# Role of circHomer1 in Synaptic Plasticity and Cocaine-Seeking Behavior

by

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ABSTRACT

Cocaine induces long-lasting changes in mesolimbic 'reward' circuits of the brain after cessation of use. These lingering changes include the neuronal plasticity that is thought to underlie the chronic relapsing nature of substance use disorders. Genes involved in neuronal plasticity also encode circular RNAs (circRNAs), which are stable, non-coding RNAs formed through the back-splicing of pre-mRNA. The Homer1 gene family, which encodes proteins associated with cocaine-induced plasticity, also encodes circHomer1. Based on preliminary evidence from shows cocaine-regulated changes in the ratio of circHomer1 and Homer1b mRNA in the nucleus accumbens (NAc), this study examined the relationship between circHomer1 and incentive motivation for cocaine by using different lengths of abstinence to vary the degree of motivation. Male and female rats were trained to self-administer cocaine (0.75 mg/kg/infusion, IV) or received a yoked saline infusion. Rats proceeded on an increasingly more difficult variable ratio schedule of lever pressing until they reached a variable ratio 5 schedule, which requires an average of 5 lever presses, and light and tone cues were delivered with the drug infusions. Rats were then tested for cocaine-seeking behavior in response to cue presentations without drug delivery either 1 or 21 days after their last self-administration session. They were sacrificed immediately after and circHomer1 and Homer1b expression was then measured from homogenate and synaptosomal fractions of NAc shell using RT-qPCR. Lever pressing during the cue reactivity test increased from 1 to 21 days of abstinence as expected. Results showed no group differences in synaptic circHomer1 expression, however, total circHomer1 expression was downregulated in 21d rats compared to controls. Lack of change in synaptic circHomer1 was likely due to trends toward different

temporal changes in males versus females. Total Homer1b expression was higher in females, although there was no effect of cocaine abstinence. Further research investigating the time course of circHomer1 and Homer1b expression is warranted based on the inverse relationship between total circHomer1and cocaine-seeking behavior observed in this study.

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# CHAPTER 1

# INTRODUCTION

Substance use disorders (SUDs) are a pervasive societal issue that has enormous human and economic consequences. Factoring in medical expenses, crime, and lost productivity, the annual cost of SUDs in the United States reaches nearly $700 billion/year (NIDA, 2022). The National Institute on Drug Abuse estimates that between 40-60% of patients treated for SUDs will experience relapse (American Psychiatric Association, 2013; NIDA, 2022). A noted contributor to relapse is a learned association of drugs with cues that are present, such as a bottle to alcohol or lighter to cigarettes. These cues acquire incentive salience as they are repeatedly associated with the drug-taking experience (Robinson & Berridge, 2001). Interestingly, the incentive motivational effects of the cues become strong enough to induce drug craving, and even strengthen their ability to do so as patients abstain from drug taking (Gawin & Kleber, 1986.) Despite the devastating consequences of SUDs, no FDA-approved treatment currently exists to help prevent relapse due to cue-induced drug craving. Thus, further research is required to parse the mechanisms involved.

To better understand the neurobiological mechanisms underlying cue-induced motivation for drug and drug-seeking behavior, preclinical researchers have developed animal models for studying this phenomena (Belin-Rauscent et al., 2016; Brownell & Gold, 2012; Smith, 2020). For example, self-administration is a powerful paradigm for studying the volitional aspects of SUDs (Belin-Rauscent et al., 2016; Markou et al., 1993; Smith, 2020). In this model, animals are trained in operant conditioning chambers to perform a task, such as pressing a lever, to obtain a drug reward. Additionally, cues such

as a light and tone are paired with drug administration such that these cues become associated with the drug-taking experience. Since these cues acquire incentive salience, they become useful tools for eliciting drug-seeking behavior. Specifically in cue-reactivity testing, animals return to operant conditioning chambers following a period of abstinence from self-administration. Animals are presented with the opportunity to press the lever, which still results in presentation of the light and tone cues, but no drug is available. Since animals promptly complete the task despite the absence of drug reward, the drug-seeking behavior is understood to occur due to the incentive salience of these reminder cues, similar to self-reports of drug-craving when people are exposed to reminder cues (Belin-Rauscent et al., 2016, p.; Markou et al., 1993; Smith, 2020). Another important pre-clinical model of drug seeking behavior to note is conditioned place preference (Huston et al., 2013; Tzschentke, 2007). In this model, animals are trained to associate a distinct side of an apparatus with a stimulus, such as injections of a drug, and another side with a control, such as saline injections. Animals are then allowed to freely roam between both sides of the apparatus, and the time spent in either side is measured. Preference for one side is thought to reflect motivation to seek out the experience conditioned to that side, and is indicative of persistent memory of that experience (McKendrick & Graziane, 2020; Tzschentke, 2007).

Models of drug-seeking behavior have been widely used to better understand the neural mechanisms of cue-induced motivation for drugs and drug seeking behavior (Lu et al., 2004; Markou et al., 1993; Smith, 2020; Wolf, 2016). These models helped successfully characterize the significant increase in cue-induced motivation that occurs with prolonged abstinence — termed ‘the incubation of craving’(Grimm et al., 2001;

Neisewander et al., 2000; Tran-Nguyen et al., 1998). The precise neurobiological mechanisms underlying the incubation of craving, however, remain obscure. Several brain regions within the cortex and limbic system have emerged as relevant targets of study. Notably, the nucleus accumbens (NAc) is implicated in drug reinforcement and motivation, and its subdivisions, the core and shell, interface with the prefrontal cortex (PFC) and other limbic regions (Kelley, 2004; Koob & Volkow, 2010). The PFC is involved in executive functioning related to drug-taking behaviors, the basolateral amygdala attributes incentive salience to reward cues, and the hippocampus is vital to contextual and long-term memory related to drug taking (Kelley, 2004; Koob & Volkow, 2010). Within this circuitry, the nucleus accumbens is thought to be an interface between limbic processing and motor output in response and is under control by glutamatergic inputs from the PFC onto mesolimbic dopamine neurons. These dopamine neurons originate in the ventral tegmental area and project to the NAc. Psychostimulants induce long-lasting changes within these mesolimbic neurons and their targets that persist far after cessation of substance use (Stuber et al., 2010). These changes include synaptic plasticity, which is a fundamental aspect of learning and memory (Sweatt, 2016). More specifically, pre and post-synaptic changes related to glutamate transmission within these circuits facilitate learning and memory of drug reward (Hyman et al., 2006; Hyman & Malenka, 2001; Kalivas, 2009; Kalivas & Volkow, 2005). To understand the incubation of craving, it is imperative to further explore the complex neurobiology involved in regulating changes in synaptic plasticity during abstinence.

**Role of Homer1 in Synaptic Plasticity and Addiction**

Repeated activity at excitatory synapses results in synaptic modifications that can influence the strength of future signaling at the synapse, otherwise known as synaptic plasticity. An example is long term potentiation (LTP), which occurs when glutamatergic activation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) depolarizes the post-synaptic membrane, which then results in unblocking of N-methyl-D-aspartate receptors (NMDARs) and the subsequent Ca++ influx initiates signaling for insertion of additional AMPARs (Huganir & Nicoll, 2013). This heightened density of receptors allows for increased glutamate binding and facilitates enhanced stimulation of the post-synaptic neuron and stronger connections specifically at the active synapses in response to future depolarization events. This mechanism, initially proposed by Donald Hebb, is thought to underly behaviors of reinforced learning and memory, including Pavlovian conditioning (Citri & Malenka, 2008; Sweatt, 2016). Paradoxically, LTP drives neuronal excitability in a positive feedback loop that, if left unchecked, would escalate and possibly de-stabilize the neuron (Turrigiano & Nelson, 2004). Homeostatic synaptic plasticity compensates for these fluctuations in activity at individuals synapses by mobilizing cell wide receptor and morphological changes to maintain homeostasis in the neuron (Turrigiano & Nelson, 2004). Synaptic scaling is an example of these homeostatic changes, wherin, neurons respond to heightened rates of synaptic activity with negative feedback mechanisms at excitatory synapses, resulting in the stabilization of action potential firing rates (Sweatt, 2016; Turrigiano, 2008). As they are intrinsically related to Hebbian plasticity, these global changes in synaptic plasticity likely play a vital

role in regulation of behavior related to learning and memory of drug reward and the salience of drug cues for predicting drug reward.

