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## Ventral hippocampal overexpression of Cannabinoid Receptor Interacting Protein 1 (CNRIP1) produces a schizophrenia-like phenotype in the rat

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### Abstract

Adolescent cannabis use has been implicated as a risk factor for schizophrenia; however, it is neither necessary nor sufficient. Previous studies examining this association have focused primarily on the role of the cannabinoid receptor 1 (CB1R) with relatively little known about a key regulatory protein, the cannabinoid receptor interacting protein 1 (CNRIP1). CNRIP1 is an intracellular protein that interacts with the C-terminal tail of CB1R and regulates its intrinsic activity. Previous studies have demonstrated aberrant CNRIP1 DNA promoter methylation in post-mortem in human patients with schizophrenia, and we have recently reported decreased methylation of the CNRIP1 DNA promoter in the ventral hippocampus (vHipp) of a rodent model of schizophrenia susceptibility. To examine whether augmented CNRIP1 expression could contribute to the pathology of schizophrenia, we performed viral-mediated overexpression of CNRIP1 in the vHipp of Sprague Dawley rats. We then tested these rats for behavioral correlates of schizophrenia symptoms, followed by electrophysiology to determine the effects on the dopamine system, known to underlie psychosis. Here, we report that overexpression of vHipp CNRIP1 induces impairments in latent inhibition and social interaction, similar to those observed in individuals with schizophrenia and in rodent models of the disease. Furthermore, rats overexpressing vHipp CNRIP1 displayed a significant increase in ventral tegmental area (VTA) dopamine neuron population activity, a putative correlate of psychosis. These data provide evidence that alterations in CNRIP1 may contribute to the pathophysiology of schizophrenia, as overexpression is sufficient to produce neurophysiological and behavioral correlates consistently observed in rodent models of the disease.

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## Keywords

Schizophrenia; cannabinoids; CB1; CNRIP1; ventral hippocampus

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## 1. Introduction

Cannabis use has been suggested to increase the risk of developing schizophrenia in individuals with an underlying genetic predisposition (Andreasson et al., 1989; Arseneault et al., 2002; Henquet et al., 2005). Likewise, animal models of the disease display an increased susceptibility to exhibiting a schizophrenia-like phenotype when exposed to synthetic cannabinoids (Aguilar et al., 2018). Moreover, individuals with schizophrenia have a higher tendency to consume cannabis (Kovaszny et al., 1997), which can precipitate symptoms of the disease (Linszen et al., 1994). The cannabinoid hypothesis of schizophrenia speculates that alterations in the endocannabinoid system may contribute to the pathogenesis of schizophrenia (Emrich et al., 1997; Fernandez-Espejo et al., 2009). Additional support for this hypothesis stems from the observation that high densities of cannabinoid receptors type 1 (CB1R) are found in regions commonly implicated in schizophrenia (i.e. prefrontal cortex, hippocampus, basal ganglia, anterior cingulate cortex) (Berding et al., 2006; Glass et al., 1997; Zavitsanou et al., 2004). Furthermore, individuals with schizophrenia display increases in CB1R binding in these key brain regions (Dalton et al., 2011; Dean et al., 2001; Zavitsanou et al., 2004); however, it remains unclear as to how, or whether, these alterations contribute to symptoms associated with schizophrenia. Indeed, it is important to note that CB1R antagonists have failed to produce beneficial effects in clinical trials (Meltzer et al., 2004).

We have recently discovered alterations in the cannabinoid receptor interacting protein 1 (CNRIP1) in the MAM (methylazoxymethanol acetate) model, and in F2 MAM rats, a novel rodent model of schizophrenia susceptibility (Neary et al., 2017; Perez et al., 2016). F2 MAM rats were obtained by mating MAM-treated rats with control rats, resulting in a proportion of the offspring (~40%) displaying a schizophrenia-like phenotype (Perez et al., 2016). Further, evidence that this is a model of susceptibility was based on observations that exposure to schizophrenia risk factors, such as adolescent cannabinoids or stress, increases the proportion of F2 MAM rats displaying a schizophrenia-like phenotype (Aguilar et al., 2018). In our characterization of this model, we observed a decrease in the methylation of the DNA promoter for CNRIP1 (Perez et al., 2016), which is predicted to translate to an increase in CNRIP1 expression. Similarly, aberrant CNRIP1 DNA promoter methylation has been observed post-mortem in human patients with schizophrenia (Wockner et al., 2014). As its name suggests, CNRIP1 interacts with the C-terminal tail of CB1R (Nie and Lewis, 2001; Niehaus et al., 2007) and is reported to alter the constitutive activity of this receptor (Niehaus et al., 2007). Thus, an increase in CNRIP1 expression is hypothesized to lead to augmented constitutive activity at the CB1R, without overt changes in CB1R expression (Smith et al., 2010). The consequences of this potential increase in CNRIP1 expression is not currently known, and will thus be investigated in this study.

Aberrant hippocampal activity has been reported in individuals with schizophrenia (Heckers, 2001, 2004; Schobel et al., 2009), as well as in rodent models of the disease (Lodge and Grace, 2007; Perez and Lodge, 2013). Additionally, increases in the activity of ventral tegmental area (VTA) dopamine neurons have been attributed to hyperactivity of the vHipp (Lodge and Grace, 2007); furthermore, the positive symptoms associated with schizophrenia are thought to arise from dysfunction of the mesolimbic dopamine system (Abi-Dargham, 2004; Laruelle and Abi-Dargham, 1999). Indeed, interactions between the cannabinoid system and the dopaminergic system are suggested to lead to psychosis, as cannabinoids transiently increase the activity of dopamine neurons (Voruganti et al., 2001). Based on these data, we posit that alterations in vHipp CNRIP1 expression may contribute to a schizophrenia-like phenotype.

## 2. Materials and Methods

All experiments were performed in accordance with the guidelines outlined in the USPH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of UT Health San Antonio.

