12-12-2019 9:30 AM

The Role of Adult Hippocampal Neurogenesis in Depression-Relevant Reward-Related Behaviours

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A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Neuroscience

Abstract

The variability in the symptomatology of depressive disorders and antidepressant treatment response has led to an increased interest in the molecular, cellular, and circuit mechanisms of many aspects of affect. Evidence suggests a reduction in adult hippocampal neurogenesis (AHN) is associated with an increase in depression-like behaviour, though much of this evidence has been from studies using aversive tests (e.g., forced swim test). Here, I used touchscreen operant chambers, which allow for non-aversive and translational testing, to test the hypothesis that AHN plays a contributing role in emotion regulation. A panel of three touchscreen tests were chosen to assess different aspects of depression-relevant reward-related behaviour, namely probabilistic reversal learning, progressive ratio, and extinction learning. Results across these tests largely indicate that AHN knockdown does not affect sensitivity to feedback information, motivation across a variety of reward strengths, and the ability to cease responding after reward withdrawal. Therefore, it seems that a role of AHN in emotion regulation may only apply in stressful, but not non-aversive, conditions.

Keywords: Neurogenesis, adult hippocampal neurogenesis, adult-born neurons, positive valence systems, negative valence systems, emotional regulation, depression, affect, mood

Summary for Lay Audience

Depressive and other mood disorders are major causes of illness and impair well-being worldwide. Despite the high occurrence of these disorders, their complexity makes determining appropriate treatment difficult as their underlying causes are largely unknown. There has been mounting evidence to suggest that the generation of new neurons (known as neurogenesis) in the hippocampus, a brain area commonly associated with learning and memory, might be involved in depressive and other mood disorders. Particularly, people with depression, and animal models of depression, often have fewer newborn neurons in this brain area and antidepressant treatment has often been found to increase the amount of neurogenesis. However, a lot of this evidence comes from animal studies using stressful methods. For my thesis, I wanted to determine if neurogenesis impacts depression-related behaviour in non-stressful situations. Considering one major characteristic of depression in humans is impaired reward responding, I used rodent touchscreen behavioural systems to test whether mice with reduced neurogenesis had changes in their responses to reward. I chose three tests, each assessing different aspects of reward-related behaviours. These aspects were (1) whether the outcome of a response would impact later responses (for example, whether getting rewarded for a response would increase the likelihood that the same response would be repeated), (2) willingness to exert effort to receive a reward, or motivation, and (3) whether responding would stop once the reward was removed. For all three tests, I did not find strong evidence for a role of decreased neurogenesis in changing reward-related behaviours, meaning that decreasing neurogenesis does not impact depression-relevant behaviours in non-stressful conditions. These results point to the importance of stress in producing depression-relevant behaviours in mice. A popular theory for the cause of depression

in humans is that there is some underlying biological cause that must be combined with an external trigger for the disorder to occur — such an underlying cause may be reduced neurogenesis and such a trigger may be stress. My thesis project shows that reducing neurogenesis, by itself, does not impact depression-relevant reward-related behaviour when stress is not involved.

Co-Authorship Statement

For all experiments included in my thesis and all manuscript preparation, I was the primary contributor. With guidance and assistance from my supervisors, Drs. Tim Bussey and Lisa Saksida, and post-doctoral advisor, Dr. Cecilia Kramar, I designed the experiments to be run, troubleshot problems that arose when conducting the experiments, ran all statistical analyses, and prepared the entirety of my thesis manuscript. However, I did receive assistance in conducting certain experiments. None of the work reported in my thesis has been submitted for publication at the time of writing. The contributions from others are as follows:

— Dr. Cecilia Kramar: Performed the Ki-67 cell counts on images I provided. Additionally, aside from my supervisors, she was the main source of guidance and provided assistance with all facets of my thesis project.

— Dr. Amy Reichelt: Ran the spontaneous location recognition test and the immunofluorescent assay for Ki-67 labelling on tissue sections I had chosen.

— Dr. Benjamin Phillips: Produced the R code for win-stay/lose-shift probability calculations for probabilistic reversal learning analysis, as well as produced the ABET schedule for this test.

— Olivia Ghosh-Sawaby: Helped run the cohort of extra mice on progressive ratio (n = 16). She ran this test on weekdays from June 22 to August 20, 2019, and I ran on the weekends.

— Rupayan Mukherjee: Helped section a cohort's worth of neural tissue (n = 11).

Acknowledgements

I would not be where I am today if it were not for the guidance, assistance, and support of many people. First and foremost, I am so grateful for my supervisors, Drs. Tim Bussey and Lisa Saksida, for seeing something in my undergrad self two and a half to three years ago and welcoming me into the Translational Cognitive Neuroscience (TCN) Lab. I am so honoured to have been part of their first cohort of graduate students at Western; I was given such a unique opportunity to be part of the process of organizing the lab space and procedures/protocols, and it has been such a pleasure to see how the lab has grown. Their guidance has been incredibly valuable and the lessons I've learned over the years working under them will stay with me for the rest of my life.

Aside from my supervisors, my post-doctoral advisor, Dr. Cecilia Kramar, has been another important figure. Her unending guidance, support, and encouragement means the world to me, as does the relationship we were able to form during our time working together. Despite having her own experiments and busy schedule, Ceci always found a way to meet with me and check in on my progress and how I was feeling; she went above and beyond, continuing to check in and look over thesis updates even after moving to McGill for another post-doctoral fellowship. I can't thank her enough for that. The lessons I've learned from Ceci and the conversations we've had have shaped the researcher, and person, I've become over the past two and a half years. Additionally, she was able to set aside time after moving to help me complete the work required for my thesis by doing the Ki-67 cell counts. Overall, I place incredibly high value on the time we spent together and simply cannot thank Tim and Lisa enough for pairing me with her upon my arrival to Western in 2017.

After Ceci moved to McGill, the other two post-doctoral fellows in the lab, Drs. Amy Reichelt and Daniel Palmer, made sure I was still receiving the support and guidance I needed to finish my thesis project. Amy was so incredibly helpful with her knowledge of immunofluorescent assays, as well as helped me schedule meetings with Tim and Lisa and create deadlines; she made sure I was on track, which I greatly appreciate. Additionally, she ran the SLR experiment and the Ki-67 assay for me so that I could focus on other work that had to be done, whether it be the (what seemed like) endless running of PR or getting my thesis manuscript organized. Near my thesis deadline, Amy was especially helpful with reviewing the Results chapter and offering valuable feedback. Dan was a wealth of knowledge when it came to touchscreen troubles, whether it be hardware-, software-, or behaviour-based, as well as statistical analyses. His input was much appreciated whenever I ran into touchscreen problems and when I needed help figuring out ABET or R code. Dan was able to revise the R script provided for analyzing rate analysis on PR - analyses that would have been included in this thesis had it not been for time limitations. Not only did Dan offer valuable practical guidance, but he also reviewed the Methods and Materials chapter for technical accuracy. Both Dan and Amy offered their time and expertise, especially in the months after Ceci had moved, despite the fact that I technically wasn't their student; as such, they've also gone above and beyond. I am so grateful for their input and assistance.

I am also incredibly grateful to those who took the time to help run experiments for my thesis. In addition to Ceci and Amy, Olivia Ghosh-Sawaby, another TCN graduate student, helped run the extra cohort of animals on PR and fed them their special food during that time. I am incredibly grateful for and appreciative of her assistance, especially given she had other

projects to work on. I'd also like to extend my gratitude to Dr. Benjamin Phillips who offered his assistance with the PRL test and its analysis, as well as the rate analysis for PR. Most of my correspondence with Ben occurred while he was finishing his PhD in Cambridge; despite never having met me, he tirelessly answered my emails, analyzed the PRL data from the first cohort I ran on the test, and was overall an indispensable resource when it came to PRL. When he visited Western in fall 2018, Ben spent at least an hour with me and Ceci to walk us through the R script he devised to calculate the win-stay/lost-shift probabilities, and spent additional time with me to go over and debug the PRL schedule script in ABET. Certainly, that particular experiment would not have gone nearly as smoothly if it weren't for his input and assistance. Additionally, Rupayan Mukherjee, an undergraduate volunteer, assisted with some tissue slicing, which I'm very grateful for. All of those who provided their time, input, and energy have most definitely not gone unnoticed nor under-appreciated as it enabled me to prioritize my energy and finish other aspects of my project.

Next, I'd like to thank the BrainsCAN rodent cognition core (RCC) technicians, research associates, and scientists who helped me with specific tasks to complete certain aspects of, or relating to, my thesis. Dr. Sara Memar, the BrainsCAN neuroinformatics specialist, recently worked with me to debug the PRL and PR rate analysis R scripts. As much as I may be familiar with R coding language, my knowledge is in its infancy and her expertise not only put my mind at ease that the codes were producing accurate data, but also helped me better understand how the codes worked. I feel so much more confident reporting and discussing the results obtained from those R analyses due to her assistance. Dr. Flavio Beraldo, BrainsCAN research associate, trained me on the confocal microscope and offered his assistance when I was optimizing our

BrdU immunofluorescent assay protocol. His knowledge of microscopy, and related knowledge about assays, helped a great deal with my optimization. Finally, I'd like to thank Matthew Cowan, Jue Fan, Chris Fodor, and, though a more recent addition to the BrainsCAN team, Grant Favell. Matt offered wonderful touchscreen and facility advice prior to our move into DSB. He also administered BrdU for a cohort of my mice, going in every day for 7 days to inject and/or feed my mice. I am very appreciative that he took the time to do this for me. Jue assisted me in many ways, though specifically she provided the nestin primers so that I could genotype my mice and offered information on how to put breeders together. Additionally, Chris and Grant offered indispensable wisdom and advice whenever I ran into PCR problems I couldn't troubleshoot myself. In particular, earlier this fall, I was having major problems with getting PCR results and their advice was extremely valuable and lead to finding a solution.

I am extremely grateful for all those who offered their time and assistance to ensure my mice remained in good condition, specifically Dr. Patti Kiser (pathologist) and Dr. Elissa Steinbock (ACVS clinical veterinarian), Raven Lake (animal technician), and the rest of ACVS. Patti and Elissa were my primary contacts when I first noticed the strange pathology in some of my mice, and they were present during a set of perfusions to do an initial gross pathology assessment and tissue collection. Patti analyzed tissue from three mice, created a pathology report, and met with me to go over the results and took the time to explain what the results meant. Though she didn't know the cause of the issue, she offered many helpful suggestions and I'm very grateful that she took the time to assess the pathology of my mice. In addition, Raven and other ACVS members were indispensable whenever I had a mouse health-related problem or needed their guidance. They were always there, and often excited, to provide assistance. In many

ways, I put the health of my mice above all else, especially considering that getting good behavioural results is largely dependent on having happy and healthy mice; these were the main people who helped me make sure they were in good shape.

I also have a huge amount of gratitude to extend for those who offered administrative assistance. Firstly, I'd like to thank Cessandra Latinovich, Tim and Lisa's (and the entire lab's) administrative assistant. From on-boarding to juggling schedules to fit meetings in to offering helpful reminders, Cass has been an absolutely amazing resource. If I didn't know how to do something admin-related or needed a university policy-related question answered, she would either find the answer for me or point in the right direction for how to find it. In many ways, she was the bridge between my daily lab life and the university as a whole. I'd also like to thank Dr. David Wasserman, who acted as the TCN lab manager until this past spring. His help with organizing lab procedures and ensuring experiments could run without a hitch (or with as little hitches as possible) was much appreciated. Finally, Susan Simpson, the neuroscience program coordinator, was always so helpful, even before I moved to Western and especially during the final few months of my MSc degree. She was always a fountain of knowledge and always put my mind at ease whenever I asked her a question or met with her. Going through graduate school, registering for courses, and keeping on top of advisory committee meetings would not have been as straightforward as it was if it weren't for her. She is truly an asset for every single neuroscience graduate student.

I'd like to take a few sentences to thank the members of my advisory committee, Drs. Elizabeth Osuch, Marco Prado, and Robert Cumming. I always left my meetings with much more knowledge and many more ideas than I went in with. When I discussed problems I was

facing, they were quick to offer possible solutions and were just as quick to recognize the effort I had put into my project. I'd especially like to thank Beth for her feedback and guidance on the Introduction chapter; she was an incredibly valuable resource when it came to the translational and mood disorder-related aspects of my project. It was important to me, as well as my supervisors, that I don't over-extend my findings or use inappropriate language when discussing depressive disorders in humans and how clinical practice differs from science. I'd also like to thank Marco for providing advice about what molecular data to put into my thesis.

Next, I want to thank the other members of TCN Lab, BrainsCAN RCC, and pertinent members of the Prado lab who have not been previously mentioned: Dr. Vania Prado, Dr. Julie Dumont, Dr. Miguel Skirzewski, Sanda Raulic, Tyler Dexter, Ornela Kljakic, Rachel Lackie, Oren Princz-Lebel, and Keon Coleman. In particular, of the Prado lab, Ornela provided protocols for some behavioural tests I ran (including buried food olfaction) and Rachel assisted with certain molecular protocol problems I faced. It was such a pleasure to be able to work with and learn from all of these people, as well as those previously mentioned. I consider myself very lucky to have met everyone who shared a lab space with me, attended conferences with me, and consistently made my days brighter through the conversations we'd have. Everyone in this lab has left me in awe or inspired, and I am grateful to have had the opportunity to work alongside them.

Finally, though definitely not least, I'd like to thank my parents and friends back home. My parents have been an endless source of support and encouragement, and I cannot possibly thank them enough for that. They always made sure I was doing well and thriving as much as possible here in London. My parents also served as wonderful proofreaders many times

throughout the past couple years, for which I am very grateful. My friends back home, specifically Sam Richmond, Viktoriya Vasylkiv, and Rachel Nickel, were reliable sources of support and encouragement. They often gave me strength when I felt much too overwhelmed, and shared in my successes when they occurred. Though we're living in different provinces, they are no less valuable to me, and no less able to help me through life.

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List of Abbreviations

η_p^2	partial eta squared
δ-HSV-TK	modified herpes simplex virus thymidine kinase
ABET	Animal Behaviour Environment Test
ABN	adult-born neuron
ACVS	Animal Care and Veterinary Services
AHN	adult hippocampal neurogenesis
ANOVA	analysis of variance
APA	American Psychiatric Association
BrdU	bromodeoxyuridine
BSA	bovine serum albumin
CBT	cognitive-behavioural therapy
CORT	corticosterone
d-SLR	dissimilar condition spontaneous location recognition
d2	discrimination ratio
DAPI	4’6-diamidino-2-phenylindole
DCX	doublecortin
DG	dentate gyrus
DNA	deoxyribonucleic acid
DREADDs	Designer Receptors Exclusively Activated by Designer Drugs
DSM-5	*Diagnostic and Statistical Manual of Mental Disorders*, 5th Edition

EDTA	ethylenediaminetetraacetic acid
eGFP	enhanced green fluorescent protein
EPM	elevated plus maze
Eq.	equation
FR	fixed ratio
FST	forced swim test
GCV	ganciclovir
GFAP	glial fibrillary acidic protein
HPA	hypothalamic-pituitary-adrenal
HSD	honestly significant difference
ICD-11	*International Classification of Diseases*, 11th Revision
IgG	immunoglobulin G
IP	intraperitoneal
IPC	intermediate progenitor cell
ITI	inter-trial interval
IV	intravenous
MRI	magnetic resonance imaging
NeuN	neuronal nuclei
NGS	normal goat serum
NIH	National Institute of Health
NIMH	National Institute of Mental Health

pAb	polyclonal antibody
PBS	phosphate-buffered saline
PBS-T	phosphate-buffered saline-Triton
PFA	paraformaldehyde
PR	progressive ratio
PRL	probabilistic reversal learning
Rb	rabbit
RDoC	Research Domain Criteria
RGL	radial glia-like cell
s-SLR	similar condition spontaneous location recognition
SEM	standard error of the mean
SGZ	subgranular zone
SLR	spontaneous location recognition
SNRI	serotonin-norepinephrine reuptake inhibitor
SSRI	selective serotonin reuptake inhibitor
SVZ	subventricular zone
TK	thymidine kinase
TK-/ctrl	no viral thymidine kinase transgene, control chow
TK+/ctrl	viral thymidine kinase trasngene, control chow
TK-/VGCV	no viral thymidine kinase transgene, valganciclovir-treated chow
TK+/VGCV	viral thymidine kinase transgene, valganciclovir-treated chow

v.	version (relating to software)
VGCV	valganciclovir
WHO	World Health Organization

1. Introduction

Depressive disorders are a leading contributor to global burden of disease. They are major contributors to disability worldwide and premature death by suicide (Ferrari et al., 2013; World Health Organization [WHO], 2017, 2018), with a prevalence rate of 4.7% in Canada (i.e., the proportion of the Canadian population diagnosed with depression [WHO, 2017]). Two major challenges in the diagnosis and investigation of depressive disorders and other mood disorders are the variability in both symptomatology and patients' response to treatment. Individuals with the same diagnosis can have differing symptom profiles and, even amongst those with similar profiles, the efficacy of a given treatment in one individual does not translate to global efficacy across all other patients. These variabilities suggest depression may more likely be an umbrella term for closely related mood disturbances that arise from different physiological and neurological dysfunctions (Sibille & French, 2013). Consequently, there has been an increased interest in the molecular, cellular, and circuit mechanisms of many aspects of mood and affect, with the goal of determining specific neurological dysfunctions that may play a role in mood disorder etiology.

1.1 Approaches to Depressive and Other Mood Disorders: The Clinical Practice Paradigm vs. The Research Domain Criteria Paradigm

To determine the differences in how depression and other mood disorders are approached in clinical practice and scientific discourse, it is useful to compare the language and organization used in diagnostic manuals, such as the *Diagnostic and Statistical Manual of Mental Disorders* (5th ed.; *DSM-5*; American Psychiatric Association [APA], 2013) or the *International Classification of Diseases* (11th Revision, v.4; *ICD-11*; WHO, 2019), and in the Research

Domain Criteria (RDoC) framework. Both the *DSM-5* (APA, 2013) and *ICD-11* (WHO, 2019) list many subtypes of depressive disorders, many of which are based on the length of time symptoms are experienced, the severity of the symptoms experienced, and/or the number of depressive episodes experienced (i.e., single episode or recurrent episodes of symptoms). The language used in these manuals is descriptive and quantitative due to their intended use, specifically that of diagnosis. The *DSM* and *ICD* are to be used by clinicians or medical professionals in order to take inventory of a client's or patient's symptoms and, based on the criteria met via clinical interview, identify the most fitting diagnosis. Nowadays, the goal of such diagnostic manuals is not to further the understanding of the underlying causes of a certain mental disorder, though, historically, this was their main purpose. These manuals are now tools to be used by mental health professionals to aid in identifying and diagnosing clients or patients. Treatment options are based on the diagnosis chosen; such treatments include specific medications (e.g., selective serotonin reuptake inhibitors [SSRIs] and serotonin-norepinephrine reuptake inhibitors [SNRIs] are common drug treatments for unipolar depressive disorders) and specific psychotherapy approaches (e.g., cognitive behavioural therapy [CBT] has been shown to be an effective treatment option for depressive disorders [Cuijpers, Cristea, Karyotaki, Reijnders, & Huibers, 2016]). The nature of this approach to depressive and other mood disorders implies that these disorders are seen as phenomenologically defined, with a single treatment plan (e.g., drug + psychotherapy combined treatment, or either one alone) for the entire constellation of symptoms experienced by a patient/client with a particular diagnosis. Thus, the *DSM*/*ICD* approach to depressive and other mood disorders has become more practically-focused due to their intended use by clinicians, offering a succinct symptomatology-based description of such

disorders. However, this approach does not take into account the heterogeneity of possible underlying causes of depressive and mood disorders, but, rather, conceptualizes these disorders as unitary, despite the variabilities present in symptom profiles across individuals/patients.

In contrast, the RDoC framework, put forward by the National Institute of Mental Health (NIMH), emphasizes furthering the understanding of the nature and potential underlying causes of mental disorders. The RDoC framework, then, is currently not a diagnostic tool to be used by practitioners, but is intended for researchers investigating the complexities of mental disorders. The RDoC framework re-conceptualizes the investigation of mental disorders by promoting a dimensional view of these disorders and, thus, represents a movement away from a phenomenological approach to mental health research (i.e., the notion that multiple symptoms can be attributed to a single cause) by categorizing research findings into six major domains related to mental disorders (e.g., those related to positive reinforcement). Each domain is comprised of the different aspects related to its function, such as behaviours, processes, and mechanisms; these are termed “constructs.” The methods used to investigate each construct in research settings, including behavioural assessment and molecular analysis, are termed “units of analysis.” At the time of writing, the six broad domains of the RDoC framework are negative valence systems (constructs include fear, anxiety, and frustrative non-reward), positive valence systems (constructs include reward responsiveness, reward learning, and reward valuation), cognitive systems (constructs include attention, perception, declarative and working memory, and language), systems for social perception (constructs include affiliation and attachment, social communication, and understanding of the self and others), arousal/regulatory systems (constructs include arousal, circadian rhythms, and sleep and wakefulness), and sensorimotor systems

(constructs include motor actions, agency and ownership, and habit). These domains are different from the symptoms listed in diagnostic manuals, though some of the domains may relate directly to symptoms experienced among the clinical population, such as symptoms related to lack of motivation (suggesting abnormal functioning of positive valence systems) and social withdrawal (suggesting abnormal functioning of systems for social perception) seen in depressive disorders. The separation of constructs related to mental health into domains is especially important for mood and depressive disorders, as it highlights the possibility that these disorders are likely caused by a multitude of factors (i.e., are not unitary) and the possibility that what is currently known as depression may be more accurately conceptualized as many different disorders with abnormalities in different combinations of constructs. For instance, there may be a fundamental difference between depressive symptoms elicited by stressful situations and those regulated by response to reward and positive situations.

Additionally, it is worthwhile to contrast the approach to treatment implicit in the RDoC framework to that implicit in the phenomenological/clinical practice paradigm. As mentioned, due to the focus on treatment plans based on a specific diagnosis given a patient's symptom profile, the clinical practice paradigm approaches depressive and other mood disorders as unitary. In contrast, the nature of the RDoC paradigm focuses on and emphasizes the possible neuropathological and pathophysiological mechanisms underlying symptoms experienced by the patient and their possible causes. Not only does this enable investigation into the underlying causes of each specific domain, but it also, though implicitly, promotes the creation of symptom-based, rather than syndrome-based, treatment plans. Instead of using an individual's symptom profile to determine a diagnosis into a unitary disease category, and then determining a treatment

plan based on the diagnosis given, the focus on the domains, and their corresponding constructs, of depressive and other mood disorders in the RDoC paradigm implicitly emphasizes the importance of conceptualizing the neuropathology/pathophysiology underlying the symptoms. Thus, each paradigm's approach to treatment differs; the clinical practice paradigm promotes, and encourages, diagnosis-based treatment (and, therefore, operates under the assumption that an individual's symptom profile is synonymous with their diagnosis), whereas the RDoC paradigm promotes, and encourages, neuropathology-based treatment (and, therefore, operates under the assumption that individual symptoms can have different underlying causes and, as such, may require different treatments). Though, historically, the goal behind diagnostic criteria and RDoC domains were the same (i.e., furthering the understanding of mental disorders), their approaches to conceptualizing mental disorders have diverged; the clinical practice paradigm, based on diagnostic manuals, focuses on external manifestations of symptoms in order to determine diagnoses and treatment plans, whereas the RDoC framework emphasizes empirical findings and the underlying pathophysiology of mental disorders.

For the purposes of my thesis, I will be referring to depression- and anxiety-like behaviours using RDoC terms and constructs. The reason for this is twofold. First, using RDoC terminology aids in avoiding the conflation of depression in the *DSM*/*ICD* diagnostic sense with the pathophysiological aspects of depression that can be investigated in a research setting. This is especially important given the fact that rodents were used as subjects, wherein mood states are already ambiguous and mood disorders, as they are understood in humans, can only be incompletely modelled. Animal models of depressive and other mood disorders cannot be used to investigate all possible underlying factors due to the relatively simpler biology (reviewed in

French & Sibille, 2013) and experiences unique to the human condition (e.g., subjective emotional experiences, rumination) that cannot be modelled. However, the relative simplicity of animal systems allows for selective and specific biological manipulations which, in turn, allows for determining how individual biological factors are related to specific emotion-related behaviours (e.g., fear). Due to the fundamental differences in mood as experienced in humans and rodents, it is important to use terms and language that do not inappropriately over-extend animal model findings to depression in humans - this is where the RDoC terminology is particularly useful as it mitigates this over-extension by keeping terms specific to the domains and constructs expected to represent neuropathophysiological changes across species. Second, using RDoC terminology will demonstrate how my work more closely aligns with contemporary mental health research. The goal of basic research, when related to mood and mental disorders, is to further the understanding of the varying aspects involved in mental disorders and how these aspects interact with each other. It is useful for such research to focus on manipulating the constructs of a limited number of domains at a time; for instance, focusing on replicating endophenotypes of depressive disorders as opposed to developing a “depressed” rodent. Through a more narrowed focus in basic research, using the RDoC as a guide, knowledge of how the neurophysiological constructs of each domain play a role in behavioural or molecular output in mental disorders will deepen. This may, eventually, lead to revisions of diagnostic criteria, but this is not necessarily the main focus of such research.

1.2 The Role of Adult-Born Neurons

The hippocampus, most known for its cognitive role in spatial learning and memory, is one of two primary regions that supports neurogenesis (i.e., the generation of new neurons)

throughout adulthood; this process is referred to as adult hippocampal neurogenesis (AHN) and results in adult-born neurons (ABNs [Eriksson et al., 1998; reviewed in Gonçalves, Schafer, & Gage, 2016; Spalding et al., 2013]). The other primary region that supports AHN is the subventricular zone (SVZ) of the lateral ventricle (Doetsch, Caillé, Lim, García-Verdugo, & Alvarez-Buylla, 1999; Eriksson et al., 1998; Mirzadeh, Merkle, Soriano-Navarro, García-Verdugo, & Alvarez-Buylla, 2008). (In rodents, the olfactory bulb is an additional region where ABNs are present due to their migration from the SVZ during their development [Carleton, Petreanu, Lansford, Alvarez-Buylla, & Lledo, 2003]). Neurogenic processes occur in the dentate gyrus (DG) region of the hippocampus, specifically in the subgranular zone (SGZ) of the DG which lies in between the granule cell layer and the hilus (reviewed in Gonçalves et al., 2016). The rate at which neurogenesis occurs in the adult brain is partially dependent on behavioural and environmental factors. For instance, neurogenesis is enhanced with exercise (Creer, Romberg, Saksida, van Praag, & Bussey, 2010; van Praag, Kempermann, & Gage, 1999), whereas stress impairs neurogenic processes and reduces the amount of neurogenesis occurring in the adult DG (reviewed in Mirescu & Gould, 2006 and Schoenfeld & Gould, 2012; Tse et al., 2014; Wu et al., 2014). Therefore, neurogenesis in the adult brain, and more specifically in the DG, is a relatively large-scale example of neural plasticity, or the change of brain structure with experience.

ABNs go through specific stages of maturation and differentiation, and each stage is marked by the presence of specific proteins (reviewed in Gonçalves et al., 2016); these proteins can then be used as biological markers of neurogenesis in adult hippocampal tissue. In the SGZ, ABNs begin as neural stem cells, previously shown to be multipotent (i.e., one neural stem cell

can differentiate into multiple cell types, such as glia and neurons [Bonaguidi et al., 2011]). One type of neural stem cell present in the SGZ is the radial glia-like cell (RGL), which can give rise to neural progenitor cells (i.e., intermediate progenitor cells [IPCs]), astrocytes, and oligodendrocytes (reviewed in Kriegstein & Alvarez-Buylla, 2009). Via fate specification and differentiation processes, IPCs give rise to newborn young neurons (i.e., immature neurons) and, through maturation processes, these newborn neurons then become fully functional granule cells. There are many types of RGLs, including nestin- and glial fibrillary acidic protein (GFAP)-expressing RGLs; nestin expression continues throughout the neural progenitor stage. Immature neurons are marked by the presence of doublecortin (DCX), whereas mature neurons are marked by the presence of neuronal nuclei (NeuN [reviewed in Berg et al., 2015]).

One of the main functional distinctions to be made is between behaviours that depend on immature and those that depend on mature neurons (i.e., behaviours that are neurogenesis-dependent and -independent, respectively). One important example of a neurogenesis-dependent cognitive process is pattern separation. Pattern separation is a putative process underlying the ability to recognize two similar stimuli as different (e.g., Bekinschtein et al., 2013). A popular analogy to illustrate pattern separation is the well-known experience of remembering where one has parked their car in the parking lot of their workplace on a daily basis. Parking in the same lot results in many of the external spatial cues being similar regardless of the specific parking spot chosen on a given day; therefore, the ability to remember where one has parked depends on pattern separation. Rodents with decreased neurogenesis have exhibited impaired ability to pattern separate between two similar locations (Clelland et al., 2009), whereas those with

increased neurogenesis due to exercise (Creer et al., 2010) or genetic manipulation (Sahay et al., 2011) have shown an enhanced ability to pattern separate.

In addition to its known role in cognition, learning, and memory, including pattern separation, the hippocampus has also been shown to play an important regulatory negative feedback role in the stress response due to its connection to the hypothalamic-pituitary-adrenal (HPA) axis (reviewed in Jankord & Herman, 2008; Snyder, Soumier, Brewer, Pickel, & Cameron, 2011). AHN and immature neurons are thought to play a key role in this regulatory function. In particular, a mouse model of neurogenesis knockdown exhibited an anxiety- and depression-like behavioural phenotype, including increased anhedonia and learned helplessness, as well as an increased concentration of corticosterone in response to a physical stressor (Snyder et al., 2011). This finding has particular implications for depressive and other mood disorders, especially as the hippocampus has been a region of interest for investigations into emotion regulation and dysregulation (Christian, Song, & Ming, 2014; Videbech & Ravnkilde, 2004) and the fact that stress, specifically psychological stress, has been shown to influence such disorders, perhaps even acting as a precipitating factor (reviewed in Yang et al., 2015). Neurogenesis, stress, and depressive behaviour are thought to interact to create a feed-forward loop (Anacker et al., 2018; reviewed in Levone, Cryan, & O'Leary, 2014; Snyder et al., 2011; Tse et al., 2014). In the presence of a stressor, glucocorticoids are released by the HPA axis and neurogenesis is reduced in the hippocampus; due to the reduction of AHN, the negative feedback of the hippocampus on the HPA axis with glucocorticoid release is inhibited which, in turn, results in enhanced stress responsivity to future stressors and perpetuates the depressive behavioural phenotype (Snyder et al., 2011). The hippocampus and AHN, then, seem not only to be crucial

for cognitive functions, such as pattern separation, but may also play a role in regulating the stress response, emotion regulation, and psychological disturbances.

1.3 The Role of the Hippocampus and Neurogenesis in Depression and Depression-Relevant Behaviours

1.3.1 Hippocampus.

Reduced hippocampal volume has been linked to depression diagnosis in humans and psychopathological behaviour in animal subjects. In a postmortem study, patients with untreated depression had smaller hippocampi than non-depressed control participants (Boldrini et al., 2009). Magnetic resonance imaging (MRI) data support the reduced hippocampal size and have further suggested hippocampal volume and lifetime duration of depression diagnosis are linked (Videbech & Ravnkilde, 2004). Further, rodent models have shown that the hippocampus is involved in emotion regulation (Christian et al., 2014), with hippocampal volume correlating with susceptibility to stress-induced psychopathology (Tse et al., 2014). Therefore, gross morphological differences exist in the hippocampi of patients with depression and animal models when compared to controls, indicating volume loss may be part of the neuropathology of depression and/or may lead to increased susceptibility to depression.

There may also be an important functional distinction between the dorsal and ventral (or posterior/anterior in humans and non-human primates) components of the hippocampus. Due to their differing network connections, the dorsal hippocampus is thought to be primarily involved in cognitive functioning (e.g., spatial learning, pattern separation) and the ventral hippocampus is thought to be primarily involved in emotion and affective functioning (reviewed in Anacker & Hen, 2017). As mentioned above, the hippocampus is connected to the hypothalamus and HPA

axis and plays an important role in buffering the stress response; the ventral hippocampus is also connected to the amygdala and prefrontal cortex which may implicate the hippocampus in fear and anxiety, as well as responses to emotional stimuli (reviewed in Kheirbek, Klemenhagen, Sahay, & Hen, 2012). There has been mounting evidence supporting a potential role for the ventral hippocampus in emotion and stress response regulation, anxiety- and depression-like behavioural phenotypes, and mood disorder diagnoses.

1.3.2 Neurogenesis.

Although there are many possible causes for the reduction in hippocampal volume in patients with depression and animal models thereof, one that is of particular interest is the possibility of a causal role for impairment in adult neurogenesis. Impaired or decreased AHN has been found in some animal models of mood disorders (Sah et al., 2012); the previously discussed involvement of AHN in regulating the stress response is consistent with this notion. Additionally, recent evidence directly implicates AHN in the ventral hippocampus in modulating stress resilience (Anacker et al., 2018). Further evidence implicating AHN as a contributing factor to depressive symptoms in humans and depression-like behaviour in animals is related to the neurological effects of antidepressant treatment. In particular, antidepressant medications, such as fluoxetine (Prozac™), have been found to increase neural progenitor levels in rodents (Popova, Castrén, & Taira, 2017), non-human primates (Wu et al., 2014), and humans (Boldrini et al., 2009).

However, the evidence supporting a causal role of AHN in depression diagnosis/ symptoms and depression-like behaviours is inconclusive due to a large number of inconclusive and/or contradictory findings (reviewed in Miller & Hen, 2015). As one example, a study in mice

found that enhancing AHN produced antidepressant behavioural effects (Hill, Sahay, & Hen, 2015), whereas another found antidepressant behavioural effects with fluoxetine administration even when the treatment did not impact AHN (Sah et al., 2012). Thus, it is difficult to draw firm conclusions about the nature of the relationship between AHN and depression. The discrepancies across studies may be due to differences in the methodologies used, including type of animal model (e.g., transgenic strains to enhance neurogenesis vs. selectively breeding for a high anxiety-like behavioural phenotype [e.g., Hill et al., 2015 and Say et al., 2012, respectively]) and testing conditions (e.g., implementing stress paradigms prior to behavioural testing on inherently stressful tasks, such as the forced swim test [e.g., Snyder et al., 2011]).

1.4 Animal Models of Depression and Assessing Depression-Relevant Constructs in Rodents

Many behaviour- or environment-based animal models of depression capitalize on the logic behind the diathesis-stress model of depression, which posits that there exists an underlying individual (e.g., biological) vulnerability (i.e., diathesis) that must be paired with an external trigger (i.e., stress) for depressive disorders to manifest. Such animal models include chronic stress paradigms to elicit anxiety- and depression-like behaviour in rodents. Stressors used can be physical (e.g., restraint [e.g., Chiba et al., 2012; Snyder et al., 2011]), social (e.g., subordination by an aggressor of the same species, or social defeat [e.g., Golden, Covington, Berton, & Russo, 2011; Venzala, García-García, Elizalde, Delagrange, & Tordera, 2012]), or physiological (e.g., injection of stress hormones, such as corticosterone in rodents [e.g., Hill et al., 2015; Zhao et al., 2008]). Animals treated with such chronic stress paradigms exhibit anxiety- and depression-like behaviour on mood-related tasks for rodents, such as the elevated plus maze (EPM) and forced

swim test (FST). Animal models of depression by these means are designed to simulate environmental factors that may be relevant to mood disorders in humans, such as chronic social defeat used as a rodent analogue to bullying or discrimination (reviewed in Björkqvist, 2001). Such behaviour- and environment-based animal models of depressive and other mood disorders are useful for determining the impacts of external factors that may lead to psychopathological behavioural phenotypes (e.g., using chronic social defeat as a model for human bullying focuses on the effects of external social factors on behaviour). However, these models, by themselves, are not as useful for determining the role neurological factors play in such behavioural changes; although such stressors do affect physiological changes, such as neurogenesis, these changes are not selective. Thus, a better way to understand the role of AHN in affect is to manipulate neurogenesis directly and selectively.

In order to determine the role of AHN in emotion regulation and depression-like behaviours, an effective approach is to use animal models. An example of a transgenic strain used for manipulating neurogenesis is the conditional knockdown nestin-thymidine kinase (TK) mouse strain. Nestin-TK mice require treatment with the antiviral drug ganciclovir (GCV), or its prodrug valganciclovir (VGCV), to induce the knockdown of neurogenesis via neural stem cell death (Singer et al., 2009; Yu et al., 2008). The nestin-TK mouse line allows for precise targeting of neurogenic processes, thereby enabling non-confounded investigation into the role of AHN in anxiety- and depression-like behavioural phenotypes.