Changes in homeostatic synaptic plasticity involve the Homer gene family, which encodes for various synaptic proteins (Bockaert et al., 2021; Clifton et al., 2019; Iasevoli et al., 2013; Shiraishi et al., 2004). These proteins are highly conserved across mammals and are expressed in corticolimbic regions related to reward, decision-making, learning, and memory (Shiraishi et al., 2004; Xiao et al., 1998). Homer protein homolog 1 (Homer1,) a widely studied member of this gene family, encodes for multiple RNA isoforms through alternative splicing (Bottai et al., 2002; Klugmann et al., 2005). These isoforms appear in two distinct variants, long and short form, such as *Homer1b/c* and *Homer1a* respectively. Long form Homer1 proteins contain a C-terminal CC coiled domain and are constituently expressed (Beneken et al., 2000; Kammermeier et al., 2000; Klugmann et al., 2005). CC domains allow for long form Homer1 proteins to self-multimerize and thus act as a cytoskeletal scaffold for type 1,5 metabotropic glutamate receptors (mGluR1,5), SH3 and multiple ankyrin repeat domains 3 (SHANK), and inositol triphosphate receptors ($IP_3$). Additionally, letting these long form Homer1 proteins influence AMPAR insertion, and act as a signal transduction molecule for mGluRs (Beneken et al., 2000; Xiao et al., 1998). Short form Homer1 proteins lack this CC domain and are activity dependent immediate early genes (IEG;Beneken et al., 2000; Kammermeier et al., 2000). Activity induced expression of these short form proteins inhibits self-multimerization, disrupting protein scaffolding of CC-Homers, therefore negatively regulating excitability of the post-synaptic membrane (Klugmann et al., 2005; Szumlinski et al., 2008). Consequently, dynamic expression of these Homer1 proteins

plays an important role in regulation of neuronal excitability and homeostatic synaptic scaling.

The Homer1 gene family is widely implicated in SUDs. A knockout of the Homer1 gene in mice induces heightened sensitivity to cocaine and enhances cocaine conditioned place preference (Szumlinski et al., 2004). Activity induced IEG-Homer1a shows a rapid, albeit transient, increase in expression in the NAc and the striatum following acute administration of psychostimulants (Hashimoto et al., 2007; Zhang et al., 2007). Withdrawal from cocaine results in decreased CC-Homer1b/c protein levels in the NAc shell of both mice and rats, and these changes are associated with mGluR expression (Ary & Szumlinski, 2007). In contrast, similar research shows an increase of CC-Homer1b/c protein levels in synaptosomal fractions of the NAc shell following withdrawal from cocaine (Ghasemzadeh et al., 2009). Following extinction training, which is a procedure of withholding reinforcement resulting in reduction of cocaine-seeking behavior, CC-Homer1b/c protein expression shows a significant decrease in both the synaptosomal fractions and total neuronal homogenate of the NAc shell (Ghasemzadeh et al., 2009). This evidence suggests a role for IEG Homer1a in synaptic scaling related to acute drug exposure and CC-Homer1b/c in maladaptive synaptic plasticity related to drug withdrawal and craving.

**Circular RNAs in the Brain**

Circular RNAs (circRNAs) are novel non-coding RNAs that are formed through pre-mRNA alternative back splicing, resulting in a single stranded, covalently bonded, closed loop structure (Hansen et al., 2013; Jeck et al., 2013; Memczak et al., 2013). Discovered in the late 1970s, these molecules were initially thought to be a specialized

product of plant viruses termed ‘viroids’ (Hsu & Coca-Prados, 1979; Sänger et al., 1976). Subsequently, several groups in the early 1990s further characterized these molecules in eukaryotes, although they were believed to be products of mis-splicing (Capel et al., 1993; Cocquerelle et al., 1993). In contrast to earlier work, recent exploration of circRNAs has revealed them to be functional, abundantly expressed across tissue types, and highly conserved among eukaryotes (Guo et al., 2014; Jeck et al., 2013; Rybak-Wolf et al., 2015). Interestingly, for many human genes, circular isoforms are expressed in higher abundance than mRNA transcripts (Ashwal-Fluss et al., 2014; Jeck & Sharpless, 2014; Salzman et al., 2012). circRNAs can be comprised completely of introns or exons but are often comprised of both. Alternative back splicing covalently links the 3’ and 5’ prime ends of the transcript, and consequently circRNAs lack a 5’ cap and Poly-A tail (Ashwal-Fluss et al., 2014; Jeck et al., 2013; Salzman et al., 2012), making circRNAs far more stable and less susceptible to exonuclease activity compared to linear RNA. These findings, amongst others, have led to a resurgence of circRNA research in the last decade.

Though the precise biological function of circRNAs remains elusive, overall they appear to act as post-transcriptional regulators of gene expression (Hansen et al., 2013; Li et al., 2015; Memczak et al., 2013). For example, some circRNAs serve as ‘sponges’ to sequester microRNAs (miRNAs) and thus disrupt miRNA binding to the 3’ UTR of mRNA (Jeck et al., 2013; Memczak et al., 2013; Rybak-Wolf et al., 2015). Although, it is disputed if most circRNAs act as miRNA sponges (Guo et al., 2014). Most circRNAs however, are derived from the same pre-mRNA as their host gene (Ashwal-Fluss et al., 2014; Pamudurti et al., 2017). Due to this shared pre-mRNA stage, circRNAs may directly regulate gene expression by their back-splicing biogenesis competing with the

canonical splicing of their linear mRNA counterparts (Ashwal-Fluss et al., 2014). Furthermore, circRNAs interact with RNA binding proteins (RBPs), which are involved in circRNA biogenesis and mRNA regulatory pathways (Conn et al., 2015; Dell'Orco et al., 2020; Knupp et al., 2021, p. 2; Zang et al., 2020).

Interestingly, circRNAs are conserved and highly expressed in the mammalian brain, are localized to dendrites, and are derived primarily from genes related to development and plasticity (Rybak-Wolf et al., 2015; You et al., 2015). circRNAs are implicated in various central nervous system disorders. Expression of circRNAs is significantly upregulated in the aging mouse brain (Gruner et al., 2016). Additionally, postmortem brain samples from the dorsolateral prefrontal cortex of patients suffering from schizophrenia show marked reduction in circRNA expression relative to healthy controls (Mahmoudi et al., 2019). Low-effort cocaine self-administration in mice dysregulates expression of circRNAs in the striatum, a region that includes the NAc (Bu et al., 2019). No study to date, however, has investigated the potential role that these molecules play in synaptic plasticity underlying the incubation of craving.