### 2.1 Western Blot

To verify that the alterations in CNRIP1 DNA promoter methylation (reported in F2 MAM-treated rats (Perez et al., 2016)) actually alters CNRIP1 protein expression, western blot was utilized. The vHipp was dissected from F2 MAM- and saline-treated rats (n= 9 per group), homogenized in ice-cold buffer (750 $\mu$ L) containing a protease inhibitor cocktail, centrifuged (14,000 r.p.m. for 2 minutes) and supernatant containing protein fractions was collected. Protein concentrations were determined using the Bradford method before incubation with Laemmli Sample Buffer containing 5% dithiothreitol (10 minutes at 90°C) and separated 150-200mA on a 10% acrylamide gel. Proteins were transferred (120 minutes at 250mA) to PVDF membranes. Membranes were then blocked for 1 hour (5% BSA in TBST) before incubation with primary antibody for CNRIP1 and CB1R (anti-CNRIP, 1:2000, Abcam, ab167087; anti-CNRI, 1:2000, Synaptic Systems, AA151-200; anti- $\beta$ -actin, 1:20,000) for 1 hour at 4°C. Membranes were washed three times in TBST (10 minutes each), followed by incubation in secondary antibody (goat anti-rabbit IgG-HRP, 1:500, Santa Cruz Biotechnology, SC-2004 or goat anti-mouse antibody IgG-HRP, 1:500, Santa Cruz Biotechnology, SC-2005) at room temperature (1 hour).

### 2.2 CB1R/CNRIP1 Co-Immunoprecipitation

To demonstrate that there is indeed a direct association between CB1R and CNRIP1 in the vHipp we performed co-immunoprecipitation. Male Sprague Dawley rats (n=4; 400-550g) were anesthetized with Fluriso™ (2-5% Isoflurane, USP with oxygen flow at 1L/min; VetOne; #502017) and quickly decapitated. The vHipp were extracted, homogenized in ice-cold buffer (750 $\mu$ L; 50mM Tris-HCl, 150mM NaCl, 0.1% Triton X-100, pH 7.4) containing a protease inhibitor cocktail (Sigma; P8340), centrifuged (14,000 r.p.m. for 2 minutes) and supernatant was collected. SureBeads™ Protein G Magnetic beads (BioRad; #1614013) were incubated for 10 minutes with anti-CB1R antibody (2 $\mu$ g; anti-CB1; Abcam; ab23703) diluted in 200 $\mu$ L TBST (15mM Tris-HCl, 137mM NaCl and 0.1% Tween 20) at room

temperature; the SureBeads™-antibody complexes were washed and incubated with 500µL of protein lysate for 15 minutes. The beads were then magnetized and washed 3 times using 500 µL TBST. Laemmli Sample Buffer (50 µL; BioRad; #161-0737) containing 5% dithiothreitol was added to the beads and heated for 10 minutes at 90°C. The immunoprecipitated protein (25µl per well) was separated (45 minutes at 150V) on Mini-Protean TGX Any kD gels (BioRad; #456-9035) in Tris/glycine/SDS buffer (Thermo Fisher Scientific; BP1341-1) and subsequently transferred to nitrocellulose/filter paper sandwiches (0.2µm; BioRad; #162-0213) with transfer buffer (25mM Tris-HCl, 192mM glycine, 20% w/v ethanol, pH 8.3) for one hour at 100V. Membranes were then blocked for 30 minutes (5% BSA in TBST) before incubation with primary antibody (anti-CB1R or anti-CNRIP1; 1:2000; Abcam; ab167087) for 1 hour at 4°C. Next, membranes were washed three times in TBST (10 minutes each), followed by incubation in Protein A - HRP (1:5000; BD Biosciences; #610438) at room temperature (1 hour). Lastly, membranes were treated with Pierce ECL Western Blotting substrate (1 minute; Thermo Fisher Scientific; #32106) and protein signal was captured with a G:BOX-XT4 Chemi system (Syngene).

### 2.3 Lentivirus overexpression of CNRIP1

Adult male Sprague Dawley rats (~350-375 g) were obtained from Envigo. All survival surgical procedures were performed under general anesthesia in a semi-sterile environment. Rats were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and placed in a stereotaxic apparatus. Anesthesia was maintained by supplemental administration of sodium pentobarbital as required to maintain suppression of limb compression withdrawal reflex. A core body temperature of 37°C was sustained by a thermostatically controlled heating pad. The skin was reflected and bore holes drilled bilaterally overlying the vHipp (A/P ± 5.3, M/L ± 5.0 from bregma and D/V -7.5 mm ventral to the brain surface). Rats were bilaterally injected with high-titer (0.75 µL/side; ~1×10<sup>9</sup> TU/mL) lentivirus particles containing vectors expressing CNRIP1 or a GFP expressing control (see Figure 2A for vector map: obtained from VectorBuilder). The skin was sutured closed and rats housed under ABSL2 conditions for 72 hours before being transferred to standard housing conditions. Rats were housed for a period of 6 weeks prior to any behavioral or electrophysiological examinations to ensure stable transgene expression.

### 2.4 Latent Inhibition

As a model of positive symptoms associated with schizophrenia we examined latent inhibition. Rats over expressing vHipp CNRIP1 or controls were randomly assigned into two groups (tone pre-exposure or no tone exposure) and placed in a conditioning chamber (30.5 × 25.4 × 30.5 cm<sup>3</sup>) with metal walls and a stainless steel grid shock floor (Coulbourn Instruments, Holliston, MA). A subset of rats were presented with a 20 second tone over 16 trials with a pseudorandom inter-trial interval (ITI) averaging two minutes, while the remaining rats remained in their home cage. All rats then underwent an established fear conditioning procedure (Green et al., 2011) in which a 20 second tone co-terminated with a mild foot shock (0.8 mA, 0.5 sec). The tone-shock pairing was presented four times with a pseudorandom ITI averaging two minutes. Twenty-four hours after conditioning, rats were returned to the conditioning chamber and re-exposed to the 20 second tone over four trials. Behavior was video recorded at each stage and freezing behavior was analyzed offline using

FreezeView (Coulbourn Instruments). Freezing behavior was defined as movement below a motion index threshold of ten, lasting at least one second. The percentage of latent inhibition was determined using the following formula:  $100 - \text{percent freezing in response to the conditioned tone (in tone pre-exposed rats / average non pre-exposed rats)}$ .