To assess the role of AHN in depression-like behaviours, animal models can be assessed on anxiety- and depression-relevant tests. The majority of evidence supporting a causal role for AHN in depression-like behavioural phenotypes and antidepressant action comes from studies

using aversive tests, which are inherently stressful. Such tests include the FST, EPM, light/dark box, and contextual fear conditioning, and fall under the negative valence systems domain of the RDoC. Due to the interplay between the stress response and AHN, the use of stressful tests introduces a major confounding variable into the investigation — namely whether the results found are due to the manipulation of neurogenesis or, instead, due to the impact of stress. Additionally, such aversive tests (or units of analysis in RDoC terminology) only take into account the negative valence systems domain and ignore the impact on the positive valence systems domain. People with depression not only show increased response to negative valence systems, such as a bias toward labelling stimuli as negative (Smith, Baxter, Thayer, & Lane, 2016; such a negativity-based cognitive bias has also been found in rats [Anderson, Munafo, & Robinson, 2013]), but they also show differences in positive valence systems. A hallmark depressive symptom, reduced motivation, is one construct under the positive valence systems domain. In a classic task designed to assess willingness to expend effort for a reward, progressive ratio (PR), patients with depression exhibited decreased effort (Hershenberg et al., 2016); patients gave up sooner during a trial, not wanting to expend as much effort as controls for a reward, indicating an impairment in reward perception. Additionally, people with depression showed blunted responses to feedback information when assessed on a gambling task (Steele, Kumar, & Ebmeier, 2007). The authors found a lack of reaction time adjustment in the trial immediately following feedback information, whereas controls increased their reaction time after negative feedback and decreased their reaction time after positive feedback. A similar pattern of results was found in the pattern of neural activity during the task; people with depression did not show activation in the areas activated in controls when given feedback information. Thus, for

multiple reasons, animal studies on emotion regulation and possible neurological underpinnings of depression-like behavioural phenotypes would benefit greatly from an expansion of the repertoire of depression-relevant tests/tasks to include assessment of positive valence systems. Not only would this increase the translational relevance of the animal tests, but the use of tests focused on positive valence systems also avoids the stress-related confounds present in aversive tests commonly used to assess negative valence systems in rodents.

Touchscreen operant chambers may provide an ideal environment with which to assess positive valence system constructs due to the increased translatability to human findings and to the decreased amount of stress involved in the touchscreen tests themselves (Nithianantharajah et al., 2015). Three of the touchscreen tests most suitable for assessing positive valence system constructs are probabilistic reversal learning (PRL), PR, and extinction learning. Each of these tests has been adapted from their non-touchscreen predecessors, oftentimes run in rodents using standard Skinner or operant boxes. PRL assesses sensitivity to feedback information (Phillips et al., 2018) and was adapted from Bari and collegues' study (2010). This test has been shown to be sensitive to serotonergic activity in the brain. Rodents exhibited behavioural change consistent with an increased sensitivity to positive (i.e., reward) feedback with increased serotonin activity via an agonist (Phillips et al., 2018) or SSRI (Bari et al., 2010), whereas a behavioural change consistent with a reduced sensitivity to positive feedback was seen following serotonergic antagonism (Phillips et al., 2018) or serotonin depletion (Bari et al., 2010). Considering serotonergic dysfunction may play a role in perpetuating depressive disorders (reviewed in Cowen & Browning, 2015), these results are consistent with those suggesting blunted response to feedback information in people with depression (Steele et al., 2007).

PR is a classic test to assess motivation or, more precisely, willingness to expend effort to receive a reward because every trial requires a greater number of responses for a reward. Traditionally, this test has been used in operant behavioural chambers (Hodos, 1961; Hodos & Kalman, 1963), but has been adapted to be run on touchscreens (Heath, Bussey, & Saksida, 2015). PR has been shown to be sensitive to dopamine activity in the brain. Treatment with a dopamine antagonist impaired performance on PR, where impaired performance was defined as less trials completed (i.e., "giving up" earlier [Heath et al., 2015]). Similar results were found in people with depression (Hershenberg et al., 2016). Additionally, studies in neurodegeneration using both mice and humans have illustrated the translational value of this approach (Heath et al., 2019).

Extinction learning is another test that may be used to assess reward-related behaviour, specifically the response to the withdrawal of reward. Extinction learning requires the cessation of responding to a previously rewarded stimulus due to the omission of reward. The use of extinction learning in the rodent touchscreen operant chambers has been previously described (Mar et al., 2013). In animal studies of affect, fear extinction is the most commonly used paradigm, often using a learned pairing of a foot shock with a conditioned stimulus that must be extinguished (e.g., Hefner et al., 2008). However, there also exists appetitive extinction (i.e., extinguishing a positive learned response to a stimulus due to its association with reward; this association was developed via appetitive conditioning), which is a paradigm much closer to the extinction learning task in the rodent touchscreen operant chambers. Dysfunctional responses to reward, like those discussed above, might be expected to impact response to reward omission as measured by the appetitive extinction test.

1.5 Rationale and Hypothesis

Emotion-related behaviours in rodents are often assessed with non-touchscreen tests utilizing aversive stimuli and/or contexts, such as tests of learned helplessness (e.g., FST, tail suspension test) and anxiety-like behaviour (e.g., EPM, light/dark box). However, these tests only take into consideration the negative valence systems domain of the RDoC. Furthermore, paradigms like the FST are used to both test for depression-like behaviour and induce a depression-like state, thereby conflating the distinction between a behavioural assay and a behavioural model (reviewed in O'Leary & Cryan, 2013). As a result, it can be unclear whether a manipulation (e.g., neurogenesis knockdown) directly affects swimming behaviour in the FST or whether such a manipulation confers susceptibility to the depressive-model aspect of the FST. Additionally, such tests are often stressful due to the use of aversive stimuli/contexts and, as mentioned previously in this chapter, neurogenesis and the stress response are closely linked: AHN modulates the stress response due to its negative feedback on the HPA axis, so the stress response is exacerbated when hippocampal neurogenesis is impaired. Therefore, the use of stressful tests to assess depression-relevant behaviours in rodent models with reduced neurogenesis may introduce the confound of stress effects on behavioural and/or physiological measures.

Due to the potential for confounding effects of stress in and conflating assay and model aspects of negative valence systems behavioural tests, I focused on the positive valence systems domain for my thesis, which is currently under-represented in the literature. Specifically, I assessed the impact of neurogenesis knockdown on tests sensitive to dysfunction in reward-related positive valence systems behaviour. I hypothesized that newborn neurons in the DG of

the adult hippocampus are a contributing factor in emotion regulation, specifically the regulation of depression-relevant reward-related behaviour under baseline (i.e., non-stressful) conditions. Using the nestin-TK mouse model of neurogenesis knockdown, I used touchscreen operant chambers to assess depression-relevant reward-related behaviours, such as response to reward/ feedback information (the PRL test), willingness to expend effort to receive reward (i.e., motivation; PR), and appetitive extinction. I predicted that neurogenesis knockdown would result in blunted or impaired sensitivity to feedback information on PRL and lowered motivation on PR. It was also expected that appetitive extinction learning may be impaired. In order to compare the results obtained from these reward-related touchscreen tests, two common negative valence systems tests were run, namely the light/dark box and EPM.

2. Method and Materials

2.1 Animals

Adult nestin-TK transgenic mice were used (Jackson Laboratory stock no. 029671; N = 117, 63 males and 54 females), where adult was defined as ≥60 days. The mice were assessed for up to approximately 14 months of age (i.e., used within the period of 60-460 days). All mice were bred on-site within the pre-existing colony and were housed 2-4 per shoebox cage under standard laboratory conditions (12-hr reverse light-dark cycle, with lights off at 09:00 and on at 21:00). Environmental enrichment included in the home cages were Enviro-Dri rodent bedding and nestlets. All experimental procedures and animal use followed the University of Western Ontario Animal Care and Veterinary Services (ACVS) guidelines.

Nestin-TK transgenic mice were used as they allow for direct manipulation of neurogenesis. The nestin-TK mouse line was generated by injecting a modified herpes simplex virus TK (*δ*-HSV-TK, which I will refer to as simply HSV-TK or viral TK), tagged with enhanced green fluorescent protein (eGFP), was injected into nuclei of fertilized murine eggs on a C57BL/6 background (Yu, Zhang, Liebl, & Kernie, 2008). Nestin is a structural protein found in many tissues, including neural stem cells, responsible for regulating intermediate filament formation and overall cell structure and organization during differentiation (Lendahl, Zimmerman, & McKay, 1990; Yu et al., 2008). To ensure region-specific expression, the transgene was driven by a neural progenitor-specific *nestin* promoter (Yu, Dandekar, Monteggia, Parada, & Kernie, 2005; Yu et al., 2008); thus, viral TK was only expressed in nestin-containing neural stem cells (i.e., co-expression of nestin and viral TK).

Neurogenesis ablation is conditional upon administration of the antiviral drug VGCV

(valganciclovir), or its active form GCV (ganciclovir [Singer, et al., 2009; Yu et al., 2008]). VGCV is a valyl ester prodrug of GCV and is rapidly hydrolyzed to GCV upon oral administration (Jung & Dorr, 1999). In human studies comparing pharmacokinetic profiles of intravenous (IV) GCV, oral GCV, and oral VGCV, oral GCV showed the lowest bioavailability and longest half-life (Jung & Dorr, 1999), with multiple doses of oral VGCV exhibiting greater exposure than IV GCV (Brown, Banken, Saywell, & Arum, 1999). Therefore, VGCV was chosen over GCV due to the greater bioavailability, increased convenience, and decreased risk of complications arising from chronic daily injections (as was seen in mice following IV GCV treatment [Boujemla et al., 2016]). Viral TK is an isoenzyme of endogenous TK, which is responsible for phosphorylating thymidine (nucleoside) prior to incorporation into the DNA backbone as thymine (nucleotide). VGCV/GCV is a high affinity ligand to viral TK, competing with the endogenous nucleoside ligand. Upon phosphorylation, VGCV/GCV is able to be incorporated into the DNA backbone as a guanine analogue (Brown et al., 1995; St. Clair, Lambe, & Furman, 1987). This analogue cannot be read by DNA polymerase and disrupts its function, terminating cellular replication (Borrelli, Heyman, Hsi, & Evans, 1988). As an antiviral medication, this mechanism results in terminating the replication of virus-infected cells; however, in the nestin-TK transgenic mice, the viral TK/VGCV mechanism only occurs in cells containing nestin. Neural stem cells no longer undergo replication, effectively knocking down neurogenesis. Nestin-TK transgenic mice have a useful advantage, namely that this neurogenesis knockdown is reversible due to the requirement of VGCV to bind to the viral TK (Singer et al., 2009).

2.2 Valganciclovir Administration

VGCV (Valganciclovir hydrochloride Pharmaceutical Secondary Standard, Sigma-Aldrich Canada, Oakville ON) was administered orally via a powdered rodent chow mixture (2018 Teklad global 18% protein rodent diet, Envigo, Indianapolis IN). VGCV was added into the chow at a dose of 0.06%, with a target consumption of 70 mg/kg/day. Animals were given VGCV-treated or control diet every weekday for 6-8 weeks before commencing behavioural assessment, and continued throughout behavioural testing. There were four genotype/drug groups, in which the experimental group was the TK+ with VGCV treatment (TK+/VGCV) animals and the three littermate control groups were the TK+ without treatment (TK+/ctrl), TK- with VGCV treatment (TK-/VGCV), and TK- without treatment (TK-/ctrl) animals. Each littermate control group served its own purpose and controlled for non-specific effects of genotype and treatment, which could confound obtained findings. The TK+/ctrl group controlled for any unforeseen influence of the addition of the HSV-TK transgene, whereas the TK-/VGCV group controlled for any non-specific effects of the drug treatment. The TK-/ctrl group served as the wild-type, untreated controls.

2.3 Spontaneous Location Recognition

A subset of naive mice ($N = 40$, 18 males and 22 females) completed the spontaneous location recognition (SLR) test, which has been described previously and shown to be sensitive to changes in neurogenesis (Bekinschtein et al., 2013). SLR was designed to assess an animal's ability to distinguish two similar locations in memory, an ability thought to be dependent on the process of pattern separation. SLR was run in a circular open field constructed with black plastic (47 cm diameter x 30 cm high). The open field was located in the centre of a black enclosure (61

cm x 61 cm x 123 cm). Three of the walls of the enclosure each had unique black and white images attached to them that served as distal spatial cues (see Figure 1A for apparatus schematic). The fourth wall served as the door, without distal cues attached. The enclosure had an open top for ease of recording animal behaviour in the open field. Standard mouse bedding (Bed-o'Cobs Combination bedding, The Andersons Inc., Delphi IN) covered the floor of the open field. An assortment of objects was used as stimuli and were made of either plastic or glass, measuring between 3-8 cm wide and 8-12 cm high. Sticky Tac (Tac'N Stick Reusable Adhesive, Elmer's, High Point NC) was used to fix the objects to the floor of the open field to prevent mice from pushing and/or moving the objects during exploration. The open field walls and objects were cleaned with 5% ethanol between animal trials and between sample and choice phases (see "SLR test" subsection below [subsection 2.3.2]). A video camera was placed directly above the open field and animal location/movement was tracked using ANY-Maze behavioural tracking software (Stoelting Company, Wood Dale IL). Videos were saved for later offline behavioural hand-scoring.

2.3.1 Habituation.

Animals were allowed to freely explore the empty arena (i.e., no objects) for 10 min per day for 5 days prior to the test phase. The purpose of the habituation phase was to acclimatize the animals sufficiently to the open field to reduce the stress associated with a novel environment, maximizing object exploration during the test phase. The total distance travelled in the arena was measured with ANY-Maze software for each day of habituation.

2.3.2 SLR test.

The test phase began the next day after the final day of habituation. A trial consisted of two phases: Sample and choice (see Figure 1B). During the sample phase, mice were exposed to three identical objects (A1, A2, and A3), each placed 7.5 cm from the arena wall, and allowed to freely explore the objects for 10 min. For the similar condition SLR [s-SLR (i.e., small separation)], objects A2 and A3 were placed 50° apart (20.5 cm separation) and object A1 was at an equal distance from each A2 and A3. For the dissimilar condition SLR [d-SLR (i.e., large separation)], all three objects were equidistant with 120° apart from each other (49 cm separation between each).

Three hours after the sample phase, mice were exposed to two new identical copies of the objects (A4 and A5) and allowed to explore the objects for 5 min. New copies of the original objects were used to prevent the use of olfactory cues. Object A4 was placed in the same location as A1 in the sample phase and served as the familiar location, whereas A5 was placed in a novel location. The novel location was defined as the position exactly in between the locations of A2 and A3 from the sample phase (i.e., the midpoint of the positions of A2 and A3 in the sample phase). Mice were run twice on the test phase, once on d-SLR and once on s-SLR, with a 72-hr period separating tests in each configuration. Different sets of objects were used for the d-SLR and s-SLR to ensure object novelty for each test and to prevent object habituation or object-specific experiences that may be transferred to the next test. Half of the mice were tested first on s-SLR followed by d-SLR and vice versa for the other half. Sex, genotype, and drug treatment were counterbalanced for all experiments.

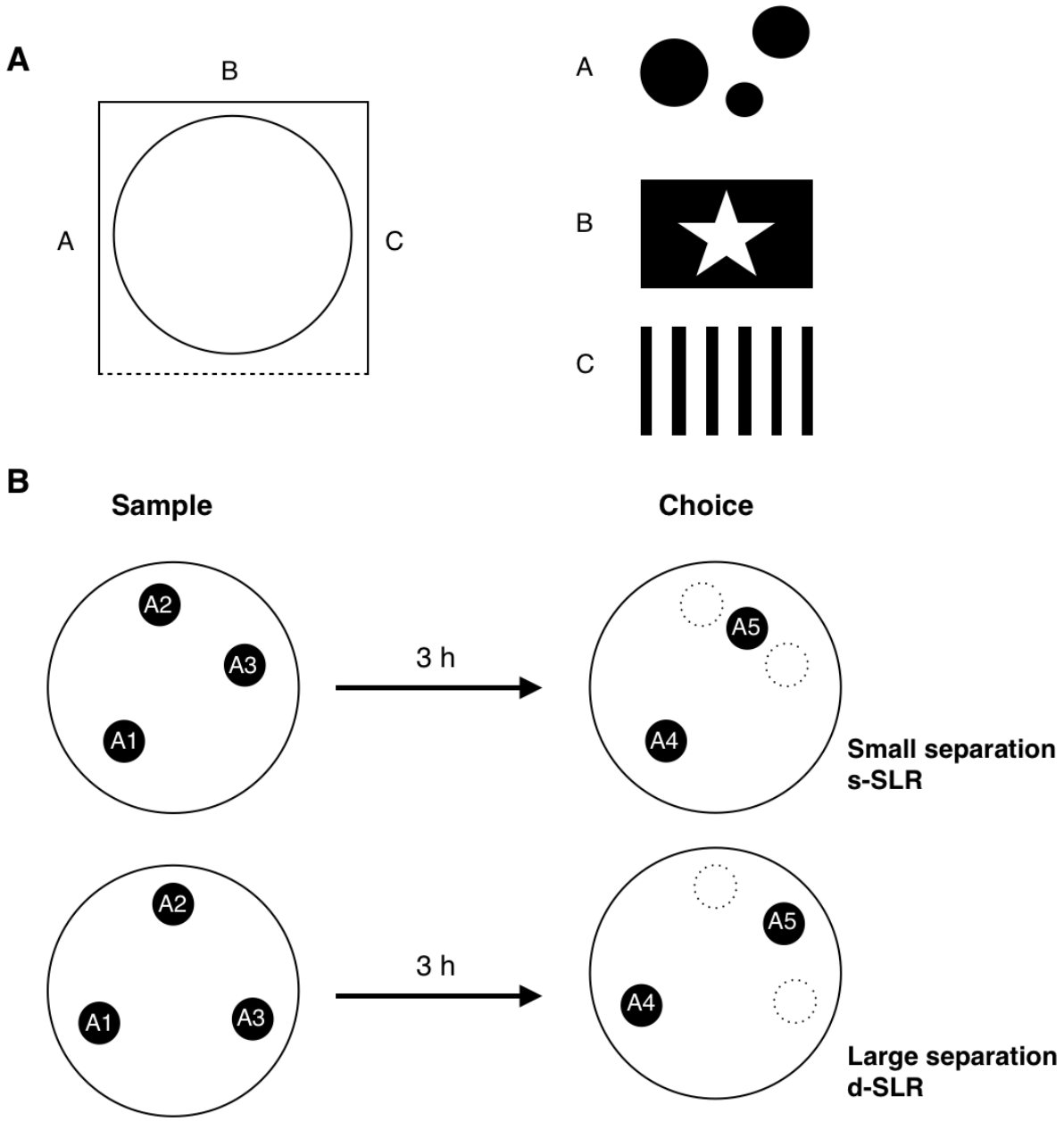

Figure 1. The spontaneous location recognition (SLR) test.
(A) Schematic of apparatus set up and distal spatial cues. The circular open field (47 cm diameter x 30 cm high) was placed in the centre of a black enclosure (61 cm x 61 cm x 123 cm). The dotted line depicts the "door" of the enclosure. All walls, except for the door, had a distal spatial cue attached. Wall A had a pattern of three circles with varying sizes (in the actual apparatus, these were white to be visible against a black wall); wall B had a rectangle with a star (in the actual apparatus, the star was painted black with a white background to be visible on the black

wall); and wall C had a rectangle with alternating white and black stripes. The apparatus had an open top for ease of capturing behaviour on video. (B) Schematic of object locations in the SLR test phase. The test phase followed five days of habituation to the open field arena. Each trial of the test phase consisted of a sample and a choice phase. The sample phase consisted of three identical objects (A1, A2, A3) placed at specific locations according to test configuration. For s-SLR, objects A2 and A3 were placed 50° apart (20.5 cm separation) and object A1 was placed at an equal distance from each A2 and A3. For d-SLR, all objects were placed equidistant to each other, 120° apart (49 cm separation between each). Three hours following the sample phase, mice were tested on the choice phase. The choice phase consisted of two new identical copies of the object (A4 and A5). Object A4 was placed in the location previously occupied by object A1 (i.e., familiar location) and object A5 was placed at the midpoint between the previous locations occupied by objects A2 and A3 (i.e., novel location). In both phases, objects were placed 7.5 cm from the arena wall. Mice were exposed to both SLR configurations and different object sets were used across configurations to maintain object novelty. Adapted from Fig. 1(A) and (B) in Bekinschtein et al., 2013, respectively.

Sample and choice phase trials were recorded for manual offline assessment of exploratory behaviour, where exploration was defined as the mouse having its nose directed at the object at a distance of <1 cm or touching the object with its nose (i.e., overt sniffing behaviour). Rearing with the nose oriented upward, climbing over the object, and sitting on the object were not.

2.4 Touchscreen Behavioural Tests

To assess depression-relevant reward-related behaviours, sixteen Bussey-Saksida mouse touchscreen operant chambers were used (see Figure 2; Lafayette Instrument Company, Lafayette IN). Behavioural task programs were run with Animal Behaviour Environment Test (ABET) II Touch software (Campden Instruments Ltd, Lafayette IN) and Whisker Server

(Cardinal & Aitken, 2010; Whisker Standard Software, Lafayette Instrument, Lafayette IN). Prior to behavioural assessment, completion of standard pre-training was required in which mice learned how to use the system. Upon completion of the pre-training stages, animals were subjected to a panel of tests, starting with probabilistic reversal learning (compares responses to reward and non-reward [PRL]), followed by progressive ratio (assesses motivation to exert effort for reward [PR]), and ending with extinction learning (assesses responses to chronic omission of reward).

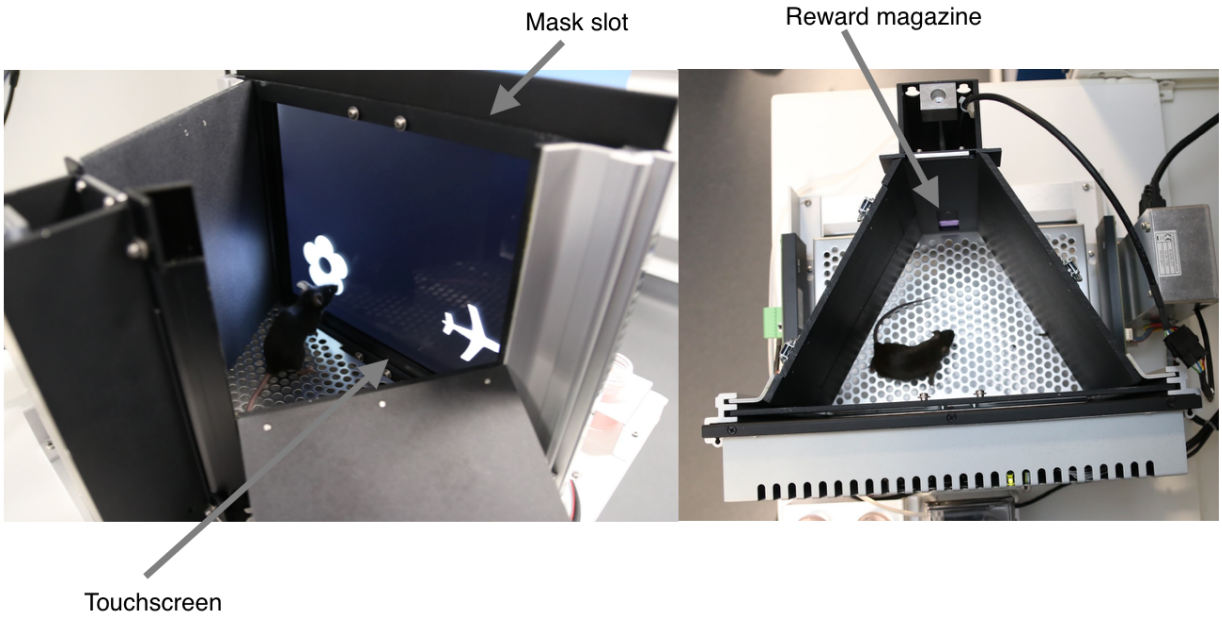

Figure 2. The Bussey-Saksida mouse touchscreen operant chamber apparatus.

2.4.1 Pre-training.

Three days prior to the start of pre-training, mice ($N = 93$, 45 males and 48 females) were put under food restriction and maintained at 85-90% of their baseline free-feeding weight. Baseline was defined as the average weight over the three days preceding food restriction. Touchscreen operant chambers rely on instrumental learning, in which rewards are used to

reinforce optimal responding. Rewards used were strawberry milkshake (Neilson Dairy Limited, Toronto ON), therefore food restriction increases the reinforcement value of the reward and enhances motivation to respond to stimuli presented on the screen. Strawberry milkshake was added to the rodent chow a minimum of three days prior to pre-training to habituate the mice to the taste of the reward.

A modified version of the standard pre-training task sequence (Horner et al., 2013) was used to familiarize the mice with operating the touchscreen system (see Figure 3 for overview). A 3-hole mask (7 cm x 7 cm per hole) was used for all stages of pre-training in preparation for PRL. For all pre-training stages that required stimulus response (Stages 2-4), the stimulus (white square) was presented in one of the two flanker positions of the mask and alternated across trials in a pseudorandom fashion such that on half the trials the stimulus appeared on the right and on the the other half the stimulus appeared on the left; the middle position always remained blank (i.e., no stimulus presented). Chambers were cleaned with 10% ethanol after each mouse.

2.4.1.1 Stage 1: Habituation.

The purpose of the habituation stage was to allow the mice to acclimatize to the chamber and reward delivery. Habituation took place incrementally over four days.

2.4.1.1.1 Habituation 1.

On the first day, mice were placed in the touchscreen chamber for 10 min. There was no reward delivery or stimulus presentation. Habituation 1 allowed familiarization with the chamber itself.

2.4.1.1.2 Habituation 2.

On the second day, reward delivery was added to the habituation paradigm to allow

familiarization with the reward collection process, including the reward magazine itself and secondary reward cues such as the tone and magazine light turning on. There were two phases of Habituation 2, the first of which required the mice to be in the chamber for 20 min (2 days) and the second of which required the mice to be in the chamber for 40 min (1 day). Upon initial nose-poke into the reward magazine, the magazine light turned off. The criterion for moving past Habituation 2, and thus Stage 1, was consumption of all milkshake delivered in the second phase.

2.4.1.2 Stage 2: Initial touch.

The purpose of this stage was to teach the mice to touch the stimulus presented on the screen and collect the consequent reward (see Figure 3). The stimulus remained on the screen for 30 s. If the mouse did not respond to (i.e., failed to touch) the stimulus after 30 s, the stimulus was removed from the screen and the standard amount of milkshake was delivered (800 ms, or ~5 µl), accompanied by a tone and the magazine light. However, if the mouse responded to the stimulus within 30 s, the stimulus was removed upon response and 3 times the usual amount of milkshake reward was delivered (2400 ms, or ~15 µl). Upon consumption of the reward and removal of the head from the magazine, the inter-trial interval (ITI) began (10 s), starting the next trial. Following the ITI, the stimulus was presented again. The criterion for Stage 2 was completing 30 correct trials in 60 min, where correct was defined as trials in which the stimulus was touched within 30 s.

2.4.1.3 Stage 3: Must touch.

The purpose of this stage was to teach the mice to respond to the stimulus for reward delivery (see Figure 3). The stimulus remained on-screen until a response was made, after which

milkshake was delivered (800 ms; ~5 µl). All other aspects of the task remained the same as in Stage 2/Initial Touch. The criterion for Stage 3 was completing 30 trials in 60 min.

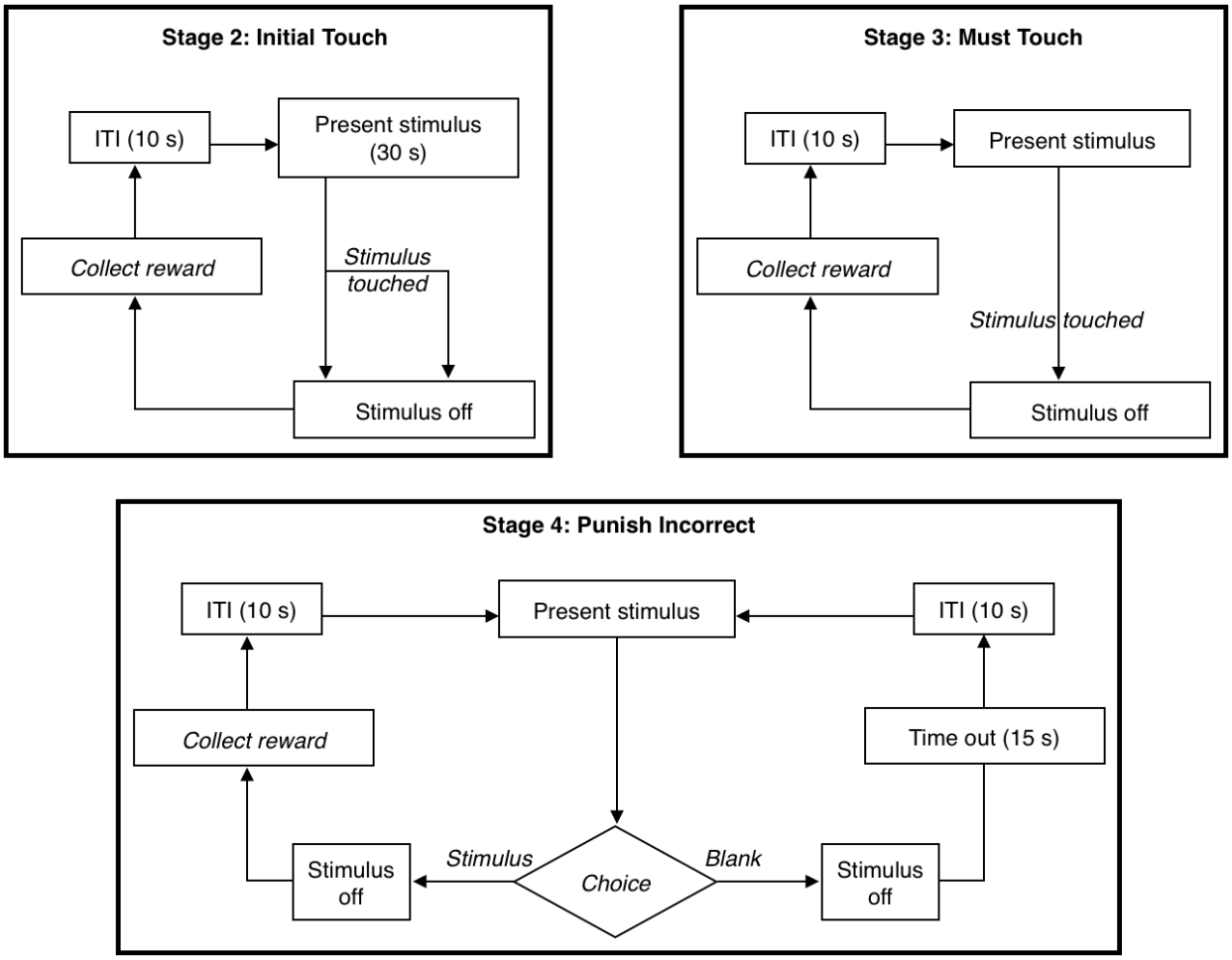

Figure 3. Flowchart overview of stages 2-4 of the touchscreen pre-training.

Initial Touch (Stage 2) followed habituation (Stage 1), in which mice were familiarized with the chamber, milkshake delivery and collection, and reward cue tones. A single white square stimulus was presented on-screen for 30 s. If the stimulus was not touched within the 30 s, the stimulus was removed and the standard amount of milkshake was delivered (800 ms; ~5 µl), together with the reward cue tone and reward magazine light. If the stimulus was touched, the stimulus is removed from the screen and triple the standard milkshake amount was delivered (2400 ms; ~15 µl) with the reward cue tone and magazine light. After reward collection, the inter-trial interval (ITI) began prior to the stimulus re-presentation. To move to the next stage,

mice were required to complete 30 correct trials in 60 min. Must Touch (Stage 3) proceeded as Initial Touch, but the stimulus remained on the screen until touched. Reward (800 ms; ~5 μl) was received only when a response to the stimulus was made. To move to the next stage, mice were required to complete 30 trials in 60 min. Punish Incorrect (Stage 4) introduced the "no reward" condition for an incorrect response (i.e., touching a blank square instead of the stimulus). If the stimulus was touched, the task proceeded as in Must Touch. If the blank square was touched, the stimulus was removed from the screen and a 15-s time-out period preceded the ITI. No milkshake was delivered, but the magazine light (a secondary reward cue) still illuminated. Successful completion of Punish Incorrect required ≥80% correct responses, or 24 correct out of 30 total trials, over two consecutive days. Adapted from Fig. 1 in Mar et al., 2013.

2.4.1.4 Stage 4: Punish incorrect.

The purpose of this stage was to enable familiarization with the consequence of an incorrect response (see Figure 3). If the mouse responded to the side opposite the stimulus (i.e., blank side), no reward was given and a 15-s time-out period preceded the ITI, but the magazine light did turn on as in the correct trials. If the stimulus was touched, the trial proceeded as in Stage 3/Must Touch. The criterion for Stage 4 was ≥80% correct responses (i.e., 24 correct trials out of 30) made over two consecutive days. If animals satisfied this criterion at different rates, those who satisfied the criterion earlier were put on maintenance, in which Punish Incorrect was only run once a week. Once all animals satisfied the criterion, and thus completed pre-training, all animals were able to move on to the touchscreen test panel, beginning with PRL.

2.4.2 Probabilistic reversal learning.

Upon completion of pre-training, mice were then tested on PRL (N = 59, 34 males and 25 females). PRL was designed to assess sensitivity to valenced (i.e., positive or negative) feedback

and was previously shown to be sensitive to depression-relevant manipulations, such as serotonin manipulation (Bari et al., 2010; Phillips et al., 2018). Each session required the mouse to complete 90 trials in 60 min. On a given trial, the mouse was presented with two white square stimuli in the flanker positions of the 3-hole mask, where one was designated correct/optimal (i.e., S+) and the other incorrect/suboptimal (i.e., S-). The middle opening always remained blank (see Figure 4A). PRL included two phases, a training phase (deterministic reversal training) and a testing phase (where probabilistic feedback was introduced).

2.4.2.1 Deterministic reversal training.

The training phase was designed to familiarize the mouse with within-session reversals. A response to the correct stimulus always resulted in milkshake delivery (800 ms; ~5 µl), whereas a response to the incorrect stimulus always resulted in milkshake being withheld, though the magazine light (secondary reward cue) turned on regardless of response type. The tone (another secondary reward cue) was only played upon a correct response (see Figure 4B). After 5 consecutive correct responses, the stimulus-reward contingencies were reversed, such that the previously correct stimulus became incorrect and vice versa (see Figure 4C). Animals continued deterministic reversal training until stable performance was reached, defined as three consecutive days of no significant difference between the number of reversals completed per session and no difference in response accuracy (as per Phillips et al., 2018). If animals satisfied this criterion at different rates, those with earlier performance stabilization were put on maintenance, in which the reversal training task was only run once a week. Once all animals satisfied the criterion, all animals were then run on reversal training to re-baseline performance.