**A Potential Role for CircHomer1**

The Homer1 gene encodes a circRNA, '*circHomer1'*. *CircHomer1* is formed via the back-splicing of exons 2-5 from the pre-mRNA of CC-containing *Homer1b* (Hafez et al., 2022; Zimmerman et al., 2020). This circRNA is highly expressed in neuronal tissues and is localized to synaptic densities, akin to its linear *Homer1b* counterpart (Iasevoli et al., 2013; You et al., 2015). Interestingly, *circHomer1* expression is significantly upregulated in hippocampal neurons following activity induced homeostatic plasticity, suggesting its role in synaptic regulation (You et al., 2015). Expression of *circHomer1* is

also significantly increased in NAc of conditioned placed preference trained mice (Li et al., 2019). Interestingly, a short hairpin-RNA (shRNA)-mediated knockdown of either *circHomer1* or *Homer1b* in the orbitofrontal cortex of mice induces increased synaptic expression of the other, respectively (Hafez et al., 2022). Indeed, this finding points to an interplay between the two at the synapse. The binding protein HuD plays a role in trafficking *Homer1b* transcripts to the synapse; *circHomer1* contains the same exons, and same binding site for HuD as *Homer1b* (Dell'Orco et al., 2020; Hafez et al., 2022; Zimmerman et al., 2020). Such evidence suggests *circHomer1* may inhibit *Homer1b* trafficking to the synapse, which would likely reduce the translation of the Homer1b protein and influence synaptic plasticity. Through this interplay between linear *Homer1b* and *circHomer1, circHomer1* may play a similar role as the IEG-Homer1a in regulating CC-Homer1b/c expression, and therefore act as a negative regulator of synaptic activity. Such impacts on synaptic activity in the NAc would likely play a role in cue-induced motivation for cocaine. Preliminary RNA-seq evidence from an environmental enrichment (EE) study in our lab, where EE animals had significantly reduced cue-induced cocaine-seeking behavior compared to isolated rats, shows that ratios of *circHomer1* to *Homer1b* expression in the NAc shell are dysregulated.

**Rationale for The Present Study**

Utilizing the cue-reactivity model to examine motivation for cocaine, the study herein aims to establish the relationship between *circHomer1* and its long form linear counterpart *Homer1b*, in the NAc shell of rats with differing degrees of cue-induced motivation for cocaine. We hypothesize, based on relevant literature and our RNA-seq data, that *circHomer1* regulates synaptic *Homer1b* expression disrupting resultant

Homer1b protein expression in the NAc shell, thereby influencing motivation for cocaine. Thus, we predict that: 1) Cue-induced motivation for cocaine is related to decreases in *circHomer1* expression, 2) these decreases in *circHomer1* expression are related to increases in *Homer1b* expression, and 3) that these changes will be more pronounced at the synapse.

Cue-induced motivation for cocaine was modulated by varying the time rats spend abstinent from cocaine following self-administration prior to cue-reactivity testing (i.e., 1 or 21 days abstinent from drug). Unlike preliminary experiments, both male and female rats were studied and compared to yoked-saline controls who received a saline infusion and exposure to cues each time his/her cocaine partner received cocaine. This design informs how expression of circHomer1 and Homer1b RNA varies across sex, drug, and abstinence. Expression of these RNAs was analyzed using RT-qPCR in both synaptosomal fractions and total homogenate of the NAc shell. This study serves as the first utilizing complex behavioral models and RT-qPCR to understand circRNA expression involved in the maladaptive synaptic plasticity underlying the incubation of craving.

# CHAPTER 2

# METHODOLOGY

## Animals

Male and female Sprague-Dawley rats (Charles River Laboratories, San Diego, CA) weighing 200-225 g upon arrival were used for all experiments. Rats were separated into standard, individual housing units (21.6 x 45.7 x 17.8 cm) in a temperature-controlled environment on a 14:10 dark:light cycle. Rats were handled daily during each of the 5 days leading up to surgery and they received *ad libitum* food and water except during initial training sessions when they were food-restricted as described below. All procedures were approved by the Institutional Animal Care and Use Committee of Arizona State University and were in accordance with institutional and NIH guidelines.

## Surgeries

Prior to surgery, rats (N = 48) were anesthetized using 2-5% isoflurane (MWI, Boise, Idaho) and underwent surgical implantation of intravenous polyurethane catheters (0.63 x 1.02 mm; Instech Laboratories Inc., Plymouth Meeting, PA, USA) into the right jugular vein as previously described (Powell et al. 2019) . Briefly, catheters were implanted into the jugular vein and then tunneled subcutaneously to a dermal exit point between the shoulder blades where they were attached to a back-pack style cannula (Instech Laboratories Inc., Plymouth Meeting, PA, USA)

Immediately following surgical procedures, rats were treated with cefazolin (100 mg/mL, IV) antibiotic, and buprenorphine (0.05 mg/kg/mL SC) and meloxicam (1 mg/kg/mL, SC) analgesics. Cefazolin (10 mg/0.1mL, IV) was dissolved in sterile saline containing heparin (70 U/mL) and was administered daily for 5 days post-surgery.

Meloxicam was administered on each of the 2 days following surgery. Catheters were flushed with sterile saline containing heparin (70 U/mL, IV) twice daily throughout self-administration to maintain patency.

**Self-administration, Forced Abstinence, and Cue Reactivity Testing**

Behavioral self-administration experiments took place in operant conditioning chambers (30 × 24 × 21 cm; Med Associates Inc, St. Albans, VT) which contained two-levers, designated as an active and inactive lever, a cue light above the active lever, and a tone generator. Pressing of the active lever resulted in presentation of the light and tone cues. Prior to self-administration, rats were transitioned onto a food-restriction schedule of 16 g standard rat chow daily, to maintain approximately 90% free -feeding body weight, with access to water remaining *ad libitum.* Rats were fed immediately following their self-administration sessions. Following surgical recovery, rats were habituated to their environment before beginning self-administration, with two, 1-hour sessions where levers were retracted in their respective operant chambers over the course of two days. Rats (N=48) were trained in 2-hour cocaine (0.75 mg/kg/0.1 mL IV) self-administration sessions daily, 6 days/week. Completion of the operant schedule of lever pressing on the active lever resulted in onset of a light and audio cue followed by an infusion of cocaine over 6-sec. The infusion pump and cues were then inactivated. Presses on the inactive lever were also recorded, though they did not produce any consequences.

Rats began self-administration on a fixed-ratio (FR) 1 schedule of cocaine reinforcement (0.75 mg/kg, IV) and progressed within session to variable ratio (VR) 2, VR3, and VR5 schedules sequentially if 7 lever presses occurred within 1 h. For FR1, infusions were capped at 50 to avoid cocaine overdose. If a rat ended 3 consecutive

sessions on a VR5 schedule of reinforcement, its starting schedule would be advanced for the next session. As the starting schedule increased from FR1 to VR5, rats were weaned from food restriction and then returned to free-feeding after 3 sessions starting on the VR5 schedule. After ending 3 consecutive sessions on VR5 within a 25% coefficient of variability for infusions, animals were transitioned into forced abstinence. Rats were assigned to either 1 or 21 days of abstinence, counterbalanced by the average infusions per session during training. Rats remained in their individual housing units and were handled daily during abstinence. Post-abstinence, animals were returned to their operant conditioning chambers for a 1-h cue reactivity test. During this test, lever presses were paired with a 7-s light and tone cue, however, no drug was available. Responses during the test reflect an animal's cue-induced motivation for cocaine, as cues are conditioned to drug stimulus. Immediately after cue reactivity testing, animals were deeply anesthetized by isoflurane and euthanized via decapitation.

**Tissue Collection**

Brains were extracted and dissected in a brain matrix with groves for a razor blade spaced 1 mm apart along the horizontal plane. Utilizing ventral landmarks on the brain obtained from the rat brain atlas, 2 mm slices containing the striatum were taken (Paxinos & Watson, 2006). A 1.2 mm punch of the NAc core was taken, followed by a 2 mm overlay to punch the NAc shell (Harris Uni-Core™). Tissue punches were homogenized in 200 μL of ice-cold homogenization buffer (0.1 mM EDTA, 0.25 mM DTT, 2 mM HEPES in a 7.5 pH, 0.32 M sucrose solution, 200) and stored on ice awaiting further processing, while the remainder of the brain was flash frozen using methyl-butane and stored at -80°C.

