as the technical replicate for statistical analysis.

Statistical Analysis

All statistical analyses and data visualization were conducted in R (version 4.5.0). For IVSA, behavioral count data were analyzed using Poisson-distributed generalized linear mixed-effects models (GLMMs) after failing to meet Gaussian assumptions and in the absence of overdispersion. Separate models were fitted for each experimental phase (acquisition, dose-response, dose reset, progressive ratio, extinction, and cue-induced reinstatement). Fixed effects included genotype, lever, day, session condition, with mouse ID included as a random intercept. Fixed effects and interactions were evaluated using likelihood ratio tests (LRTs). Post-hoc pairwise comparisons were performed using estimated means with false discovery rate (FDR) correction. Sex was not included as a factor due to insufficient power to detect sex differences. PR breakpoint, which consisted of a single count measure per subject, was compared between genotypes using Welch's t-test. RT-qPCR relative expression values were also analyzed using Welch's t-test for each gene of interest. CPP data, which met Gaussian assumptions, were analyzed using a linear mixed model (LMM) to assess fixed effects of genotype, dose, and change in preference for the fentanyl-paired chamber. Similarly, fentanyl-induced locomotion (average speed) was analyzed using an LMM including genotype and dose as fixed effects. The percentage of Fos-positive nuclei relative to total DAPI-positive nuclei was modeled with a GLMM to compare the fixed effect of genotype, where mice and nested slices were considered random effects. Statistical significance was defined as $p < 0.05$. Exact test statistics and degrees of freedom are reported in Supplemental Table 2-6.

Results

Reln haploinsufficiency does not alter the acquisition of fentanyl

To determine whether reduced *Reln* expression alters opioid reinforcement or relapse-like responding, *Reln*+/− and wild-type (WT) littermates were assessed in a multi-phase fentanyl IVSA paradigm, including acquisition, dose-response, dose reset, progressive ratio (PR), extinction, and cue-induced reinstatement (Figure 1A).

During a four-day acquisition phase (15 ug/kg/infusion), both genotypes acquired fentanyl self-administration and displayed a clear preference for the active over the inactive lever. Although a significant lever × genotype × day interaction was detected ($\chi^2(3) = 28.259$, $p < 0.0001$, Table 2.01), post hoc comparisons revealed that this effect was driven by transient genotype differences in inactive lever responding on day 2 ($z = 2.17$, adjP = 0.030) and day 4 ($z = -2.40$, adjP = 0.016) without differences detected on the active lever (Figure 1B, Table 2.02). Importantly, there was no main effect of genotype on infusions during acquisition (Figure 1C, Table 2.03), indicating a preservation of the initial fentanyl reinforcement learning in *Reln*+/− mice. Together, these data indicate that *Reln* haploinsufficiency does not modify baseline opioid reinforcement.

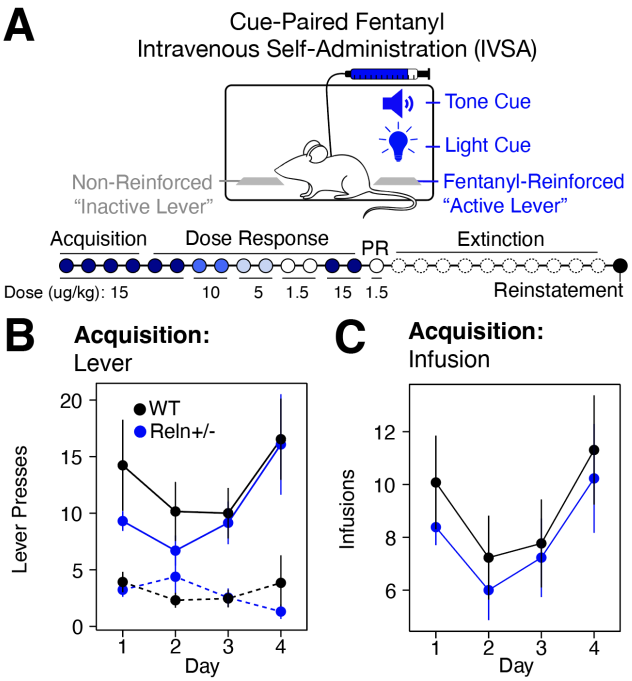

Figure 1 | Assessing Reln haplosufficiency within a fentanyl IVSA paradigm. A Schematic of fentanyl IVSA comparing Reln+/- (n=13) and WT (n=13) mice. **B-C** Active (solid line) and inactive lever presses (dashed line; **B**) or infusions (**C**) for acquisition sessions Day 1-4.