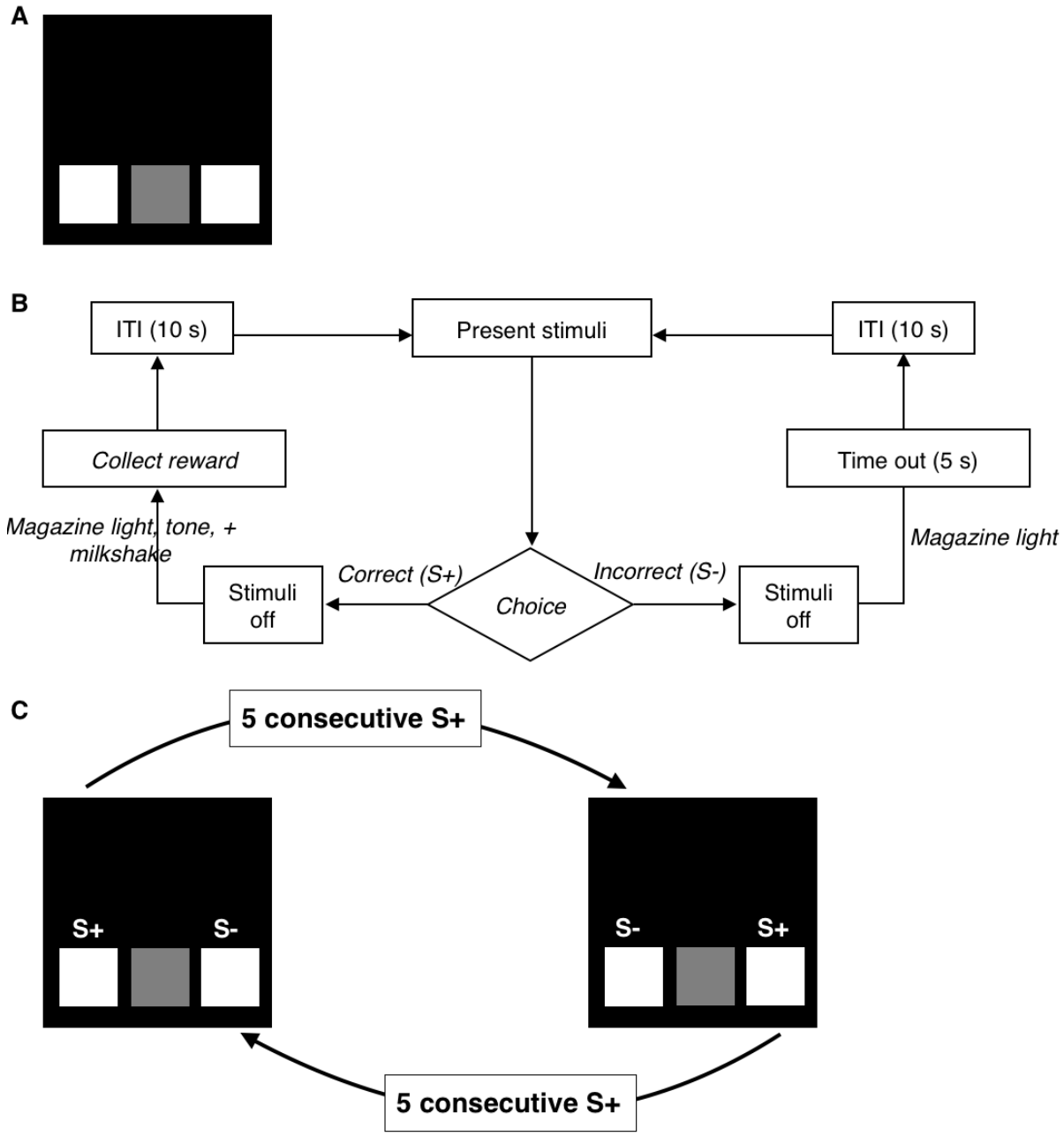

Figure 4. The probabilistic reversal learning (PRL) test.
(A) The 3-hole mask was used for PRL. Each opening measured 7 cm x 7 cm. The white square stimuli were presented in the flanker positions of the mask (i.e., openings 1 and 3) and the middle opening remained blank. (B) Flowchart of choice outcomes. For a response to the stimulus designated as correct/optimal (i.e., S+), milkshake was delivered (800 ms; ~5 µl) in conjunction with two other secondary reward cues (i.e., magazine light and tone). For a response to the stimulus designated as incorrect/suboptimal (i.e., S-), no milkshake was delivered and no

tone sounded, but the magazine light turned on. The response was followed by a 5 s time out. The mouse was required to insert their head into the magazine as if a reward was present. The 10-s inter-trial interval (ITI) began once the mouse removed their head from the magazine. During the within-session serial reversal training phase, these outcomes were deterministic (i.e., S+ responses always resulted in reward and S- responses always resulted in no reward). During the PRL test proper, choice outcomes were probabilistic, where a response to S+ resulted in reward 80% of the time and no reward the other 20% and vice versa for an S- response. For both test phases, the mice were required to complete 90 trials within 60 min. Adapted from Fig. 3(B) in Mar et al., 2013. (C) Schematic of within-session serial reversals. For both the training phase and the test proper, 5 consecutive responses to the stimulus designated as S+ resulted in reward contingency reversal (i.e., the stimulus that was the S+ was now the S- and vice versa). Adapted from Fig. 2(A) in Phillips et al., 2018.

2.4.2.2 Introduction of probabilistic feedback.

Following stable performance on the reversal training phase, probabilistic feedback was introduced (see Figure 4B-C). A response to the optimal stimulus resulted in milkshake delivery 80% of the time and no milkshake delivery the other 20% (i.e., the same feedback as an incorrect response). Similarly, a response to the suboptimal stimulus resulted in milkshake being withheld 80% of the time and milkshake delivery the other 20% (i.e., the same feedback as a correct response, including all secondary reward cues). All other aspects of the task were retained (i.e., within-session reversals after 5 consecutive correct trials). The endpoint criterion was stable performance across 3 consecutive days, where stable performance was defined as above.

2.4.3 Progressive ratio.

Upon completion of PRL, the same animals progressed on to PR ($N = 93$, 45 males and 48 females). A subset of these animals ($N = 16$ females) were previously tested on SLR and not

PRL. PR is a classic test for assessing motivation; previous use in touchscreen operant chambers has shown reduced performance with administration of dopamine antagonists and increased performance with amphetamine treatment (Heath et al., 2015). A 5-hole mask (4 cm x 4 cm per hole) was used, with the white square stimulus presented in the centre hole (see Figure 5A); all others remained blank. When multiple responses were required, a response resulted in the brief removal of the stimulus (500 ms), accompanied by a click, and milkshake was delivered (800 ms; ~5 µl) following the response that satisfied the task ratio. To encourage fast responding, the ITI was reduced to 4.5 s. PR included four phases, namely training for repeated responding to the same stimulus, basic PR-4, high demand PR, and uncapped FR-5. Testing occurred on consecutive days both across and within phases. Task breakdown is displayed in Figure 5 (fixed ratio: 5B; progressive ratio: 5C).

2.4.3.1 Repeated responding training.

In order to train the requirement of repeated responding for one reward, the mice were run through 4 fixed ratio (FR) schedules. The first was FR-1, wherein one response to the stimulus resulted in reward. Second was FR-2, wherein two responses to the stimulus were required for milkshake delivery. FR-2 was followed by FR-3, wherein three responses were required for reward. For each of FR-1, -2, and -3, the criterion for moving on to the next schedule was completing 30 trials in 60 min. Following FR-3 was three days of FR-5, wherein five responses were required for reward. In order to move on to basic PR-4 testing, the mice were required to complete 30 trials within 60 min on the final day of FR-5, at minimum.

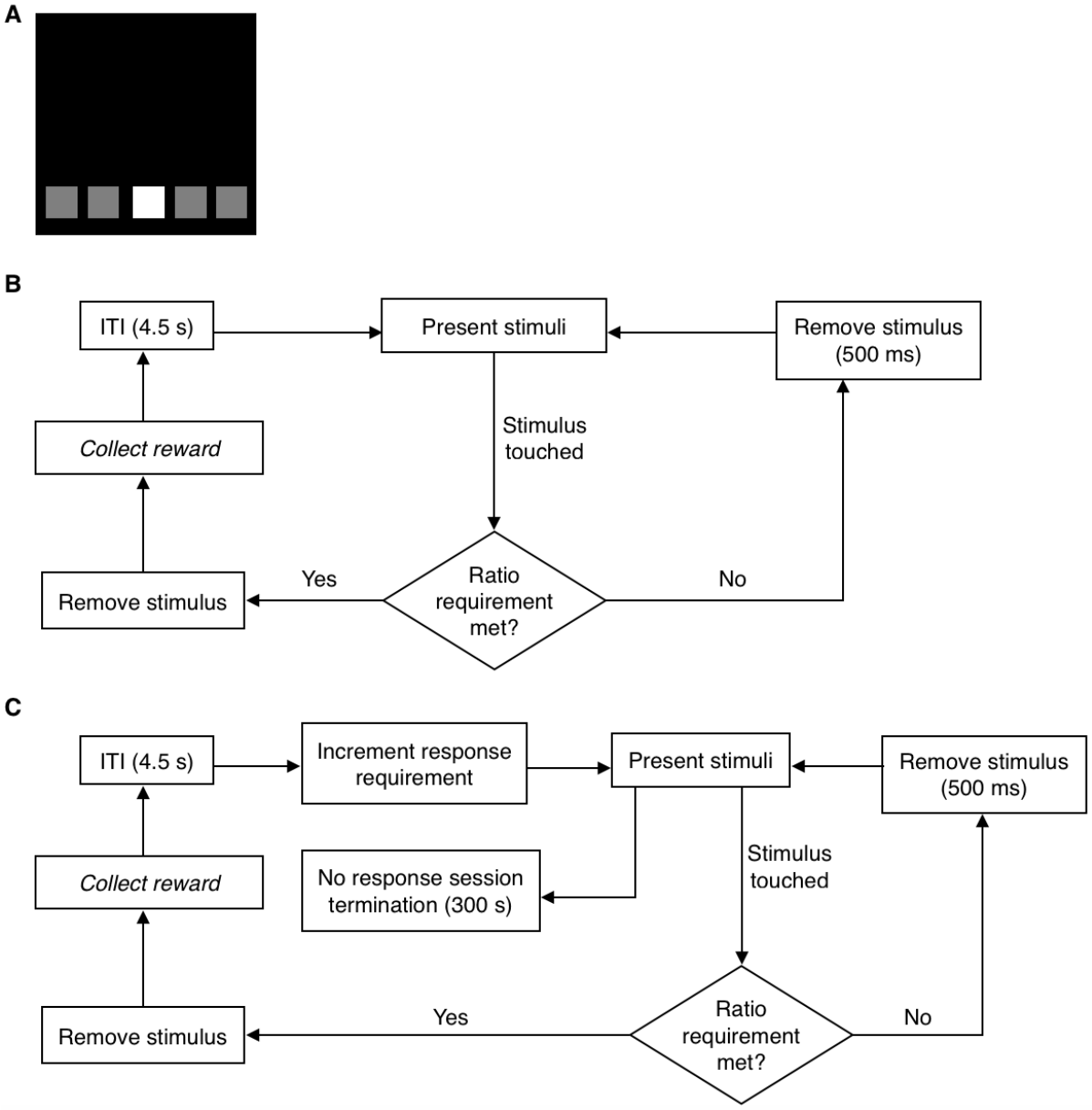

Figure 5. The fixed ratio (FR) and progressive ratio (PR) tasks.
(A) The 5-hole mask was used. Each opening measured 4 cm x 4 cm. The white square stimulus was presented in the middle opening (i.e., opening 3) and all others remained blank. (B) Flowchart of the FR protocol. FR required a fixed number of responses to the stimulus to initiate milkshake delivery. For example, an FR-5 schedule required 5 responses to the stimulus for reward delivery. If less than 5 responses had occurred, the stimulus was briefly removed and presented again. If exactly 5 responses had occurred, the stimulus was removed and milkshake was delivered (800 ms; ~5 µl). Upon reward collection, a 4.5-s inter-trial interval (ITI) began.

The ITI was reduced from the standard 10 s to promote a quick response rate. After every response to the stimulus, there was a click-like sound. This sound in conjunction with the brief 500-ms stimulus removal indicated that a response had occurred and another was required. Adapted from Fig. 4 in Health et al., 2016. (C) Flowchart of the PR protocol. The response requirement increased on a linear ramp with every trial in a PR session. For example, the response requirement on a PR-4 schedule increased by 4 with every trial (i.e., 1 response for the first trial, 5 responses for the second trial, 9 responses for the third, and so on). The logistics of the task were identical to FR, with two exceptions. The first exception was the increase in response requirement between trials and the second was the criterion for session termination. After 300 s (or 5 min) of no response to the stimulus, the task terminated. Adapted from Fig. 5 in Heath et al., 2016.

2.4.3.2 Basic PR-4.

Basic PR-4 included two blocks of PR-4 separated by one block of FR-5. Each block consisted of three testing days (i.e., nine days total; six days PR-4 and three days FR-5). PR-4 required the mice to respond to the stimulus 4 times more than the previous trial, following a linear ramp. For instance, trial 1 required 1 response, trial 2 required 5 responses, trial 3 required 9 responses, and so on. The session ended either after 60 min had passed or the mouse was inactive for 5 min consecutively. The FR-5 block between the two PR-4 blocks was included to maintain and reinforce multiple responding to the stimulus. Mice were required to complete the 30 trials of FR-5 within the 60-min session.

2.4.3.3 High demand PR.

Immediately following the nine days of basic PR-4, high demand PR started, in which the response ratio increased with each testing day. Similar in structure to basic PR-4, there were two blocks of high demand PR interleaved with one block of FR-5. Blocks were three days each. The

sequence of response ratios for high demand was PR-4, followed by PR-8, and then PR-12, one on each day for each block. This sequence was repeated for both blocks. The baseline FR-5 block was run as above.

2.4.3.4 Uncapped FR-5.

Immediately following the final day of high demand PR (i.e., PR-12 of the second block), mice were tested on uncapped FR-5 across three days. The task required the mice to complete as many trials as possible within 60 min. Uncapped FR-5 can serve as a control to assess whether altered responding on PR tasks might be driven by effects on satiation.

2.4.3.5 Reward Strength Timelines.

As the effect of neurogenesis deficits on PR performance has been previously shown to be affected by reward strength (Karlsson et al., 2018), PR was repeated using three milkshake concentrations: 20%, 50%, and 100% (undiluted). Milkshake was diluted with tap water to the appropriate concentration. To control for the order of exposure to each concentration, mice were separated into two groups so both ascending and descending concentration exposures were assessed. The first group followed Timeline A, wherein the full PR protocol was followed for 100%, followed by 50%, and then 20% milkshake. Another round of high demand with 100% milkshake was run after exposure to all three concentrations (i.e., three days of 30 trials on FR-5, high demand PR as above, and three days of uncapped FR-5 as above). The second group followed Timeline B, wherein PR was repeated for 20%, followed by 50%, and then 100% milkshake. As in Timeline A, another round of high demand with 20% milkshake was run after completion of the three rounds of PR. The addition of a second round of high demand PR with

the initial concentration was to assess for training effects on responding with repeated PR testing.

2.4.4 Extinction learning.

Five out of six cohorts of mice previously tested on PR were run on extinction learning following completion of their full PR timeline (N = 77, 45 males and 32 females). The excluded cohort (n = 16 females) was used to equalize group numbers on the PR task. Extinction learning involved the withdrawal of reward for a previously rewarded stimulus. The same mask and stimulus that were used for PR were used for extinction learning (i.e., 5-hole mask, with white square stimulus presented in the middle opening; see Figure 6A). The extinction learning paradigm used was modified from that previously described by Mar et al. (2013). Mice were first run on an FR-1 schedule with undiluted milkshake reward, in which 30 trials were to be completed within 60 min, to obtain a baseline (i.e., stable performance). Stable performance was defined as no significant difference between the time taken to finish the 30 trials across three consecutive days. This period of stabilization is equivalent to the acquisition phase described by Mar et al. (2013).

Once performance was stabilized, extinction began, wherein a response to the stimulus did not result in reward delivery, or any secondary reward cues (magazine light and tone; see Figure 6B). Once the stimulus was presented, it remained on the screen for 10 s, or until a response was made. If one of those conditions was met, the stimulus turned off and the ITI (10 s) began. Mice were run on the extinction test for a minimum of 10 days, after which the endpoint criterion was followed. The endpoint criterion was two consecutive days with ≥77% omissions, or 23 out of 30 trials in which the stimulus was not touched (as per Mar et al., 2013).

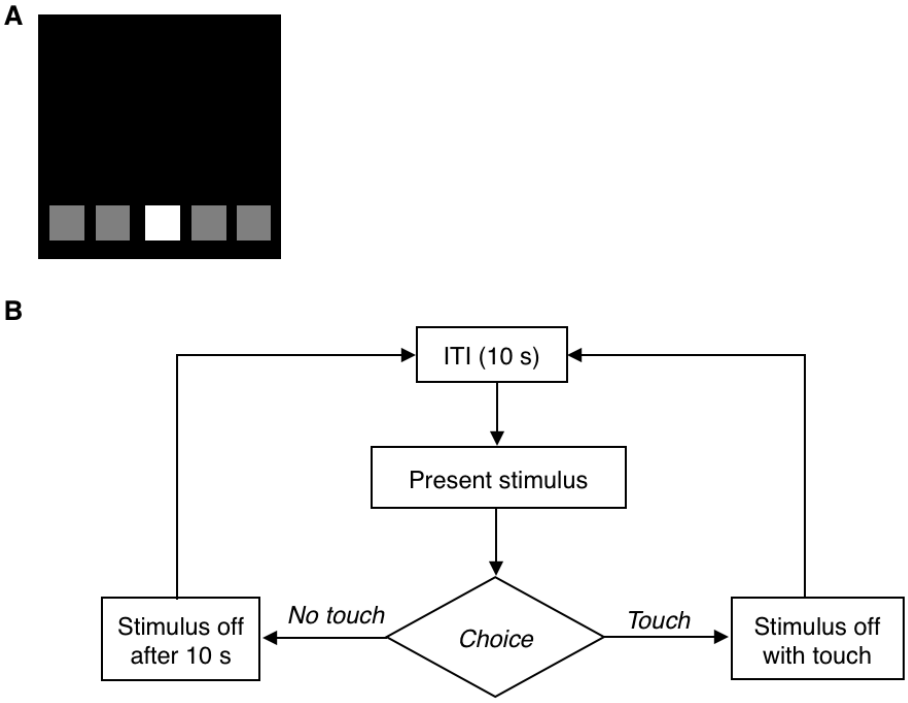

Figure 6. The extinction learning test.
(A) The 5-hole mask was used, which was identical to that used for FR and PR tasks. Each opening measured 4 cm x 4 cm. The white square stimulus was presented in the middle opening (i.e., opening 3) and all others remained blank. (B) Extinction learning required the mouse to cease responding to a previously rewarded stimulus, hence the same stimulus set-up as in FR/PR. If the mouse responded to the stimulus, the stimulus was removed and the 10-s inter-trial interval (ITI) began. There was no reward delivery and no secondary reward cues (i.e., magazine light, tone). If the mouse did not respond to the stimulus within 10 s (i.e., an omission), the stimulus was removed and the ITI began. Adapted from Fig. 2(C) in Mar et al., 2013.

2.5 Reward Concentration Preference Test

All mice that completed extinction learning ($N = 77$, 45 males and 32 females) continued on to a choice-based reward concentration preference test in order to verify the assumption that 100% milkshake was the most rewarding (i.e., strongest reward) milkshake concentration, followed by 50%, and 20% the least rewarding (i.e., weakest reward). Vacuum vials (13 ml

capacity) with a hole cut into the rounded end were used as liquid dispensers. The vacuum seal prevented excess liquid from dripping out of the vial, but allowed liquid to be dispensed when disturbed, such as when a mouse licked at the liquid. In this way, error due to extraneous spillage/dripping of liquid was minimized. Animals were placed in new cages for the duration of the 2-hr test and replaced in their home cages after the test. The task included habituation and test phases, for a total of five days.

2.5.1 Habituation phase.

Habituation occurred over two days and was designed to allow acclimatization to drinking from the vacuum vials and the new cage. Each animal had access to two vials, one on each side of the cage top, for 2 hr. For habituation, the vials were filled with tap water. To minimize effects of location, the vial positions were switched on the second day of habituation. Vials were weighed before and after the 2-hr habituation.

2.5.2 Test phase.

Preference testing occurred over three days, wherein animals were exposed to a different binary choice of concentrations each day (e.g., Day 1: 100% vs. 50%, Day 2: 100% vs. 20%, Day 3: 50% vs. 20%). The side on which the higher concentrated milkshake was located was counterbalanced across days to control for location effects (i.e., as in habituation above, the vials were switched each day). Additionally, the order of binary choice exposure was counterbalanced across animals to control for possible order effects on drinking behaviour. Vials were weighed before the test and four times throughout the test, specifically after 10 min, 30 min, 60 min, and

120 min (test endpoint). Vial weights were taken at these four time points to obtain a consumption curve throughout test duration.

2.6 Negative Valence Systems Tests

Following the reward concentration preference test, all mice ($N = 77$, 45 males and 32 females) were then tested on two classic tests to assess negative valence systems (e.g., anxiety- and fear-related responses). The first was the light/dark box, which was followed by the EPM.

2.6.1 Light/dark box.

The light/dark box is a classic test of anxiety-like behaviour in rodents based on the innate aversion to brightly lit areas (Bourin & Hascoët, 2003). The apparatus was built on-site and the dimensions used were informed by those previously reported, as was the protocol followed (Hascoët & Bourin, 2009; Serchov, van Calker, & Biber, 2016). The dark chamber took up 1/3 of the box (black plastic walls and floor, with a black plastic lid) and the light chamber took up 2/3 (white plastic walls and floor, no lid) with an opening between the chambers (4 cm x 4 cm). The dimensions of the light/dark box were 46 cm x 27 cm x 30 cm (l x w x h). The test was run during the dark cycle, so a lamp directed at the light chamber was used to make the chamber bright. After a 30-min habituation to the test room, animals were placed in the dark chamber and allowed to freely explore the chambers for 5 min. In between each animal, the apparatus was cleaned with 10% ethanol. There was only one test day. Behaviour was recorded for offline scoring using ANY-Maze software. The zone entry criteria for the light compartment were at least 85% of the mouse's body area had to be in the zone to be scored as an entry and at least 75% of the mouse's body had to remain in the zone to not be scored as an exit. As the dark

compartment was a hidden zone (i.e., the mouse was not visible when in this zone), only a zone exit criterion was stipulated; at least 85% of the mouse's body area had to become visible to be scored as an exit. Immobility detection was enabled in ANY-Maze, with 65% immobility sensitivity and a minimum immobility period of 2000 ms before the mouse was considered immobile. Freezing detection was also enabled, with a minimum freeze duration of 1000 ms before the mouse was considered to be freezing. The values used for freezing on and off thresholds remained as default (i.e., on threshold = 30, off threshold = 40).

2.6.2 Elevated plus maze.

The EPM is another classic test of anxiety-like behaviour based on rodents' innate aversion to heights and bright open areas. The apparatus was built on-site and the dimensions used were informed by those previously used, as was the protocol followed (Komada, Takao, & Miyakawa, 2008; Leo & Pamplona, 2014; Walf & Frye, 2007). The apparatus consisted of two types of arms, open and closed, for a total of two of each arm type with 180° between the arms of the same type (i.e., the opening of a closed arm faced the opening of the other closed arm and vice versa for the open arms). There was a centre platform at the junction of the four arms (5 cm x 5 cm). The arms (30 cm x 5 cm) had a white plastic floor. The closed arm walls were black plastic (15 cm), whereas the open arms had white plastic bumpers along the edges (0.5 cm) to prevent an animal from falling off the edge. The maze itself was 50 cm tall, from ground to the floor of the maze. Similar to the light/dark box, EPM was run during the dark cycle, so a lamp was directed at the maze in order to illuminate the open arms. After a 30-min habituation to the test room, animals were placed on the centre platform facing the open arm opposite the experimenter and allowed to freely explore for 10 min. In between each animal, the apparatus

was cleaned with 10% ethanol. There was only one test day. Behaviour was recorded for offline scoring with ANY-Maze software. The zone entry criteria for both the open and closed arms were at least 85% of the mouse's body area had to be in the zone to be scored as an entry and at least 75% of the mouse's body had to remain in the zone to not be scored as an exit. For the centre zone, the mouse was considered to be in this zone when it was not in any other zone. Immobility and freezing detection were enabled with the same criteria used for the light/dark box.

2.7 Buried Food Olfaction Test

After completing EPM testing, all mice ($N = 77$, 45 males and 32 females) were assessed on the buried food olfaction test. Considering ABNs also exist in the rodent olfactory bulb (Carleton et al., 2003), the buried food olfaction test was run to ensure that olfaction impairments were not present and did not confound behavioural output observed on previous tests. The buried food olfaction test protocol was modified from the olfaction-based foraging task previously described (Ferguson et al., 2000). The test took two days to complete, one for odour and taste habituation and one for the test itself. For odour and taste habituation, mice were brought into the test room and put into new cages. They were then given one Teddy Graham cookie each (Honey flavour Teddy Grahams, Christie of Mondelēz International Inc., Deerfield IL) to avoid neophobia during the test. They were left in the test room until their cookies had been completely consumed, after which they were replaced in their home cages. As the animals were still under restricted feed due to treatment administration via food, a food deprivation day was not necessary. The test occurred approximately 20 hr after daily feeding.

On the test day, after a 30-min habituation to the testing room, mice were placed into a new cage that had 2-3 cm of standard bedding. The mice had a period of 5 min to acclimatize to the new cage, with cage lid open. Mice were then removed from the test cage and a Teddy Graham cookie was buried in one of the four corners of the cage until it was no longer visible. The mice were then returned to the test cage, with cage lid still open. Once the mice found the cookie and began eating it, the test ended. Eating was defined as the mouse holding the cookie with both forepaws and nose oriented toward the cookie (i.e., overt feeding behaviour). Mice remained in the test cages, with lid closed, until the entire cookie was consumed before being replaced in their home cages. The mice had a maximum of 15 min to complete the test. Behaviour was recorded for offline hand-scoring.

2.8 Tissue Extraction

Upon completion of all behavioural tasks, mice were euthanized and tissue collected. Mice were anesthetized via intraperitoneal (IP) injection (100 mg/kg ketamine; 10.8 mg/kg xylazine) and perfused first with 1X phosphate-buffered saline (PBS) with 0.02% 0.5M ethylenediaminetetraacetic acid (EDTA) for approximately 5 min, or until the liver turned colour from deep purple to light beige/brown. EDTA was added to the PBS due to its anticoagulant properties. The initial wash with PBS/EDTA solution cleared the vasculatory system of blood as blood is auto-fluorescent and would increase the noise present in later immunofluorescent labelling. Following the PBS/EDTA wash, the mice were then perfused with 4% paraformaldehyde (PFA; in 1X PBS) for 15-20 min. Following fixation with PFA, the brains were extracted and put into 4% PFA solution. Approximately 24 hr later, the brains were put into a 20% sucrose solution (dissolved in 1X PBS) in preparation for sectioning. Brains were sliced

with a cryostat (Leica CM3050 S, Leica Biosystems Inc., Concord ON), with a slice thickness of 30 μm.

2.9 Ki-67 Immunofluorescent Labelling

Ki-67 is a nuclear protein and an endogenous marker of proliferation present during all phases of the cell cycle, except early G_1 phase and resting (G_0) phase (du Manoir, Guillaud, Camus, Seigneurin, & Brugal, 1991; Gerdes et al., 1984); therefore, Ki-67 can be used to assess neurogenesis (Kee, Sivalingam, Boonstra, & Wojtowicz, 2002). A free-floating staining method was used, with no more than 6 brain slices in a single well of a 24-well plate to maximize antibody binding (3 dorsal DG and 3 ventral DG slices were chosen). All incubation steps were run on a rocking apparatus to enable equal exposure of the solutions to all slices present in a single well. Hippocampal slices were first washed in 1X PBS 3 times, 10 min each, and then treated with blocking buffer for 2 hr at room temperature. The blocking buffer recipe was as follows: 0.1% bovine serum albumin (BSA [lyophilized powder, crystallized, ≥98.0% (GE), 05470-5G, Sigma-Aldrich, St. Louis MO; or heat shock fraction, pH 7.0, ≥98, A7906-100G, Sigma-Aldrich, St. Louis MO]) and 4% normal goat serum (NGS [Normal Goat Serum Blocking Solution, S-1000, Vector Laboratories Inc., Burlingame CA]) in PBS-T (1X PBS with 0.1% Triton [Triton X-100 (Electrophoresis), BP151-100, Fisher BioReagents by Fisher Scientific, Hampton NH]). PBS-T was used as Triton is a detergent that permeabilizes cell membranes to enable penetration of the primary and secondary antibodies. The purpose of the blocking step was to prevent non-specific binding of the secondary antibody in later steps (i.e., reduce background staining), therefore components used in the blocking buffer were dependent on the host of the secondary antibody used. Following tissue blocking, slices were treated with the

primary antibody solution for ~40 hr at 4°C. The primary antibody solution was composed of 1:1000 Ki-67 primary antibody (Rb pAb to Ki67, ab15580, Abcam, Cambridge UK) in a 1:1 solution of 1X PBS:blocking buffer.

Following primary antibody incubation, slices were washed three times with 1X PBS, 10 min each. Following these washes, tissue was incubated with the secondary antibody solution for 2 hr at room temperature. This solution was composed of 1:500 secondary antibody (AlexaFluor 488 goat anti-rb IgG, A11008, Life Technologies of Thermo Fisher Scientific, Waltham MA) in a 1:1 solution of 1X PBS:blocking buffer. The secondary antibody acted as an indirect fluorescent marker as it bound to the primary antibody which, in turn, was bound to the protein of interest. Due to the light-sensitive nature of fluorescence, the incubation with secondary antibody, as well as all following steps, were completed with the well plate covered/wrapped in tinfoil. Following secondary antibody incubation, tissue was washed three times with 1X PBS, 10 min each. Tissue was then mounted on SuperFrost Plus slides (Fisher Scientific, Waltham MA) with hardset compound containing 4’6-diamidino-2-phenylindole (DAPI), a DNA/nuclei marker (Antifade Mounting Medium with DAPI (hardset), ENZ-53003-M010, Enzo Life Sciences Inc., Ann Arbor MI). Slides were left to dry in the dark overnight before being refrigerated. Slides were imaged with the EVOS cell imaging system (EVOS FL Auto 2, Invitrogen of Thermo Fisher Scientific, Waltham MA).

2.10 Analysis

All data were analyzed using SPSS (v.26; IBM, Armonk NY). For PRL, win-stay and lose-shift probabilities were calculated using R (v.3.4.4; open-source). Immunofluorescent images were processed with ImageJ Fiji (v1.51s; National Institute of Health [NIH] USA). Ki-67

cell counts were done manually using the Cell Counter plug-in for ImageJ. All graphs were produced using GraphPad Prism (v.8; GraphPad Software, San Diego CA). Data on graphs represent mean ± standard error of the mean (SEM); the x-axes represent independent variables and the y-axes represent values of the dependent variables. Effect sizes computed were partial eta squared (η_p^2) and magnitude benchmarks used followed Cohen's (1969) guidelines: $\eta_p^2 = .0099$ as small, $\eta_p^2 = .0588$ as medium, and $\eta_p^2 = .1379$ as large (cited in Richardson, 2011).

For all analysis of variance (ANOVA) tests performed, tests of homogeneity were run. If Mauchly's Test of Sphericity was violated in a repeated measures ANOVA (i.e., $p < .05$), the Greenhouse-Geisser-corrected *p* values were reported. For post-hoc analyses, uncorrected Tukey's honestly significant difference (HSD) comparisons were used. Only post-hoc comparisons of interaction effects between three or more factors were Bonferroni corrected for multiple comparisons. Across all analyses, no outliers were removed. There were no general exclusion criteria used across all tasks (i.e., removing an animal's entire dataset from all analyses); test-specific exclusion criteria are specified when necessary.

2.10.1 Spontaneous location recognition.

The final *N* used for analysis was the same as mentioned in the task description ($N = 40$, 18 males and 22 females; TK+/VGCV = 11, TK+/ctrl = 6, TK-/VGCV = 11, TK-/ctrl = 12). Distance travelled in the arena for each day of habituation were compared across groups using a 4-way repeated measures ANOVA, where the within-subjects factor was test day (1 through 5) and the between-subjects factors were genotype (TK+, TK-), treatment (VGCV, control), and sex (male, female). If main and/or interaction effects were found in the omnibus ANOVA, post-hoc comparisons were run.

For the choice phase of the test, discrimination ratios (d2) were calculated (Eq. 1).

$$d2 = (time_{A5} - time_{A4}) / (time_{A5} + time_{A4})$$

Equation 1

where $time_{A5}$ is the exploration time for object A5 (i.e., novel location object) and $time_{A4}$ is the exploration time for object A4 (i.e., familiar location object), therefore measuring degree of preference for the object in the novel location relative to the object in the familiar location. Discrimination ratios for each SLR configuration (i.e., d-SLR and s-SLR) were analyzed separately using 3-way univariate ANOVAs, with between-subjects factors genotype (TK+, TK-), treatment (VGCV, control), and sex (male, female). If interaction effects were found in the omnibus ANOVA, post-hoc comparisons were run to determine simple main effects.

2.10.2 Probabilistic reversal learning.

Data were averaged across the first 7 days of testing. If a mouse failed to complete all 90 trials or failed to accomplish at least 1 reversal in a session, an extra session was run. If a mouse failed to attain the minimum requirements (i.e., 7 days of usable data), then that subject's data was removed from the analyses of all measures (n = 3 females). Thus, the final N used for statistical analyses was 55 (31 males, 24 females; TK+/VGCV = 16, TK+/ctrl = 16, TK-/VGCV = 13, TK-/ctrl = 10).

The measures of interest were the number of reversals completed, trials per reversal, overall accuracy, and win-stay/lose-shift probabilities. Trials per reversal was calculated by dividing the number of trials completed by the number of reversals made in a session. For determining win-stay and lose-shift probabilities, trial-by-trial analyses were conducted, which examined the response made on a trial given feedback information (i.e., reward vs. no reward)

from the previous trial. Win-stay was defined as the probability a mouse would respond to the same stimulus if that stimulus was rewarded in the previous trial. Conversely, lose-shift was defined as the probability a mouse would shift their response to the alternate stimulus if the stimulus chosen in the previous trial was not rewarded.

Number of reversals completed, trials required per reversal, and overall accuracy were analyzed using 3-way univariate ANOVAs, with between-subjects factors genotype (TK+, TK-), treatment (VGCV, control), and sex (male, female). If interaction effects were found in the omnibus ANOVA, post-hoc comparisons were run to determine simple main effects. The probabilities of win-stay and lose-shift behaviour were analyzed using a 4-way repeated measures ANOVA, where the within-subjects factor was behaviour (win-stay vs. lose-shift) and the between-subjects factors were genotype (TK+, TK-), treatment (VGCV, ctrl), and sex (male, female). If interaction effects were found, post-hoc comparisons were run.

2.10.3 Progressive ratio.

The exclusion criterion for PR was premature death (n = 2 males). Thus, the final N used for statistical analyses was 91 (43 males, 48 females; TK+/VGCV = 29, TK+/ctrl = 23, TK-/VGCV = 20, TK-/ctrl = 19). Due to the large number of factors involved in the PR task, multiple analyses were conducted at varying levels of statistical complexity.

2.10.3.1 Overall analysis.

In order to maximize power to detect any group differences on PR performance, all reward concentration and timeline conditions were collapsed and data were averaged across all conditions (N = 91). The primary measures of interest for basic PR-4 and high demand PR were

breakpoint, number of target touches, and number of blank touches, and for uncapped FR-5 were number of trials completed and blank touches made. For basic PR-4 and uncapped FR-5, 3-way univariate ANOVAs were run on each measure of interest, with between-subjects factors genotype (TK+, TK-), treatment (VGCV, ctrl), and sex (male, female). For high demand PR, 4-way repeated measures ANOVAs were run on each measure of interest, with the within-subjects factor schedule (PR-4 vs. PR-8 vs. PR-12) and between-subjects factors genotype (TK+, TK-), treatment (VGCV, ctrl), and sex (male, female). For all ANOVAs run, if main or interaction effects were found, post-hoc comparisons were run to determine simple main effects.

2.10.3.2 Overall analysis by reward concentration.

To determine whether any group differences occurred on PR performance due to reward strength, data from each reward concentration were collapsed across exposure timeline ($N = 91$). The measures of interest were the same as listed above. The exact same analyses were run here as were run for the overall analysis, but were repeated three times for each reward concentration condition (100%, 50%, 20%).

2.10.3.3 Timeline analyses.

It is possible that each reward concentration exposure schedule/timeline influenced behaviour differently and, if this were the case, combining the timelines together may have masked differences between groups. Thus, behaviour for each reward concentration during each timeline was analyzed separately. Timeline A followed a descending order of concentration exposure (100%, 50%, 20%, then a second round of high demand PR and uncapped FR-5 with 100%), whereas Timeline B followed an ascending order of concentration exposure (20%, 50%,

100%, then a second round of high demand PR and uncapped FR-5 with 20%). No further exclusion criteria existed for Timeline A (*N* = 58, 28 males and 30 females; TK+/VGCV = 17, TK+/ctrl = 13, TK-/VGCV = 13, TK-/ctrl = 15), but only TK+ mice were analyzed for Timeline B (*N* = 34, 15 males and 19 females; TK+/VGCV = 13, TK+/ctrl = 10, TK-/VGCV = 7, TK-/ctrl = 4). The removal of TK- mice from the Timeline B analysis was due to the small number of TK- mice. The reduced amount of TK- mice for this timeline was likely due to a breeding error that resulted in fewer TK- pups per litter. Thus, the final *N* used for Timeline B analyses, as well as the following PR analyses, was 22 (11 male, 11 females; TK+/VGCV = 12, TK+/ctrl = 10).

The same measures of interest, as listed above, were used. Additionally, the exact same analyses were run for each task phase as for the overall analysis; however, these analyses were repeated for each timeline, and repeated for each reward concentration within each timeline. The only exception was that analyses for Timeline B excluded the factor of genotype, so 2-way univariate ANOVAs were run for basic PR-4 and uncapped FR-5 measures and 3-way repeated measures ANOVAs were run for high demand PR measures.