**Crude Synaptosomal Isolation**

Crude synaptosomal fractions were isolated as described previously (Boese et al., 2016; Rao & Steward, 1991). Of the initial 200 µL of homogenized tissue, 50 µL representing total homogenized sample (TOT) was pipetted into a separate tube and stored at -80°C. The remaining samples of 150 µL were spun at 2,000 x *g* for 2 min. This resulted in a small, cloudy pellet representing cellular nuclei and debris (P1). The remainder of the supernatant was collected in a separate tube (S1b). P1 was then resuspended in 100µL fresh homogenization buffer and centrifuged at 2,000 x *g* for 2 minutes. Additional supernatant was added to S1b.

Combined supernatants (S1b) were spun at 17,000x*g* for 10 minutes to isolate crude synaptosomal fractions. The supernatant was removed and discarded; the resultant pellet (P2) constituted the crude synaptosome. All samples were then stored at -80°C.

**cDNA Synthesis and RT-qPCR**

RNA was isolated from homogenized tissue samples using Trizol® (Invitrogen, # 15596026) extraction and ethanol precipitation in the presence of GlycoBlue™ (Invitrogen, # AM9516). RNA concentrations were determined using the Qubit™ RNA HS Assay Kit (Invitrogen, # Q32852). For all samples, approximately 35 ng of RNA were used to prepare cDNA using SuperScript™ III First-Strand Synthesis System (Invitrogen, # 18080051). Random hexamer primers, a mixture of oligonucleotides representing all possible hexamer combinations which are capable of annealing to RNA species lacking a poly-A tail, were utilized for circRNA detection. Oligo-dT primers, which are specialized to anneal with fully mature poly-A tail containing mRNA, were utilized for linear mRNA detection. Primers were used in conjunction with cDNA and

xiTaq™ Universal SYBR® Green Supermix (BioRad, #1725121) for RT-qPCR. *circHomer1* primers were developed to specifically avoid targeting and amplifying linear *Homer1b* mRNA sequences. Expression of target sequences was quantified by the comparative $2^{-\Delta CT}$ method using GAPDH as a control (Litvak & Schmittgen, 2001)

***Table 1. Primer Targets and Sequences for RT-qPCR***

| Target | Forward Sequence | Reverse Sequence |
|---|---|---|
| *circ-Homer1* | TCAATGGGACAGATGATGAG | TTGTGTTTGGGTCGATCTGG |
| *Homer1b* | ACACCCGATGTGACACAGAA | CACTGCTTCACGTTGGCAGT |
| *Gapdh* | CTCTCTGCTCCTCCCTGTTC | TACGGCCAAATCCGTTCACA |

***Statistical Analyses***

Statistical analyses were performed in R-4.1.2 and Graph-Pad Prism 8. Behavioral analyses were analyzed with repeated measures ANOVAs (type III). For all analyses, saline-yoked controls were combined into a single group across 1- and 21-day abstinence lengths as there were no significant differences between these groups. Expression values greater than two standard deviations from the mean of their respective group were treated as outliers. If there were multiple outliers within the same group, then neither were removed. RT-qPCR data were analyzed using a mixed-effects ANOVA (type III) with two fixed effects (sex and group) and one random effect (cohort) to account for variability between cohorts. The output from these ANOVAs can be found in the Appendix 1.

# CHAPTER 3

# RESULTS

## Acquisition of Cocaine Self-Administration

Rats underwent training on a variable ratio schedule of cocaine reinforcement during which they learned to associate an active lever response and cues with an infusion of cocaine; controls received the same cues and an infusion of saline each time their yoked partner received cocaine. Cue reactivity was measured after a 1- or 21-day abstinence period as lever presses on the active lever, which resulted in the cue presentation but no cocaine (see Figure 1A for timeline). A repeated measures ANOVA was used to analyze active and inactive lever pressing in cocaine and saline-yoked control rats during their last three sessions of self-administration since all cocaine rats were exhibiting <25% variance in reinforcement rates at this time. An outlier was removed from the cocaine inactive lever presses analysis due to exhibiting extreme stereotypic behavior (lever presses > 800 per session). The ANOVA revealed a significant effect of session ($F_{1.5,130.9} = 4.1$, $p < 0.05$), drug ($F_{1,89} = 87.0$, $p < 0.001$), and lever ($F_{1,89} = 79.3$, $p < 0.001$), and an interaction effect of drug by lever ($F_{1,89} = 102.7$, $p < 0.0001$). *Post hoc* Tukey's test of the interaction effect found that for each session, cocaine-trained rats had significantly higher active lever presses than inactive lever presses ($p < 0.05$), but there was no difference in active versus inactive lever presses in saline-yoked controls (Figure 1B) and both measures were similar to inactive lever presses of cocaine-trained rats. This higher response rate of cocaine rats on the active lever is indicative of successful lever discrimination and demonstrates operant conditioning. Low lever pressing on both levers in saline-yoked rats is indicative that no

such conditioning occurred. Upon completion of self-administration, rats were sorted into either 1- or 21-day abstinence groups that were counterbalanced based on cocaine intake during the last three self-administration sessions. A repeated measures ANOVA verified that there were no significant differences between sessions in infusion rates due to sex, nor abstinence length across all three days (Figure 1C), ensuring that any differences between these groups in cue-reactivity or gene expression were not due to differences in self-administration history.

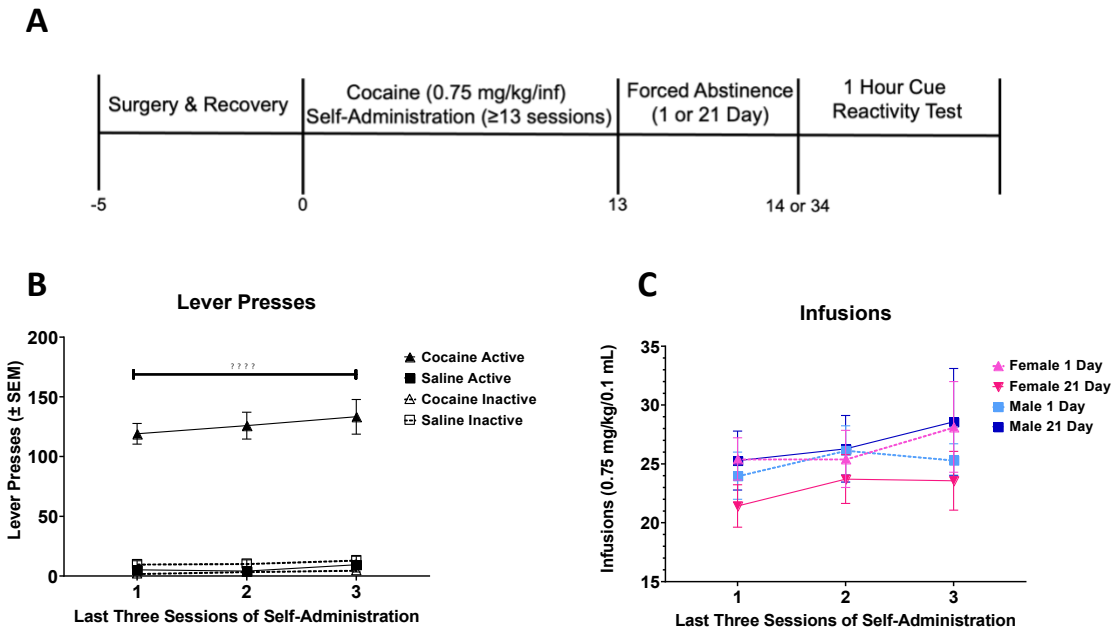


***Figure 1. (A)*** Experimental timeline and ***(B)*** active and inactive lever presses (mean±SEM) in cocaine self-administering rats and their saline-yoked controls during the last three training sessions under a VR5 schedule of cocaine reinforcement (Cocaine; n = 27) or yoked saline administration (Saline; n = 21). * Indicates that active lever response rate is significantly higher for each session compared to inactive lever response rate and response rates on both levers for saline-yoked controls (Tukey test, $p<0.05$). ***(C)*** Cocaine infusions (0.75 mg/kg/0.1mL) per session during the last three training sessions in male and female rats assigned to 1 day (Female, n = 7; Male, n = 6) or 21 days (Female, n = 7; Male, n = 7) abstinence.