## 2.5 Social Interaction

To evaluate negative symptoms associated with schizophrenia we examined social behavior (as described previously (Cecchi et al., 2002)) in rats over expressing vHipp CNRIP1 or controls. In brief, rats were placed singly in a testing arena ( $100 \times 100 \times 40$  cm) for 15 minutes per day for a period of two days prior to testing to habituate to the arena. On the testing day, experimental rats were placed in the arena with a weight matched “stimulus” rat. Testing was video recorded for five minutes by video camera for offline analysis by a blind experimenter. The dependent measure was the time the test animal spent actively engaged in social activity (sniffing, grooming, climbing on, following, or wrestling) with the stimulus rat.

## 2.6 In Vivo Extracellular Recordings

Rats over expressing vHipp CNRIP1 or controls were anesthetized with 8% chloral hydrate (400 mg/kg, i.p.), as this anesthetic does not significantly depress dopamine neuron activity (Hyland et al., 2002), and placed in a stereotaxic apparatus. Anesthesia was maintained by supplemental administration of chloral hydrate as required to maintain suppression of limb compression withdrawal reflex. A core body temperature of  $37^{\circ}\text{C}$  was sustained by a thermostatically controlled heating pad. Extracellular glass microelectrodes (impedance 6-14 M $\Omega$ ) were lowered into the VTA (A/P  $\pm 5.3$ , M/L  $\pm 0.6$  from bregma and D/V  $-6.5$  to  $-9.0$  mm from the surface of the brain) using a hydraulic micropositioner to measure dopamine neuron activity. Spontaneously active dopamine neurons were identified with open filter settings (low pass: 30 Hz; high pass: 30 kHz) using previously established electrophysiological criteria (Grace and Bunney, 1983), which have recently been reviewed and evaluated to support the reliability of these types of recordings (Ungless and Grace, 2012). Once isolated, dopamine neuron activity was recorded for 2-3 minutes. Three parameters of dopamine neuron activity were measured: population activity (defined as the number of spontaneously active dopamine neurons encountered while making six to nine vertical passes, separated by 200  $\mu\text{m}$  in a predetermined pattern to sample equivalent regions of the VTA); basal firing rate; and burst firing (proportion of action potentials occurring in bursts).

## 2.7 Quantitative PCR

To verify CNRIP1 overexpression we performed qPCR. vHipp tissue was taken from a subset of rats following electrophysiology and processed using an RNA isolation kit (RNAqueous®-4PCR Total RNA Isolation Kit; Thermo Fisher Scientific; AM1914). Briefly, tissue samples were homogenized in Lysis/Binding solution and RNA was precipitated and separated by filtration. The concentration of RNA was determined by absorbance at 260 nm with a NanoDrop Spectrometer (ThermoFisher Scientific) and converted to single stranded cDNA by using a High Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific; #4368814) and thermal cycler. Real-time PCR was performed with FAM-labeled

TaqMan primers (Thermo Fisher Scientific) targeting either CNRIP1 (Rn01434074\_ml), CB1R (CNR1; Rn02758689\_sl), CB2R (CNR2; Rn03993699\_sl) or GAPDH (Rn01775763\_gl).

## 2.8 Data Analysis

Electrophysiological analysis of dopamine neuron activity was performed using commercially available computer software (LabChart version 7.1; ADInstruments, Chalgrove, Oxford, UK). Detection of FAM-labeled DNA was performed by a CFX384 Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA). Co-Immunoprecipitation blots were captured with a G:BOX-XT4 Chemi system (Syngene; Frederick, MD, USA). Western blots of F2 tissue were scanned and optical density measured using ImageJ. Data are represented as the mean  $\pm$  s.e.m. unless otherwise stated, with n values representing the number of neurons recorded or number of animals per experimental group where indicated. All statistics were calculated using Prism software (GraphPad Software, San Diego, CA, USA) or SigmaPlot (Systat Software, Chicago, IL, USA). Data was analyzed by Unpaired-t test, one-way ANOVA or two-way ANOVA and significance determined at  $P < 0.05$ .

## 2.9 Materials

Fluriso<sup>TM</sup> was purchased from VetOne (Boise, ID, USA). Chloral hydrate, sodium pentobarbital and protease inhibitor cocktail were purchased from Sigma-Aldrich (St. Louis, MO, USA). Lentiviral vectors were produced and packaged by VectorBuilder (Santa Clara, CA, USA). The Pierce ECL Western Blotting Substrate, Tris/glycine/SDS buffer RNAqueous<sup>®</sup>, 4PCR Total RNA Isolation Kit and High Capacity cDNA Reverse Transcriptase Kits, as well as FAM-labeled TaqMan Primers and Gene Expression Master Mix were purchased from ThermoFischer Scientific (Waltham, MA, USA). Anti-CB1R and anti-CNRIP1 antibodies were purchased from Abcam (Cambridge, MA, USA). Anti-CNR1 antibody (used in western blot) was purchased from Synaptic Systems (Goettingen, Germany). HRP Protein A was purchased from BD Biosciences (San Jose, CA, USA). Anti- $\beta$ -actin was purchased from Chemicon. Goat anti-rabbit IgG-HRP and goat anti-mouse IgG-HRP were purchased from Santa Cruz Biotechnology (Dallas, TX, USA). SureBeads<sup>TM</sup> Protein G Magnetic beads, Laemmli Sample Buffer, Mini-Protean TGX Any kD gels and nitrocellulose/filter paper sandwiches were supplied from BioRad (Hercules, CA, USA). All other chemicals and reagents were of either analytical or laboratory grade, and were purchased from various suppliers.