2.10.3.4 Training effect analysis.

Repeated testing on PR might have altered baseline responding levels. In other words, with increased repetitions of the task, there may have been a greater tendency for repeated responding. It is conceivable that effects of neurogenesis knockdown may be baseline responding-dependent. To test for the presence of training effects, the number of trials completed during uncapped FR-5 were compared for the repeated concentration on each timeline (i.e., 100% for Timeline A and 20% for Timeline B). For Timeline A, a 4-way repeated measures ANOVA was run, with the within-subjects factor timepoint (first vs. second exposure) and

between-subjects factors genotype (TK+, TK-), treatment (VGCV, ctrl), and sex (male, female). If interaction effects were found, post-hoc comparisons were run. Additionally, data were collapsed across between-subjects factors and a t-test was run to compare the trials completed at the first and second exposures of 100% milkshake.

The same analyses were run on Timeline B data, except, as above, the factor of genotype was removed as only TK+ mice were analyzed. Thus, the repeated measures ANOVA was 3-way.

2.10.3.5 Timeline comparison.

As previously mentioned, it is conceivable that prior reward experiences may impact PR performance differently. In other words, behaviour with a weak reward (i.e., 20%) may differ if it was the first reward experienced or if it was the third reward experienced, and vice versa for a strong reward. To determine if concentration order influenced responding when receiving the weakest and strongest rewards, the breakpoints achieved during high demand PR and the trials completed during uncapped FR-5 were compared across timelines. As group differences were not necessarily the focus of this analysis, the small number of TK- mice that underwent Timeline B were left in ($N = 91$).

To compare behaviour with 100% milkshake, breakpoints achieved during high demand PR on each timeline were analyzed using a 5-way repeated measures ANOVA, with the within-subjects factor schedule (PR-4 vs. PR-8 vs. PR-12), and between-subjects factors timeline (A, B), genotype (TK+, TK-), treatment (VGCV, ctrl), and sex (male, female). Trials completed during uncapped FR-5 were analyzed using a 4-way univariate ANOVA, with the same between-subjects factors as the 5-way repeated measures ANOVA. If any main or interaction effects were revealed, post-hoc comparisons were run to determine simple main effects.

The same analyses were run to compare behaviour with 20% milkshake across each timeline.

2.10.4 Extinction.

Mice that did not reach endpoint criterion within 20 days were dropped from analyses (*n* = 3, 2 males and 1 female). Therefore, the final *N* used for analyses was 72 (40 males, 32 females; TK+/VGCV = 25, TK+/ctrl = 22, TK-/VGCV = 13, TK-/ctrl = 12). The primary measures of interest were the percent of omissions made across the first 10 days of the task (i.e., extinction curve) and the number of days to reach criterion. The extinction curves were compared across groups using a 4-way repeated measures ANOVA, with the within-subjects factor session number (1 through 10) and between-subjects factors genotype (TK+, TK-), treatment (VGCV, control), and sex (male, female). If the omnibus ANOVA revealed significant main or interaction effects, post-hoc comparisons were run to determine simple main effects. The days to criterion measure was compared across groups using a 3-way univariate ANOVA, with genotype (TK+, TK-), treatment (VGCV, control), and sex (male, female) as factors. Post-hoc comparisons were run if the omnibus ANOVA revealed significant interaction effects.

2.10.5 Reward concentration preference test.

One cohort of mice was not included in analyses (*n* = 18) and one premature subject death occurred post-touchscreen testing (female), therefore the final *N* used for analyses was 55 (31 males, 24 females; TK+/VGCV = 18, TK+/ctrl = 18, TK-/VGCV = 10, TK-/ctrl = 9). The primary measures of interest were the the total amount of each concentration consumed during the entire 2-hr test and the preference index for the more concentrated option in each choice. The

preference index was calculated using Equation 2 below (as per Snyder et al., 2011).

$$\text{Preference Index} = [(\Delta\text{weight}_{C1}) / (\Delta\text{weight}_{C1} + \Delta\text{weight}_{C2})] \times 100$$

Equation 2

where Δweight_{C1} is the amount of the more concentrated milkshake consumed within the 2-hr test and Δweight_{C2} is the amount of the less concentrated milkshake consumed within the 2-hr test. Therefore, the preference index expresses the degree to which the more concentrated reward was preferred over the less concentrated reward as a percentage.

For each measure of interest, a 3-way univariate ANOVA was run, with between-subjects factors genotype (TK+, TK-), treatment (VGCV, ctrl), and sex (male, female). If any interaction effects were revealed, post-hoc comparisons were then run. Additionally, the amount consumed of each concentration at the test endpoint were compared with a 4-way repeated measures ANOVA, with the within-subjects factor concentration (higher vs. lower) and the between-subjects factors the same as listed previously. Again, if any interaction effects were found, post-hoc comparisons were run.

2.10.6 Negative valence systems tests.

2.10.6.1 Light/dark box.

The same cohort mentioned above (n = 18) was also not included here, along with another cohort (n = 17). The second cohort was excluded because they were subjected to a sub-optimal version of the test, wherein the mice began in the light chamber instead of the dark. Therefore, the final N used for analyses was 39 (22 males, 17 females; TK+/VGCV = 10, TK+/ctrl = 13, TK-/VGCV = 9, TK-/ctrl = 7).

The primary measures of interest were latency to exit the dark compartment, time spent in the dark compartment, and number of transitions made between compartments. The time spent in the dark compartment was expressed as a percentage relative to the time spent in the light compartment. All measures were analyzed using 3-way univariate ANOVAs, with the between-subjects factors genotype (TK+, TK-), drug (VGCV, control), and sex (male, female). If interaction effects were found in the omnibus ANOVA, post-hoc comparisons were run to determine simple main effects.

2.10.6.2 Elevated plus maze.

The same cohort as mentioned for the prior two tests was not included along with one premature death (male) left $N = 54$ to be used for analyses (39 males, 24 females; TK+/VGCV = 17, TK+/ctrl = 18, TK-/VGCV = 10, TK-/ctrl = 9).

The primary measures of interest were the number of entries made into the open arms (expressed as a percentage of open entries relative to closed entries) and time spent in the closed arms (expressed as percentage of closed arm time relative to open arm + centre time). Discrimination ratios (Eq. 1) were also calculated using the time spent in each arm type such that the d2 ratios represented degree of preference for the closed arms. The same statistical analyses used for the light/dark box data (above) were used here for all measures.

2.10.7 Buried food olfaction.

The same cohort as mentioned for the prior three tests was also not included here ($n = 18$). Therefore, the final N used for analysis was the same as for the EPM ($N = 54$, 39 males and 24 females; TK+/VGCV = 17, TK+/ctrl = 18, TK-/VGCV = 10, TK-/ctrl = 9). The only variable

of interest was the time it took for the mouse to find and begin eating the cookie (i.e., latency to feed). Latency to feed was compared across groups using the same 3-way univariate ANOVA mentioned above. If interaction effects were found in the omnibus ANOVA, post-hoc comparisons were run to determine simple main effects.

2.10.8 Ki-67 cell counts.

Tissue was selected from a random subset of mice so there were 6 mice from each genotype/treatment group (N = 18; 14 males, 10 females). Each group had tissue from an equal number of males and females with the exception of the TK-/ctrl group, which had tissue from only one female. Six sections were chosen so there were 3 dorsal sections and 3 ventral sections per mouse. Cell counts, blind to group conditions, were taken for only one hemisphere. For a cell to be counted as labelled, it was required to be within the blades of the DG (i.e., within the granule cell layer, not the hilus). The restricted counting area minimized the likelihood of false positive counts as microglia can also undergo proliferation (Hua, Schindler, McQuail, Forbes, & Riddle, 2012); Ki-67-positive cells outside the DG granule cell layer (i.e., the DG blades) are more likely to be proliferating microglia cells. Cell counts were summed across all 6 sections to get a total count, as well as across the 3 dorsal and 3 ventral sections to obtain region-specific counts. These sums were then multiplied by six as, when tissue was sliced, they were separated out into a 1 in 6 series (i.e., a single well contained one section every 6 sections, with 180 μm between each section). The resultant values were taken as the total, dorsal, and ventral DG Ki-67 cell counts for a single hemisphere.

The three main measures of interest were Ki-67 cell counts for the total DG, dorsal DG, and ventral DG. Additionally, it was of interest to determine if these counts differed across dorsal

and ventral subregions. For each main measure, a 3-way univariate ANOVA was run, with between-subjects factors the same as above. If any interaction effects were revealed, post-hoc comparisons were run to determine simple main effects. To compare dorsal to ventral counts, a 4-way repeated measures ANOVA was run, with the within-subjects factor region (dorsal vs. ventral) and the same between-subjects factors as stated above. If any interaction effects occurred between the within-subjects factor of region and any other factor, then post-hoc comparisons were run to determine simple main effects.

3. Results

3.1 Spontaneous Location Recognition

SLR was run as a behavioural verification of neurogenesis knockdown. The small separation condition (s-SLR; see Figure 1B) requires the ability to pattern separate, an ability that has been repeatedly found to be dependent on hippocampal neurogenesis (Bekinschtein et al., 2013; Clelland et al., 2009; Creer et al., 2010; McHugh et al., 2007; Niibori et al., 2012; Sahay et al., 2011). The main measure of performance is the discrimination (d2) ratio, which indicates the degree of preference an animal has for the object in the novel location during the test phase. Positive d2 ratios signify a preference for the object in the novel location, whereas negative scores signify a preference for the object in the familiar location. For the large separation (d-SLR) condition, there were no group differences between d2 ratios, indicating neurogenesis knockdown did not impact the ability to discriminate between dissimilar locations (see Figure 7A). In contrast, for s-SLR, there was a genotype by treatment interaction, $F(1,32) = 14.966$, $p = .001$, $\eta_p^2 = .319$ (see Figure 7B), with the TK+/VGCV group exhibiting lower d2 ratios than the TK+/ctrl ($p < .0001$) and TK-/VGCV groups ($p < .0001$). There were no differences between any control groups on s-SLR d2 ratios. Given the evidence mentioned above that the s-SLR condition relies on intact neurogenesis, the lower d2 ratios for the TK+/VGCV group suggests the presence of neurogenesis knockdown in the hippocampi of these mice. This effect was not due to differences in activity level as there were no group differences between TK+/VGCV and control groups in the amount of distance travelled during the habituation phase of the task (see Appendix A, Figure A1).

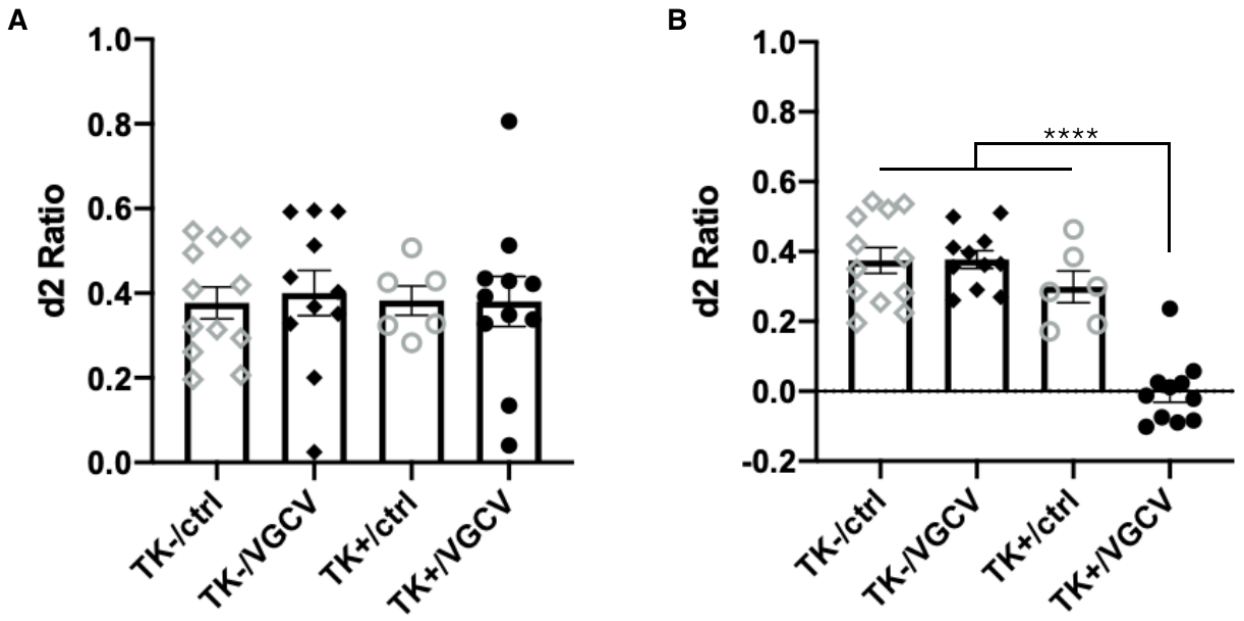

Figure 7. Performance during the spontaneous location recognition (SLR) test phase. (A) Performance on the d-SLR (i.e., large separation) condition. There were no differences between groups in ability to distinguish between the familiar and novel locations. (B) Performance on the s-SLR (i.e., small separation) condition. The TK+/VGCV group was significantly impaired in the ability to differentiate between familiar and novel locations, suggesting adult hippocampal neurogenesis is necessary for pattern separation. ****$p < .0001$

3.2 Probabilistic Reversal Learning

PRL assesses sensitivity to feedback information. Performance on this test has previously been shown to be affected by serotonergic drugs both outside touchscreens (Bari et al., 2010) and, more recently, inside touchscreen chambers (Phillips et al., 2018). There were no group differences on any measure of PRL performance (see Figure 8). The accuracy, number of reversals made, and trials made per reversal did not differ across groups (see Figure 8A-C). The main behavioural measure of PRL is the probability of win-stay and lose-shift behaviours. There were no group differences in the probabilities of both win-stay and lose-shift behaviours,

suggesting neurogenesis knockdown does not impact sensitivity to feedback information (see Figure 8D). However, all groups showed a greater probability for win-stay behaviour compared to lose-shift behaviour, $F(1,47) = 597.016$, $p < .0001$, $\eta_p^2 = .927$, suggesting rewards (i.e., positive reinforcement or positive feedback information) are stronger predictors of future behaviour than non-rewards (i.e., negative reinforcement or negative feedback information).

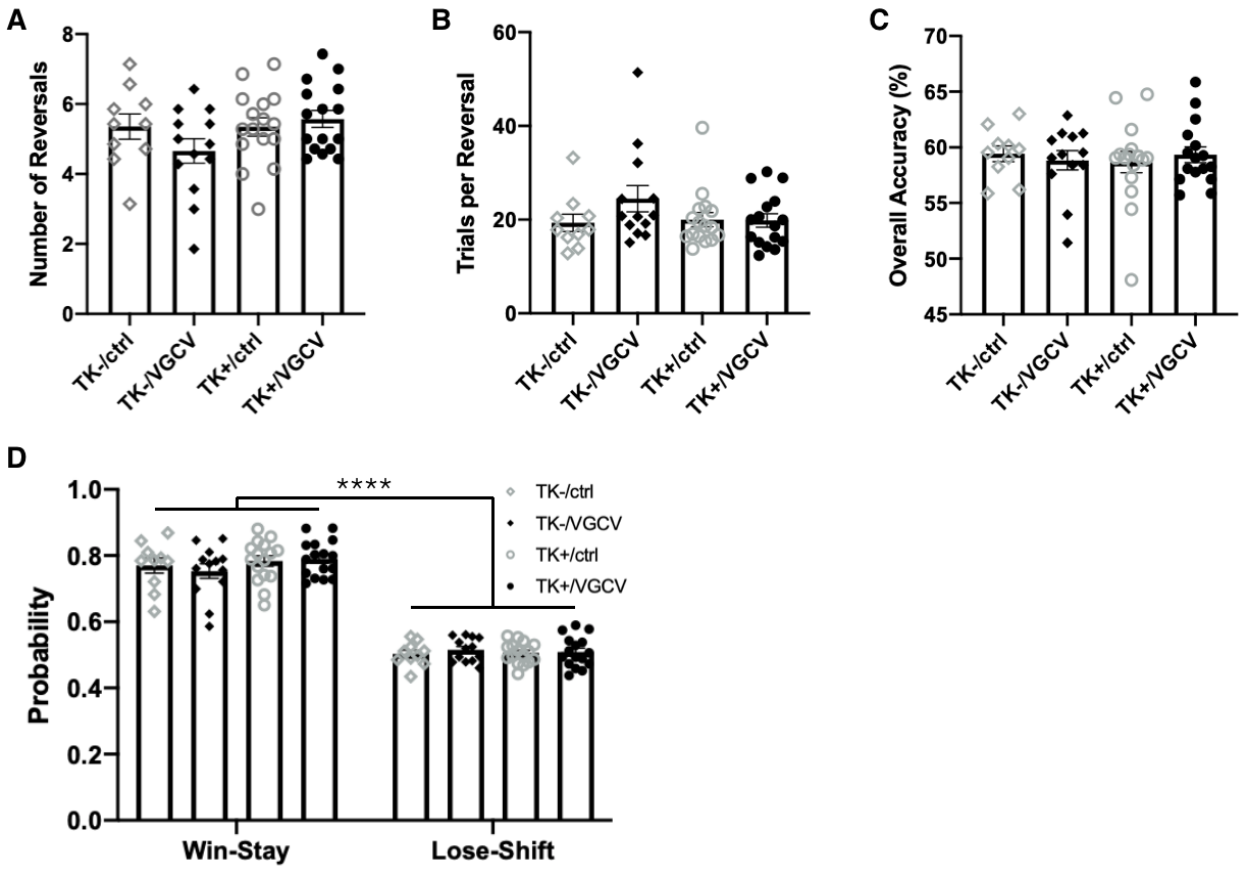

Figure 8. Performance on probabilistic reversal learning (PRL).
There were no group differences on (A) the number of reversals made during a session, (B) the number of trials required per reversal, (C) the overall accuracy (i.e., percentage of correct responses), or (D) the probability of win-stay and lose-shift behaviours. (D) All groups exhibited higher probabilities for win-stay behaviour than lose-shift behaviour. Neurogenesis knockdown does not seem to impact sensitivity to feedback information. ****$p < .0001$

3.3 Progressive Ratio

3.3.1 Overall analysis.

Initially, in order to maximize power to detect any impairments in performance, all reward concentration conditions were collapsed and data were averaged across each reward concentration. The primary measures of interest were breakpoint, number of target touches, and number of blank touches for basic PR-4 and high demand PR, or number of trials completed and number of blank touches for uncapped FR-5. Across all task phases, there were no significant differences between the TK+/VGCV group and all control groups on any measure (see Figure 9A-E). During high demand PR, however, there was a main effect of PR schedule on breakpoints achieved, $F(2,166) = 224.446$, $p < .0001$, $\eta_p^2 = .730$ (see Figure 9C), and target touches made, $F(2,166) = 150.746$, $p < .0001$, $\eta_p^2 = .645$ (see Figure 9D). With increasing demand, there were higher breakpoints achieved and less target touches were made ($p < .0001$ for all pairwise comparisons on both measures).

However, there was a main effect of treatment on uncapped FR-5, $F(1,83) = 6.623$, $p = .012$, $\eta_p^2 = .074$ (see Figure 9F). VGCV-treated mice completed fewer trials than control-fed mice, suggesting there were non-specific effects of VGCV treatment. Additionally, for all task phases, females made more blank touches than males, $F(1,83) = 8.821$, $p = .004$, $\eta_p^2 = .096$ (basic PR-4), $F(1,83) = 4.884$, $p = .030$, $\eta_p^2 = .056$ (high demand PR), $F(1,83) = 10.017$, $p = .002$, $\eta_p^2 = .108$ (uncapped FR-5; see Figure A2A-C). The increased number of blank touches made for females across all task phases suggests females may, in general, have a higher baseline activity level than males or may have a higher tendency for hyperactivity and non-specific responding than do males.

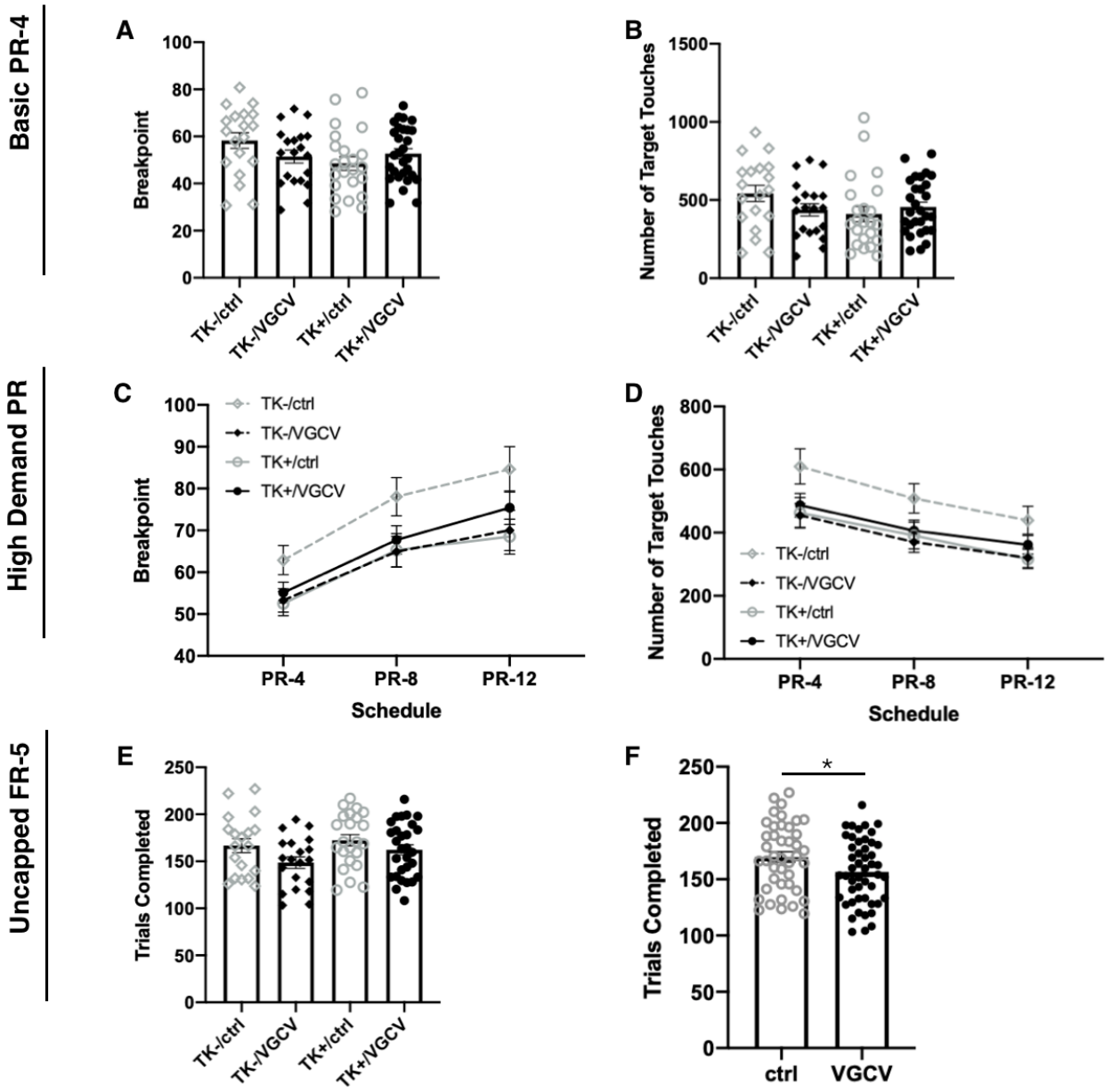

Figure 9. Overall performance on progressive ratio (PR), averaged across all milkshake concentration conditions.

(A) The TK+/VGCV group was not different from any other group for breakpoints achieved and (B) the number of target touches made during basic PR-4. However, the TK+/ctrl group achieved lower breakpoints than the TK-/ctrl group. (C) The breakpoints achieved and (D) the number of target touches made for the TK+/VGCV group during high demand PR did not differ from any control group. However, the TK-/ctrl group achieved higher breakpoints than the TK+/ctrl and TK-/VGCV groups, as well as made more target touches than the TK+/ctrl and TK-/VGCV groups. Additionally, all groups achieved higher breakpoints and fewer target touches with

increasing demand. (E) There were no group differences on the number of trials completed during uncapped FR-5. (F) Mice that received VGCV treatment completed fewer trials than did mice that received control chow. *$p < .05$

Differences between TK+ and TK- control groups were found during basic PR-4 and high demand PR. For basic PR-4, there was an interaction between genotype and treatment on breakpoint, $F(1,83) = 4.504$, $p = .037$, $\eta_p^2 = .051$ (see Figure 9A). TK-/ctrl mice achieved higher breakpoints than the TK+/ctrl mice ($p = .013$), suggesting a non-specific effect of genotype may have occurred. For high demand PR, there were genotype by treatment interaction effects on breakpoint, $F(1,83) = 4.948$, $p = .029$, $\eta_p^2 = .056$, and target touches, $F(1,83) = 4.605$, $p = .035$, $\eta_p^2 = .053$ (see Figure 9C-D). On both measures, the TK-/ctrl group achieved higher breakpoints and made more target touches than both the TK+/ctrl ($p = .015$ and .019, respectively) and the TK-/VGCV groups ($p = .025$ and .018, respectively), suggesting there were non-specific effects for both TK genotype and VGCV treatment on high demand PR performance.

3.3.2 Overall analysis by reward concentration.

High (100%), medium (50%), and low (20%) reward concentrations were tested as Karlsson et al. (2018) found impairments following neurogenesis knockdown only when reward strength was low. Data from each reward concentration were collapsed across concentration exposure timeline to determine if there were overall differences in behaviour between reward strengths. As above, the primary measures of interest were breakpoint, number of target touches, and number of blank touches for basic PR-4 and high demand PR, or number of trials completed and number of blank touches for uncapped FR-5.

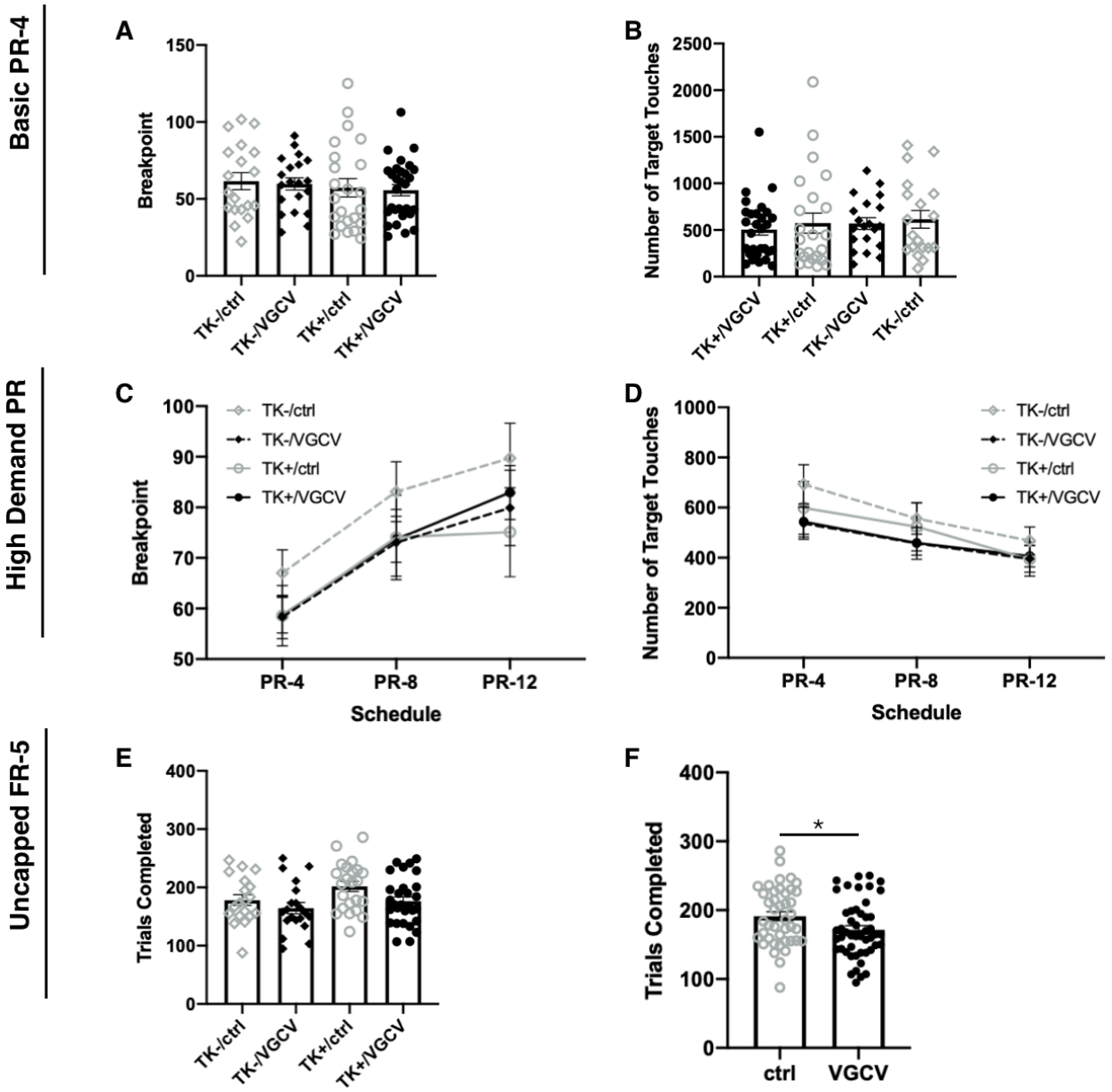

Figure 10. Overall performance on progressive ratio (PR) for 100% milkshake concentration. During basic PR-4, there were no differences between groups on (A) breakpoint achieved and (B) number of target touches. During high demand PR, there were also no group differences seen on (C) breakpoint and (D) target touches. (E) There were no group differences on trials completed during uncapped FR-5. (F) VGCV-treated mice completed less trials than control-fed mice during uncapped FR-5, suggesting a possible non-specific effect of VGCV treatment.
*$p < .05$

3.3.2.1 Strong reward condition.

There were no group differences observed across all task phases for all measures of interest (see Figure 10A-E). As above, there was a main effect of PR schedule found during high demand PR for both breakpoint, $F(2,166) = 105.264$, $p < .0001$, $\eta_p^2 = .559$ (see Figure 10C), and target touches, $F(2,166) = 54.477$, $p < .0001$, $\eta_p^2 = .396$ (see Figure 10D). Higher breakpoints were achieved and fewer target touches were made with increasing demand ($p < .0001$ for all pairwise comparisons on both measures). Also as seen in the overall data, there was a main effect of treatment on the number of trials completed on uncapped FR-5, $F(1,83) = 5.233$, $p = .025$, $\eta_p^2 = .059$ (see Figure 10F). Mice treated with VGCV completed fewer trials than control-fed mice, suggesting a non-specific effect of VGCV treatment. Again, as above, there was an effect of sex on blank touch data across task phases, $F(1,83) = 11.251$, $p = .001$, $\eta_p^2 = .119$ (basic PR-4), $F(1,83) = 8.944$, $p = .004$, $\eta_p^2 = .097$ (high demand PR), and $F(1,83) = 7.490$, $p = .008$, $\eta_p^2 = .083$ (uncapped FR-5; data not shown).

3.3.2.2 Medium reward condition.

The TK+/VGCV group did not differ from any of the control groups across all task phases for all measures analyzed (see Figure 11A-E). Again, during high demand PR, there was a main effect of PR schedule on breakpoints achieved, $F(2,166) = 97.334$, $p < .0001$, $\eta_p^2 = .540$ (see Figure 11C), and number of target touches made, $F(2,166) = 33.893$, $p < .0001$, $\eta_p^2 = .290$ (see Figure 11D). Higher breakpoints were achieved and fewer target touches were made with increasing demand ($p < .0001$ for all pairwise comparisons on both measures, with the exception PR-8 vs. PR-12 $p > .05$). The main effect of treatment seen on trials completed during uncapped FR-5 for the overall data and 100% data above was not replicated in this condition. The main

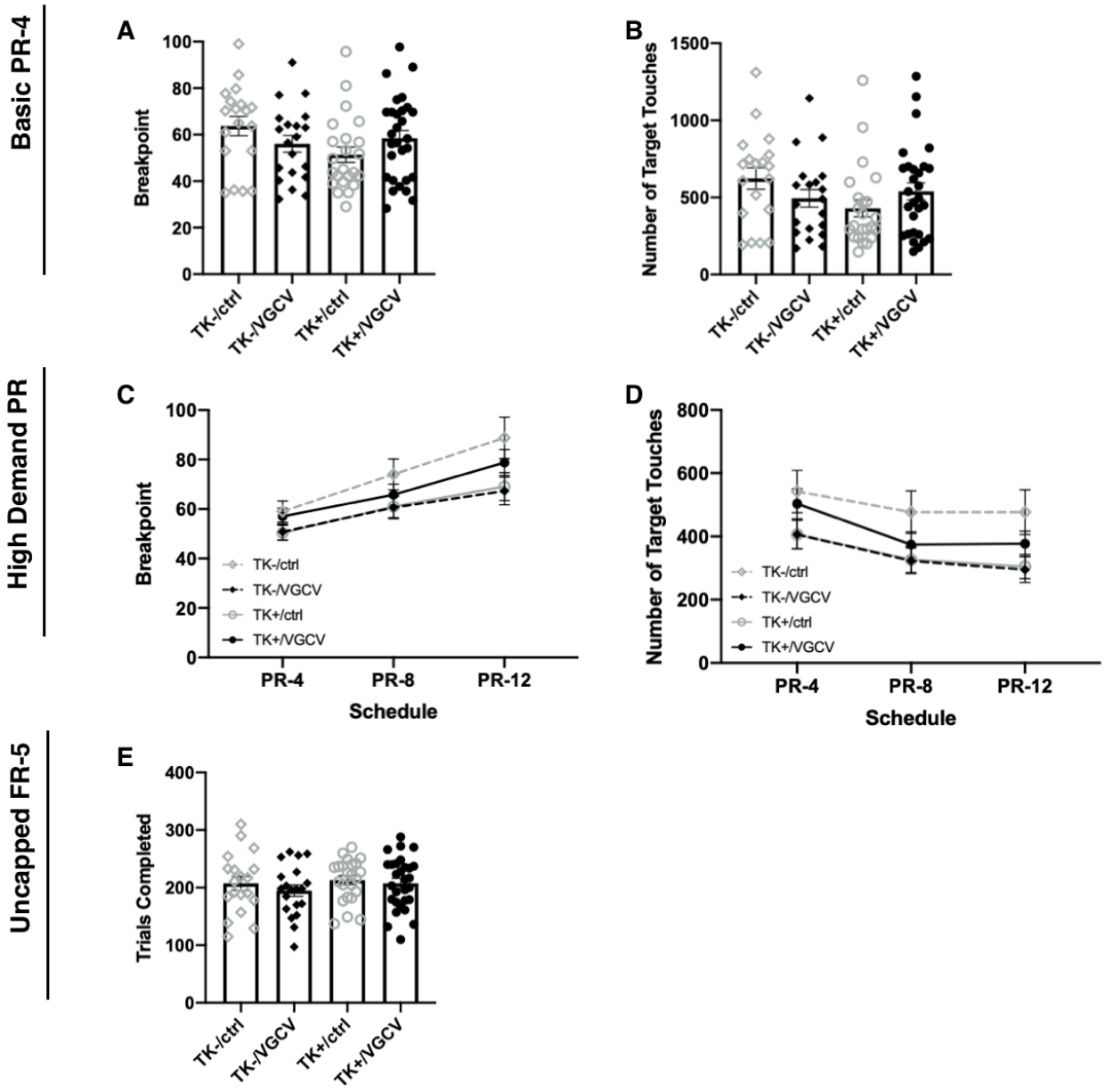

Figure 11. Overall performance on progressive ratio (PR) for 50% milkshake concentration. During basic PR-4, there were no differences seen between the TK+/VGCV group and any control group on (A) breakpoints achieved and (B) number of target touches made. However, TK-/ctrl mice achieved higher breakpoints and made more target touches than TK+/ctrl mice, suggesting a potential behavioural difference between genotypes. During high demand PR, there were no differences between the TK+/VGCV group and any control group on (C) breakpoints achieved and (D) number of target touches made. However, TK-/ctrl mice achieved higher breakpoints and made more target touches than both the TK+/ctrl and TK-/VGCV mice,

suggesting non-specific effects of both TK genotype and VGCV treatment on PR performance. (E) No group differences were found on the number of trials completed during uncapped FR-5.

effect of sex on number of blank touches was present only for basic PR-4, $F(1,83) = 4.884$, $p = .030$, $\eta_p^2 = .056$, with females making more blank responses than males (data not shown).