Genotype-dependent differences emerge during fentanyl dose manipulation

To further determine whether *Reln* haploinsufficiency alters sensitivity to fentanyl reinforcement under changing reinforcement contingencies, we implemented a de-escalating dose response. During the dose-response phase, both genotypes exhibited the expected inverse relationship between unit dose and active lever presses (Figure 2A). Active lever presses exhibited a significant genotype × dose interaction ($\chi^2(3) = 7.962$, $p < 0.0001$, Table 2.04), and this effect was also present when analyzed by individual test day ($\chi^2(7) = 29.984$, $p < 0.0001$, Table 2.06). Fentanyl infusions followed a similar pattern, increasing as unit dose decreased (Figure 2B). The infusion numbers also showed a significant genotype × dose interaction (Figure 2C, $\chi^2(3) = 36.164$, $p < 0.0001$, Supplemental Table 2.08), indicating that *Reln*+/− mice adjusted their fentanyl intake differently across changing dose conditions. This interaction remained significant when modeled by individual test day (genotype × day: $\chi^2(7) = 62.957$, $p < 0.0001$; Supplemental Table 2.10). Although a downward shift in response was observed in the *Reln*+/- mice during the de-escalating dose-response phase, post hoc pairwise comparisons did not reveal significant genotype differences at individual doses (Supplemental Tables 2.05, 2.07, 2.09, 2.11). Total fentanyl intake varied significantly as a function of dose (dose: $\chi^2(3) = 295.585$, $p < 0.0001$, Supplemental Table 2.12), creating an inverted U-shaped function when collapsed across doses (Supplemental Figure 1). Intake also differed significantly across individual test days (day: $\chi^2(7) = 304.671$, $p < 0.0001$, Supplemental Table 2.13). No genotype effects were detected for intake (Supplemental Tables 2.12-2.13). When the dose was reset to the acquisition dose (15 µg/kg/infusion), following dose-response, mice adapted with reduced responses (Figure 2A,B). No genotype differences were observed for presses (Supplemental Table 2.14) or infusions (Supplemental Table

2.16), indicating that performance at the acquisition dose was maintained despite changes during the dose-response (Figure 2A,B).

Together, these findings indicate genotype-dependent differences in dose-dependent responding, despite comparable overall fentanyl intake.

Reln haploinsufficiency does not alter motivation for fentanyl intake

Following the two dose-reset sessions at the acquisition dose (15 ug/kg/infusion), motivation for fentanyl was assessed under a linear PR schedule conducted at the lowest dose in the dose-response (1.5 ug/kg/infusion). Mice showed strong lever bias overall (lever: $\chi^2(1) = 154.04$, $p < 0.0001$; Figure 2C; Supplemental Table 2.17), and there was a significant lever × genotype interaction ($\chi^2(1) = 19.625$, $p < 0.0001$; Figure 2C; Supplemental Table 2.17), indicating that the degree of lever discrimination differed by genotype. However, post hoc comparisons did not detect genotype differences on lever presses (Supplemental Table 2.18). Consistently, the breakpoint did not differ between genotypes (Figure 2D, Table 2.19). Overall, these results suggest that *Reln* haploinsufficiency does not alter reduce motivation for fentanyl intake.

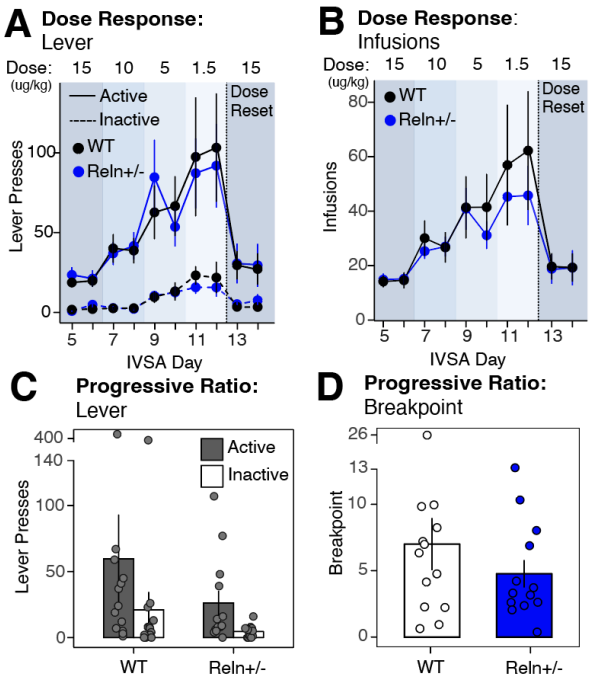

Figure 2 | Measuring genotypic effect during fentanyl IVSA dose response and progressive ratio. A-B Dose response and reset measurements of lever (**A**) or infusions (**B**) per genotype. **C-D** Lever presses (**C**) or breakpoint (**D**) during a linearized progressive ratio session.