## 3. Results

### 3.1 Western Blot and Co-Immunoprecipitation

We have previously shown that F2 MAM-treated rats display a decrease in CNRIP1 DNA promoter methylation (Perez et al., 2016) that likely translate to an increase in CNRIP1 expression. To examine whether F2 generation MAM- and saline-treated rats exhibit altered CNRIP1 and CB1R expression in the vHipp, we performed western blot analysis. Indeed, CNRIP1 expression in the vHipp is elevated in F2 MAM rats (~20% increase compared to F2 saline rats; n= 9 rats per group) without a change in the expression of CB1R (Figure 1A).



In addition, further data analysis showed a significant positive correlation between CNRIP1 and CB1R expression in control rats ( $R^2= 0.5254$ ;  $P< 0.05$ ) that was completely lost in F2 MAM rats ( $R^2= 0.0074$ ;  $P= 0.83$ ; Figure 1B). In order to verify an association between CB1R and CNRIP1 in the vHipp, we performed co-immunoprecipitation (Figure 1C,D) and found that CNRIP1 was co-immunoprecipitated with CB1R, as determined by the presence of a 17kD band, consistent with previous reports of an association between CNRIP1 and the CB1R.

### 3.2 Quantitative PCR.

The over-expression of CNRIP1 in the vHipp was confirmed post-mortem by quantitative PCR. Rats that received lentivirus particles containing vectors expressing CNRIP1 ( $n= 6$ ; Figure 2A) displayed robust expression of vHipp CNRIP1 mRNA (Figure 2B). Statistical analysis was not performed, as CNRIP1 expression levels in control rats were below the limits of detection. Importantly, GAPDH mRNA expression was observed in equivalent amounts in both groups (relative quantity; control:  $1.00\pm 0.50$ ; Lenti:  $1.42\pm 0.24$ ; Unpaired t test;  $t= 0.75$ ;  $P= 0.47$ ). In addition, there was not a significant difference between the levels of CB1R mRNA (CNR1; control:  $1.00\pm 0.51$ ; Lenti:  $1.08\pm 0.30$ ) and CB2R mRNA (Figure 2C; CNR2; control:  $1.00\pm 0.50$ ; Lenti:  $1.01\pm 0.29$ ; two-way ANOVA;  $P= 0.93$ ).

### 3.3 Latent Inhibition

Latent inhibition can be measured across species and deficits are observed in individuals with schizophrenia (Swerdlow et al., 2003). Latent inhibition is the observation that a conditioned or familiar stimulus takes more time to become meaningful when compared to a new stimulus. Individuals with schizophrenia display decreases in latent inhibition, which is thought to reflect an inability to ignore irrelevant stimuli (Weiner and Arad, 2009). Here we demonstrate that rats overexpressing CNRIP1 in the vHipp displayed a trend ( $p=0.05$ ) for a deficit in latent inhibition ( $n= 6$ ;  $-57.54 \pm 51.71\%$  latent inhibition) when compared to control rats ( $n=6$ ; bilaterally injected with lentivirus particles containing vectors expressing GFP;  $+64.42 \pm 20.37\%$  latent inhibition; Unpaired t test;  $t=2.19$ ,  $p=0.05$ ; Figure 3A).

### 3.4 Social Interaction

Individuals with schizophrenia often display negative symptoms, such as social avoidance (Hansen et al., 2009). Rodents display a relatively consistent social behavior that is disrupted in putative models of the disease (Donegan et al., 2017; Sams-Dodd, 1995, 1996). Thus in this study we used the social interaction test as a correlate of these symptoms. CNRIP1 over-expression in the vHipp reduced social interaction time ( $n=11$ ;  $74.45 \pm 6.88$  sec) when compared to control rats ( $n=12$ ;  $95.00\pm 5.70$  sec; Unpaired t test;  $t=2.32$ ,  $p<0.05$ ; Figure 3B), which is a consistent observation in various other rodent models of schizophrenia (Donegan et al., 2017; Flagstad et al., 2004; Seillier and Giuffrida, 2009).

### 3.5 In Vivo Extracellular Recordings

Over-expression of CNRIP1 in the vHipp caused downstream changes in dopamine neuron activity within the VTA. Control rats ( $n= 6$ ;) displayed an average of  $1.00 \pm 0.06$  spontaneously active dopamine neurons per track, consistent with previous findings in

untreated rats (Boley et al., 2014; Lodge and Grace, 2007; Perez et al., 2014; Perez and Lodge, 2012; Perez et al., 2013; Shah and Lodge, 2013). Rats over-expressing CNRIP1 in the vHipp (n= 10) had a significant increase in the number of spontaneously active dopamine neurons ( $1.98 \pm 0.11$ ) when compared to control rats (one-way ANOVA;  $P < 0.001$ ; Figure 4A). No significant differences were observed in the firing rate or percent burst firing of VTA dopamine neurons (n= 36-118; one-way ANOVA; Figure 4B,C).

#### 4. Discussion

Recent post-mortem studies of individuals with schizophrenia suggest that epigenetic modifications, such as differential DNA methylation, are important in the etiology of the disease (Connor and Akbarian, 2008; Nishioka et al., 2012; Wockner et al., 2014). Aberrant DNA methylation of the promoter for the CB1 receptor interacting protein, CNRIP1, has been observed in individuals with schizophrenia (Wockner et al., 2014) and in F2-MAM rats (Perez et al., 2016), a rodent model of heritable susceptibility. Surprisingly, there is very little information about the role of CNRIP1, which is particularly striking given the extensive literature on the cannabinoid system. Here, we demonstrate that overexpression of CNRIP1 in the vHipp of rodents leads to downstream changes in dopamine neuron activity and behavioral deficits analogous to positive and negative symptoms commonly observed in individuals with schizophrenia.