Differences were seen between control groups during basic PR-4 and high demand PR. For basic PR-4, an interaction was seen between genotype and treatment on breakpoint achieved, $F(1,83) = 4.460$, $p = .038$, $\eta_p^2 = .051$ (see Figure 11A), and target touches made, $F(1,83) = 4.372$, $p = .040$, $\eta_p^2 = .050$ (see Figure 11B). The TK-/ctrl group achieved higher breakpoints and made more target touches than the TK+/ctrl group ($p = .024$ and .032, respectively), suggesting a non-specific effect of VGCV treatment. For high demand PR, an interaction between genotype and treatment was seen on breakpoint, $F(1,83) = 5.363$, $p = .023$, $\eta_p^2 = .061$ (see Figure 11C), and target touches, $F(1,83) = 5.933$, $p = .017$, $\eta_p^2 = .067$ (see Figure 11D). The TK-/ctrl mice achieved higher breakpoints and made more target touches than both the TK+/ctrl ($p = .038$ and .024, respectively) and TK-/VGCV mice ($p = .045$ and .032, respectively), suggesting possible non-specific effects of both TK genotype and VGCV treatment on PR performance when receiving medium strength reward.

3.3.2.3 Weak reward condition.

There was a genotype by treatment interaction during basic PR-4 for the breakpoints achieved, $F(1,83) = 12.504$, $p = .001$, $\eta_p^2 = .131$ (see Figure 12A), and number of target touches made, $F(1,83) = 14.292$, $p < .0001$, $\eta_p^2 = .147$ (see Figure 12B). TK+/VGCV mice made *more* target touches than TK+/ctrl mice ($p = .031$) and trended toward achieving *higher* breakpoints than TK+/ctrl mice ($p = .054$), indicating that neurogenesis knockdown may potentiate

motivation (i.e., multiple responding) when the reward is weak. This effect, however, was not replicated during high demand PR as no differences were seen between the TK+/VGCV group and any control group for all measures of interest (see Figure 12C-D). As was seen previously, during high demand PR, there was a main effect of PR schedule on both breakpoints achieved, $F(2,166) = 58.539$, $p < .0001$, $\eta_p^2 = .414$ (see Figure 12C), and target touches made, $F(2,166) = 27.200$, $p < .0001$, $\eta_p^2 = .247$ (see Figure 12D). With increasing demand, higher breakpoints were achieved ($p < .0001$ for all pairwise comparisons, except PR-8 vs. PR-12 $p = .014$) and fewer target touches were made (PR-4 vs. PR-8 $p = .016$, PR-4 vs. PR-12 $p < .0001$, PR-8 vs. PR-12 $p = .001$). As has occurred multiple times in previous analyses, there were no differences between groups on the number of trials completed during uncapped FR-5 (see Figure 12E). The previous sex difference seen on number of blank touches was not present here.

In addition to the potentially neurogenesis knockdown-related differences mentioned above during basic PR-4, there were also differences between control groups. For basic PR-4 and high demand PR, TK-/ctrl mice achieved higher breakpoints and made more target touches than TK+/ctrl (basic PR-4: $p = .001$ and $p < .0001$, respectively; High demand PR: $p = .001$ on both measures) and TK-/VGCV mice (basic PR-4: $p = .004$ and .003, respectively; High demand PR: $p = .020$ and .014, respectively). These control group differences suggest the presence of non-specific effects of TK genotype and VGCV treatment on PR performance when given a weak reward.

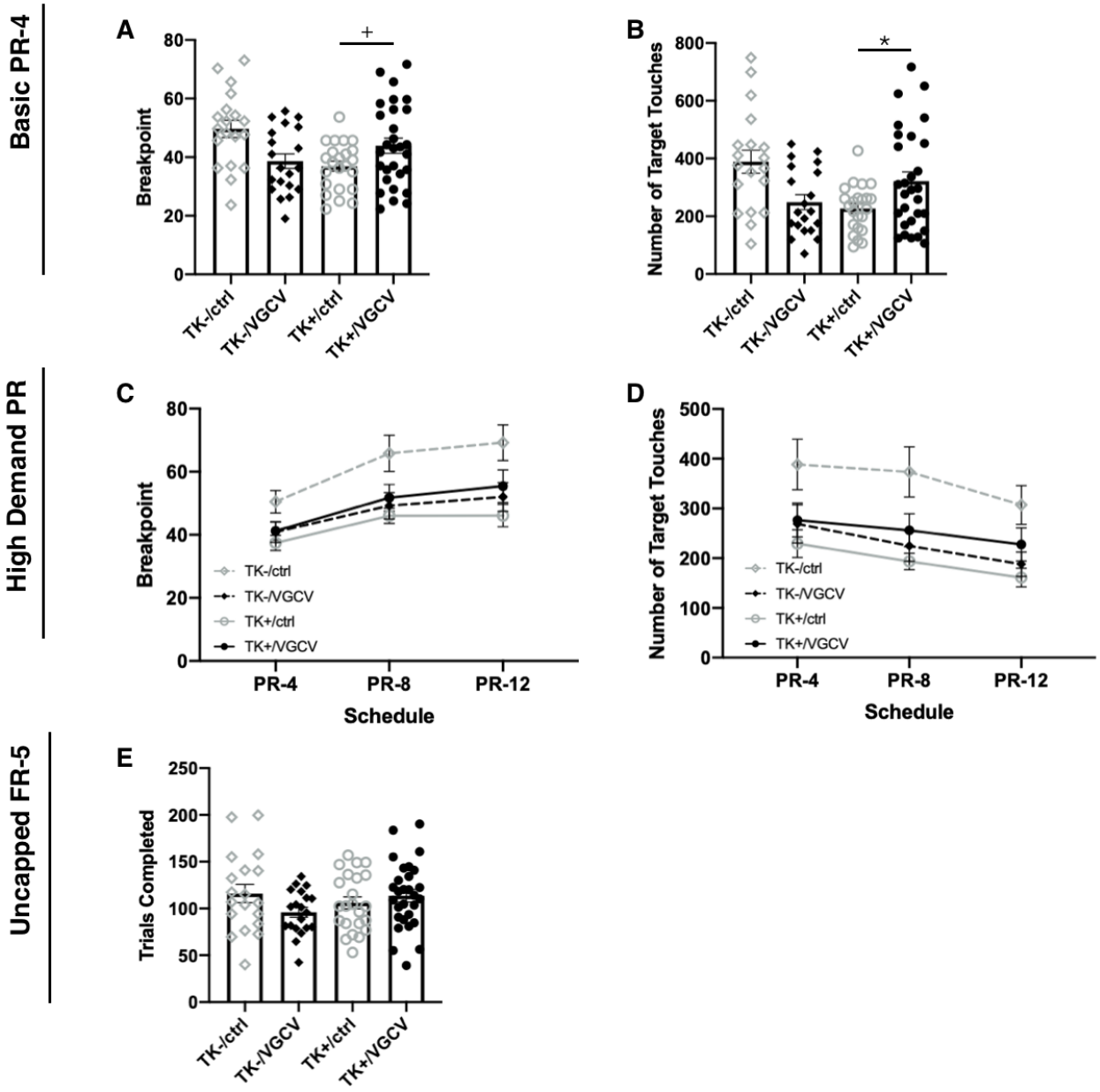

Figure 12. Overall performance on progressive ratio (PR) for 20% milkshake concentration. During basic PR-4, the TK+/VGCV group (A) trended toward higher breakpoints and (B) made more target touches than the TK+/ctrl group, suggesting *increased* motivation with neurogenesis knockdown. However, the TK-/ctrl group achieved higher breakpoints and made more target touches than the TK+/ctrl and TK-/VGCV groups. The differences between control groups suggests potential non-specific effects of VGCV treatment and/or genotype on behaviour. Neurogenesis knockdown seemed to bring the performance of the TK+/VGCV mice closer to baseline (i.e., the performance of the TK-/ctrl group). During high demand PR, the TK+/VGCV

group did not differ from any control group on (C) breakpoint achieved and (D) number of target touches made. However, the differences between control groups were replicated. Additionally, with increasing demand, higher breakpoints were achieved and fewer target touches were made. (E) There were no group differences on the number of trials completed during uncapped FR-5. $^{+}p < .065$, $*p < .05$

3.3.2.4 Summary for both overall analyses.

When data were collapsed across reward concentration conditions there were no differences found between the TK+/VGCV group and any control group on PR performance. Breaking down the data further into each milkshake concentration condition revealed slightly more nuanced results, with the TK+/VGCV group exhibiting more target touches made and a trend toward higher breakpoints during basic PR-4 only when the reward was weak. However, given the far more replicated effects found were those between control groups, it cannot be said that this difference is due to neurogenesis knockdown; it is possible that the confounding factors of TK genotype and VGCV treatment interacted to produce the changes in PR performance seen in the TK+/VGCV mice with weak reward.

3.3.3 Timeline analyses.

The three milkshake concentrations were tested in two different orders/timelines to control for order effects within a given timeline. It is conceivable that neurogenesis knockdown might have an effect during one order of testing, but not another. If this were to be the case, combining the orders for analysis, as above, might have masked such differences. Thus, the data were broken down further for each timeline to be analyzed separately. The two orders were Timeline A, which followed a schedule of descending concentration (i.e., 100%, 50%, then 20%,

followed by another 100%), and Timeline B, which followed a schedule of ascending concentration (i.e., 20%, 50%, then 100%, followed by another 20%). The main measures of interest were the same as listed above for the previous two levels of analyses. As mentioned in the Methods and Materials (see Chapter 2, specifically 2.10.3.3), for Timeline B data, only the TK+ groups were analyzed (i.e., TK+/VGCV and TK+/ctrl).

3.3.3.1 Timeline A (descending milkshake concentrations).

3.3.3.1.1 Strong reward: First exposure of 100% milkshake.

There was an interaction effect between genotype and treatment on breakpoint during high demand PR, $F(1,50) = 7.183$, $p = .010$, $\eta_p^2 = .126$ (see Figure 13B). TK+/VGCV mice achieved *higher* breakpoints than TK+/ctrl mice ($p = .045$). This interaction was only trending during basic PR-4, $F(1,50) = 3.924$, $p = .053$, $\eta_p^2 = .073$ (see Figure 13A), with TK+/VGCV mice trending toward *higher* breakpoints than TK+/ctrl mice ($p = .062$). These effects may suggest that, if any effect does exist, neurogenesis knockdown may potentiate/support multiple responding to a single stimulus. There were no other differences between the TK+/VGCV group and any control group across all task phases for all remaining measures (see Figure A3A-C). As previously seen, during high demand PR, there was a main effect of PR schedule on breakpoints achieved, $F(2,100) = 45.250$, $p < .0001$, $\eta_p^2 = .475$ (see Figure 13A), and target touches made, $F(2,100) = 39.839$, $p < .0001$, $\eta_p^2 = .443$ (see Figure A3B). With increasing demand, higher breakpoints were achieved ($p < .0001$ for all pairwise comparisons, except PR-8 vs. PR-12 $p = .001$) and fewer target touches were made ($p < .0001$ for all pairwise comparisons). Additionally, there was a main effect of treatment on the number of trials completed during uncapped FR-5, $F(1,50) = 5.620$, $p = .022$, $\eta_p^2 = .101$ (see Figure 13C), where VGCV treatment resulted in fewer

trials completed than controls. As was seen in the overall data, there was a recurring sex effect indicating females made more blank touches than males, $F(1,50) = 13.472$, $p = .001$, $\eta_p^2 = .212$ (basic PR-4), $F(1,50) = 9.928$, $p = .003$, $\eta_p^2 = .166$ (high demand PR), and $F(1,50) = 4.295$, $p = .043$, $\eta_p^2 = .079$ (uncapped FR-5; data not shown). There was also a sex effect seen for basic PR-4 breakpoint, $F(1,50) = 13.784$, $p = .001$, $\eta_p^2 = .216$ (data not shown), and target touch data, $F(1,50) = 13.658$, $p = .001$, $\eta_p^2 = .215$ (data not shown), with females achieving higher breakpoints and making more target touches than males.

Other than the potentially neurogenesis knockdown-related group differences seen, there were also differences between certain control groups. For basic PR-4 and high demand PR, TK-/ctrl mice achieved higher breakpoints than TK+/ctrl mice (basic PR-4: $p < .0001$ [see Figure 13A]; High demand PR: $p = .001$ [see Figure 13B]). For high demand PR, there was also an interaction effect between genotype and treatment on target touches, $F(1,50) = 5.081$, $p = .029$, $\eta_p^2 = .092$ (see Figure A3B). TK-/ctrl mice made more target touches than TK+/ctrl mice ($p = .004$).

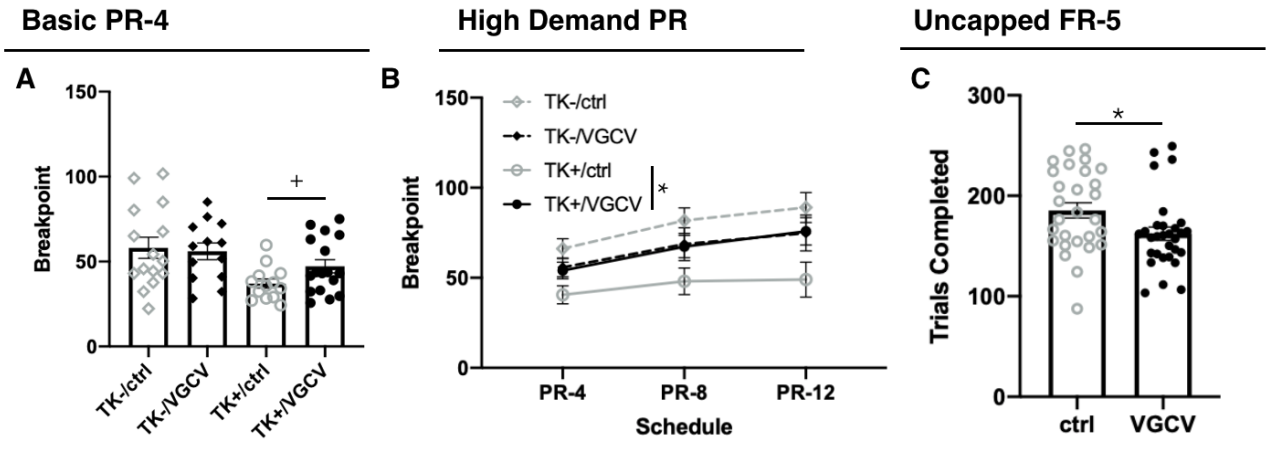

Figure 13. Progressive ratio (PR) performance with the first exposure of 100% milkshake for mice that underwent Timeline A of milkshake concentration exposure.
(A) During basic PR-4, TK+/VGCV mice trended toward *higher* breakpoints than TK+/ctrl mice.

However, TK-/ctrl mice achieved higher breakpoints than TK+/ctrl mice. (B) During high demand PR, TK+/VGCV mice achieved *higher* breakpoints than TK+/ctrl mice. However, TK-/ctrl mice also had higher breakpoints than TK+/ctrl mice. Higher breakpoints were seen with increasing demand. (C) During uncapped FR-5, VGCV-treated mice completed fewer trials than control-fed mice. $^{+}p < .065$, $^{*}p < .05$

3.3.3.1.2 Medium reward: 50% milkshake.

There was an interaction between genotype and treatment for breakpoint during basic PR-4, $F(1,50) = 4.338$, $p = .042$, $\eta_p^2 = .080$ (see Figure 14A). TK+/VGCV mice trended toward *higher* breakpoints than TK+/ctrl mice ($p = .062$), suggesting that, if any effect does exist, neurogenesis knockdown may potentiate multiple responding and may support increased motivation. The trend toward increased breakpoints seen with neurogenesis knockdown did not translate to differences in number of target touches made between TK+/VGCV and TK+/ctrl groups (see Figure A4A) and was not replicated during high demand PR (see Figure A4B-C). However, during high demand PR, there was a main effect of PR schedule observed on breakpoint, $F(2,100) = 78.698$, $p < .0001$, $\eta_p^2 = .611$ (see Figure A4B), and target touches, $F(2,100) = 27.983$, $p < .0001$, $\eta_p^2 = .359$ (see Figure A4C). With increasing demand, higher breakpoints were achieved ($p < .0001$ for all pairwise comparisons) and fewer target touches were made ($p < .0001$ for all pairwise comparisons, except PR-8 vs. PR-12 $p > .05$). During uncapped FR-5, there was no overall effect of group (see Figure A4D), but there was a three-way interaction between sex, genotype, and treatment on number of trials completed, $F(1,50) = 5.947$, $p = .018$, $\eta_p^2 = .106$ (see Figure 14B), with TK+/VGCV females completing fewer trials than TK+/VGCV males ($p = .020$). These data suggest that neurogenesis knockdown and/or VGCV

treatment may differentially affect behaviour across sexes. The sex effect previously seen across all task phases where females made more blank touches than males was only present during basic PR-4 with 50% milkshake, $F(1,50) = 6.706$, $p = .013$, $\eta_p^2 = .118$ (data not shown).

In addition to the differences related to the TK+/VGCV group mentioned above, there were also difference seen between certain control groups. For basic PR-4, TK-/ctrl also achieved higher breakpoints than TK+/ctrl ($p = .013$; see Figure 14A). There was also a genotype by treatment interaction on target touches, $F(1,50) = 4.338$, $p = .043$, $\eta_p^2 = .080$ (see Figure A4A). TK-/ctrl mice made more target touches than TK+/ctrl mice ($p = .024$). For high demand PR, there were genotype by treatment interactions on breakpoints achieved, $F(1,50) = 6.653$, $p = .013$, $\eta_p^2 = .117$ (see Figure A4B), and target touches made, $F(1,50) = 7.509$, $p = .008$, $\eta_p^2 = .131$ (see Figure A4C). TK-/ctrl mice achieved higher breakpoints and made more target touches than TK+/ctrl ($p = .008$ and .004, respectively) and TK-/VGCV mice ($p = .040$ and .023, respectively).

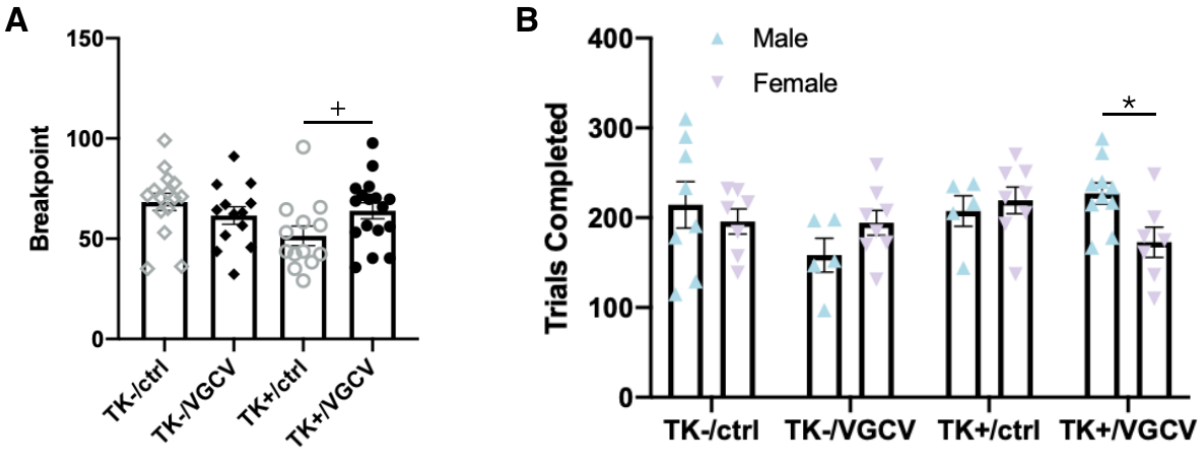

Figure 14. Progressive ratio (PR) performance with 50% milkshake for mice that underwent Timeline A of milkshake concentration exposure.
(A) During basic PR-4, TK+/VGCV mice trended toward achieving higher breakpoints than TK+/ctrl mice. However, TK-/ctrl mice achieved higher breakpoints than TK+/ctrl mice,

suggesting a possible non-specific effect of genotype. (B) During uncapped FR-5, TK+/VGCV females completed fewer trials than TK+/VGCV males, suggesting VGCV treatment and/or neurogenesis knockdown impacts each sex differently. $^{+}p < .065$, $*p < .05$

3.3.3.1.3 Weak reward: 20% milkshake.

There was an interaction between genotype and treatment in the breakpoint data during basic PR-4, $F(1,50) = 12.543$, $p = .001$, $\eta_p^2 = .201$ (see Figure 15A), with TK+/VGCV mice achieving *higher* breakpoints than TK+/ctrl mice ($p = .005$). Unlike prior milkshake concentrations, this neurogenesis knockdown effect was seen in target touch data, $F(1,50) = 14.019$, $p < .0001$, $\eta_p^2 = .219$ (see Figure A5A), where TK+/VGCV mice made more target touches than TK+/ctrl mice ($p = .004$) and trended toward a greater number of target touches than TK-/VGCV mice ($p = .055$). There were no differences between the TK+/VGCV group and any control group on all measures during high demand PR (see Figure A5B-C). However, there was the effect of PR schedule on breakpoint, $F(2,100) = 30.656$, $p < .0001$, $\eta_p^2 = .380$ (see Figure A5B) and target touches, $F(2,100) = 29.223$, $p < .0001$, $\eta_p^2 = .369$ (see Figure A5C). With increasing demand, higher breakpoints were achieved ($p < .0001$ for all pairwise comparisons, except PR-8 vs. PR-12 $p > .05$) and fewer target touches were made ($p < .0001$ for all pairwise comparisons). Additionally, unseen previously, there was an interaction between genotype and treatment on number of trials completed during uncapped FR-5, $F(1,50) = 4.155$, $p = .047$, $\eta_p^2 = .077$ (see Figure 15B). TK+/VGCV mice trended toward completing more trials than TK-/VGCV mice ($p = .060$). The effect of sex on blank touches previously seen was only found during uncapped FR-5 with a three-way interaction between sex, genotype, and treatment, $F(1,50) = 6.804$, $p = .012$, $\eta_p^2 = .120$ (data not shown). Here, females only made more blank touches than

males when both were TK+/VGCV ($p < .0001$), indicating neurogenesis knockdown may affect responding behaviour differently across sexes.

In addition to the possibly neurogenesis knockdown-related effects mentioned above, there were also differences between certain control groups seen during basic PR-4, as well as during high demand PR and uncapped FR-5. For basic PR-4, TK-/ctrl mice achieved higher breakpoints and made more target touches than TK+/ctrl ($p = .003$ and .002, respectively) and TK-/VGCV mice ($p = .041$ and .026, respectively [see Figures 15A and A5A, respectively]). For high demand PR, there was a genotype by treatment interaction observed on breakpoint, $F(1,50) = 4.209$, $p = .045$, $\eta_p^2 = .078$ (see Figure A5B) and target touches, $F(1,50) = 5.727$, $p = .021$, $\eta_p^2 = .103$ (see Figure A5C). The TK-/ctrl mice achieved higher breakpoints and made more target touches than the TK+/ctrl mice ($p = .004$ and .003, respectively). For uncapped FR-5, TK-/ctrl mice completed more trials than TK-/VGCV mice ($p = .025$; see Figure 15B).

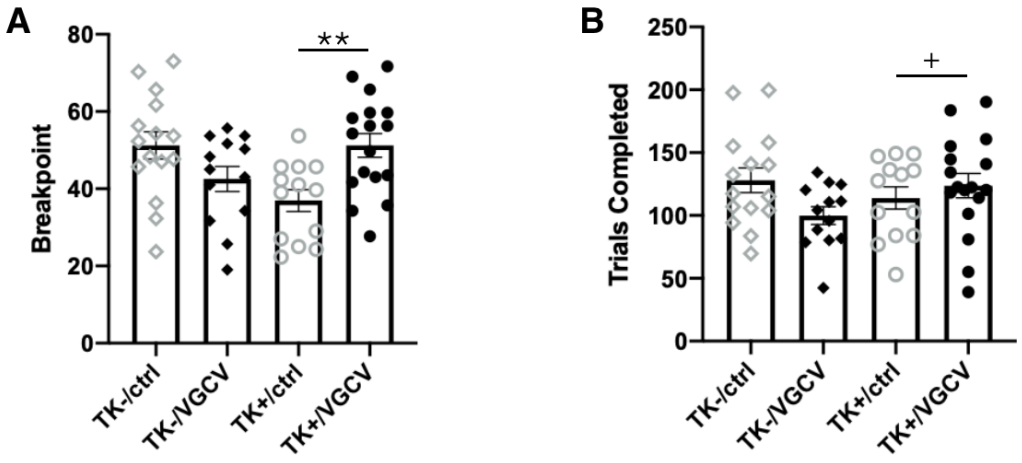

Figure 15. Progressive ratio (PR) performance with 20% milkshake for mice that underwent Timeline A of milkshake concentration exposure.
(A) During basic PR-4, TK+/VGCV mice achieved higher breakpoints than TK+/ctrl mice. However, TK-/ctrl mice achieved higher breakpoints than TK+/ctrl and TK-/VGCV mice, suggesting the presence of non-specific effects of genotype and treatment. It may be that

neurogenesis knockdown allows behaviour to return to baseline levels (i.e., TK-/ctrl levels). (B) During uncapped FR-5, TK+/VGCV mice trended toward completing more trials than TK+/ctrl mice. However, TK-/ctrl mice completed more trials than TK-/VGCV mice, implicating non-specific VGCV effects on behaviour. $^{+}p < .065$, $^{**}p < .01$

3.3.3.1.4 Strong reward: Second exposure of 100% milkshake.

During high demand PR, there was a three-way interaction between sex, genotype, and treatment, $F(1,50) = 4.762$, $p = .034$, $\eta_p^2 = .087$ (see Figure 16A), with TK+/VGCV males showing *greater* breakpoints achieved than TK+/ctrl males ($p = .016$) and TK-/VGCV males ($p = .038$). Additionally, TK+/VGCV males achieved higher breakpoints than TK+/VGCV females ($p = .028$). Female TK+/VGCV mice did not differ from any control group females. Similarly, there was the same three-way interaction seen in the target touch data, $F(1,50) = 4.239$, $p = .045$, $\eta_p^2 = .078$ (see Figure A6A), with TK+/VGCV males making *more* target touches than TK+/ctrl males ($p = .011$) and TK-/VGCV males ($p = .043$), as well as more target touches than TK+/VGCV females ($p = .021$). These data add to the evidence of neurogenesis knockdown having differential behavioural affects with sex. Additionally, the main effect of PR schedule was seen on both measures, $F(2,100) = 4.036$, $p < .0001$, $\eta_p^2 = .492$ (breakpoint; see Figure A6B) and $F(2,100) = 51.664$, $p < .0001$, $\eta_p^2 = .508$ (target touches; see Figure A6C). With higher demand, higher breakpoints were achieved ($p < .0001$ for all pairwise comparisons, except PR-8 vs. PR-12 $p = .028$) and fewer target touches were made ($p < .0001$ for all pairwise comparisons) There were no group differences on trials completed during uncapped FR-5 (see Figure A6D), but VGCV-treated mice completed less trials than control-fed mice, $F(1,50) = 9.120$, $p = .004$, $\eta_p^2 = .154$ (see Figure 16B). This treatment effect indicates a non-specific effect of VGCV

administration on behaviour. The tendency toward hyperactivity in females seen previously was only found during uncapped FR-5, with females completing more trials than males, $F(1,50) = 2.575$, $p = .038$, $\eta_p^2 = .083$ (see Figure A6E).

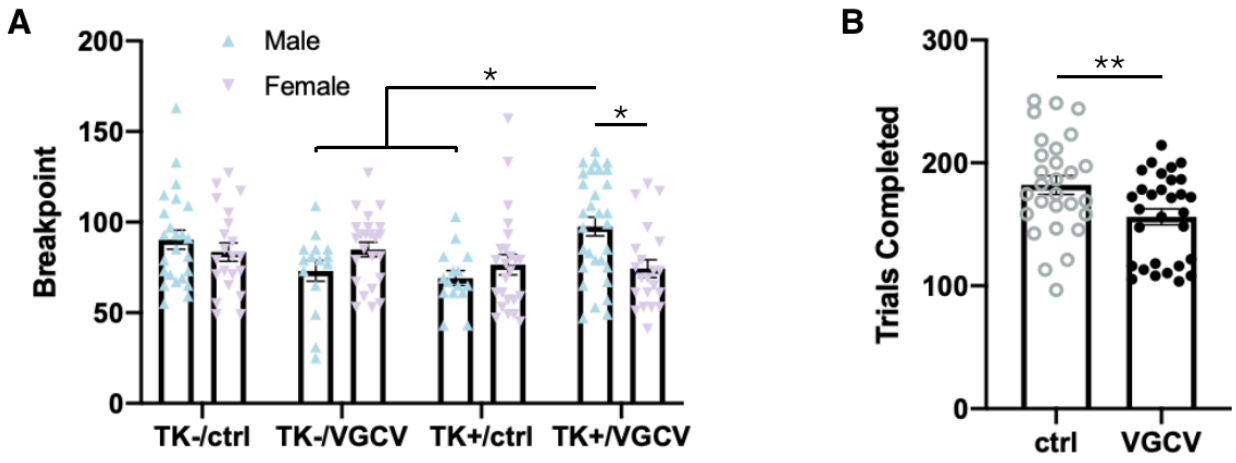

Figure 16. Progressive ratio (PR) performance with the second exposure of 100% milkshake for mice that underwent Timeline A of milkshake concentration exposure.
(A) During high demand PR, TK+/VGCV males achieved higher breakpoints than both TK+/ctrl and TK-/VGCV males. Additionally, TK+/VGCV males achieved higher breakpoints than TK+/ VGCV females, suggesting neurogenesis knockdown differentially affects behaviour across sex. (B) During uncapped FR-5, VGCV-treated mice completed fewer trials than control-fed mice, indicating a non-specific effect of VGCV treatment on behaviour. *$p < .05$, **$p < .01$

3.3.3.1.5 Timeline A: Summary.

Overall, when exposed to descending milkshake concentrations, there was some evidence for a neurogenesis knockdown effect on motivation. However, in light of the recurrent differences between control groups, suggesting effects of genotype and/or treatment alone on behaviour, as well as the three-way interactions seen between sex, genotype, and treatment, it cannot be concluded that neurogenesis knockdown is sufficient to cause an increase in motivation at any reward strength.

3.3.3.2 Timeline B (ascending milkshake concentrations).

3.3.3.2.1 Weak reward: First exposure of 20% milkshake.

There were no group differences seen across all task phases for each measure of interest (see Figure A7A-E). There was, however, the main effect of PR schedule during high demand PR seen previously on breakpoint, $F(2,36) = 27.126$, $p < .0001$, $\eta_p^2 = .601$ (see Figure A7C), and target touches, $F(2,36) = 5.620$, $p = .008$, $\eta_p^2 = .238$ (see Figure A7D). With increasing demand, higher breakpoints were achieved ($p < .0001$ for all pairwise comparisons, except PR-8 vs. PR-12 $p > .05$) and fewer target touches were made (PR-4 vs. PR-12 $p = .003$; PR-8 vs. PR-12 $p = .009$). The tendency toward hyperactivity and/or non-specific responding seen for females in the overall PR analyses and Timeline A analysis was not seen.

3.3.3.2.2 Medium reward: 50% milkshake.

There were no group differences seen across all task phases for each measure of interest (see Figure 8A-E). The main effect of PR schedule was observed during high demand PR for breakpoint, $F(2,36) = 26.398$, $p < .0001$, $\eta_p^2 = .595$ (see Figure A8C), and target touches, $F(2,36) = 5.715$, $p = .015$, $\eta_p^2 = .241$ (see Figure A8D). With increasing demand, higher breakpoints were achieved ($p < .0001$ for all pairwise comparisons, except PR-8 vs. PR-12 $p = .003$) and fewer target touches were made (PR-4 vs. PR-12 $p = .006$; PR-8 vs. PR-12 $p = .019$). The increase in blank touches made in females seen previously in the overall PR analyses and Timeline A analysis was not seen here.

3.3.3.2.3 Strong reward: 100% milkshake.

There were no group differences seen on basic PR-4 on breakpoints achieved and target

touches made (see Figure A9A-B). However, there was an effect of treatment seen during high demand PR on breakpoints achieved, $F(1,18) = 5.535$, $p = .030$, $\eta_p^2 = .235$ (see Figure 17A), and number of target touches made, $F(1,18) = 5.600$, $p = .029$, $\eta_p^2 = .230$ (see Figure A9D), with TK+/VGCV mice having higher breakpoints and making more target touches than TK+/ctrl mice. It was also found that sex interacted with the effect of neurogenesis knockdown on breakpoint achieved, $F(1,18) = 5.098$, $p = .037$, $\eta_p^2 = .221$ (see Figure 17B), with neurogenesis knockdown suppressing the breakpoint for TK+/VGCV females compared to TK+/ctrl females ($p = .004$). In other words, the breakpoint suppression seen with neurogenesis knockdown is only present in females, suggesting neurogenesis knockdown has differential effects on each sex. The same effect of PR schedule during high demand PR was also observed here on breakpoint, $F(2,36) = 62.631$, $p < .0001$, $\eta_p^2 = .777$ (see Figure 17A), and target touches, $F(2,36) = 14.438$, $p < .0001$, $\eta_p^2 = .445$ (see Figure A9D). With increasing demand, higher breakpoints were achieved ($p < .0001$ for all comparisons, except PR-8 vs. PR-12 $p = .055$) and fewer target touches were made (PR-4 vs. PR-8 $p = .017$; PR-4 vs. PR-12 $p < .0001$; PR-8 vs. PR-12 $p = .002$). No group differences were seen during uncapped FR-5 (see Figure A9E), though females were found to complete more trials than males, $F(1,18) = 4.671$, $p = .044$, $\eta_p^2 = .206$ (see Figure A9F). The sex effect previously seen on blank touches made was here only present as a sex by treatment effect during basic PR-4, $F(1,18) = 4.595$, $p = .046$, $\eta_p^2 = .203$ (see Figure A9C). Neurogenesis knockdown may suppress the increased blank responses seen in females, with TK+/VGCV females making fewer blank touches than TK+/ctrl females ($p = .004$).

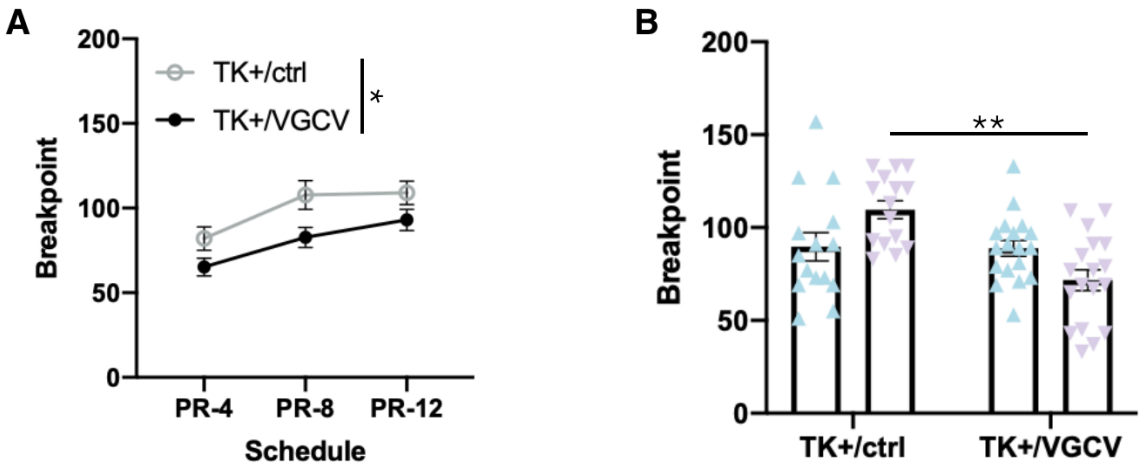

Figure 17. Progressive ratio (PR) performance with 100% milkshake for mice that underwent Timeline B of milkshake concentration exposure.
(A) During high demand PR, TK+/VGCV mice achieved lower breakpoints than TK+/ctrl mice, suggesting neurogenesis knockdown may decrease motivation in this condition. This difference between groups may be driven by underlying sex differences, (B) as it is only TK+/VGCV females that achieved lower breakpoints than TK+/ctrl females. There is no such difference for males. In general, higher breakpoints were achieved with increasing demand. *$p < .05$, **$p < .01$

3.3.3.2.4 Weak reward: Second exposure of 20% milkshake.

The treatment effect seen during high demand PR with 100% milkshake was replicated here. TK+/VGCV mice achieved lower breakpoints than TK+/ctrl mice, $F(1,18) = 7.626$, $p = .013$, $\eta_p^2 = .298$ (see Figure 18), and made fewer target touches than TK+/ctrl mice, $F(1,18) = 7.653$, $p = .013$, $\eta_p^2 = .298$ (see Figure A10A). However, the three-way interaction with sex seen with 100% milkshake on breakpoints achieved was only nearing significance here ($p = .060$; see Figure A10B). There were no group differences on the number of trials completed during uncapped FR-5 (see Figure A10C). As with 100% milkshake, the sex effect on the number of trials completed was found, with females completing more trials than males, $F(1,18) = 9.402$, $p = .007$, $\eta_p^2 = .343$ (see Figure A10D). As seen in the first exposure to 20% and the 50% conditions,

there was also no effect of sex on blank touches made seen here that was found in previous PR analyses.