***Cue reactivity tests after abstinence***

To assess cocaine-seeking behavior following abstinence, rats underwent cue-reactivity testing, wherein active lever presses resulted in light/tone cue presentations but no cocaine or saline infusions. If a value exceeded two standard deviations from the mean, it was considered an outlier. Two outliers were removed prior to analysis of behavior, one from the male saline and one from the female saline group. A two-way ANOVA of sex and abstinence was performed to analyze active lever presses during cue-reactivity testing. The ANOVA revealed a significant effect of sex ($F_{1, 40} = 5.0$, $p < 0.05$; Figure 2B), a significant effect of abstinence ($F_{2, 40} = 49.9$, $p < 0.0001$), however, no significant sex by abstinence interaction. Tukey's *post-hoc* tests of the abstinence effect revealed that each group significantly differed from the other two groups ($p < 0.001$), these results are consistent with the "incubation of craving" effect (Figure 2A).

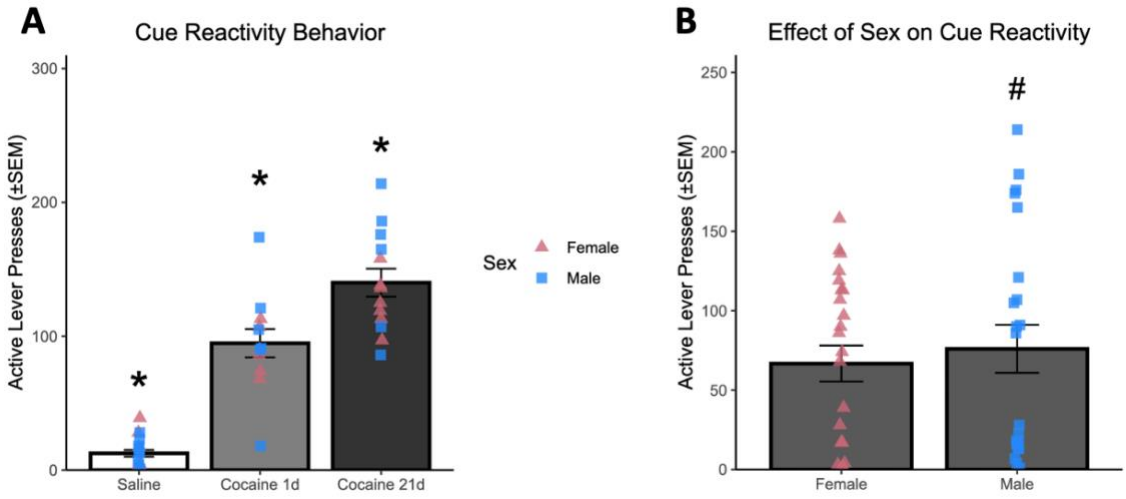


***Figure 2.*** (**A**) Mean active lever presses (±SEM) during cue-reactivity testing. Rats completed cocaine self-administration or were saline-yoked controls, and underwent 1 (n = 13) or 21 days (n = 14) of forced abstinence. Saline-yoked controls were combined into a control group (n = 21). (**B**) Main effect of sex on mean active lever presses (±SEM) during cue reactivity (Male; n = 23, Female; n = 23). * Indicates a significant difference from all other groups, Tukey test, $p<0.05$, # Indicates main effect of sex, $p <0.05$.

**circHomer1 Expression**

To understand the relationship between increased cue-induced motivation for cocaine and *circHomer1* expression, total neuronal and crude synaptosomal *circHomer1* levels in the NAc shell were quantified using RT-qPCR. To analyze RNA expression data, a mixed effects ANOVA with two fixed effects (group and sex) and one random effect (cohort) was used. Prior to analysis of total *circHomer1* expression, one male outlier was removed from the 21-day cocaine group. The ANOVA found no significant effect of sex, however, there was a significant effect of cohort ($p < 0.0005$), and of group ($F_{2, 39} = 4.46$, $p < 0.05$) on total *circHomer1* expression. *Post-hoc* Tukey's tests of the group effect indicated a significant decrease in *circHomer1* expression in the 21-day cocaine group when compared to the saline group ($p < 0.05$) but found no significant difference between the 1-day cocaine abstinence group and the other two groups (Figure 3A.) Prior to analysis of crude synaptosomal *circHomer1* expression, a male and a female outlier from the saline group were removed. A mixed effects ANOVA indicated no significant effects of group or of sex, however, it did reveal a significant effect of cohort ($p < 0.0001$) and a sex by group interaction ($F_{2, 38} = 4.78$, $p < 0.01$) on synaptosomal *circHomer1* expression. *Post-hoc* Tukey's tests of the interaction effect, however, failed to reveal a significant source of the interaction as there were no group differences (Figure 3B.) Pearson's correlation tests of both total and synaptosomal *circHomer1* expression with active lever pressing during cue-reactivity were conducted, however, showed no significant effects.

**Homer1b Expression**

Akin to *circHomer1* RNA, total homogenate and crude synaptosomal *Homer1b* mRNA expression in the NAc shell was quantified using RT-qPCR. One outlier was removed from the male saline group prior to analysis of total *Homer1b* expression. A mixed effects ANOVA revealed significant effects of cohort ($p < 0.05$) and of sex ($F_{1, 39} = 10.71$, $p < 0.005$), however, no effect of group, nor interaction of sex by group for total *Homer1b* expression (Figure 3C). Prior to the analysis of crude synaptosomal *Homer1b* expression, two male and female outliers were removed from the saline group. A mixed effects ANOVA revealed no significant effects of cohort, sex, abstinence, nor an interaction effect on synaptosomal *Homer1b* expression (Figure 3D). Additionally, Pearson's correlation tests of both total and synaptosomal *Homer1b* expression to active lever pressing during cue reactivity were conducted, however, they showed no significant effects.