Cannabinoid receptors (i.e. CB1R) are G-protein-coupled receptors (GPCRs) that play a role in central nervous system function by mediating the effects of tetrahydrocannabinol (THC; the principal psychoactive constituent of cannabis) and endocannabinoids (Howlett et al., 2002). Expression levels of CNRIP1 do not appear to influence the expression of CB1R or ligand binding affinity (Niehaus et al., 2007; Smith et al., 2015). Similarly, here we demonstrate that the overexpression of CNRIP1 mRNA does not affect the expression of CB1 or CB2 receptors, as measured by qPCR. Western blot analysis performed in vHipp tissue revealed a 20% increase in CNRIP1 protein expression in F2 MAM-treated rats when compared to controls, with no change in CB1R protein expression. Additionally, evidence supports an interaction between CNRIP1 and CB1R (Blume et al., 2013; Ludanyi et al., 2008), such that CNRIP1 may play a regulatory role in the cannabinoid system by modulating CB1R activation (Smith et al., 2015). Whether CB1R activity contributes to, or is affected by schizophrenia, is not currently known and was the focus of this study. Here we confirm an association between CB1R and CNRIP1 in the vHipp by co-immunoprecipitation and demonstrate a significant correlation between CB1R and CNRIP1 protein expression. This correlation was completely lost in F2 MAM rats, where increases in CNRIP1 were independent of CB1 levels, suggesting a possible dysregulation of CB1R function in these animals.

In rats that over expressed CNRIP1 in the vHipp, we performed the latent inhibition behavioral assay to model the positive symptoms associated with schizophrenia. Latent inhibition is disrupted in patients with schizophrenia (Gray et al., 1995b), as patients typically have difficulty ignoring irrelevant stimuli. Similarly, rodents with increased mesolimbic dopaminergic activity display deficits in latent inhibition (Moore et al., 2006; Young et al., 1993). Typical antipsychotics have been shown to enhance latent inhibition



preclinically, as well as in patients (Gray et al., 1995a; Gray et al., 1995b; Leumann et al., 2002; Young et al., 1993). Rats that over-expressed CNRIP1 in the vHipp exhibited a deficit in latent inhibition when compared to their respective controls, mimicking positive symptoms associated with schizophrenia. We were not able to directly correlate the latent inhibition with our readout of dopamine neuron activity (population activity); however, studies have demonstrated that latent inhibition relies on dopamine system function (Gray et al., 1995a; Young et al., 1993). Additionally, as a marker of negative symptoms, we measured social interaction time. Individuals with schizophrenia often display deficits in social functioning or behaviors (Blanchard et al., 1998). Rodent models of the disease display similar deficits in the social interaction task, as they spend less time interacting when introduced to a novel rat (Flagstad et al., 2004; Sams-Dodd, 1995; Seillier and Giuffrida, 2009). Likewise, we observed a decrease in the time spent interacting in rats with over-expression of CNRIP1 in the vHipp. Most commonly associated with negative symptoms is the medial prefrontal cortex (mPFC), which is innervated by the vHipp (Carr and Sesack, 1996; Jay et al., 1989; Wolkin et al., 1992). Indeed, we have previously demonstrated that rodent behavioral correlates of negative symptoms can be reversed by normalizing activity in the vHipp (Donegan et al., 2017). Thus, we posit that increases in vHipp CNRIP1 may cause deficits in social interaction due to aberrant innervating of the mPFC.

Interestingly, CNRIP1 over-expression in the vHipp was sufficient, in and of itself, to induce downstream changes in VTA dopamine neuron population activity, such that the number of spontaneously active dopamine neurons was significantly increased, without any change to the average firing rate, or burst firing pattern. This not surprising as previous studies have demonstrated that the vHipp can regulate VTA dopamine neuron activity, such that aberrant activity in the vHipp leads to a significant increase in population activity (Lodge and Grace, 2007). Increases in dopamine neuron population activity are commonly observed in rodent models of schizophrenia [i.e. MAM, chronic PCP, and F2-MAM models (Aguilar, 2014; Lodge and Grace, 2007; Perez et al., 2016; Perez et al., 2014; Perez and Lodge, 2012, 2013; Perez et al., 2013)] and attributed to aberrant regulation of the mesolimbic dopamine system by the vHipp (Aguilar, 2014; Lodge and Grace, 2007). Further, studies have shown that the firing rates and burst firing activity of individual dopamine neurons are affected by manipulations to the pedunclopontine tegmental nucleus, medial prefrontal cortex, and lateral dorsal tegmentum, without altering the population activity of dopamine neurons (Floresco et al., 2003; Murase et al., 1993). Thus, CNRIP1 over-expression accurately recapitulates the schizophrenia-like phenotype of increased population activity observed in various rodent models of the disease (Aguilar, 2014; Perez et al., 2016; Perez et al., 2014; Perez and Lodge, 2012, 2013; Perez et al., 2013). Measuring cells per track has been shown to be a reliable method to study *in vivo* changes in dopamine neuron population activity, as well as differences in average firing rate and burst firing patterns (Grace and Bunney, 1983; Ungless and Grace, 2012). Although it has been suggested that ‘silent’ dopamine neurons may not be detected using this method because of low firing rates and/or low electrotonic length (Dai and Tepper, 1998), evidence from more recent studies suggests that this is a reliable measure of the activity of the population of dopamine neurons in the VTA (Choong and Shen, 2004; Shen and Choong, 2006; Ungless and Grace, 2012).

The exact mechanism as to how this over-expression of vHipp CNRIP1 yields a schizophrenia-like phenotype is unclear; however, as mentioned previously alterations in cannabinoid signaling have been observed in individuals with schizophrenia (De Marchi et al., 2003; Leweke et al., 1999). Pre-clinically, studies have demonstrated that administration of CB1R agonists, in F2 MAM susceptible rats, can significantly increase the proportion of rats that exhibit a schizophrenia-like phenotype (Aguilar et al., 2018). Specifically, those that develop the phenotype display increases in population activity and an increased sensitivity to psychomotor stimulants (a marker of positive symptoms) (Aguilar et al., 2018). A class of fast-spiking containing PV, a calcium binding protein, have been associated with aberrant activity in the vHipp and downstream dysfunction of VTA dopamine neurons (Boley et al., 2014; Perez and Lodge, 2014; Shah and Lodge, 2013). Further, protein expression of parvalbumin (PV) in the vHipp was significantly decreased by adolescent cannabinoid exposure and negatively correlated with VTA dopamine neuron population activity, such that those rats that had lower levels of PV expression displayed a higher population activity (Aguilar et al., 2018). It is possible that over-expressing CNRIP1, leads to aberrant constitutive activity of the CB1R, which may alter the function of PV interneurons; however, this remains to be established.