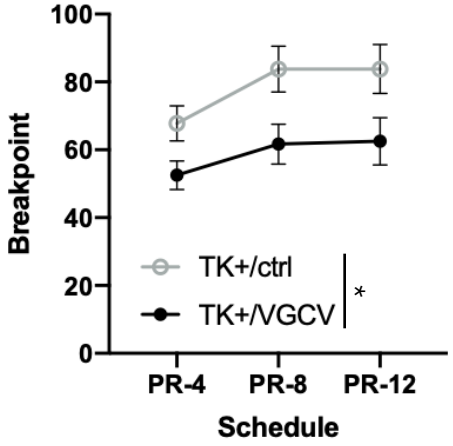

Figure 18. Progressive ratio (PR) performance with the second exposure of 20% milkshake for mice that underwent Timeline B of milkshake concentration exposure.
During high demand PR, TK+/VGCV mice achieved lower breakpoints than TK+/ctrl mice, suggesting neurogenesis knockdown may decrease motivation in this condition. In general, higher breakpoints were achieved with increasing demand. *$p < .05$

3.3.3.2.5 Timeline B: Summary.

Overall, there was little effect of neurogenesis knockdown on motivation when mice were exposed to ascending milkshake concentrations. There were a few instances that suggested mice with neurogenesis knockdown exhibited reduced motivation, but only once the strongest reward was experienced and/or after two full repetitions of the task. This reduction in motivation with neurogenesis knockdown may also only apply to females as an interaction with sex was seen with 100% milkshake; however, the second exposure of 20% milkshake did not reveal this effect. When evidence of reduced motivation due to neurogenesis knockdown was observed, it occurred only under specific conditions and not most others, indicating neurogenesis knockdown does not cause robust effects on motivation.

3.3.4 Training effect analysis.

Repeated testing on the PR task might alter baseline levels of responding; specifically, there may be a greater tendency to respond to the stimulus with each repetition of the task. To determine whether such an effect was present, the number of trials completed during uncapped FR-5 were compared for the repeated concentration in each timeline (i.e., 100% for Timeline A and 20% for Timeline B). Here, a training effect might manifest as an increased amount of trials completed with the second exposure as multiple responding to the stimulus becomes increasingly habitual for the animal. For Timeline A, there was no difference found between number of trials completed for the first exposure and the second exposure of 100% milkshake (see Figure 19A). However, as expected from the analysis of Timeline A, an effect of treatment was found with VGCV-treated mice completing fewer trials, in general, than control-fed mice, $F(1,50) = 11.481$, $p = .001$, $\eta_p^2 = .187$ (see Figure 19B). To focus on the effect of time, the data were collapsed across groups and subjected to a paired-subjects t-test, which also indicated no difference between exposures (see Figure A11A). For Timeline B, mice completed more trials during the second exposure of 20% milkshake than the first, $F(1,18) = 8.790$, $p = .008$, $\eta_p^2 = .328$ (see Figure 19C). There was also an interaction with sex, $F(1,18) = 8.790$, $p = .009$, $\eta_p^2 = .328$ (see Figure 19D), where males completed more trials than females during the second exposure of 20% milkshake; males may have an increased susceptibility to training effects with repeated testing than females. When collapsed across groups, the finding that mice completed more trials during the second exposure when compared to the first was confirmed, $t(21) = -4.569$, $p < .0001$ (see Figure A11B). Therefore, a training effect only occurred for mice who experienced Timeline B, but not for mice who experienced Timeline A.

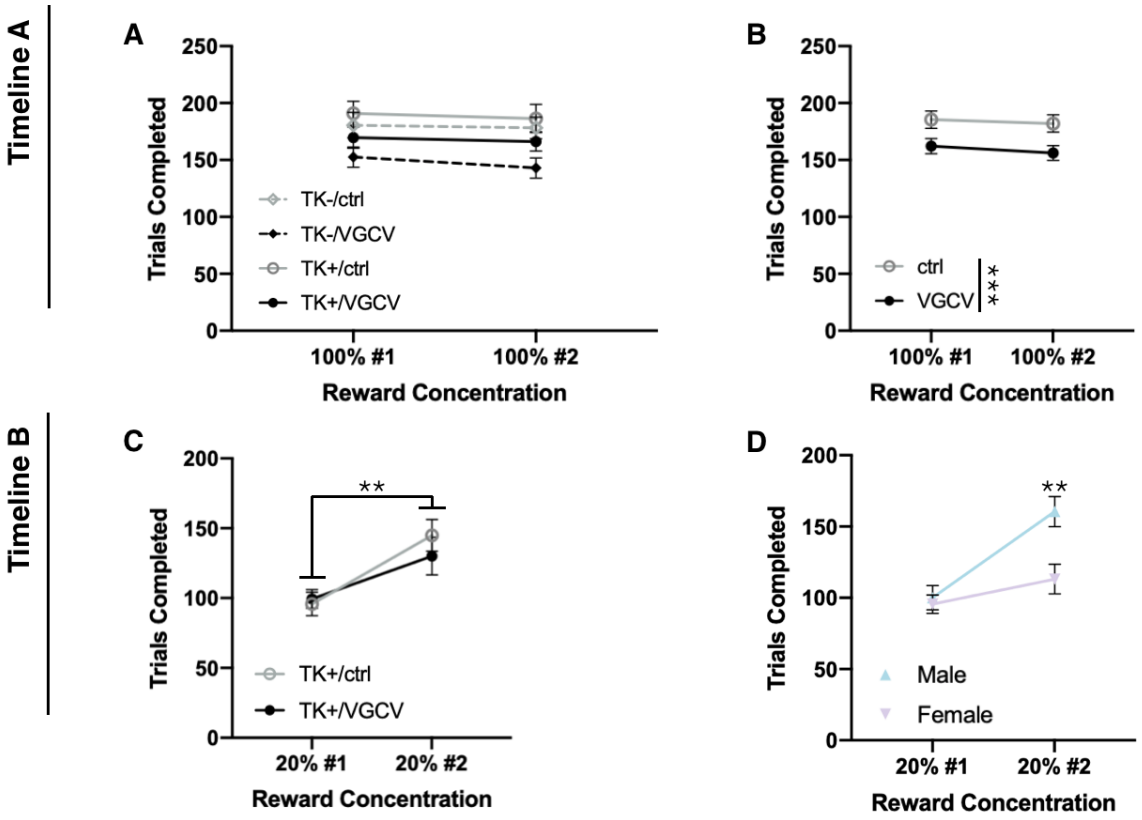

Figure 19. Determination of the presence of training effects in each milkshake concentration timeline of the progressive ratio (PR) task.

For Timeline A, milkshake concentrations were exposed in descending order, with 100% repeated; for Timeline B, milkshake concentrations were exposed in ascending order, with 20% repeated. (A) For mice that underwent Timeline A, there were no group differences between the number of trials completed with the first exposure and those completed with the second exposure of 100% milkshake during uncapped FR-5. No change in trials completed across both timepoints suggests a training effect was not present for these mice. (B) For mice that underwent Timeline A, VGCV-treated mice completed fewer trials than control-fed mice at both timepoints, suggesting a possible non-specific treatment effect on behaviour. (C) For mice that underwent Timeline B, a greater number of trials was completed during the second exposure of 20% milkshake when compared to the first during uncapped FR-5 for both groups. The increase in the number of trials completed at the second timepoint suggests the presence of a training effect. (D) For the mice that underwent Timeline B, a sex difference was only seen with the second exposure of 20% milkshake, with males completing more trials than females at this timepoint.

These data may indicate that males and females have different tendencies toward training effects due to repeated testing on the PR schedule. $^{**}p < .01$, $^{***}p \leq .001$

3.3.5 Timeline comparison.

To determine whether exposure order (i.e., ascending vs. descending milkshake concentrations) impacted behaviour, high demand breakpoint and uncapped FR-5 trials completed data were compared for 100% (Timeline A: First exposure, first time through PR task; Timeline B: Third time through PR task) and 20% milkshake (Timeline A: Third time through PR task; Timeline B: First exposure, first time through PR task). As these same data were already analyzed for group differences, groups were collapsed for this analysis. Collapsing across group here had the added benefit of highlighting the overall effect of timeline.

For timeline comparisons with 100% milkshake, Timeline B mice were found to have greater breakpoints than Timeline A mice during high demand PR for each schedule, $F(2,150) = 4.895$, $p = .017$, $\eta_p^2 = .061$ (see Figure A12A), and in general, $F(1,75) = 17.336$, $p < .0001$, $\eta_p^2 = .188$ (see Figure A12B). Mice that underwent Timeline B were also found to have completed more trials on uncapped FR-5 than those that underwent Timeline A, $F(1,75) = 4.736$, $p = .033$, $\eta_p^2 = .059$ (see Figure A12C). This difference between timelines, however, is likely due to the training effect seen in Timeline B mice; the training effect present in the Timeline B data confounds the comparison between timelines with 100% milkshake.

For timeline comparisons with 20% milkshake, Timeline A was found to have greater breakpoints than Timeline B during high demand PR, $F(1,75) = 13.160$, $p = .001$, $\eta_p^2 = .149$ (see Figure A12D), and more trials were completed during uncapped FR-5 for Timeline A than B, $F(1,75) = 12.777$, $p = .001$, $\eta_p^2 = .146$ (see Figure A12E). From the training effect analyses, it

was found that one was not present for Timeline A mice, therefore it is unlikely the differences found between timelines is due to a training effect. However, it may be that the previous experience with stronger rewards preceding exposure to 20% milkshake for mice that underwent Timeline A made it easier for these mice to achieve higher breakpoints during high demand PR and complete more trials during uncapped FR-5. For mice that underwent Timeline B, 20% milkshake was their first experience with the PR task; as 20% milkshake was the weakest reward and no past experience of the task with stronger rewards allowed for increased responding, these mice completing fewer trials is understandable.

3.4 Extinction Learning

Extinction learning was run after PR to assess whether the withdrawal of reward for a previously rewarded stimulus was impacted by neurogenesis knockdown. The two measures were amount of omissions made and the days required to reach the endpoint criterion. The extinction curve represents the amount of omissions made (i.e., a correct lack of response to the stimulus) over the first 10 days. There were no differences between groups on omissions made on each day of the extinction curve. All groups exhibited an increase in omissions made across sessions, $F(9,576) = 32.258$, $p < .0001$, $\eta_p^2 = .335$ (see Figure 20A), where more omissions were made on the first day when compared to the tenth ($p < .0001$). Similarly, there were no group differences in the days required to reach the endpoint criterion of two consecutive days of ≥77% omissions made (see Figure 20B). These data suggest neurogenesis knockdown does not influence the ability to withhold responses to a stimulus that is no longer rewarded.

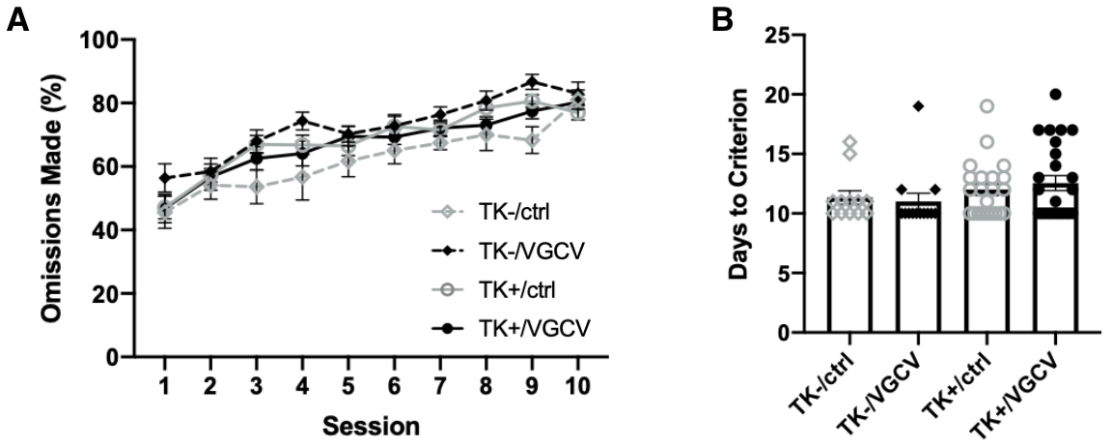

Figure 20. Performance on extinction learning.
(A) There were no group differences on the amount of omissions made for each day of the extinction curve. All groups showed an increase in the amount of omissions made across sessions, indicating all groups learned to cease responding with time. (B) There were no group differences on the days required to reach endpoint criterion (i.e., two consecutive sessions of ≥77% omissions). These data suggest there was no effect of neurogenesis knockdown on extinction learning.

3.5 Reward Concentration Preference Test

The reward concentration preference test was run to verify the assumption, underlying the PR tests reported above, that 100% (or undiluted) milkshake was a stronger reward than 50% milkshake, which in turn was a stronger reward than 20% milkshake. Additionally, as each reward concentration had different levels of calories and sweetness, this test may potentially be used as an indicator of anhedonic behaviour as an analogue of the sucrose preference test. The main measures for each binary choice between concentrations were the total amount of each concentration consumed during the 2-hr test and the preference indices for the milkshake of higher concentration. (See Appendix B for additional results from only Timeline B mice)

3.5.1 100% vs. 50%.

There were no group differences on the total amount of each concentration consumed, nor were there any differences on the preference indices for 100% milkshake. All groups consumed more 100% than 50% milkshake over the course of the test, $F(1,47) = 28.186$, $p < .0001$, $\eta_p^2 = .375$ (see Figure 21A), suggesting all groups preferred 100% to 50% milkshake. The preference for 100% milkshake was further indicated by the preference indices being over 50% (see Figure 21B). These data support the assumption that 100% milkshake had stronger reinforcer value than 50% milkshake and are consistent with a conclusion that neurogenesis knockdown itself does not induce anhedonic-like behaviour.

3.5.2 100% vs. 20%.

As with the 100% vs. 50% choice, there were no group differences on the amount of each concentration consumed over time, nor were there differences on the preference indices for 100% milkshake. All groups consumed more 100% than 20% milkshake over the course of the test, $F(1,47) = 188.800$, $p < .0001$, $\eta_p^2 = .801$ (see Figure 21C), suggesting all groups preferred 100% milkshake over 20%. The preference for 100% milkshake was further indicated by the preference indices being over 50% (see Figure 21D). These data support the assumption that 100% milkshake was a stronger reward than 20% milkshake and are consistent with a conclusion that neurogenesis knockdown itself does not induce an anhedonic behavioural effect.

3.5.3 50% vs. 20%.

In line with the results of the previous two binary choices, there were no group differences on the total amount of each concentration consumed, nor were there differences on the preference indices for 50% milkshake. All groups consumed more 50% than 20% milkshake

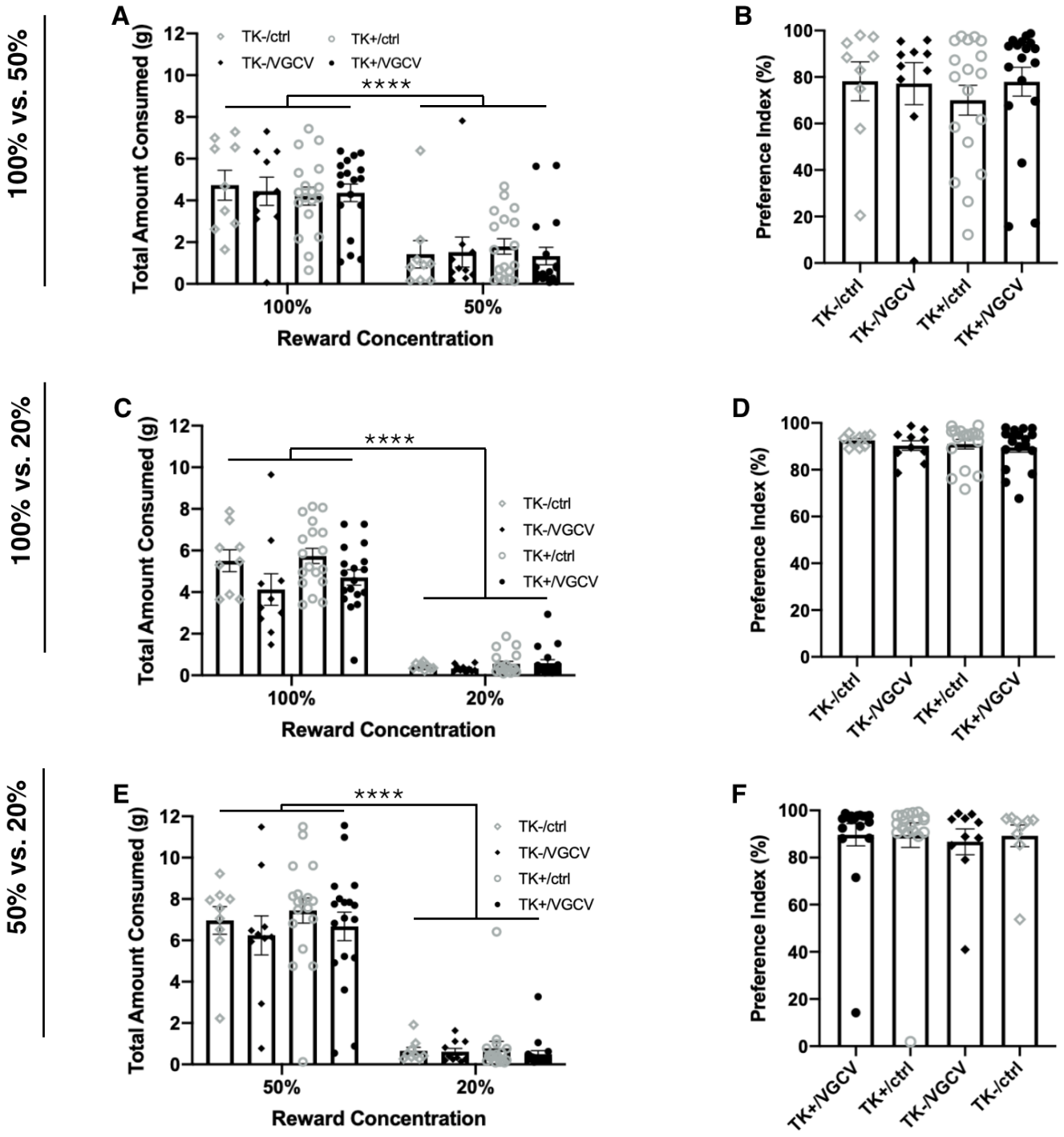

Figure 21. Reward concentration preference test performance.

For the 100% vs. 50% choice, there were no differences between groups on (A) the amount of each concentration consumed over the 2-hr test period, (B) nor were there group differences on the preference indices for 100% milkshake. All groups showed a preference for 100% milkshake over 50%, verifying the assumption that 100% milkshake was a stronger reward than 50%. For

the 100% vs. 20% choice, there were no group differences on (C) the amount of each concentration consumed over the 2-hr test period, (D) nor were there differences on the preference indices for 100% milkshake. All groups showed a preference for 100% milkshake over 20%, verifying the assumption that 100% milkshake was a stronger reward than 20%. For the 50% vs. 20% choice, there were no differences between groups on (E) the amount of each concentration consumed over the 2-hr test period, (F) nor were there group differences on the preference indices for 50% milkshake. All groups showed a preference for 50% milkshake over 20%, verifying the assumption that 50% milkshake was a stronger reward than 20%. Together, these results suggest neurogenesis knockdown did not induce anhedonia as all groups exhibited a preference for the milkshake of higher concentration with each binary choice. ****$p < .0001$

over the 2-hr test, $F(1,47) = 127.435$, $p < .0001$, $\eta_p^2 = .731$ (see Figure 21E), suggesting all groups preferred 50% over 20% milkshake. The preference for 50% milkshake was further indicated by the preference indices being over 50% (see Figure 21F). These data support the assumption that 50% milkshake had stronger reward value than 20% milkshake and, in combination with the results of the previous two binary choices, suggest that neurogenesis knockdown by itself was not sufficient to induce an anhedonic behavioural profile.

3.6 Negative Valence Systems Tests

3.6.1 Light/dark box.

The light/dark box is a classic test that assesses anxiety-like behaviour in rodents. The main measures of interest were the amount of time spent in the light chamber, the amount of entries into the light chamber, and latency to enter the light chamber from the dark chamber. There were no differences overall between the TK+/VGCV group and any control group across all measures. However, there was a three-way interaction for time spent in the light chamber, $F(1,31) = 5.844$, $p = .022$, $\eta_p^2 = .159$ (see Figure 22A), with female TK+/VGCV mice spending

less time in the light chamber than female TK+/ctrl mice (p = .012) and male TK-/VGCV mice spending less time in the light chamber than male TK-/ctrl mice (p = .001). The reduced time spent in the light chamber for female TK+/VGCV and male TK-/VGCV likely drove the main effect of treatment seen, $F(1,31) = 9.225$, p = .005, $\eta_p^2 = .229$ (see Figure 22B), where VGCV treatment in general lead to reduced time in the light chamber. Therefore, due to the non-specific anxiogenic effect of VGCV treatment in TK- males, it cannot be concluded that the reduced time in the light chamber for the TK+/VGCV females was due to neurogenesis knockdown. The potential non-specific effect of VGCV treatment is further supported by the finding that mice treated with VGCV made fewer entries into the light chamber than mice given control chow, $F(1,31) = 7.124$, p = .012, $\eta_p^2 = .187$ (see Figure 22C).

Additionally, there was a two-way interaction between sex and treatment for latency to enter the light chamber, $F(1,31) = 5.597$, p = .024, $\eta_p^2 = .153$ (see Figure 22D). Males given control chow showed longer latencies to enter the light chamber than did females given control chow (p = .041), but males given VGCV showed much shorter latencies than control-fed males (p = .001). On the measure of latency, VGCV only induced a behavioural difference in males and not females, seeming to reduce the amount of time taken for males to enter the light chamber. In other words, VGCV treatment seemed to have had an anxiolytic effect in males on the measure of latency, which is the opposite effect of VGCV seen for time spent in the light chamber. It is possible that the anxiogenic effect of neurogenesis knockdown, if one exists, may be masked by the confound of the non-specific anxiogenic effect VGCV treatment on its own may elicit. Thus, the effect of neurogenesis knockdown on light/dark box anxiety-like behaviour remains inconclusive.

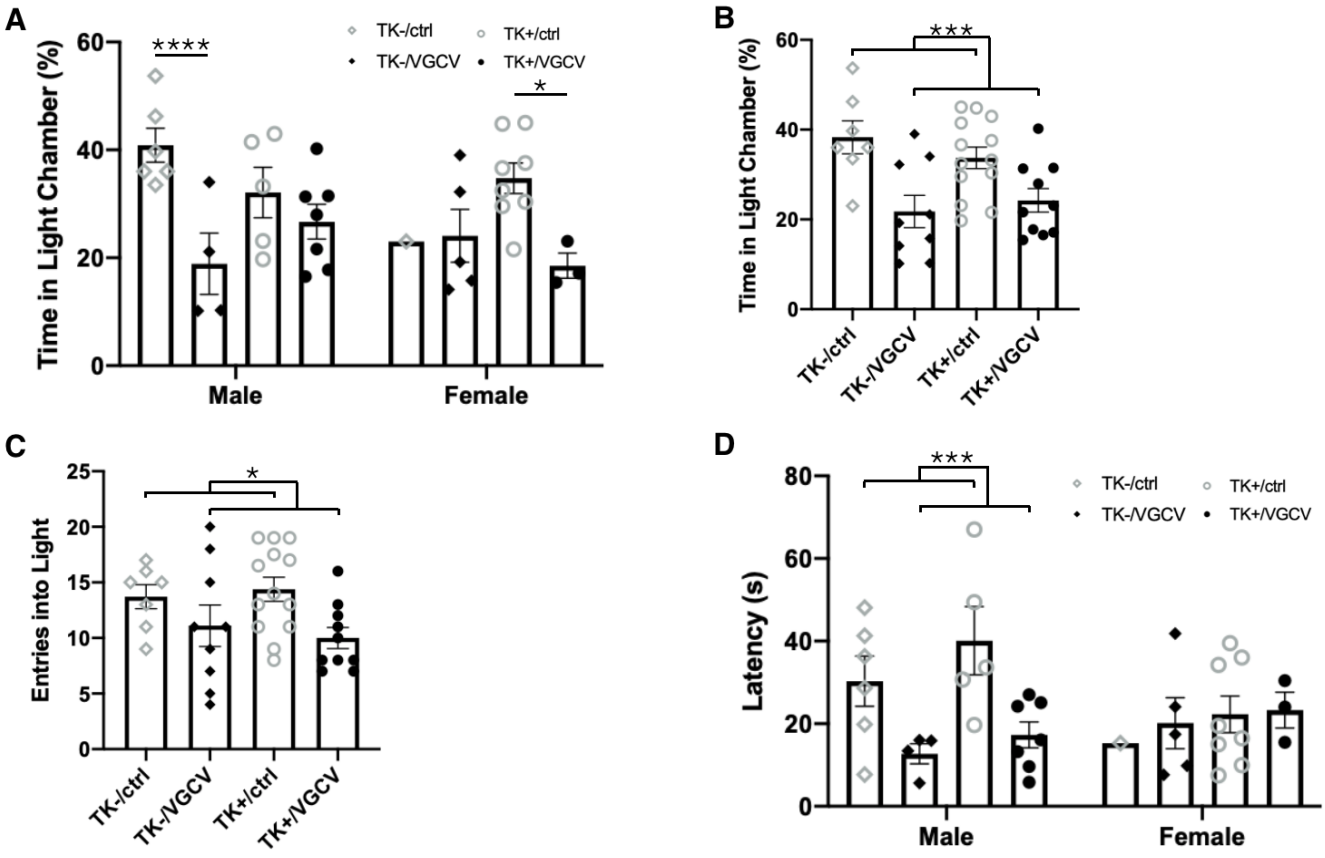

Figure 22. Performance on the light/dark box.

(A) TK+/VGCV females spent less time in the light chamber than TK+/ctrl females, and TK-/ctrl males spent less time in the light chamber than TK-/VGCV males. (B) VGCV-treated mice spent less time in the light chamber than control-fed mice, suggesting a non-specific anxiogenic behavioural effect of VGCV treatment. (C) VGCV-treated mice made less entries into the light chamber than control-fed mice, offering further evidence for the non-specific anxiogenic behavioural effect of VGCV. (D) Males treated with VGCV took less time to first enter the light chamber than males fed control chow, suggesting a non-specific anxiolytic effect of VGCV treatment for males on the measure of latency. On previous measures, VGCV treatment seemed to induce an anxiogenic-like effect, particularly for females, whereas here VGCV treatment seems to cause an anxiolytic-like effect for males. Across all measures, there is no evidence of neurogenesis knockdown itself, however, influencing anxiety-like behaviour on the light/dark box. *$p < .05$, ***$p \leq .001$, ****$p < .0001$

3.6.2 Elevated plus maze.

The EPM is another of the classic tests for assessing anxiety-like behaviour in rodents. The main measures of interest were the number of entries made into the open arms, the amount of time spent in the closed arms, and the d2 ratio indicating the degree of preference for closed arms over open arms. On all measures analyzed, there were no group differences in behaviour on the EPM (see Figure 23A-C). However, there was a main effect of genotype on the number of entries made, $F(1,46) = 8.830$, $p = .005$, $\eta_p^2 = .161$ (data not shown, however can consult Figure 23A). TK+ mice made more entries into the open arms than TK- mice, suggesting a non-specific anxiolytic-like effect of genotype. Overall, there is no evidence to suggest an effect of neurogenesis knockdown on anxiety-like behaviour when tested on the EPM.

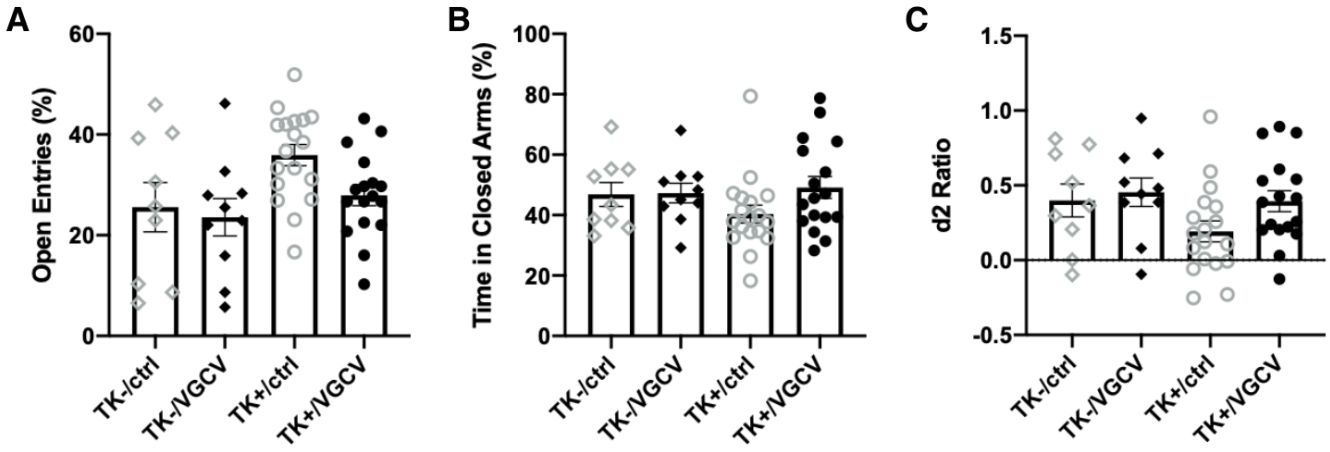

Figure 23. Performance on the elevated plus maze (EPM).
(A) There were no group differences on the amount of open arm entries made. However, TK+ mice made more entries than TK- mice, suggesting a possible non-specific anxiolytic-like effect of genotype. (B) There were no group differences on the amount of time spent in the closed arms. (C) There were no differences between groups on the d2 ratio, indicating no differences in the degree of preference each group had for closed over open arms (i.e., a more positive d2 ratio would indicate greater preference for time spent in the closed arms, whereas a more negative d2

ratio would indicate a greater preference for time spent in the open arms). Taken together, the EPM data suggest neurogenesis knockdown does not impact anxiety-like behaviour.

3.7 Buried Food Olfaction

Changes in olfaction could affect perception and/or valuation of reward and, therefore, performance on the reward-related tests. ABNs are present in the rodent olfactory bulb, so VGCV treatment in the TK+ mice may have altered perception and/or valuation of reward. Therefore, the buried food olfaction test was run to determine whether any gross deficits in olfaction were present in mice with neurogenesis knockdown. There were no group differences on this test according to the measure of latency to find the Teddy Graham cookie (see Figure 24), suggesting neurogenesis knockdown itself does not impair olfactory ability.

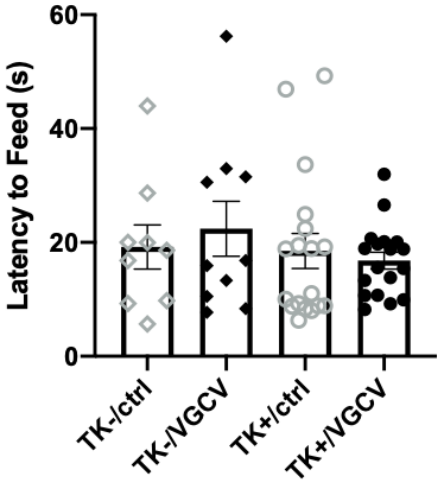

Figure 24. Buried food olfaction test performance.
There were no group differences on the latency to eat the Teddy Graham cookie, indicating gross olfactory ability was not impaired with neurogenesis knockdown.

3.8 Immunofluorescent Analysis of Neurogenesis

Ki-67 is an immunohistochemical marker for proliferating cells. The main measures of interest were Ki-67 cell counts for the entire DG, counts for the dorsal DG, and counts for the ventral DG, as well as the comparison of Ki-67 cell counts between the dorsal and ventral DG. A representative image of a Ki-67-labelled cell is reported in Figure 25A. There was a genotype by treatment effect for the Ki-67 cell counts for the total DG, $F(1,16) = 18.064$, $p = .001$, $\eta_p^2 = .530$ (see Figure A13), where the TK+/VGCV group had fewer Ki-67 counts than controls ($p < .0001$); the dorsal DG, $F(1,16) = 15.099$, $p = .001$, $\eta_p^2 = .486$ (see Figure 25B), where the TK+/VGCV group had fewer Ki-67 cell counts than TK+/ctrl ($p < .0001$) and TK-/VGCV ($p = .004$); and the ventral DG, $F(1,16) = 5.753$, $p = .029$, $\eta_p^2 = .264$ (see Figure 25C), where the TK+/VGCV group had fewer Ki-67 cells than the TK+/ctrl group ($p = .004$) and the TK-/VGCV group ($p = .004$). These data verify that neurogenesis was indeed knocked down in TK+/VGCV mice. This finding, taken in addition to the TK+/VGCV impairment seen on s-SLR, increases the confidence in the conclusion that VGCV treatment caused neurogenesis ablation in TK+ mice. Additionally, both the molecular and behavioural neurogenesis verifications do not indicate non-specific effects of VGCV treatment in TK- mice.

There were no differences between Ki-67 counts for the dorsal and ventral DG, indicating that the level of neurogenesis knockdown and the level of proliferating neural cells remain consistent throughout the adult DG.

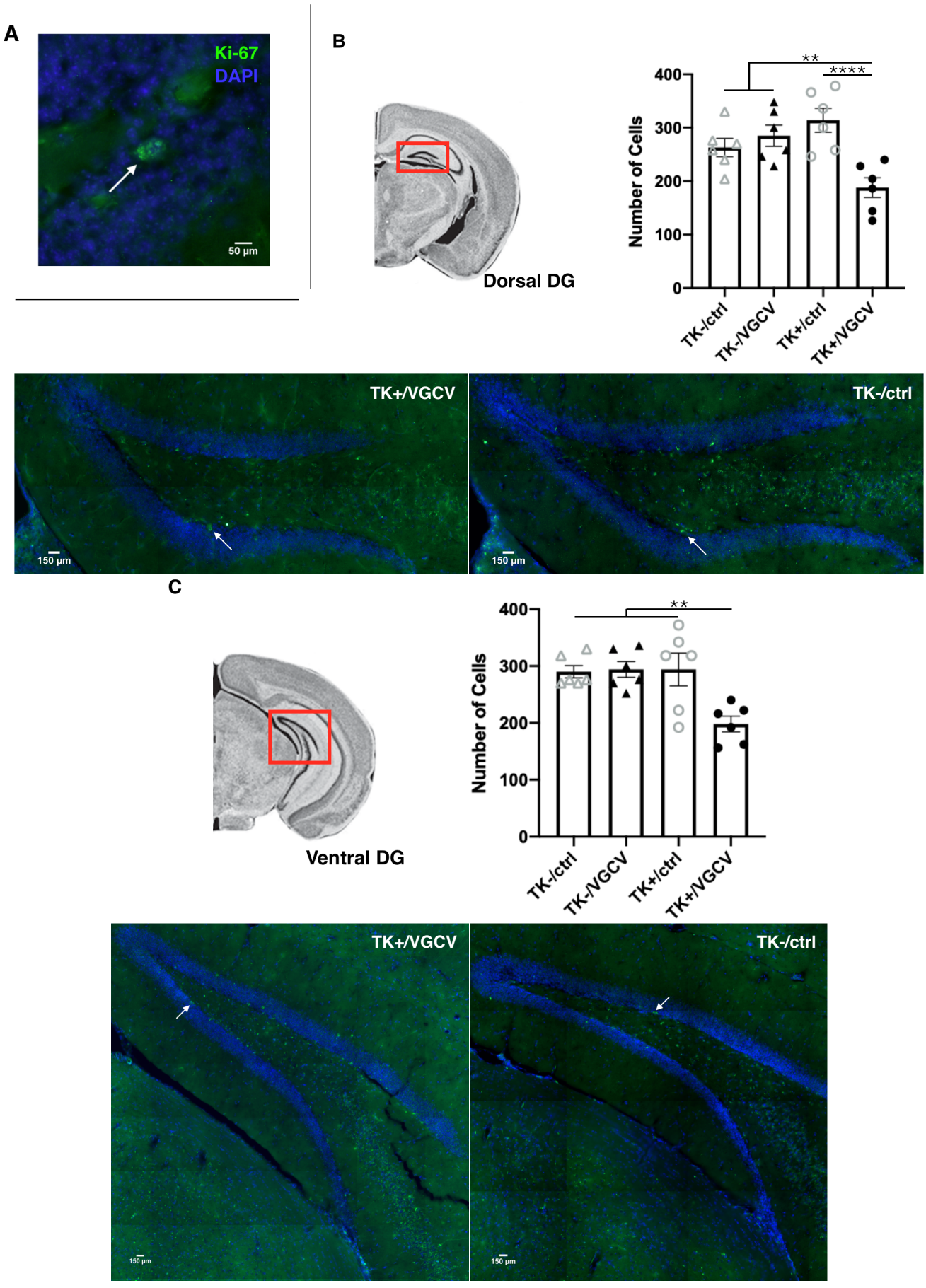
A
Ki-67
DAPI
50 µm
B
Dorsal DG
Number of Cells
400
300
200
100
0
**
****
TK-/ctrl
TK-/VGCV
TK+/ctrl
TK+/VGCV
TK+/VGCV
TK-/ctrl
150 µm
150 µm
C
Ventral DG
Number of Cells
400
300
200
100
0
**
TK-/ctrl
TK-/VGCV
TK+/ctrl
TK+/VGCV
TK+/VGCV
TK-/ctrl
150 µm
150 µm

Figure 25. Ki-67 expression in the dorsal and ventral dentate gyrus for one hemisphere. (A) Representative Ki-67-labelled cell. (B)-(C) TK+/VGCV mice exhibited fewer Ki-67 cells in the dorsal and ventral dentate gyrus in comparison to controls, indicating a reduction in proliferating neural cells across both subregions. Coronal section diagrams of dorsal and ventral hippocampus adapted from Barfield et al. (2017). $^{**}p < .01$, $^{****}p < .0001$

4. Discussion

Adult hippocampal neurogenesis (AHN), specifically that in the ventral hippocampal dentate gyrus (DG), has been repeatedly implicated in mood and depressive disorders, or depression- and anxiety-like behaviour in animal subjects. The evidence for this view, however, largely comes from studies using aversively-motivated behaviours, such as the forced swim test (e.g., Li et al., 2008; Mateus-Pinheiro et al., 2013; Snyder et al., 2011) and contextual fear conditioning (e.g., McHugh et al., 2007; Sahay et al., 2011; reviewed in Kheirbek et al., 2012). My thesis project investigated the role of AHN in depression-relevant reward-related behaviours (i.e., behaviours related to positive valence systems) under non-stressful conditions. Initial tests with the nestin-TK model of neurogenesis knockdown revealed a clear effect of immature neuron ablation on the similar condition of the SLR test, replicating the finding that neurogenesis knockdown, across a variety of methods, leads to impaired pattern separation (Bekinschtein et al., 2013; Clelland et al., 2009; Creer et al., 2010; McHugh et al., 2007; Niibori et al., 2012; Sahay et al., 2011). The behavioural verification of neurogenesis knockdown on the SLR test was confirmed by the reduction in Ki-67-labelled cells in the DG of TK+/VGCV mice in comparison to control mice. The reduction of Ki-67-labelled cells was seen in both the dorsal and ventral DG, indicating neurogenesis knockdown was not region-specific. However, neurogenesis knockdown had a very limited effect on performance on three touchscreen tests of depression-relevant reward-related behaviours, namely probabilistic reversal learning (PRL), progressive ratio (PR), and appetitive extinction learning. These findings are consistent with the notion that AHN plays a regulatory role in depression-relevant behaviours primarily under conditions of chronic stress (e.g., Hill et al., 2015).