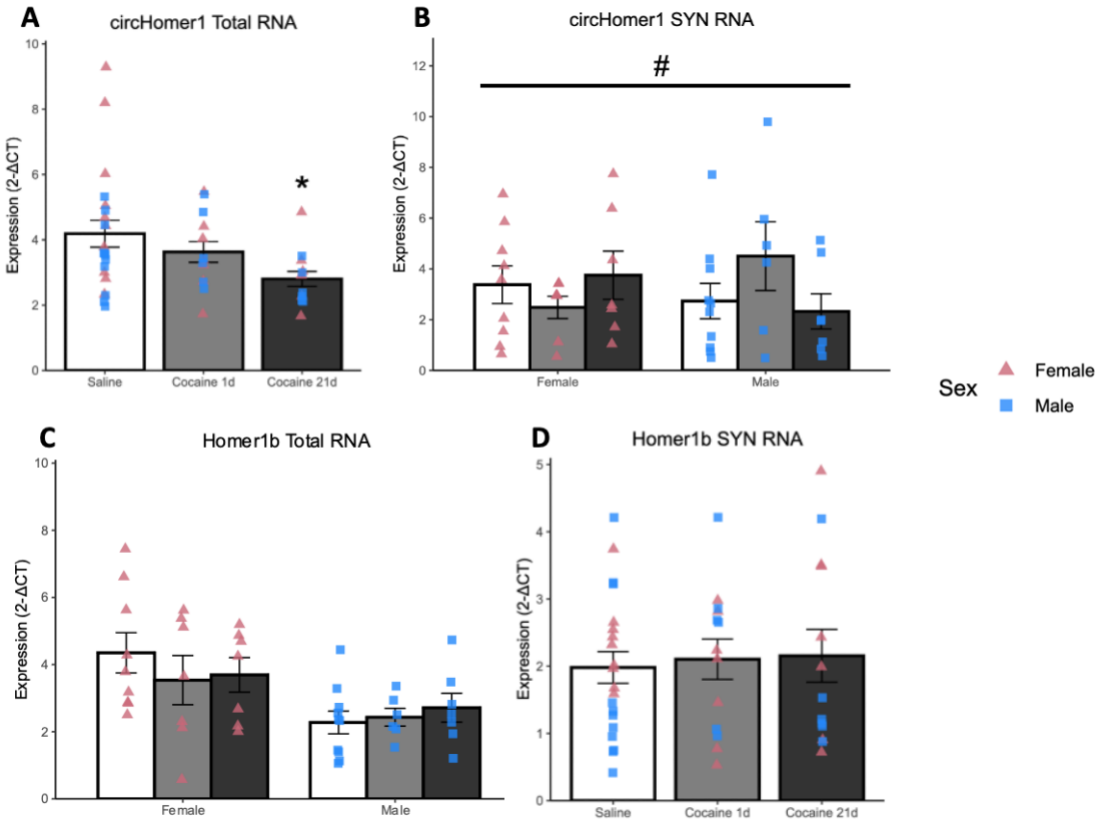


***Figure 3.*** *circHomer1* RNA and *Homer1b* mRNA measured in NAc shell homogenate (**A, C,** respectively) and in crude synaptosomal fractions of NAc shell (**B, D**, respectively) using RT-qPCR. Values are presented as 2-ΔCT (+SEM) with GAPDH as a control (Litvak & Schmittgen, 2001) and are graphed separately for males and females for analyses revealing an effect of sex. * Indicates significant difference from saline, *post-hoc* Tukey's test, $p < 0.05$. # Indicates interaction effect of group by sex. (**A**, **D** Saline, n = 20-21; Cocaine 1d, n = 13; Cocaine 21d, n = 13-14) (**B**, **C** Female Saline, n = 9-10; Female Cocaine 1d, n = 7; Female 21d, n = 7; Male Saline, n = 10; Male 1d, n = 6; Male 21d, n = 7).

**Ratio of Homer1b mRNA RNA and circHomer1 Expression**

The relative expression of *Homer1b* to *circHomer1* transcripts in both the total homogenate and crude synaptosomal fractions were analyzed. For each subject, *Homer1b* ($2^{-\Delta CT}$) was divided by *circHomer1* expression ($2^{-\Delta CT}$) expression. If ratio values exceeded two standard deviations from the mean they were considered an outlier and excluded prior to analysis. For the ratio of total NAc shell expression, three outliers were removed prior to analysis. A mixed effects ANOVA revealed no significant effect of

cohort, a significant main effect of sex ($F_{1, 39} = 8.09$, $p < 0.01$), but no effect of abstinence, nor interaction of sex by abstinence in the ratios of total expression. Prior to analysis of transcript ratios in crude synaptosomal fractions of the NAc shell, three outliers were removed. A mixed effects ANOVA revealed a significant effect of cohort ($p = 0.0002$), however, there was no significant effect of sex, abstinence, nor an interaction effect in the ratio of synaptosomal expression. Pearson's correlation tests between expression ratios in both total and synaptosomal tissues and active lever pressing during cue reactivity were conducted, however, neither showed significance.

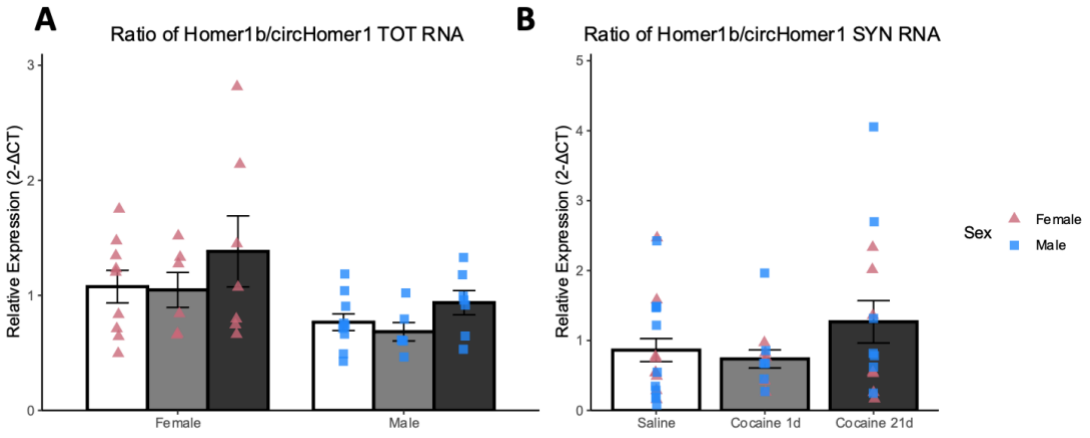


***Figure 4.*** Ratio of *Homer1b* mRNA to *circHomer1* RNA measured in NAc shell homogenate (**A,** Female Saline, n = 9; Female Cocaine 1d, n = 7; Female Cocaine 21d, n = 7; Male Saline, n = 10; Male Cocaine 1d, n = 6; Male Cocaine 21d, n = 7) and in crude synaptosomal fractions of NAc shell (**B,** Saline; n = 21, Cocaine 1d; n = 13, Cocaine 21d; n = 14) using RT-qPCR. All ratio values were computed by dividing *Homer1b* 2-ΔCT expression by *circHomer1* 2-ΔCT expression.

CHAPTER 4

# DISCUSSION

In this study we utilized a cue-reactivity model to assess motivation for cocaine and its relationship to the expression of the novel circRNA *circHomer1*. We focused on *circHomer1* because it is derived from a gene family which is highly implicated in the synaptic plasticity related to SUDs based on preclinical animal models (Ary & Szumlinski, 2007; Ghasemzadeh et al., 2009; Szumlinski et al., 2006, 2008). Indeed, *Homer1b* encodes for a protein that is positively associated with increases in motivation for cocaine and modulates glutamatergic signaling in the NAc shell (Ghasemzadeh et al., 2009; Ghasemzadeh et al., 2009). Recent evidence has emerged which indicates that *circHomer1* expression interplays with *Homer1b* mRNA as they are derived from the same pre-mRNA and contain the same binding sites for HuD, which is involved in their synaptic trafficking (Dell'Orco et al., 2020; Hafez et al., 2022; Zimmerman et al., 2020). We hypothesized that *circHomer1* regulates synaptic expression of *Homer1b* and acts to disrupt resultant Homer1b protein expression in the NAc shell and influence motivation for cocaine. Thus, we predicted that: 1) increases in motivation for cocaine would be inversely related to *circHomer1* expression in the NAc shell, 2) that these decreases in *circHomer1* expression would be related to increases in linear *Homer1b* expression, and 3) that these effects would be pronounced at the synapse. Our findings did not support our hypothesis as detailed below, however, there is still much to be understood about the role of *circHomer1* in cue induced motivation for cocaine.