Reln haploinsufficiency does not alter extinction or reinstatement of fentanyl self-administration

During extinction (Figure 3A), lever discrimination decreased across sessions with a detectable, significant interaction between genotype × lever × day ($\chi^2(9) = 92.201$, $p < 0.0001$; Supplemental Table 2.20). Post hoc comparisons did not detect significant changes within specific days (Supplemental Table 2.21).

During cue-induced reinstatement, in the presence of fentanyl-associated cues without fentanyl delivery, mice exhibited robust reinstatement of active lever responding, compared to the last extinction session, (Figure 3B). This was demonstrated by a significant session condition × lever interaction ($\chi^2(1) = 57.681$, $p < 0.0001$; Supplemental Table 2.22). Post hoc analysis showed a difference between active and inactive lever presses for reinstatement ($z = 5.8638$, $p < 0.001$) in contrast to a lack of lever discrimination during extinction (Supplemental Table 2.23). Additionally, post hoc analysis detected differences between the active ($z = -22.4218$, $p < 0.0001$) or inactive ($z = -6.6972$, $p < 0.0001$) lever presses when comparing extinction and reinstatement conditions (Supplemental Table 2.23). However, there was no main effect of genotype and no significant genotype interactions (Supplemental Table 2.22). Consistent with the absence of genotype-dependent behavioral differences during reinstatement, the expression of IEGs (*Fos, Arc, Egr1, Npas4*), measured after reinstatement in the dorsal striatum, did not differ between genotypes (Figure 3C, Supplemental Table 3).

Together, these findings demonstrate that *Reln* haploinsufficiency does not alter relapse-like cue-induced seeking, consistent with a stable baseline opioid reinforcement in *Reln*+/− mice.

Reln haploinsufficiency enhances acute fentanyl-induced locomotion and dorsal striatal *Fos* expression

To examine opioid-evoked responses independent of instrumental contingencies, we evaluated passive fentanyl exposure using CPP, locomotor analysis, and Fos immunoreactivity in the dorsal striatum as a marker of neuronal activation.

CPP doses (20 or 80 µg/kg, i.p.) were selected to approximate contingent intake observed during IVSA at the lowest and highest unit doses, respectively (Figure 4A). Reln+/- mice did not alter fentanyl CPP at either dose (Figure 4B; Supplemental Table 4).