For my thesis project, I also evaluated mice with neurogenesis knockdown on two classic tests of anxiety-like behaviour in rodents (i.e., negative valence systems tests), namely the light/ dark box and EPM. These tests are considered to be at least moderately aversive for the animals and behaviour on these tests has previously been found to be affected by neurogenesis knockdown (Mateus-Pinheiro et al., 2013; Revest et al., 2009). Due to variability across control groups, and the inconsistent differential effects of VGCV treatment across sexes, interpretation of the results from these tests remains inconclusive. On the basis of the depression-relevant reward-related behavioural touchscreen tests, I tentatively conclude that AHN plays a regulatory role in mood and depression- and anxiety-like behaviours only under stressful conditions, but does not play a significant role in reward-related behaviours under non-stressful conditions.

4.1 Probabilistic Reversal Learning

There was no evidence for an effect of neurogenesis knockdown on any measure analyzed for PRL. Specifically, there were no differences in the number of reversals made, number of trials required per reversal, and accuracy achieved. Of particular interest, there were also no group differences on win-stay and lose-shift probabilities, all groups exhibiting a higher probability of win-stay behaviour than lose-shift behaviour. These findings strongly suggest that neurogenesis knockdown, on its own, does not impact sensitivity to feedback information.

The lack of differences between groups seen in the present study is not because the test itself is not sensitive enough to detect differences. PRL performance has previously been found to be affected by serotonergic activity. Increased serotonin activity via chronic SSRI (Bari et al., 2010) or agonist administration (Phillips et al., 2018) resulted in an increased probability of win-stay behaviour, whereas decreased serotonin activity via neurochemical depletion (Bari et al.,

2010) or chronic antagonist administration (Phillips et al., 2018) reduced the probability of win-stay behaviour. Therefore, PRL is sensitive enough to detect differences in sensitivity to feedback information where such differences exist.

4.2 Progressive Ratio

In general, neurogenesis knockdown did not significantly affect behaviour during PR, regardless of reward concentration condition. There were, however, effects of neurogenesis knockdown under certain conditions. For instance, with 20% milkshake, when mice from each timeline were analyzed together, TK+/VGCV mice made more target touches than TK+/ctrl mice on basic PR-4. During Timeline A, TK+/VGCV mice achieved higher breakpoints than TK+/ctrl mice with 20% milkshake reward during basic PR-4, and TK+/VGCV males achieved higher breakpoints than TK+/ctrl and TK-/VGCV males during high demand PR with the second exposure of 100% milkshake. Alone, these data might suggest that neurogenesis knockdown increases propensity toward increased responding, or higher motivation, when given a weak reward after experiencing stronger rewards (especially for males), with the effect returning once the strong reward is re-introduced. However, non-specific effects of VGCV treatment and differences between the three control groups were seen multiple times, therefore drawing any conclusions from these results is difficult due to the likely confounding factor of VGCV treatment and/or the presence of the TK transgene. It was often seen in the Timeline A mice that the TK+/ctrl and TK-/VGCV groups had lower breakpoints and/or target touches than the TK+/VGCV and TK-/ctrl groups. There are many possibilities for this recurrent pattern, though one may be that both VGCV treatment and TK+ genotype, when alone, suppress repeated responding. The behavioural difference of the TK+/VGCV group, when seen, may in actuality be

due to factors other than neurogenesis knockdown, such as potential side effects, per se, of VGCV treatment interacting with the TK+ genotype.

During Timeline B, TK+/VGCV females achieved lower breakpoints than TK+/ctrl females during high demand PR with 100% milkshake, and TK+/VGCV mice achieved lower breakpoints than TK+/ctrl mice during high demand PR with the second exposure of 20% milkshake. Alone, these data suggest that neurogenesis knockdown results in lower breakpoints (i.e., reduced motivation) when given strong reward after previously experiencing weak rewards, with the effect re-emerging once the weak reward is re-introduced. This effect may be exacerbated in females due to the interaction of sex with treatment found under the 100% milkshake condition, wherein TK+/VGCV females achieved lower breakpoints than TK+/ctrl females during high demand PR; this behavioural difference was not seen between treatment groups in males. However, the lack of TK- controls to compare with the TK+ data makes drawing firm conclusions about these specific effects difficult. Additionally, in light of the knowledge that VGCV treatment itself can impact behaviour (see above), it is not clear whether the reduced motivation seen in TK+/VGCV mice is a true effect of neurogenesis knockdown or is a result of confounding effects of VGCV treatment. Regardless, the potential effects of neurogenesis knockdown on motivated behaviour seen during both PR timelines are condition-specific, occurring only for certain concentrations and sometimes only for one sex, and, therefore, are not strong enough evidence to base a firm conclusion of the role of AHN in motivation.

The results found in the present study were not due to satiation effects during PR sessions, inability to distinguish weaker rewards from stronger rewards, and insensitivity of the

task. The primary goal of the uncapped FR-5 phase was to test for satiation effects, as it is possible that breakpoints achieved during PR could be a result of cessation of responding due to satiation. However, all mice completed many more trials (i.e., received many more rewards) during uncapped FR-5 than during PR. Mice generally completed 100s of trials during uncapped FR-5, whereas mice generally only completed 10s of trials on any given PR schedule. Therefore, behavioural differences seen in breakpoint were not due to differences in satiation, at any reward concentration. It is also possible that behaviour may have been impacted by impaired olfaction in TK+/VGCV mice, or differences in the perception of reward palatability. The buried food olfaction test was run to assess gross olfactory ability and there were no group differences on this test, indicating gross olfactory ability was not influenced by neurogenesis knockdown. Additionally, all groups showed a preference for the stronger rewards over the weaker rewards in the reward concentration preference test, indicating that mice with neurogenesis knockdown had normal perception of and preferences for higher reward concentrations. Further, the PR task itself is sensitive enough to elucidate group differences, if any exist. The PR touchscreen task for rodents has been used many times previously and performance on PR has been shown to be affected by manipulations of the dopamine system (Heath et al., 2015), known to be involved in motivation and reward systems.

A recent study by Karlsson et al. (2018) also examined the effects of neurogenesis knockdown on PR with different reward strengths. These authors used GFAP-TK rats and mice, with sucrose as a weak reward and balanced food pellets as a strong reward (as determined by a reward preference test). The authors concluded that, in both rats and mice, neurogenesis knockdown affected motivation for weak, but not strong, rewards. This conclusion is similar to

that of the present study, specifically that neurogenesis knockdown may alter responding in PR, but only under certain specific conditions. In the present study, however, these sporadic effects were not specific to reward concentration. Indeed, I found some evidence of altered responding in PR with both weak and strong rewards, but these effects were not consistent across conditions.

There were a number of methodological differences between my thesis project and that of Karlsson et al. (2018). First, even though nestin-TK and GFAP-TK mice are both models of neurogenesis knockdown and share many similarities, there may be underlying gene interaction and/or physiological differences that may produce differences in behaviour or differences in potential non-specific effects of VGCV treatment and of TK. Second, Karlsson et al. (2018) did not have the full complement of control groups for either species tested. For rats, the authors tested TK+/VGCV and TK-/VGCV, then had a single cohort of TK+/ctrl and TK-/ctrl rats to test for non-specific transgene effects (though sample sizes were not given); for mice, the authors only tested TK+/VGCV and TK-/VGCV. The omission of control-fed control groups for mice leaves open the possibility that VGCV treatment might be influencing behaviour above and beyond the effect of neurogenesis knockdown, which may be of particular importance given the present study found recurrent treatment effects and differences between the TK-/ctrl group and other control groups. Further, the assumption in the Karlsson et al. (2018) study that the lack of non-specific effects of genotype in rats can be applied or inferred for mice is questionable. Despite similarities between the species, the micro-environments may differ drastically. Third, the Karlsson et al. (2018) study only used male rats and mice, therefore sex differences could not be determined. The lack of female subjects may be particularly important given the present study revealed sex differences on PR behaviour on both milkshake concentration exposure timelines.

Fourth, the PR schedule differed between my thesis project and Karlsson et al. (2018). I used three different PR schedules, all of which increased demand for each trial following a linear ramp (e.g., +4 responses each trial), whereas Karlsson et al. (2018) used a single PR schedule that increased the demand of each trial following a non-linear ramp (e.g., 1, 2, 4, 6, 9, 12, 15, 20, etc.). The PR schedules used in the present study, therefore, were much more difficult as the demand increases much more rapidly, which may also lead to the differences seen across this study and Karlsson et al. (2018).

As a final consideration, the difference between TK+/VGCV and TK-/VGCV mice with weak reward reported by Karlsson et al. (2018), though significant, is rather small. The TK+/VGCV mice did indeed complete less trials than TK-/VGCV mice, but the difference between the trials completed for each group is less than five. Therefore, taking together the results of my thesis and those of Karlsson et al. (2018), one conclusion that can be safely drawn is that neurogenesis knockdown may cause some alterations in motivation, but these changes can be bidirectional and occur only under certain specific conditions. Overall, it seems there is not strong evidence for a role of AHN on motivation in a non-stressful environment.

4.3 Extinction Learning

There was no evidence of an effect of neurogenesis knockdown on extinction learning, all groups extinguishing responses to the stimulus at similar rates (i.e., no group differences in the extinction curve data). Additionally, there were no group differences in the number of days required to reach criterion. Therefore, the ability to cease responding to a stimulus that was previously rewarded is not affected by neurogenesis knockdown.

The lack of group differences found on the extinction learning test is not due to the test being insensitive to detect such differences if they exist. For example, the metabotropic glutamate receptor 5 knockout mouse model of schizophrenia was shown to be impaired on the extinction learning test, indicated by fewer omissions made (i.e., higher degree of response perseveration) when compared to controls (Zeleznikow-Johnston et al., 2018; Lim et al., 2019). Therefore, the test was sensitive enough to observe a higher propensity for response perseveration in a mouse model of schizophrenia.

4.4 Negative Valence Systems Tests

Much like the the touchscreen tests, there was little convincing evidence for an effect of neurogenesis knockdown on the classic rodent behavioural tests of negative valence systems, specifically the light/dark box and EPM. Behaviour on the light/dark box seemed to be primarily influenced by VGCV treatment itself and its interaction with genotype and sex. VGCV-treated mice spent less time in and made less entries into the light chamber when compared to control-fed mice, indicating a non-specific effect of VGCV treatment. Further, TK+/VGCV females spent less time in the light chamber than TK+/ctrl females, and TK-/VGCV males spent less time in the light chamber than TK-/ctrl males. Alone, these data may suggest that neurogenesis knockdown induces an anxiety-like behavioural phenotype for females, and that the non-specific effects of VGCV treatment may be exacerbated in males. However, these effects are specific to the measure of time spent in the light chamber and are not replicated in the other measures. Specifically, the measure of latency showed contradictory evidence, with VGCV-treated males entering the light chamber more quickly than control-fed males whereas there was no difference between treatment conditions in females. Here, VGCV treatment seemed to induce an anxiolytic

effect for males, whereas VGCV treatment seemed to induce an anxiogenic effect for time spent in and entries into the light chamber. The sex-specific difference between treatment groups is, however, in agreement with the implication that males may be more susceptible to non-specific treatment effects on the measure of time spent in the light chamber. As a whole, the light/dark box data remain inconclusive due to the possible confounding non-specific effects of VGCV treatment and the potential that these non-specific effects interact within each sex differently.

The results from the EPM were much less complex. On this test, there were no group differences on any measure analyzed, specifically entries made into the open arms, time spent in the closed arms, and the d2 ratio. All groups exhibited behaviour consistent with a general preference for the closed arms over the open arms. Therefore, neurogenesis knockdown did not induce an anxiety-like behavioural phenotype when tested on the EPM.

The conclusion that neurogenesis knockdown does not alter behaviour on these tests contradicts previous studies that found reduced anxiety-like behaviour on the EPM in a different mouse model of neurogenesis ablation (Tsai, Tsai, Arnold, & Huang, 2015), as well as previous studies indicating increased anxiety-like behaviour on these tests (Mateus-Pinheiro et al., 2013; Revest et al., 2009), but is in agreement with Snyder et al.'s (2011) finding that neurogenesis knockdown in GFAP-TK mice did not impact time spent in the open arms of the EPM when not under a chronic stress paradigm. Therefore, the variable findings in my thesis and in the literature as a whole from these two putative tests of anxiety-like behaviour means that a definitive conclusion about the regulating role of AHN in anxiety-like behaviour cannot yet be drawn.

4.5 Sex Differences

Effects of sex recurred across many of the tests in my thesis. Females, in multiple phases

and reward conditions of PR, often made more blank touches than males, suggesting females have a higher baseline of activity than males or potentially have a higher tendency for hyperactivity than males. Higher activity levels in females have been reported previously (e.g., Konhilas et al., 2015).

In the PR data, the only positive results (i.e., an effect of neurogenesis knockdown was observed) often occurred differentially across sexes. For instance, with mice that underwent Timeline A (i.e., descending concentration exposure), TK+/VGCV males completed more trials than TK+/VGCV females on uncapped FR-5 with 50% milkshake, and TK+/VGCV males achieved higher breakpoints than TK+/ctrl and TK-/VGCV males on high demand PR with the second exposure of 100% milkshake; with mice that underwent Timeline B (i.e., ascending concentration exposure), VGCV-treated females achieved lower breakpoints than control-fed females on high demand PR with 100% milkshake, and males completed more trials on uncapped FR-5 than females with 100% and the second exposure of 20% milkshakes. The impairment in motivation, as well as lower responding in uncapped FR-5, seen in females on Timeline B may suggest that females are more likely to exhibit a negative affect with neurogenesis knockdown. Females being more prone to decreased motivation with neurogenesis knockdown is not entirely unlikely given depressive disorders are more common in human females than males (WHO, 2017, 2018). However, the conclusions about these differences must be tempered due to the lack of TK- control groups in the Timeline B experiments. Furthermore, there was some evidence for the opposite effect: In Timeline A of PR, for example, males with neurogenesis knockdown often exhibited some evidence of higher responding, whereas there were no group differences for females.

There was also evidence for a potential sex effect of VGCV treatment itself, where VGCV may affect each sex differently. For instance, on the light/dark box, VGCV-treated males exhibited lower latencies than control-fed males, whereas females did not exhibit this difference across treatment groups. Sex differences have been found in the pharmacokinetics of VGCV; in other words, sex differences have been found in how the body affects VGCV once administered. Specifically, in humans, females showed higher clearance levels than males, and a lower central volume of distribution (i.e., the female body eliminates VGCV quicker than the male body and the blood concentration of VGCV is lower in females than males [Perrottet et al., 2009]). The higher clearance in females, if translatable to the mouse, may explain the sex/VGCV interactions seen in the light/dark box. If females excrete VGCV faster than males, there may be less chance for VGCV to have non-specific effects in females.

4.6 Limitations

My thesis project has three main limitations, namely the lack of strong stressors, only a single immunofluorescent marker for neurogenesis, and the presence of non-specific effects of VGCV treatment. No strong stressors or chronic stress paradigms were employed because the primary goal behind my thesis was to determine the effect of neurogenesis knockdown on positive valence systems and reward-related behaviour without the presence of aversive stimuli. However, given that it has been found previously that a depression- and anxiety-like behavioural phenotype occurs only when under conditions of chronic stress (Hill et al., 2015 [see also Lehmann, Brachman, Martinowich, Schloesser, & Herkenham, 2013; Surget et al., 2011]) and that neurogenesis regulates the stress response (Anacker et al., 2018; Snyder et al., 2011; Tse et al., 2014), the present study would have benefitted from a stress condition. It would have been

useful, for example, to compare behaviour between mice under a chronic stress paradigm and non-stressed mice on the reward-related depression-relevant tests used.

As previously mentioned, the nestin-TK neurogenesis knockdown model was verified by the Ki-67 data, with TK+/VGCV mice showing fewer Ki-67-labelled cells than controls in both the dorsal and the ventral DG. However, additional markers of neurogenesis would increase the confidence in this knockdown verification. Doublecortin (DCX) labelling, a marker of immature progenitor cells (as opposed to Ki-67 marking the presence of cell proliferation), was also completed, but the antibodies did not bind properly to the tissue; it was not possible to differentiate between labelled cells and background. The challenges with the DCX immunofluorescence may be due to some of the non-specific effects of VGCV treatment on physiology.

The non-specific effects of VGCV treatment observed throughout the results confounds any neurogenesis knockdown effect seen, making it difficult to draw meaningful conclusions and have not been reported in literature using TK mice and VGCV treatment. However, the effect of VGCV on behaviour may be an important finding in itself. Previous studies using the TK neurogenesis model often do not use control-fed TK- mice, opting to only use and/or report VGCV-treated TK+ and TK- mice (Karlsson et al., 2018; Lehmann et al., 2013; Schloesser, Lehmann, Martinowich, Manji, & Herkenham, 2010; Snyder et al., 2011), so non-specific effects may indeed be present but are unnoticed/undetectable. There may be many reasons behind the non-specific treatment effects seen in the present study. One may be the length of time the animals were on VGCV treatment: Mice began VGCV treatment in adulthood (i.e., ≥60 days old) and continued until the end of behavioural testing. This meant that mice were on VGCV

treatment upwards of eight months, the longest of which being 16 months (64 weeks). No other study using the TK transgenic strains have reported using mice on VGCV treatment for that long, with a range of 3-19 weeks (Karlsson et al., 2018; Lehmann et al., 2013; Schloesser et al., 2010; Snyder et al., 2011). There may be detrimental physiological complications of such long-term VGCV treatment. Although not detailed in this thesis, a small subset of VGCV-treated mice exhibited extreme enlargement and necrosis of the liver, as well as the spleen in one known case (n = 3 males and 3 females, 3 of which died as a result), strongly suggesting that VGCV treatment disrupts normal physiological functioning with extended exposure. Anecdotally, it was also noticed during perfusions and tissue collection that the blood of the mice was abnormal, irrespective of treatment or genotype (though the effect may have been exacerbated for TK+ mice). The blood was much darker than usually seen in other mouse strains, appearing a deep burgundy colour, and was much more prone to coagulation. These irregularities seen in the blood may be attributed to, or exacerbated by, VGCV treatment as GCV treatment has previously been shown to have haematological toxicity in mice, specifically reducing the number of hemoglobin (i.e., red blood cells), neutrophils, and platelets (Boujemla et al., 2016). The irregular properties of the blood may have impacted the antibody binding during immunofluorescent labelling and perhaps may explain the lack of success with the DCX labelling previously mentioned.

4.7 Future Directions

There are five primary future directions for the present study, two of which are already underway. First, as AHN has been shown to regulate the stress response (Anacker et al., 2018; Snyder et al., 2011; Tse et al., 2014), running a stress test and assessing serum corticosterone (CORT) levels seems like an obvious next step and is currently underway. As per Rodgers et al.

(1999), mice are being run on the EPM (though conceivably any test of negative valence systems could be used) and, 20-30 min after EPM test onset, blood samples are being taken to assess serum CORT levels. It is predicted CORT levels will be higher in mice with neurogenesis knockdown.

Second, further immunofluorescent assessment of neurogenesis would be beneficial, such as bromodeoxyuridine (BrdU) labelling and co-labelling of nestin and GFP. BrdU is a uridine analogue and is incorporated into the DNA of mitotic cells at the time of administration, thus BrdU can be used as a marker of cell survival (reviewed in Kee et al., 2002). In a mouse with neurogenesis knockdown, there would be less BrdU-labelled cells when compared to controls because there would be fewer mitotic cells present at any given time. Nestin/GFP co-labelling would provide a further molecular verification of neurogenesis knockdown, with the added benefit of verifying genotype. The TK transgene was tagged with enhanced GFP (eGFP), therefore TK+ mice would show nestin cells co-labelled with GFP whereas TK- mice would not. Further, TK+/VGCV mice would show fewer nestin/GFP co-labelled cells than TK+/ctrl mice due to neurogenesis knockdown. Imaging and cellular count assessment of these two immunofluorescent assays is currently ongoing.

Third, assessing these mice on other depression-relevant reward-related tests, or modifications of such tests used in my thesis, would be beneficial. For instance, PRL was run without a punishment condition. Given that a punishment condition would increase the aversiveness of the test, it would be interesting to determine if replacing the non-reward outcome with a punishment outcome (e.g., bright light) would impact behaviour, in line with the idea that ABNs are important for affect-related behaviour under more aversive, stressful conditions (Hill

et al., 2015; Lehmann et al., 2013; Surget et al., 2011; Wu & Hen, 2014). Additionally, other tests that may be run are those assessing for cognitive/judgement bias (e.g., the go/no-go test paradigm) and reward valuation over time (e.g., delay discounting). Humans with depression have shown a negative emotional bias which manifests cognitively as attentional biases toward negative stimuli (Erickson et al., 2005; Smith et al., 2016). In general, patients with depression, both medicated and non-medicated, did not show signs of generalized cognitive impairment/bias, but attentional biases and impairments manifested only when affective/valenced stimuli were used. In order to maintain the focus on positive valence systems and depression-relevant reward-related behaviour, such biases could be assessed in animals using reward-based stimuli, as seen in the cross-species trial initiation go/no-go test (Hintze et al., 2018). In this test, effective for mice, rats, and horses, there are five spatial locations and animals must learn that one extreme flanker location is positive (i.e., rewarded) and must be responded to, whereas the other is negative (i.e., not rewarded) and must not be responded to and a new trial must be initiated. The three middle locations were ambiguous, so whether a response was made (i.e., "go") or a new trial initiated (i.e., "no-go") was indicative of judgement bias; animals, in general, were found to make graded judgments across all five locations, with more no-go responses made for the negative location and incrementally decreased as locations neared the positive location. This test could be easily modified for use in the mouse touchscreen operant chambers, where the five-hole mask used in PR and extinction learning could be used. The two extreme flanker holes would be deterministic (i.e., one coded as always not rewarded and the other as always rewarded), with the three middle holes coded incrementally (i.e., the one adjacent to the negative location would be not rewarded 75% of the time and rewarded the other 25%, the middle location would be 50/50

not rewarded and rewarded, and the location adjacent to the positive location would be 75% rewarded and 25% not rewarded). This test would be similar to PRL in its probabilistic outcomes to responses, but may provide different results due to the increased options available and greater number of probabilistic/ambiguous feedback conditions.

Another potential test to use would be delay discounting. Delay discounting has already been modified into an established rodent touchscreen operant task (DD [e.g., Abela & Chudasama, 2014]) and is depression-relevant. Humans with depression were more likely to subjectively de-value large rewards with increased delay, preferring short-term rewards, and the degree of reward devaluation positively correlated with severity of hopelessness on Beck's Depression Inventory (Pulcu et al., 2014). The fact that choices made on a delay discounting test correlated with severity of hopelessness for patients with depression may suggest that the rodent touchscreen equivalent may be able to give similar behavioural results to the highly aversive FST or tail suspension test, which are classic tests of learned helplessness and inescapable stress in rodents (reviewed in Bai, Li, Clay, Lindstrom, & Skolnick, 2001).

Fourth, the addition of a stress condition or an antidepressant condition would be beneficial. The addition of a stress condition was mentioned above (see "Limitations," subsection 4.6). Potential stress paradigms that have been shown to be neurogenesis-relevant are unpredictable chronic mild stress (Culig et al., 2017; Surget et al., 2011), chronic social defeat (Anacker et al., 2018; Tse et al., 2014), restraint stress (Snyder et al., 2011), and repeated injections of CORT (Hill et al., 2015). Replicating the present the study with the addition of a chronic stress paradigm may further the understanding of the regulatory role AHN plays on the stress response and how they may interact to influence behaviour in reward-related depression-

relevant tests. Additionally, antidepressant treatment has been shown to enhance neurogenesis (Boldrini et al., 2009; David et al., 2009; Popova et al., 2017; Wu et al., 2014) and rescue depression- and anxiety-like behavioural phenotypes when subjects were under a chronic stress paradigm (David et al., 2009; Rygula et al., 2008; Strekalova, Gorenkova, Schunk, Dolgov, & Bartsch, 2006). Therefore, it would be interesting to replicate the present study in wild-type mice under chronic antidepressant treatment to determine if enhancements and behavioural rescue could be seen.

Lastly, using another model of neurogenesis knockdown would allow for behavioural and molecular assessment free of non-specific VGCV effects on behaviour and physiology. Models available include other transgenic strains (e.g., tamoxifen-inducible *Tbr2* gene knockout mouse, which reduces neurogenesis in the DG but not other neurogenic areas [Tsai et al., 2015]), chemical suppression of DG proliferation (e.g., four-cycle treatment with DNA-alkylating agent temozolomide [Garthe, Behr, & Kempermann, 2009; Niibori et al., 2012]), Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) models (e.g., inhibitory DREADD in *i-hM4Di* mice in adult-born granule cells of the ventral DG silences the activity of these cells following clozapine-N-oxide administration [Anacker et al., 2018]), and optogenetic manipulation (e.g., photo-inhibition of DG ABNs impaired location discrimination on the difficult separation in touchscreens, replicating the finding that neurogenesis is required for pattern separation [Zhuo et al., 2016]; similar effects were found when GABAergic afferents to DG parvalbumin interneurons were photo-activated and further found that the resulting decrease in neural stem cell activation occurred to a greater degree in the ventral than dorsal DG [Bao et al., 2017]). Some of these alternative neurogenesis knockdown models may be preferred over the

nestin-TK model due to the increased regional specificity, as is the case in the *Tbr2* knockout mouse used by Tsai et al. (2015), the DREADD model used by Anacker et al. (2018), and the optogenetic model used by Bao et al. (2017), not to mention the benefit of neurogenesis knockdown not dependent on long-term VGCV treatment that any of these alternatives offer.

4.8 General Conclusions

Across all tests, there was no strong evidence for a role of AHN in depression-relevant reward-related behaviours. Even when different reward strengths were used, as in Karlsson et al. (2018), the effects observed were highly condition- and context-specific, suggesting that the effect of neurogenesis knockdown on depression-relevant reward-related behaviour under non-stressful conditions is nowhere as strong and replicable as the effect of neurogenesis on, for instance, pattern separation, a finding again replicated in my thesis.

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Appendices

Appendix A: Supplemental Results Figures

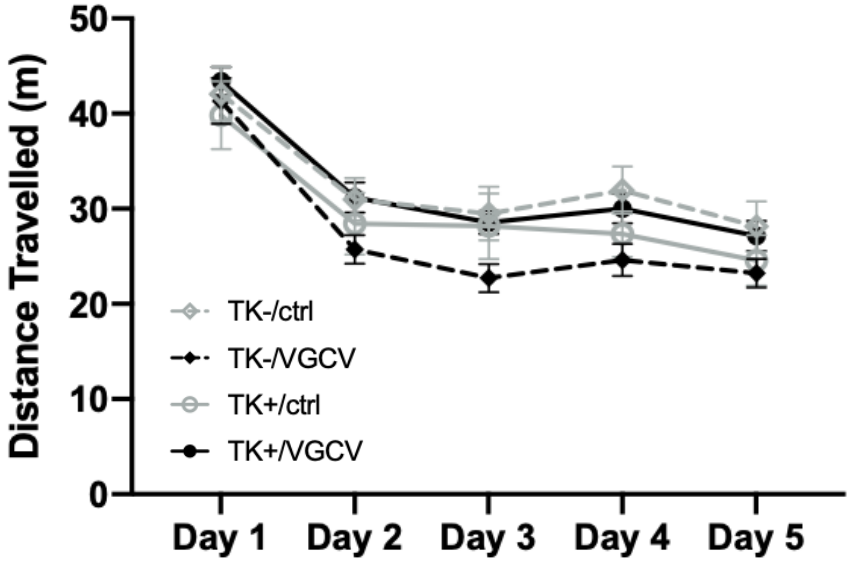

Figure A1. Distance travelled during the spontaneous location recognition (SLR) habituation phase. There was no difference between groups, indicating neurogenesis knockdown did not impact general motor ability or baseline activity levels.

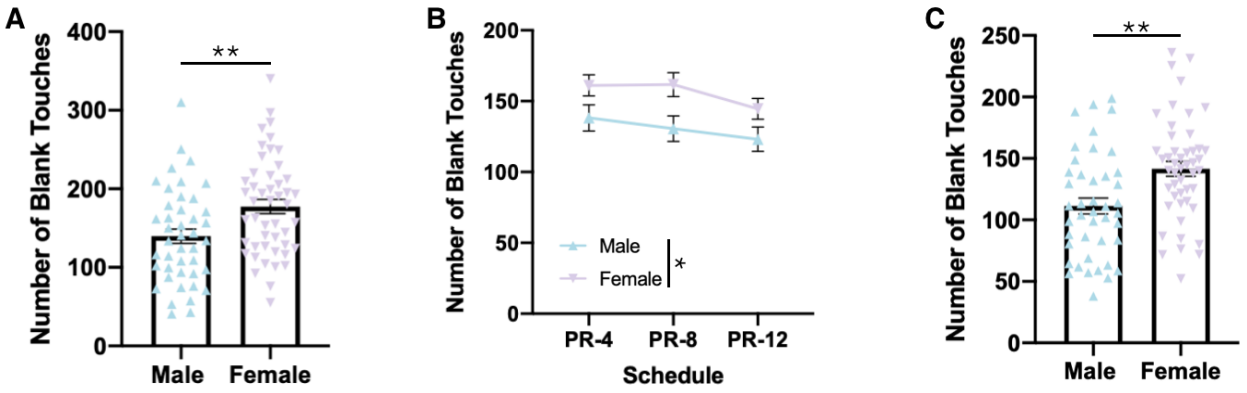

Figure A2. Sex differences in number of blank touches made in the overall progressive ratio (PR) analysis, when averaged across all milkshake conditions. Females made more blank touches during (A) basic PR-4, (B) high demand PR, and (C) uncapped FR-5. Females may have a higher level of baseline activity or an increased tendency for hyperactive behaviour and/or non-specific responding than do males. *$p < .05$, **$p < .01$

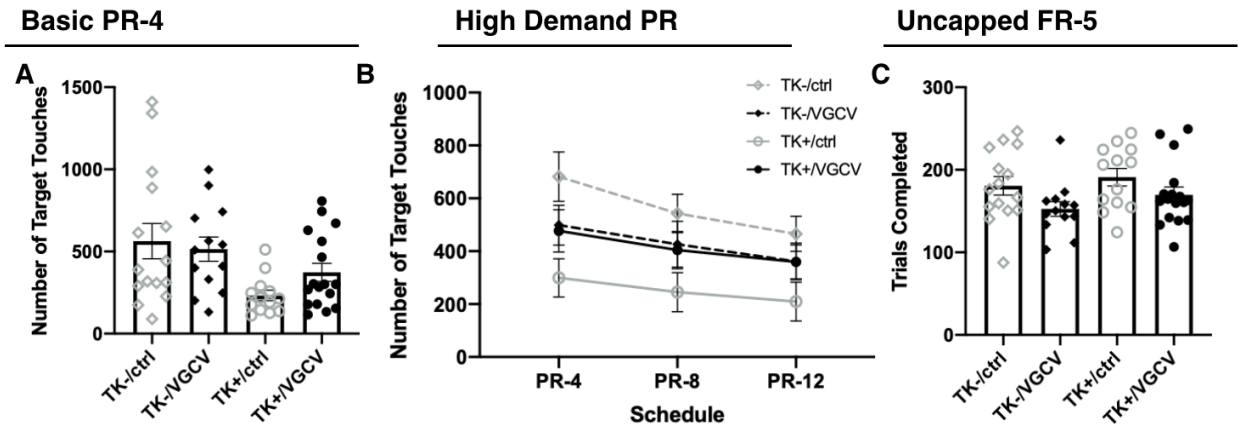

Figure A3. Progressive ratio (PR) performance with the first exposure of 100% milkshake for mice that experienced the descending order of reward concentration, or Timeline A. (A) During basic PR-4, TK+/VGCV mice did not differ from any control group on target touches made. (B) During high demand PR, TK+/VGCV mice did not differ from any control group on number of target touches. However, TK-/ctrl mice made more target touches than TK+/ctrl mice. Mice generally made less target touches as demand increased. (C) During uncapped FR-5, there were no differences between groups on the number of trials completed.

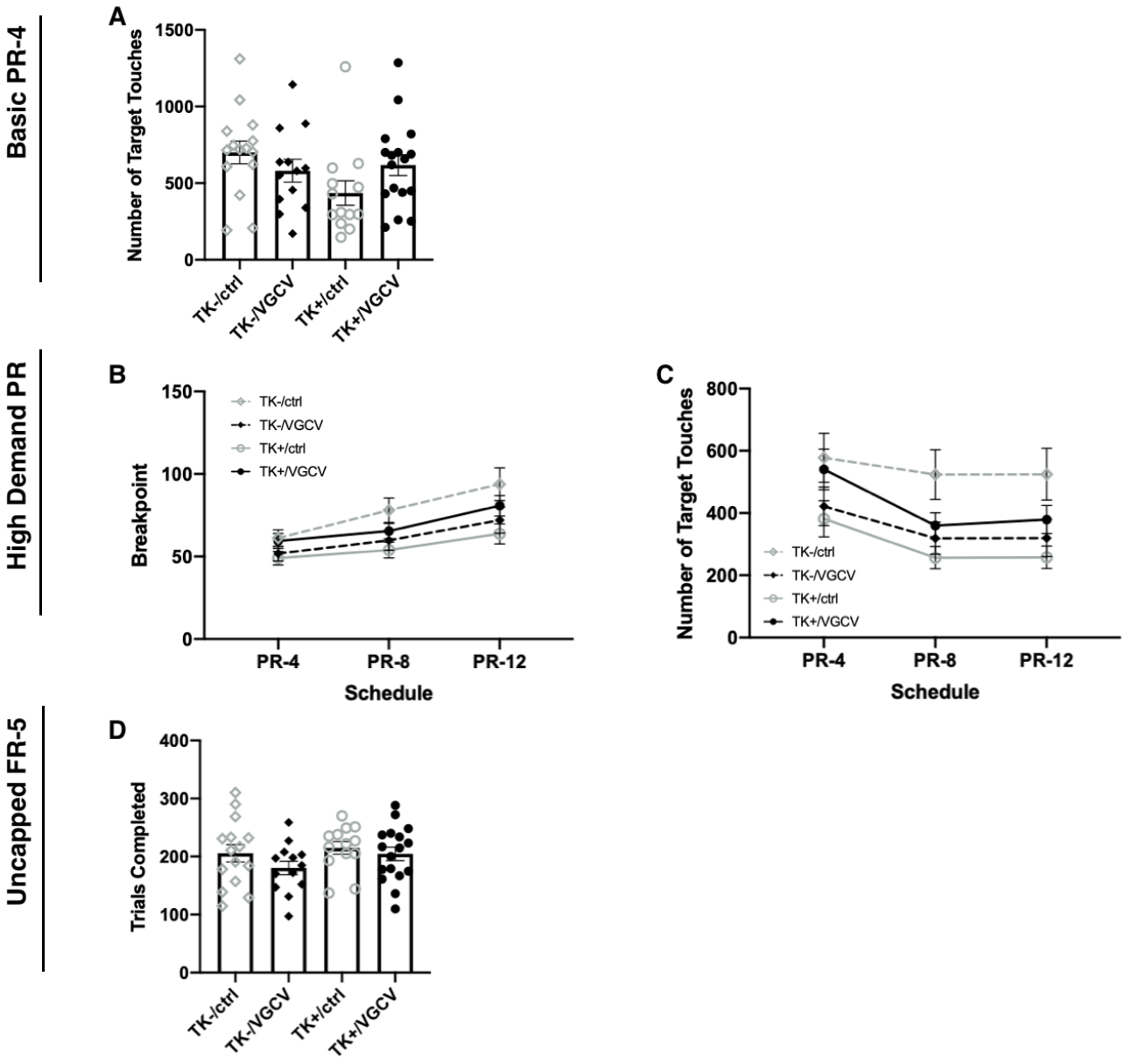

Figure A4. Progressive ratio (PR) performance with 50% milkshake for mice that experienced the descending order of reward concentration, or Timeline A. (A) During basic PR-4, there were no differences between the TK+/VGCV group and any control group on target touches made. However, TK-/ctrl mice made more target touches than TK+/ctrl mice, suggesting a non-specific effect of genotype. During high demand PR, the TK+/VGCV group did not differ from any control group on (B) breakpoints achieved and (C) number of target touches made. However, TK-/ctrl mice achieved higher breakpoints and made more target touches than TK+/ctrl and TK-/VGCV mice, suggesting non-specific effects of both genotype and treatment. In general, there

were higher breakpoints and fewer target touches with increasing demand. (D) During uncapped FR-5, there were no differences seen between groups.