In general, modeling symptoms of schizophrenia is challenging, as this disease truly is human in nature. However, given that we don't completely understand the pathophysiology of the disease, rodent models are essential to provide information to guide novel therapies. Here, we demonstrate that alterations in CNRIP1 expression could contribute to the pathophysiology of schizophrenia. Indeed, altered CNRIP1 expression may increase the susceptibility of an individual to develop schizophrenia. This is supported by preclinical data demonstrating that adolescent cannabinoid agonist administration increases the proportion of F2 MAM rats displaying a schizophrenia-like phenotype (Aguilar et al., 2018). In our model of vHipp CNRIP1 overexpression, there were downstream changes in dopamine neuron population activity, which contribute to positive symptoms of the disease, as well as behaviors analogous to positive and negative symptoms associated with the disease. These data are consistent with observations made in various other rodent models of schizophrenia. Further, this model will serve as a useful tool to further investigate interactions between CNRIP1 and CB1R and the role of hippocampal CNRIP1 in developing schizophrenia; thus, allowing us to develop new targets for therapeutic interventions for the treatment of schizophrenia.

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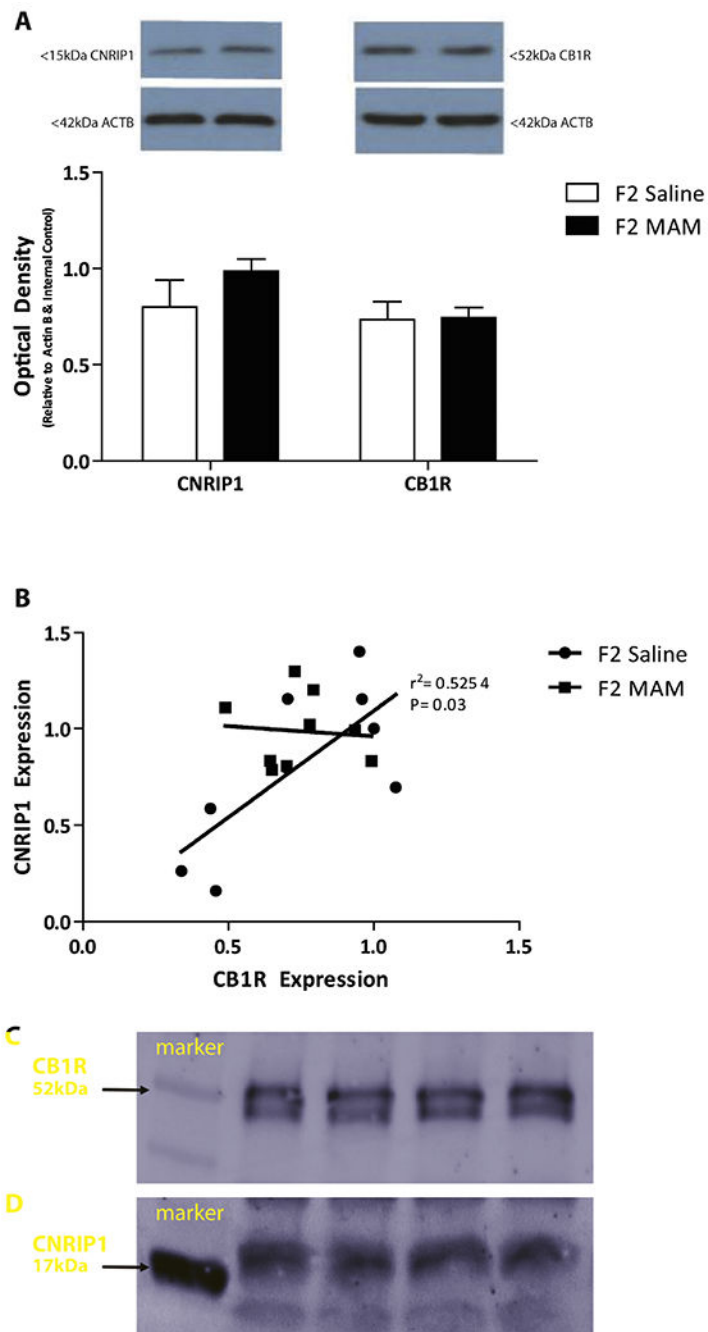
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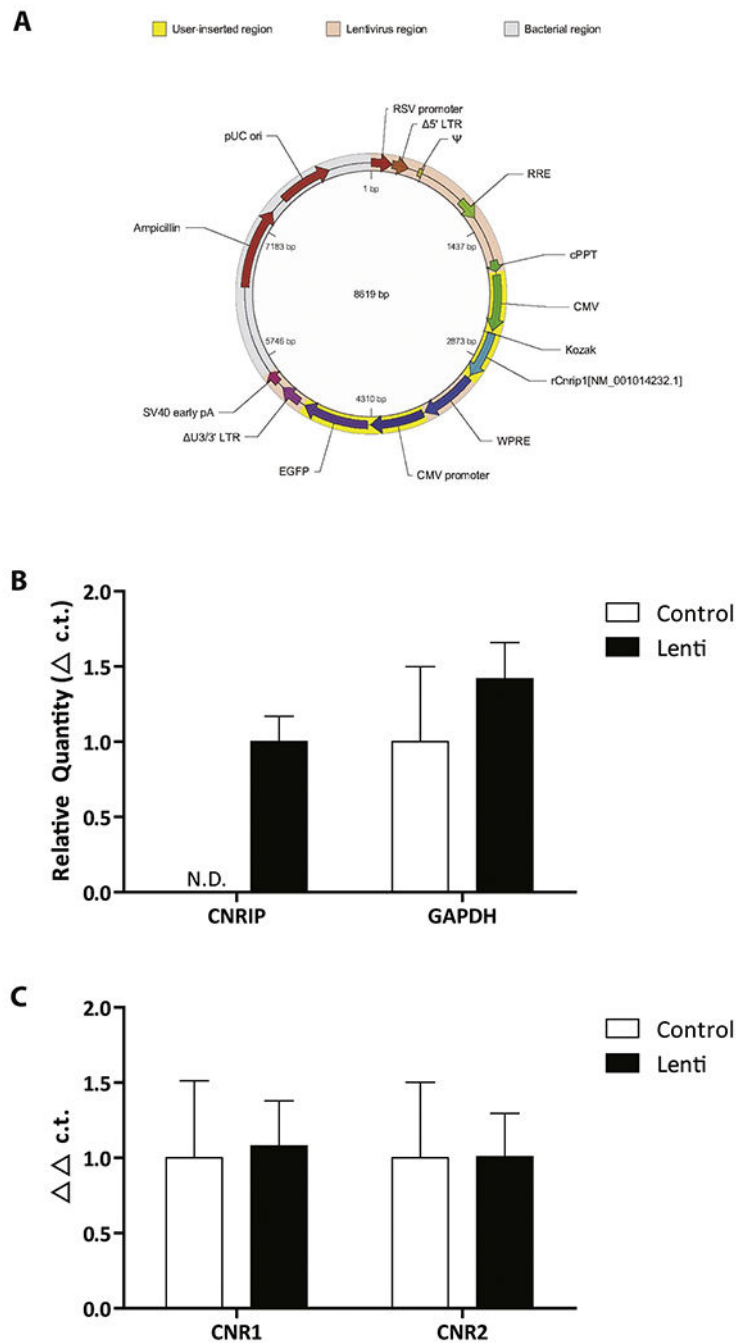
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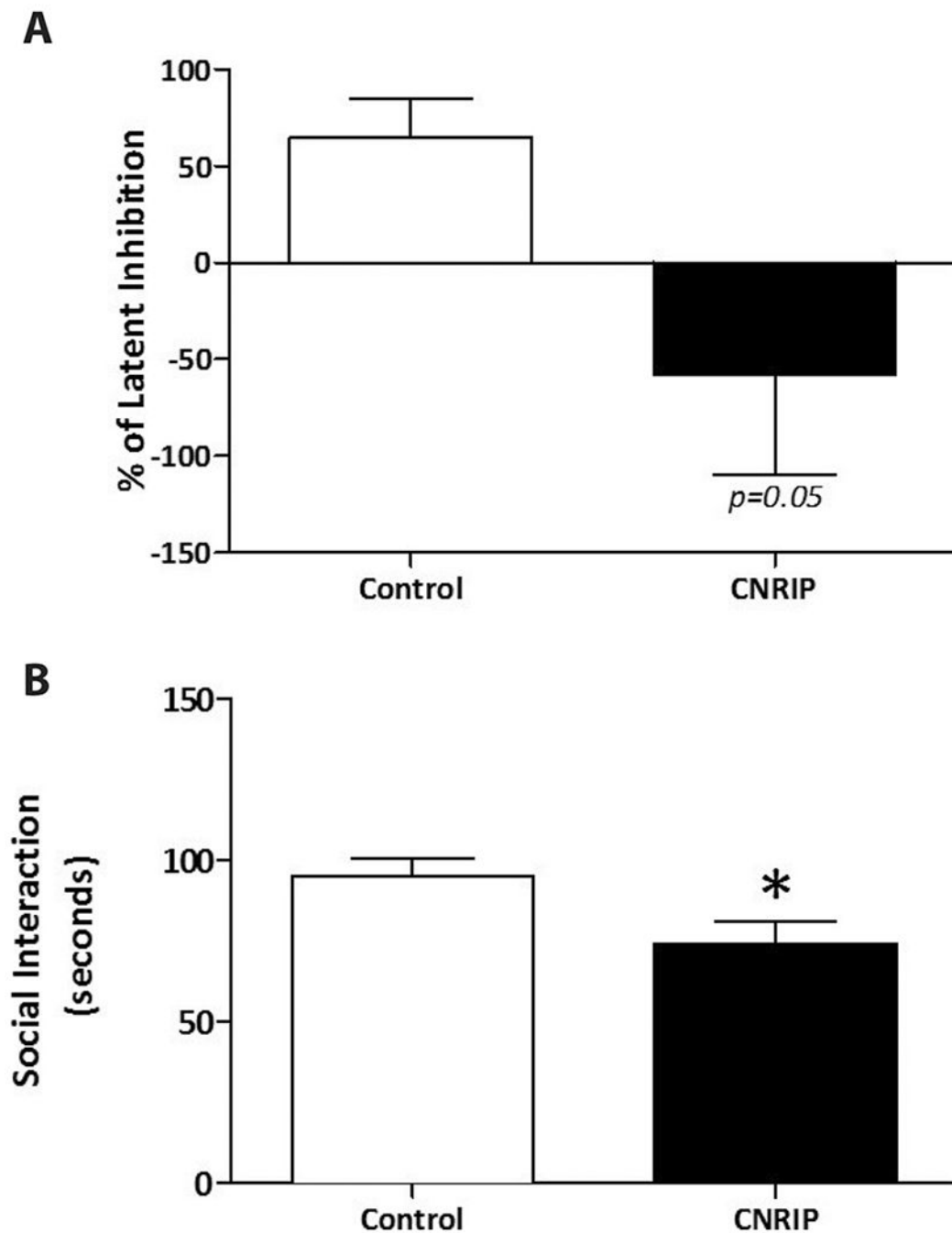


**Figure 1. F2 MAM-treated rats display an increase (~20%) in the expression of ventral hippocampal (vHipp) cannabinoid receptor interacting protein 1 (CNRIP1).** Quantification of CNRIP1 and cannabinoid 1 receptor (CB1R) protein expression in the vHipp of F2 MAM- and saline-treated rats (A). There is a significant correlation between CNRIP1 and CB1R in the vHipp of control rats and not in F2 MAM- treated rats (B; Pearson correlation;  $R^2 = 0.5254$ ). In normal rats, co-immunoprecipitation revealed an association between CB1R and CNRIP1 (n = 4 rats). Representative films demonstrating CNRIP1 (C) and CB1R (D) expression.