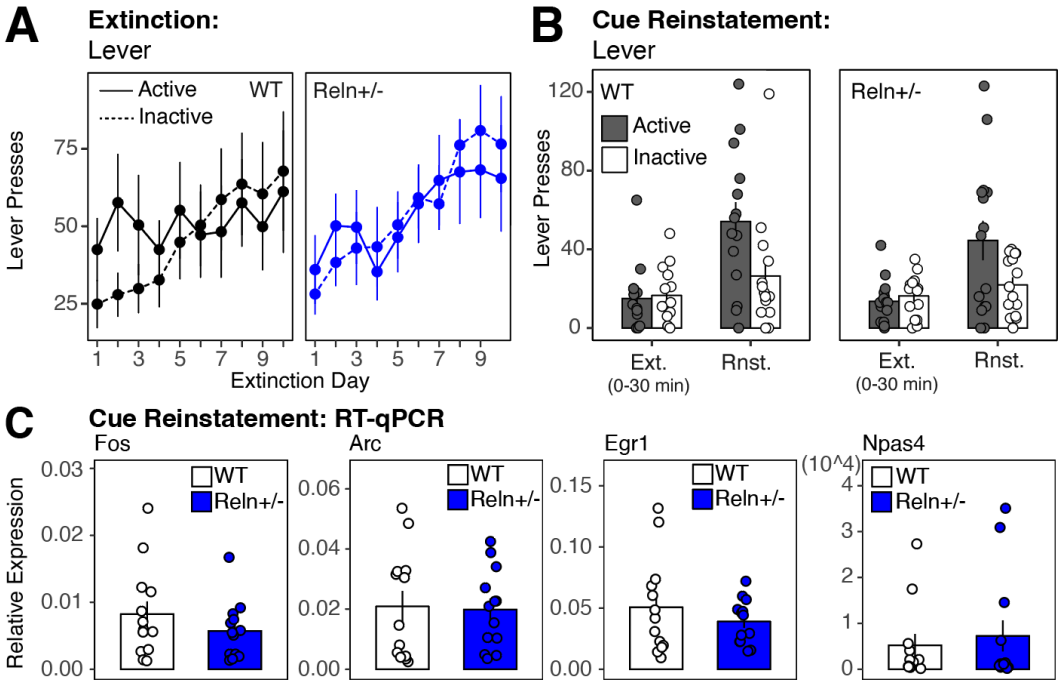

Figure 3 | Measuring genotypic response during intravenous fentanyl self-administration paradigm and subsequent quantification of fentanyl seeking IEG expression. A Lever presses during extinction phase (Extinction Day 1-10). **B** Comparison active and inactive lever presses during the first 30 minutes from the previous extinction session (Extinction Day 10) for comparison with the 30 minute session of cue-induced reinstatement. **C** Within the dorsal striatum, relative expression (2^-ΔCt) of Fos, Arc, Egr1, and Npas4 gene expression between cue-induced reinstatement when using Gapdh as the reference gene.

In contrast, during the first fentanyl CPP conditioning session, a main effect of genotype ($\chi^2(1) = 5.0185$, $p < 0.0251$) was detected on fentanyl-induced locomotion. While a trend for increased locomotion was observed, there was no detectable interaction between genotype x dose (Figure 4C; Supplemental Table 5). This result suggests that *Reln* haploinsufficiency may enhance acute behavioral sensitivity to fentanyl.

To determine whether this behavioral difference was accompanied by altered neuronal activation, a separate cohort of mice received an acute fentanyl injection at the low dose (20 µg/kg, i.p.), and Fos immunoreactivity was quantified 90 minutes later in the dorsal striatum (Figure 4D). Quantifying the population of Fos-positive (Fos+) cells relative to the total cellular population (Dapi+ cells), *Reln*+/- mice showed a significant increase in Fos+ cells compared to WT mice (main genotype effect, ($\chi^2(1) = 4.1873$, $p < 0.041$; Figure 4E; Supplemental Table 6).

Together, these findings demonstrate that *Reln* haploinsufficiency enhances acute fentanyl-induced locomotion and neuronal activation in the dorsal striatum, without affecting passively learned reinforcing properties of fentanyl.

Discussion

This study provides the first characterization of Reelin in the context of opioid-related behaviors. Using complementary contingent and non-contingent paradigms, we found that *Reln* haploinsufficiency did not alter baseline opioid reinforcement or relapse-like seeking, but it modulated how striatal neurons respond to changes in reinforcer magnitude and acute fentanyl exposure.

Reln function in opioid reinforcement

Prior work has demonstrated that reducing *Reln* expression alters behavioral and cellular responses to psychostimulants (de Guglielmo *et al.* 2022; Brida *et al.* 2025). In a cocaine IVSA paradigm, the reduction in *Reln* expression has been associated with decreased drug intake, motivation, and striatal activation patterns (Brida *et al.* 2025). With cell-type-specific transcriptomic profiling, Brida et al. (2025) also identified *Reln*-expressing neurons among cocaine-responsive neuronal ensembles. These findings suggested that Reelin may broadly regulate drug reinforcing mechanisms.