**circHomer1 Expression is Significantly Down-regulated After Abstinence**

RT-qPCR analysis of total RNA samples showed that *circHomer1* expression was significantly decreased at 21 days of abstinence when compared to the saline control group. Behaviorally, this 21-day abstinence group showed the highest cue-induced motivation for cocaine, significantly higher than both the 1-day abstinence group and the saline group. This inverse relationship between total *circHomer1* expression and cue-induced motivation for cocaine supports our overarching hypothesis that *circHomer1* plays a protective role in these behaviors. Interestingly, the literature that exists on *circHomer1* expression in relation to psychostimulant motivation is mixed. Akin to our findings, methamphetamine conditioned place preference, a model of approach toward an environment associated with methamphetamine experience, is higher in mice expressing decreased levels of *circHomer1* in the NAc compared to control mice (Li et al., 2020). Contrastingly, a study with similar methodology found a near opposite effect, where expression of *circHomer1* is significantly higher in the NAc of methamphetamine-conditioned mice than in control mice (Li et al., 2019). Bu et al. (2019) report that a microarray analysis in the dorsal striatum of mice following cocaine self-administration did not detect *circHomer1*, though they note that the array used was not equipped with a probe for *circHomer1* (Bu et al., 2019). An additional study using a microarray and RT-qPCR to investigate circRNA expression in the orbitofrontal cortex of heroin self-administering rats did not report detecting *circHomer1* (Floris et al., 2022). In this case, it is unclear if the array was equipped with a probe for *circHomer1* or not. Although, the lack of effect in rats self-administering heroin is not surprising because the Homer1 gene family is not widely implicated in opioid use disorders (Szumlinski et al., 2008).

Considering these findings, circHomer1 *'s* function in SUDs is far from conclusive, and further research is required to understand its role.

Much of the available literature on *circHomer1* indicates that its expression is localized to the synapse, hence we analyzed crude synaptosomal fractions of these same samples (Dube et al., 2020; Hafez et al., 2022; You et al., 2015; Zimmerman et al., 2020). Surprisingly, analysis of synaptosomal *circHomer1* expression did not reveal the same significant effect as observed with total homogenate, however, we did observe a significant sex by group interaction. *Post-hoc* tests did not find significant differences among these groups; however, males and females abstinent from cocaine for 1 day did show opposite trends toward a change of expression (Figure 3B). Females show a trend toward increases in synaptic *circHomer1* expression from 1 to 21 days of abstinence and male expression show a trend toward decreases akin to the significant effect seen in the total samples. These results suggest that synaptic circHomer1 levels may be differentially regulated in males and females during cocaine self-administration and in abstinence, but additional research is needed to verify this possibility. A single study to date has identified sex differences in *circHomer1* expression, in which, *circHomer1* is downregulated in postmortem samples of the entorhinal cortex of human female patients suffering from Alzheimer's disease, but not in males when compared to healthy controls (Urdánoz-Casado et al., 2021). However, no study to my knowledge has specifically examined whether there are sex differences in *circHomer1* expression. Sex differences, although understudied, play an important role in synaptic plasticity and in SUDs. Baseline differences in dendritic spine morphology exist between male and female rats, and are often exacerbated by environmental stimuli (Hyer et al., 2018). Female rats even

show enhanced LTP in CA1 neurons during proestrus (Warren et al., 1995). These differences can have profound impacts on synaptic communication and are thought to be mediated by differences in steroid hormones (Gall et al., n.d.; Hyer et al., 2018). In female rodents, estrus cycle phase and associated hormones also mediate many aspects of drug-taking behavior (Johnson et al., 2019; Lynch & Taylor, 2005; Roberts et al., 1989). One study indicates that cues acquired during estrus result in higher motivation for cocaine and even upregulate c-fos expression in striatum when compared to both diestrus controls and male rats (Johnson et al., 2019). Indeed, cycle phase and ovarian hormone levels are an important factor to consider in neuronal plasticity related to drug-seeking behavior. Future work should further parse these possible sex differences in synaptic expression of *circHomer1* in the NAc associated with higher motivation for cocaine.

**Homer1b Expression & Expression Relative to circHomer1**

Previous literature related to cognition, learning, and memory has indicated that regulation of *circHomer1* and *Homer1b* expression are inter-related as they contain similar exonic sequences, are derived from the same pre-mRNA, and likely compete for binding with the RNA binding protein HuD for synaptic trafficking (Dube et al., 2020; Hafez et al., 2022; Zimmerman et al., 2020). RT-qPCR analysis of *Homer1b* mRNA in total homogenate of NAc shell revealed only a difference of sex, wherein total *Homer1b* expression was higher in females than males regardless of abstinence condition. We were unable to detect any differences in *Homer1b* expression at the synaptic level. Additionally, as we hypothesized that *circHomer1* and *Homer1b* expression are inter-related we examined the ratio of mRNA to circRNA as an aggregate measure of their interplay. Apart from a significant sex difference in total expression, we were unable to

detect significant differences in the ratio expression of *Homer1b* to *circHomer1*. These results are contrary to well-established research on Homer1b/c protein expression in relation to motivation for cocaine in rats. For example, following a 21-day period of withdrawal from a repeated administration regimen to induce cocaine behavioral sensitization, rats show significant increases in Homer1b/c expression in the NAc shell. Conversely in a model of extinction meant to reduce motivation for cocaine, Homer1b/c expression is decreased in both total and synaptosomal fractions of the NAc shell following extinction of cocaine-seeking behavior (Ghasemzadeh et al., 2009; Ghasemzadeh al., 2009). These findings strongly suggest that Homer1b/c expression in the NAc shell is positively linked to increases in motivation for cocaine.

The reason that *Homer1b* was not upregulated in the present study after 21 days abstinence when cocaine-seeking behavior is high is not clear. One possible cause for this could be that the 21 days abstinence was not the optimal time point to analyze changes in *Homer1b* expression during abstinence. mRNA expression changes are often transient, depending largely on active transcription and nuclease degradation, unlike protein, in which differences in expression are often longer lasting. Thus, it is possible that any transcriptional dysregulation of *Homer1b* that preceded changes in protein expression may have stabilized by the 21d abstinence time point giving rise to the non-significant differences shown in our study. As we did not observe significant effects in transcriptional *Homer1b* expression while manipulating cue-induced motivation for cocaine, future studies should measure Homer1b/c protein levels to further probe the regulatory relationship between *circHomer1* and Homer1b/c.

**Future Directions and Limitations**

We have established that *circHomer1* is downregulated in rats exhibiting elevated cocaine-seeking behavior following abstinence, and that sex differences may exist in synaptic *circHomer1* expression during abstinence. It remains in question if modulating *circHomer1* expression then influences cocaine-seeking behavior, and if this mechanism is mediated by *circHomer1*'s regulation of *Homer1b* expression. As *circHomer1* is downregulated at 21 days of abstinence in total tissue samples of both males and females, future studies should look to rescue this effect by overexpressing *circHomer1* at the onset of abstinence. Use of lentiviral vectors, akin to previous work in our lab manipulating RNA expression *in vivo*, would be a promising medium to carry out this manipulation in the CNS (Bastle et al., 2018). Separately, this work should aim to establish if acute over-expression of *circHomer1* does indeed regulate *Homer1b* transcript levels in the NAc of rats without any behavioral manipulation. Previous studies have used shRNA lentiviral vectors to build evidence for a bidirectional relationship between these transcripts in the orbitofrontal cortex of mice, however, such an effect remains to be validated in the NAc of rats (Hafez et al., 2022). These experiments would help to further test our hypothesis that *circHomer1* mediates cue induced motivation for cocaine via regulation of *Homer1b*. Additionally, we suspect that such changes in protein expression induced by *cicHomer1* would result in morphological changes and negatively regulate activity in the neurons akin to the function of Homer1a (Bockaert et al., 2021; Jia Hua Hu et al., 2010). Future studies should perform electrophysiological assays and qualitative analysis of dendritic spine morphology to understand if dysregulated *circHomer1* levels correspond to changes in activity or morphology of neurons in the NAc shell.