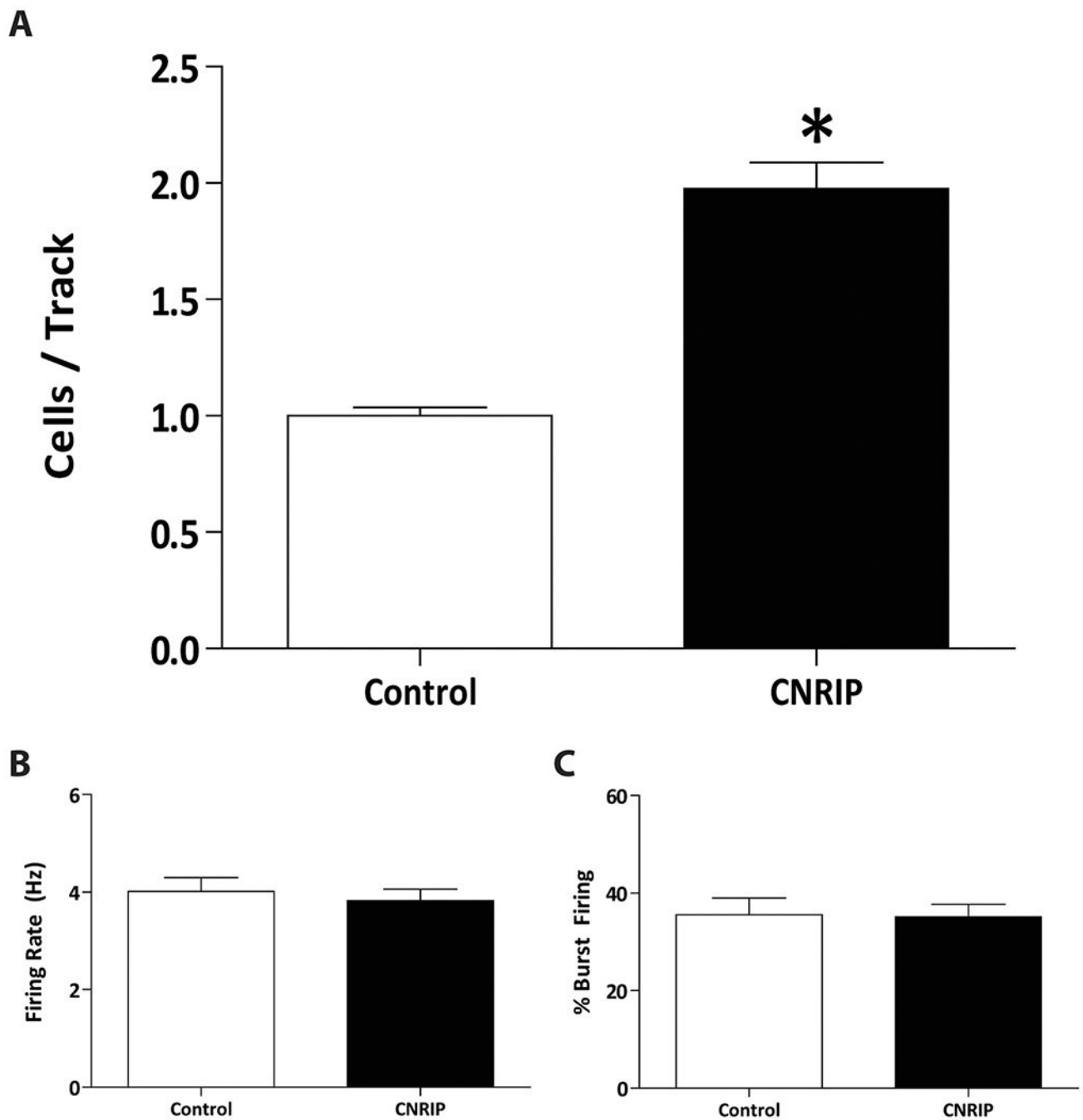


**Figure 2. Vector map for cannabinoid receptor interacting protein 1 (CNRIP1) over-expression (A) and validation of ventral hippocampal (vHipp) CNRIP1 overexpression.**

Over-expression of CNRIP1 in the vHipp was confirmed using RT-PCR (B; n= 6 rats per group). Since the levels of CNRIP1 in control rats were not detectable (N.D.), no statistical analyses were performed. Importantly, the expression of GAPDH was not different between groups. mRNA expression levels of the cannabinoid 1 receptor (CNR1) and the cannabinoid 2 receptor (CNR2) were not affected by the over expression of CNRIP1 (C; n= 6 rats per group).



**Figure 3. Cannabinoid receptor interacting protein 1 (CNRIP1) over-expression in the ventral hippocampus (vHipp) impairs latent inhibition and causes deficits in social interaction.** Rats in which CNRIP1 is over-expressed in the vHipp display a deficit in latent inhibition, a behavioral correlate of positive symptoms, when compared to control rats (**A**; n= 6 rats per group). \*p=0.05. CNRIP1 overexpression in the vHipp reduced time spent interacting time when compared to control rats (**B**; n= 11-12 rats per group), a marker of negative associated symptoms. \*p<0.05.



**Figure 4. Ventral hippocampal (vHipp) over-expression of cannabinoid receptor interacting protein 1 (CNRIP1) causes changes in ventral tegmental area (VTA) dopamine neuron activity.** Specifically, we observed significantly increases in dopamine neuron population activity (**A**; n= 6-10 rats per group) without altering the average firing rate (**B**; n= 36-118 cells per group) or percent burst firing (**C**; n= 36-118 cells per group). \*p<0.001.