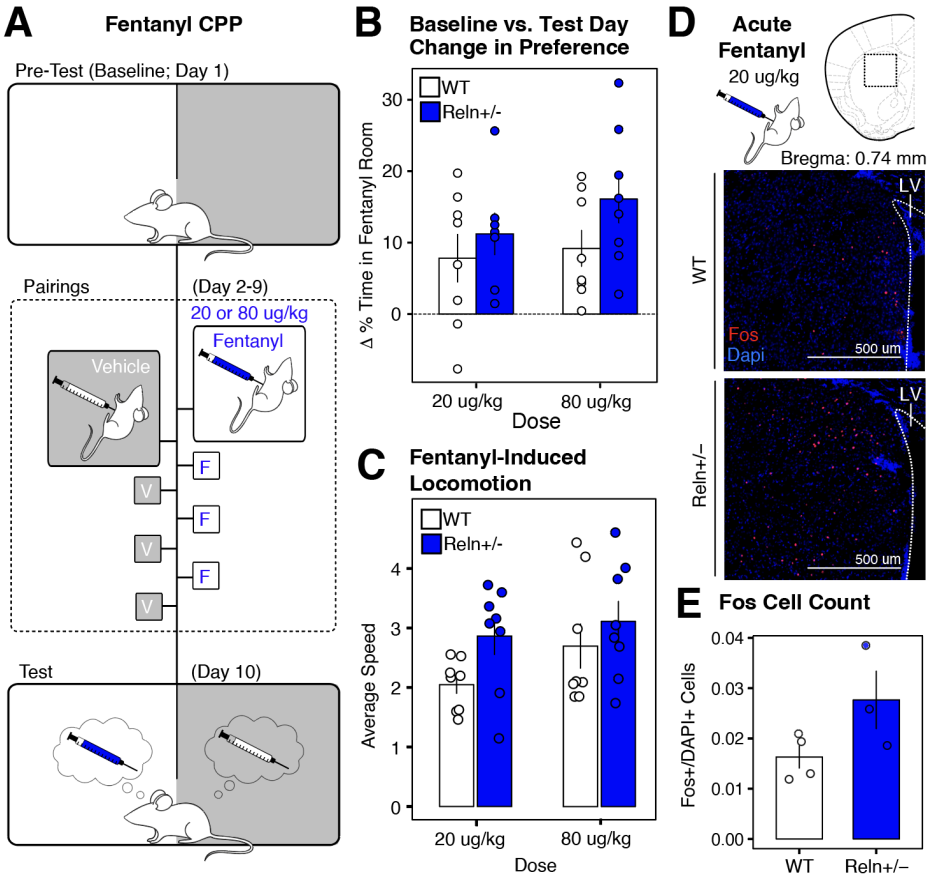

Figure 4 | Dose-Considerate Fentanyl CPP, Fentanyl-Induced Locomotion and Fos Immunoreactivity in the Dorsal Striatum. A Schematic of CPP paradigm. **B** Quantification of change in percent of time spent in the fentanyl (80 ug/kg or 20 ug/kg) paired room (as opposed to the saline paired room) on the test day compared to the baseline. **C** Fentanyl-induced locomotion (speed = total distance / total time) after acute fentanyl (20 or 80 ug/kg) i.p. Injection. **D** Immunohistochemistry for Fos+/Dapi+ cells in the dorsal striatum following acute fentanyl (20 ug/kg) intraperitoneal injection. **E** Quantification of Fos+/Dapi+ cells in the dorsal striatum.

In contrast, the present data indicate that *Reln* haploinsufficiency does not significantly alter baseline fentanyl reinforcement or relapse-like cue-induced seeking. Across multiple IVSA phases, *Reln*+/− mice exhibited intact acquisition and reinstatement, and total fentanyl intake did not differ by genotype during dose-response testing. Moreover, PR breakpoint, the primary operational measure of motivation, was unchanged. This divergence indicates that *Reln*-dependent modulation of reinforcement may not generalize across drug classes and may instead reflect substance-specific circuit engagement.

The divergence from cocaine IVSA findings may also reflect differences in experimental strategies. The present study used germline *Reln* haploinsufficient mice, in which reduced *Reln* expression occurs throughout development and across brain regions. In contrast, prior cocaine IVSA experiments used adult, region-specific knockdown within the nucleus accumbens of rats (Brida *et al.* 2025). Therefore, differences in regional specificity and developmental compensation may underlie the distinct behavioral outcomes observed here.

Perturbed *Reln* expression affects cost-dependent drug reinforcement

Despite similarity in baseline reinforcement during acquisition, *Reln*+/− mice exhibited genotype-dependent differences during de-escalating dose-response testing. Importantly, these effects emerged in infusion number and lever pressing but not in total fentanyl intake, indicating preserved overall reinforcement while response patterns differed across doses. These results suggested that *Reln* may regulate flexibility in cost valuation for opioids when reinforcer magnitude changes. Moreover, extinction and cue-induced reinstatement were not affected by *Reln* haploinsufficiency, further suggesting Reelin signaling may have a role in regulating response to changes in dose and reinforcement schedule without effects on acquisition, maintenance, and recall of drug responses.