Interestingly, in this study we were able to find evidence of a trend towards opposite synaptic expression of *circHomer1* in male and female rats between 1 and 21 days of abstinence. Male synaptic expression seems to follow the same trend of decreasing across abstinence as in the total expression, however, females show the opposite effect and trend towards increasing at 21d of abstinence. Future studies should investigate these differences further by tracking estrous cycle phases in female rats during self-administration and cue reactivity to better understand if endogenous hormones may be mediating this effect. Such differences seen in our study could prove to be a novel sex specific mechanism for *circHomer1* in synaptic regulation and its relationship with motivation for drug reward.

**Concluding Remarks**

The chronic relapsing nature of SUDs remains a contributing factor to their prevalence in society and poses a significant challenge in providing effective treatment solutions to patients. Long-lasting changes in synaptic plasticity within the 'reward circuitry' of the brain related to learning and memory underly the increases in motivation for drug following abstinence that occur in both SUDs and in animal models (Gawin & Kleber, 1986; Grimm et al., 2001; Kalivas, 2009; Kalivas & Volkow, 2005; Neisewander et al., 2000; Tran-Nguyen et al., 1998). Non-coding RNA plays an important role in regulation of gene expression in neuropsychiatric disorders, with circRNAs increasingly implicated in those involving synaptic plasticity (Earls et al., 2014; Spadaro & Bredy, 2012; You et al., 2015).

This study set out to explore the role of *circHomer1* expression in the synaptic plasticity underlying the incubation of craving utilizing a cue-reactivity model of cocaine

self-administration. While our results relating *Homer1b* to *circHomer1* expression were inconclusive, the results provide the first evidence for *circHomer1's* role in cue-induced motivation for cocaine using a robust model of cocaine-seeking behavior. Additionally, we have shown a trend towards possible sex specific differences in synaptic expression of *circHomer1* related to motivation for cocaine that needs to be explored further. The study of *circHomer1*, amongst other circRNAs, is still in its infancy in the fields of neuroscience and substance use disorders. These RNAs hold great promise of adding a layer of regulation to the dynamic neurobiological changes involved in synaptic plasticity underlying complex behaviors such as drug seeking behavior. Future work should further examine other circular RNAs derived from genes associated with SUDs and their potential to regulate the expression of other transcripts within their host gene.

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# APPENDIX A

# MIXED EFFECTS ANOVA TABLE

| | | Random Effects | | Fixed Effects | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| ANOVA | | Variance | P | | Estimate | SE | df | t-value | P |
| 1. circHomer1 TOT: Sex * Group + (1\|Cohort) | Cohort | 0.9373 | 0.0002904 | Intercept | 3.362406 | 0.586052 | 2.034553 | 5.737 | 0.0279 |
| | | | | Sex | 0.209296 | 0.173471 | 39.265659 | 1.207 | 0.2348 |
| | Residual | 1.3011 | | Group1 | -0.043122 | 0.253249 | 39.172362 | -0.170 | 0.8657 |
| | | | | Group2 | -0.576371 | 0.255803 | 39.296869 | -2.253 | 0.0299 |
| | | | | Sex x Group1 | -0.358717 | 0.250885 | 39.0525 | -1.430 | 0.1607 |
| | | | | Sex x Group2 | 0.008376 | 0.252595 | 39.145364 | 0.033 | 0.9737 |
| 2. circHomer1 SYN: Sex * Group + (1\|Cohort) | Cohort | 3.822 | 2.925e-07 | Intercept | 2.89870 | 1.15174 | 2.00498 | 2.517 | 0.12790 |
| | | | | Sex | -0.22916 | 0.22978 | 38.15384 | -0.997 | 0.32491 |
| | Residual | 2.260 | | Group1 | -0.05141 | 0.33497 | 38.09394 | -0.153 | 0.87882 |
| | | | | Group2 | 0.22209 | 0.32928 | 38.11499 | 0.674 | 0.50409 |
| | | | | Sex x Group1 | -0.96953 | 0.33017 | 38.01384 | -2.936 | 0.00561 |
| | | | | Sex x Group2 | 0.80471 | 0.32709 | 38.08841 | 2.460 | 0.01854 |
| 3. Homer1b TOT: Sex * Group + (1\|Cohort) | Cohort | 0.448 | 0.03067 | Intercept | 3.0691 | 0.4314 | 2.0578 | 7.115 | 0.01777 |
| | | | | Sex | 0.6283 | 0.1920 | 39.7860 | 3.273 | 0.00221 |
| | Residual | 1.606 | | Group1 | -0.2960 | 0.2805 | 39.4589 | -1.055 | 0.29764 |
| | | | | Group2 | 0.1867 | 0.2763 | 39.6416 | 0.676 | 0.50313 |
| | | | | Sex x Group1 | -0.1083 | 0.2775 | 39.1173 | -0.390 | 0.69848 |
| | | | | Sex x Group2 | -0.1299 | 0.2745 | 39.4809 | -0.473 | 0.63860 |
| 4. Homer1b SYNl: Sex * Group + (1\|Cohort) | Cohort | 0.02376 | 0.798 | Intercept | 2.07366 | 0.19665 | 2.11738 | 10.545 | 0.00727 |
| | | | | Sex | 0.15372 | 0.17460 | 39.83686 | 0.880 | 0.38390 |
| | Residual | 1.32588 | | Group1 | 0.02764 | 0.25445 | 39.38012 | 0.109 | 0.91406 |
| | | | | Group2 | 0.05327 | 0.25520 | 39.95620 | 0.209 | 0.83571 |
| | | | | Sex x Group1 | -0.43771 | 0.25388 | 38.50503 | -1.724 | 0.09272 |
| | | | | Sex x Group2 | 0.29453 | 0.25432 | 39.21069 | 1.158 | 0.25383 |
| 5. Homer1b/ circHomer1 TOT: Sex * Group + (1\|Cohort) | Cohort | 0.0000 | 1 | Intercept | 0.98213 | 0.06554 | 39.00000 | 14.985 | < 2e-16 |
| | | | | Sex | 0.18638 | 0.06554 | 39.00000 | 2.844 | 0.00706 |
| | Residual | 0.1863 | | Group1 | -0.11640 | 0.09732 | 39.00000 | -1.196 | 0.23888 |
| | | | | Group2 | 0.17722 | 0.09344 | 39.00000 | 1.897 | 0.06530 |
| | | | | Sex x Group1 | -0.00457 | 0.09732 | 39.00000 | -0.047 | 0.96278 |
| | | | | Sex x Group2 | 0.03640 | 0.09344 | 39.00000 | 0.390 | 0.69898 |
| 6. Homer1b/ circHomer1 SYN: Sex * Group + (1\|Cohort) | Cohort | 0.4050 | 0.000214 | Intercept | 1.070625 | 0.381598 | 1.975922 | 2.806 | 0.108 |
| | | | | Sex | -0.014141 | 0.103021 | 38.334610 | -0.137 | 0.892 |
| | Residual | 0.4415 | | Group1 | -0.188537 | 0.150317 | 38.048183 | -1.254 | 0.217 |
| | | | | Group2 | 0.204471 | 0.146549 | 38.200157 | 1.395 | 0.171 |
| | | | | Sex x Group1 | -0.005894 | 0.149782 | 38.009478 | -0.039 | 0.969 |
| | | | | Sex x Group2 | -0.124750 | 0.144723 | 38.089916 | -0.862 | 0.394 |