Neural circuits within the dorsal striatum integrate reward magnitude and effort-related information to regulate response output and behavioral adaptation (Cromwell & Schultz 2003; Everitt & Robbins 2005; Gan *et al.* 2009; Lüscher & Malenka 2011). Given the established role of Reelin signaling in regulating synaptic plasticity and activity-dependent gene expression (Chen *et al.* 2005; Qiu *et al.* 2006; Niu *et al.* 2008; Rogers *et al.* 2011; Lee *et al.* 2014, 2023; Bosch *et al.* 2016; de Guglielmo *et al.* 2022), reduced *Reln* expression may alter striatal activation in a manner that affects how animals recalibrate operant responding when reinforcement parameters shift.

Future studies combining projection-specific activity recordings, cell-type–specific manipulation of *Reln* expression within dorsal striatal circuits, and transcriptomic profiling following dose de-escalation will be required to directly test whether altered synaptic plasticity mechanisms underlie the observed cost-dependent behavioral phenotype.

***Reln*-dependent modulation of acute fentanyl-induced locomotor and striatal activation**

In non-contingent paradigms, *Reln*+/− mice exhibited enhanced fentanyl-induced locomotion regardless of dose and increased dorsal striatal Fos immunoreactivity following acute exposure at a lower dose of fentanyl (20 ug/kg). Importantly, increased *Fos* expression was not detected after cue-induced reinstatement of fentanyl seeking. This dissociation suggests that *Reln* preferentially modulates acute pharmacological activation of opioids in the striatum rather than learned cue-driven activation.

These findings extend prior work linking Reelin signaling to drug-induced neuronal activation and provide the first evidence that Reelin signaling influences opioid-evoked recruitment of striatal populations. Notably, similar phenotypes have been reported in psychostimulant models. De Guglielmo et al. (2022) demonstrated that *Reln* haploinsufficient mice exhibit enhanced cocaine-induced hyperlocomotion accompanied by increased *Fos* expression in the dorsal striatum, without a significant alteration in cocaine conditioned place preference. This suggests that *Reln* has a specific role in modulating drug-induced responses, separate from modulating drug-free recall of drug-paired learning.

However, *Reln*-dependent drug effects are not uniformly expressed across substances or doses. For example, methamphetamine-induced hyperlocomotion was attenuated in *Reln* haploinsufficient mice in a dose-dependent manner (Matsuzaki et al., 2007), underscoring the importance of reinforcer dose and pharmacological mechanism when interpreting *Reln*-dependent modulation of psychomotor activation. Moreover, nucleus accumbens-specific *Reln* knockdown did not alter cocaine-induced locomotion but reduced cocaine CPP (Brida et al., 2025). These findings highlight that the influence of Reelin signaling on drug-evoked activation depends on pharmacological context, method of manipulation and circuit specificity.

Study Limitations

Several limitations warrant consideration. The use of *Reln* haploinsufficient mice precludes dissociation of developmental versus adult roles of Reelin signaling. Given the critical role of Reelin signaling in neural development (D'Arcangelo *et al.* 1995; Ikeda & Terashima 1997; Rice & Curran 2001), we cannot exclude the possibility that the observed phenotypes reflect developmental adaptations rather than solely adult *Reln* function. Moreover, given that *Reln*+/− mice exhibit lifelong, global reduction of *Reln* expression, future studies using circuit- and cell type-specific manipulations will be required to distinguish dorsal striatal *Reln*-dependent effects from broader network-level adaptations.

Additionally, molecular analyses were restricted to selected IEGs and single time points, which do not capture the full transcriptional or epigenetic landscape of opioid exposure (Egervari *et al.* 2017; Gaddis *et al.* 2022).

Finally, although both male and female mice were included, the study was not powered to detect sex-dependent effects. Therefore, future studies specifically designed to test sex as a biological variable will be necessary to determine whether *Reln*-dependent modulation of opioid reinforcement differs between males and females.

Conclusions

Collectively, these findings position Reelin as a modulatory factor in opioid-induced plasticity and behavioral adaptation rather than a primary driver of opioid reinforcement. These experiments provide a foundation for future studies to define how Reelin signaling shapes underlying cellular activity within opioid-responsive circuits.

Authors contributions

Conceptualization: C.L., F.T.; Investigation: C.L., A.M.L., S.D., T.H., L.L., A.C.; Formal analysis: C.L.; Visualization: C.L.; Writing – original draft: C.L.; Writing – review & editing: F.T., C.L., S.D.; Supervision: F.T.

Competing interests

The authors declare that they have no competing interests.

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