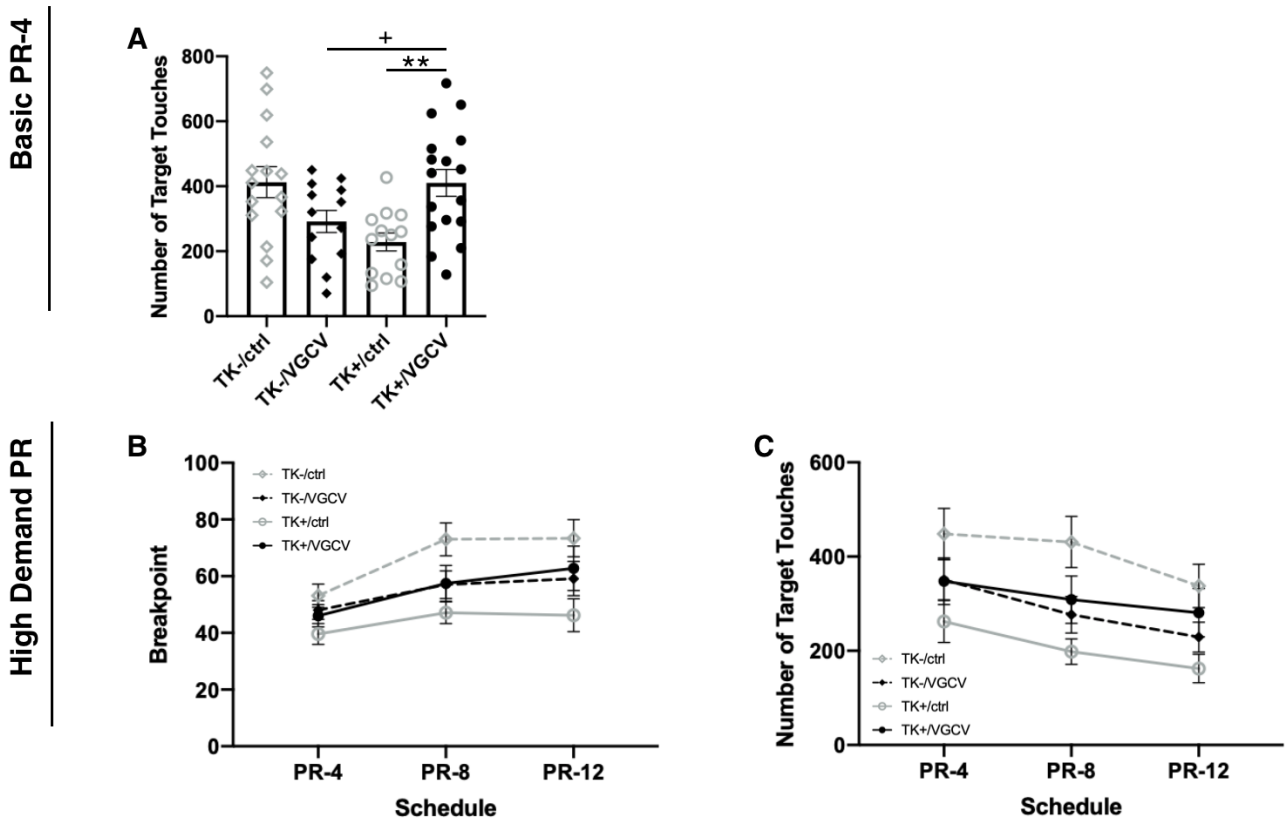

Figure A5. Progressive ratio (PR) performance with 20% milkshake for mice that experienced the descending order of reward concentration, or Timeline A. (A) During basic PR-4, TK+/VGCV mice made more target touches than TK+/ctrl mice and trended toward making more target touches than TK-/VGCV mice. However, TK-/ctrl mice made more target touches than TK+/ctrl mice and TK-/VGCV mice, suggesting the presence of non-specific effects of genotype and treatment. During high demand PR, there were no differences between the TK+/VGCV group and any control group on (B) breakpoints achieved and (C) target touches made. However, TK-/ctrl mice achieved higher breakpoints and made more target touches than TK+/ctrl mice, suggesting genotype itself affects behaviour. In general, higher breakpoints were achieved and fewer target touches were made with increasing demand. $^{+}p < .065$, $^{**}p < .01$

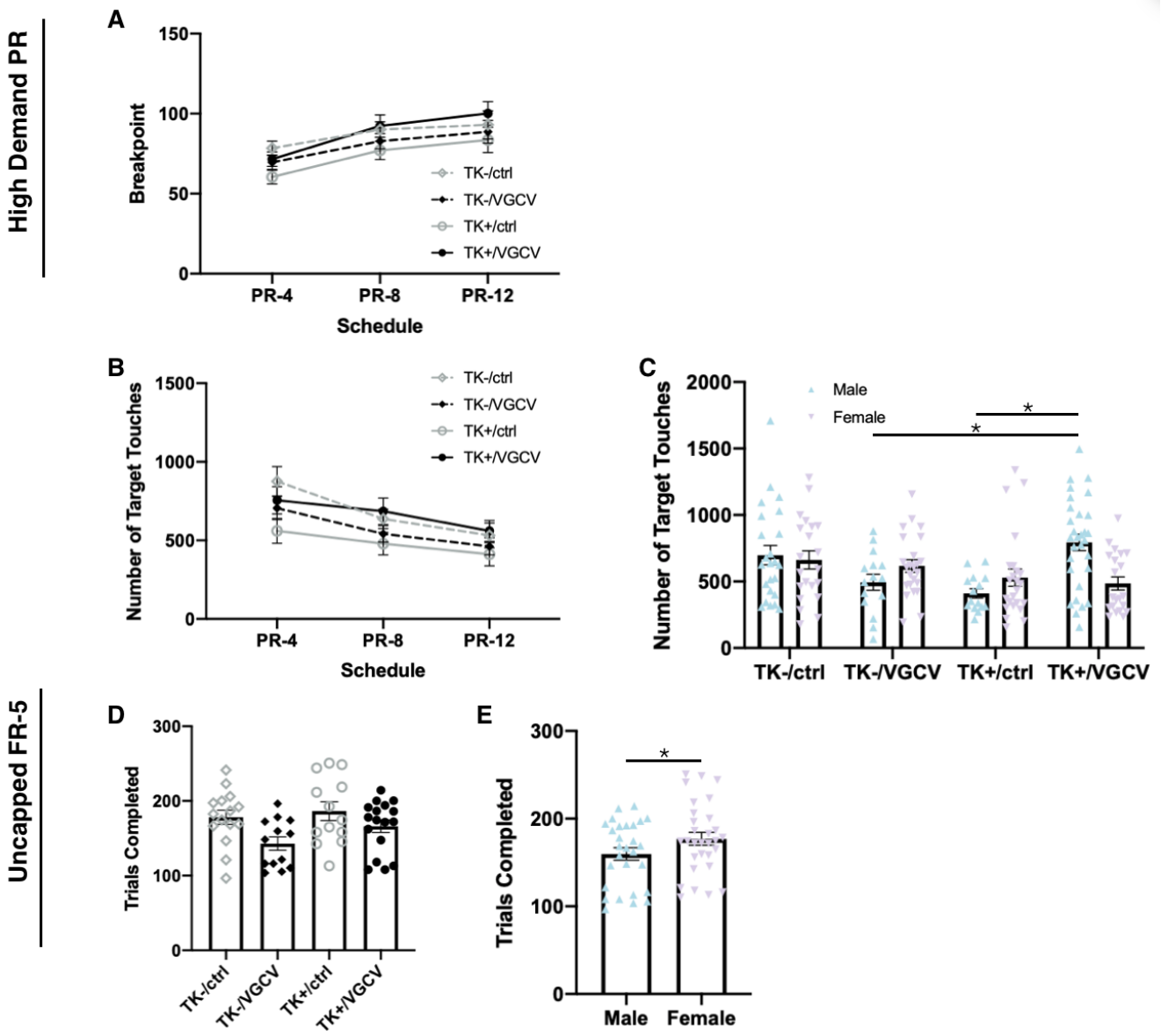

Figure A6. Progressive ratio (PR) performance with the second exposure of 100% milkshake for mice that experienced the descending order of reward concentration, or Timeline A. (A) During high demand PR, there were no overall differences between the TK+/VGCV group and any control group on (A) breakpoints achieved and (B) number of target touches made. In general, higher breakpoints were achieved and fewer target touches were made with increasing demand. (C) During high demand PR, TK+/VGCV males made more target touches than TK+/ctrl and TK-/VGCV males. Additionally TK+/VGCV males made more target touches than TK+/VGCV females. These data suggest neurogenesis knockdown and/or treatment affects behaviour

differently for each sex. (D) During uncapped FR-5, there were no group differences in the number of trials completed, but (E) females, in general, completed more trials than males. *$p < .05$

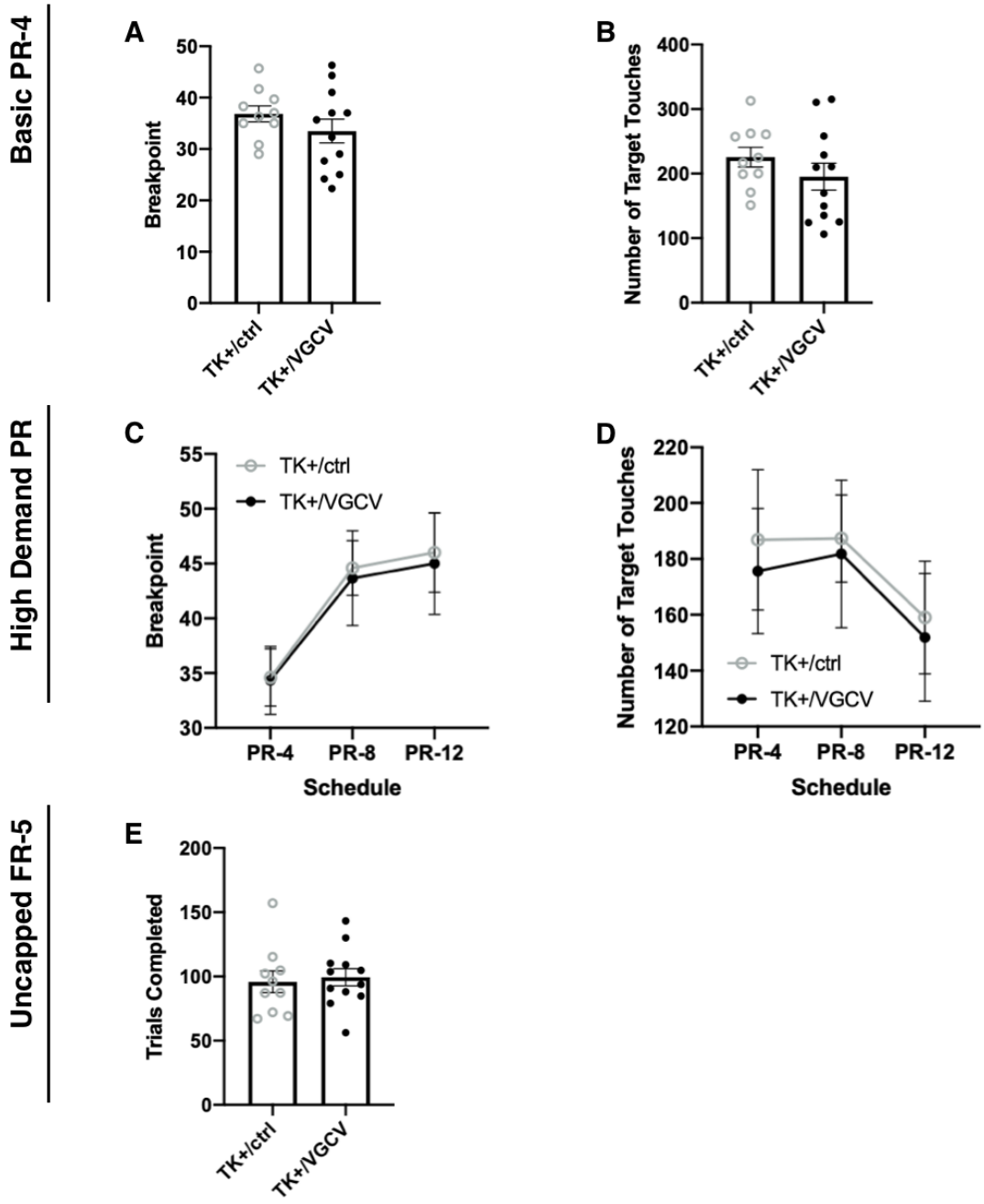

Figure A7. Progressive ratio (PR) performance with the first exposure of 20% milkshake for mice that experienced the ascending order of reward concentration, or Timeline B. During basic PR-4, there were no group differences on (A) breakpoints achieved and (B) target touches made. During high demand PR, there were no group differences on (C) breakpoints achieved and (D) target touches made. In general, higher breakpoints were achieved and fewer target touches were made with increasing demand. (E) During uncapped FR-5, there were no group differences on number of trials completed.

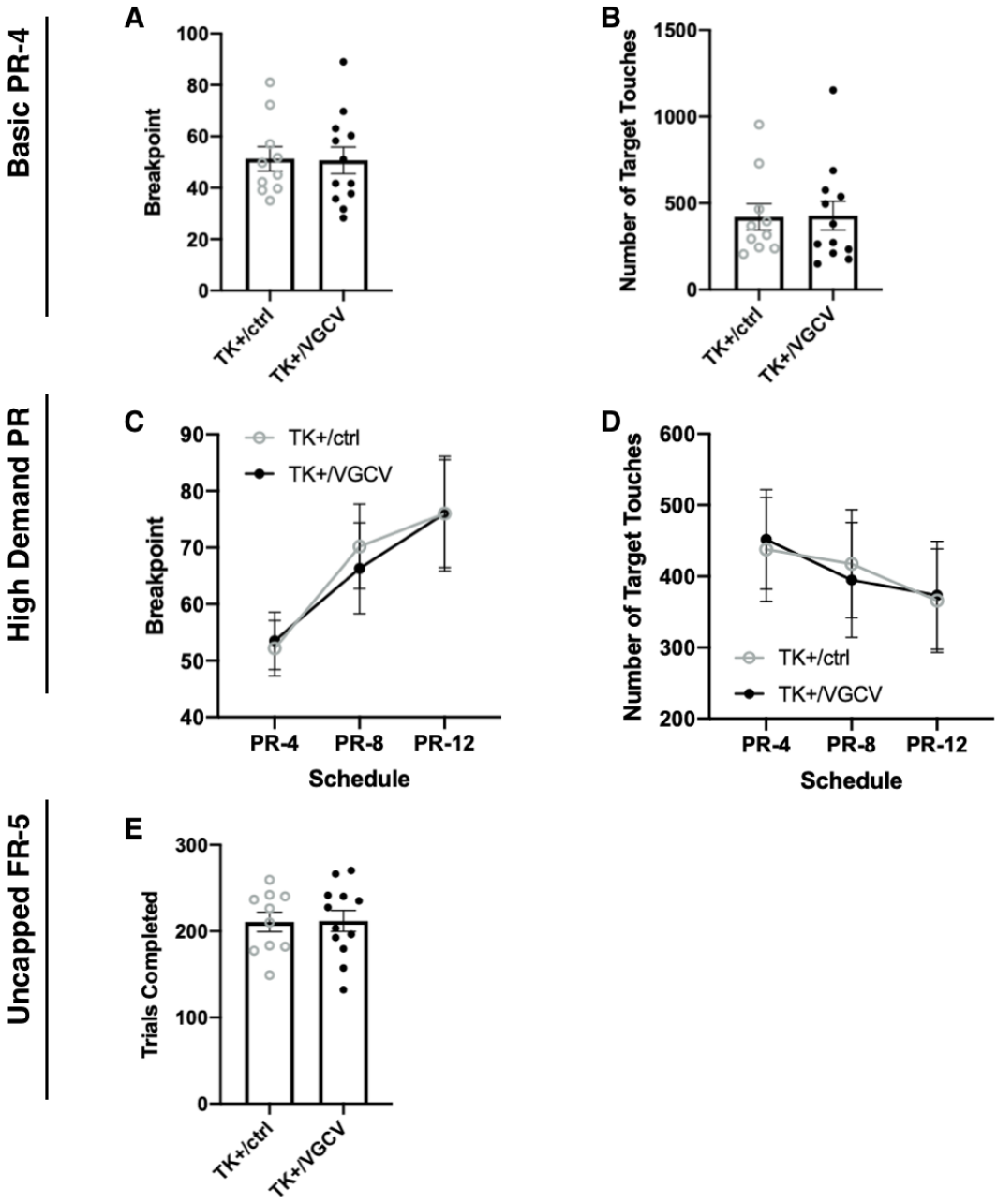

Figure A8. Progressive ratio (PR) performance with 50% milkshake for mice that experienced the ascending order of reward concentration, or Timeline B. During basic PR-4, there were no group differences on (A) breakpoints achieved and (B) target touches made. During high demand PR, there were no group differences on (C) breakpoints achieved and (D) target touches made. In general, higher breakpoints were achieved and fewer target touches were made with increasing demand. (E) During uncapped FR-5, there were no group differences on number of trials completed.

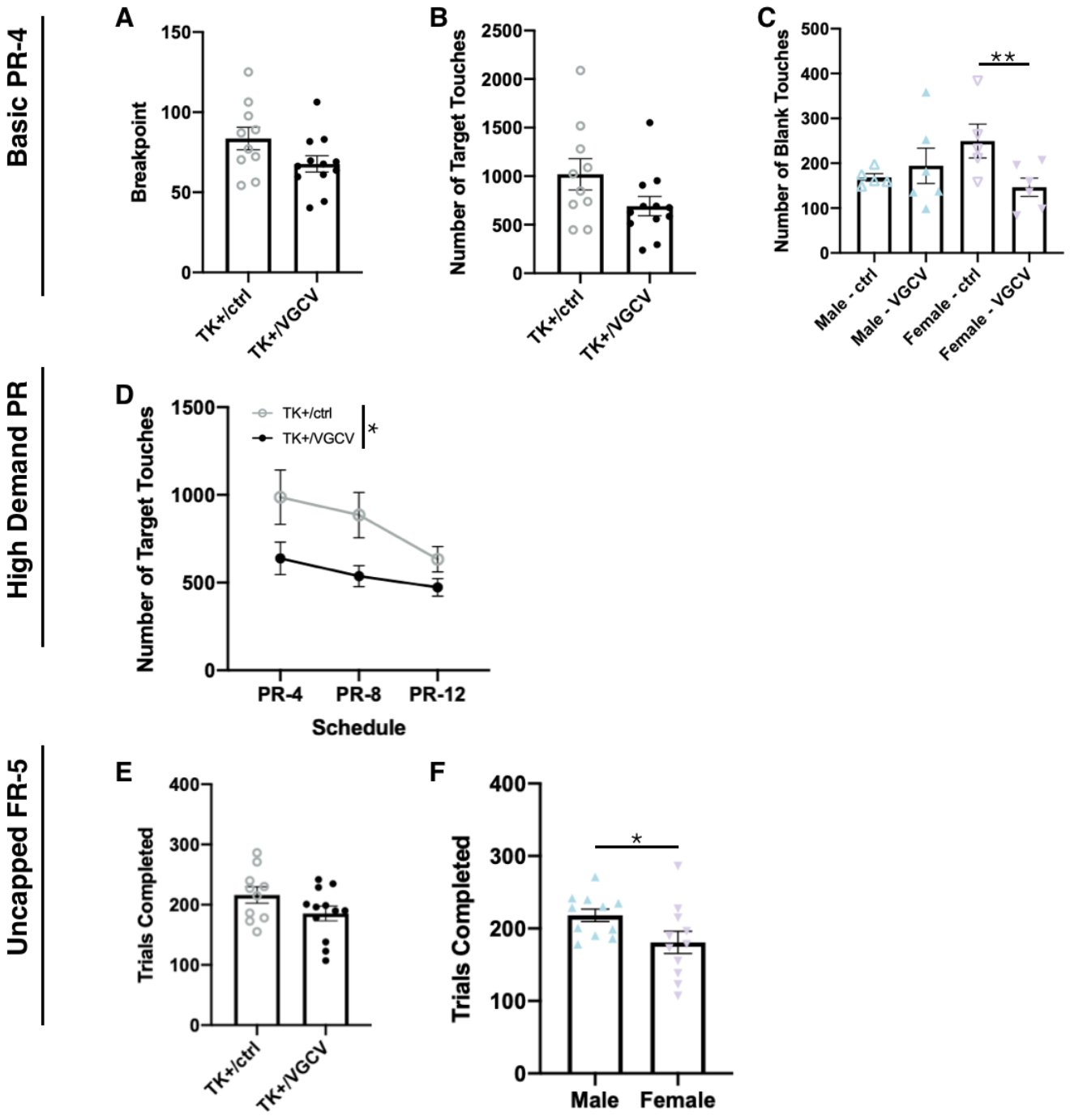

Figure A9. Progressive ratio (PR) performance with 100% milkshake for mice that experienced the ascending order of reward concentration, or Timeline B. During basic PR-4, there were no group differences on (A) breakpoints achieved and (B) number of target touches made. (C) During high demand PR, TK+/VGCV females made fewer blank touches than TK+/ctrl females, suggesting VGCV treatment suppressed the increased number of blank responses seen in other

PR conditions. (D) During high demand PR, TK+/VGCV mice made fewer target touches than TK+/ctrl mice, suggesting a possible suppression of responding to the stimulus with neurogenesis knockdown. In general, fewer target touches were made with increasing demand. (E) During uncapped FR-5, there were no group differences in the number of trials completed, but (F) males completed more trials than females. *$p < .05$, **$p < .01$

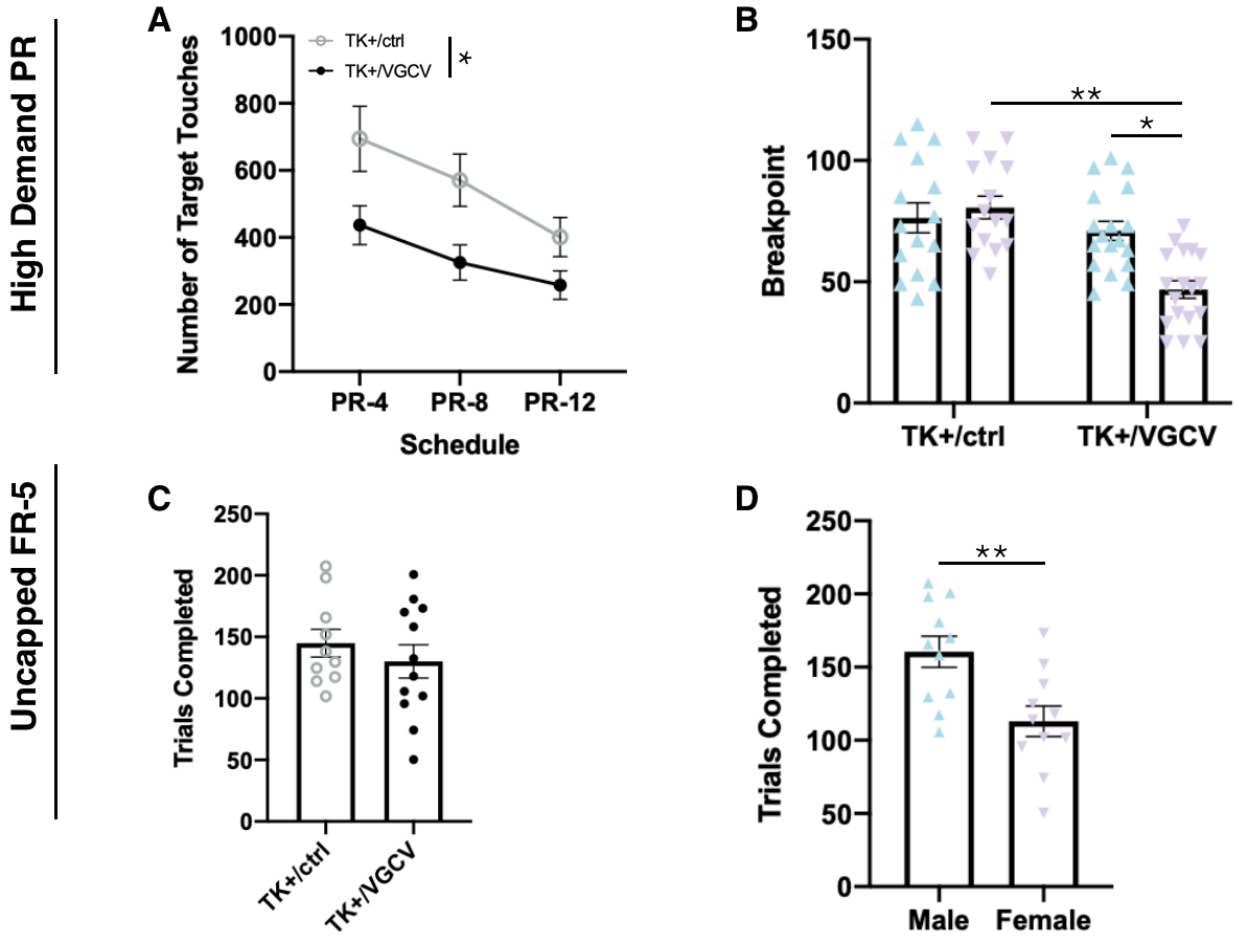

Figure A10. Progressive ratio (PR) performance with the second exposure of 20% milkshake for mice that experienced the ascending order of reward concentration, or Timeline B. During high demand PR, (A) TK+/VGCV mice made fewer target touches than TK+/ctrl mice, suggesting neurogenesis knockdown suppressed responding to the stimulus. In general, fewer target touches were made with increasing demand. (B) Though the interaction effect was only trending, TK+/VGCV females achieved lower breakpoints than TK+/ctrl females and TK+/VGCV males. There were no group differences seen in males. Neurogenesis knockdown may affect the behaviour for each sex differently. (C) During uncapped FR-5, there were no group differences on the number of trials completed, but (D) males completed more trials than females. $^{+}p < .065$, $*p < .05$, $**p < .01$

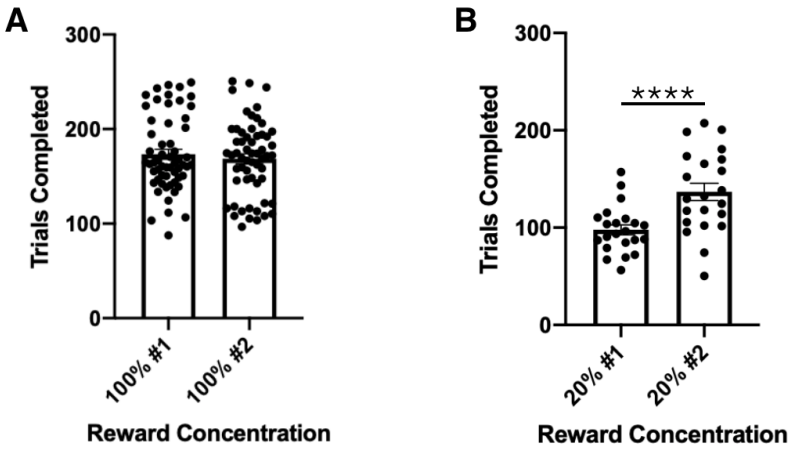

Figure A11. Determination of the presence of training effects in each milkshake concentration exposure timeline of the progressive ratio (PR) task. (A) On Timeline A, there were no differences in the number of trials completed between the first and second exposures of 100% milkshake during uncapped FR-5. (B) On Timeline B, more trials were completed with the second exposure of 20% milkshake than the first during uncapped FR-5, suggesting the presence of a training effect with repeated PR testing. ****$p < .0001$

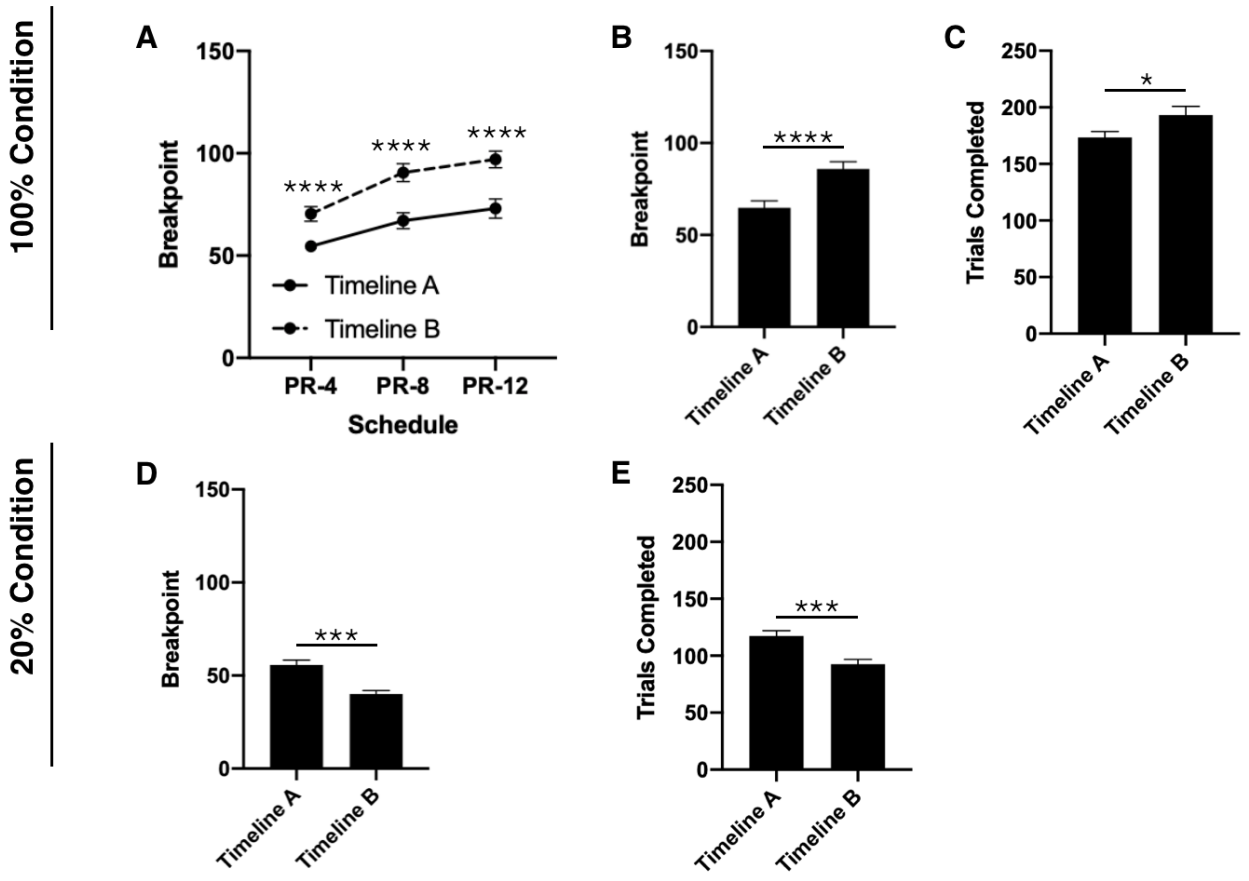

Figure A12. Comparisons between Timeline A and Timeline B on progressive ratio (PR) for 100% and 20% milkshake. For timeline comparisons with 100% milkshake, (A) Timeline B mice achieved higher breakpoints than Timeline A mice for each PR schedule and (B) in general during high demand PR, though this may be confounded by the training effect seen in Timeline B mice. (C) A similar effect was seen during uncapped FR-5, with Timeline B mice completing more trials than Timeline A mice. For timeline comparisons with 20% milkshake, (D) Timeline A mice, in general, achieved higher breakpoints than Timeline B mice during high demand PR. (E) A similar effect was seen during uncapped FR-5, with Timeline A mice completing more trials than Timeline B mice. Though, as Timeline A mice did not exhibit training effects, the difference between timelines with 20% milkshake may be a result of Timeline A mice having previously experienced stronger rewards. *$p < .05$, ***$p \leq .001$, ****$p < .0001$

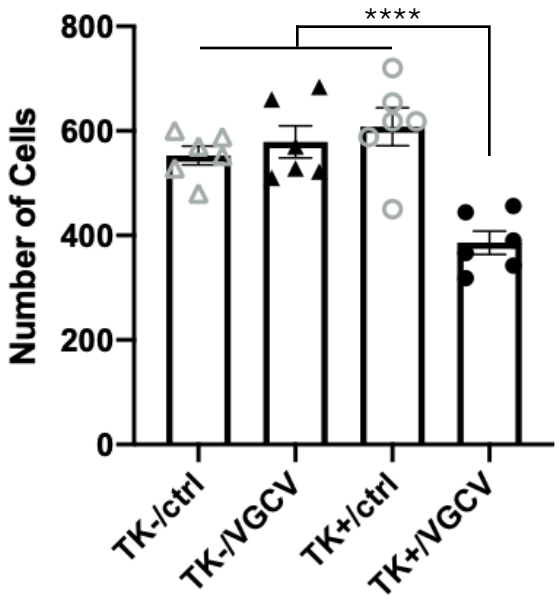

Figure A13. Number of Ki-67-labelled cells in the whole dentate gyrus for one hemisphere. TK+/VGCV mice had fewer Ki-67-labelled cells than all control groups, indicating decreased proliferating cells/neurons and verifying neurogenesis knockdown occurred. ****$p < .0001$

Appendix B: Reward Concentration Preference Test Data from Timeline B Mice

Due to the decreased breakpoint and target touches seen for the TK+/VGCV mice in the high demand phase of the 100% and the second 20% conditions in Timeline B PR data, the reward preference test data for these mice were separated out and analyzed separately. These additional analyses indicate neurogenesis knockdown effects for only the 100% vs. 50% choice. There were no group differences found on total amount of 100% milkshake consumed, but there was a difference on total amount of 50% consumed, $F(1,14) = 5.776$, $p = .031$, $\eta_p^2 = .292$, with TK+/VGCV mice drinking more 50% milkshake than TK+/ctrl mice (see Figure B1A). Additionally, there was an interaction between concentration and treatment nearing significance, $F(1,14) = 4.472$, $p = .053$, $\eta_p^2 = .239$; this near-significant effect was driven by TK+/ctrl mice showing an increased consumption of 100% milkshake in comparison to 50% milkshake ($p < .0001$), whereas TK+/VGCV mice did not exhibit a difference. These results are corroborated by the preference index data; TK+/VGCV mice showed a lower preference for 100% milkshake than TK+/ctrl mice, $F(1,14) = 4.830$, $p = .045$, $\eta_p^2 = .257$ (see Figure B1B). These data suggest that, for the mice that underwent Timeline B of the PR task, neurogenesis knockdown caused a reduced preference for the 100% milkshake over the 50% milkshake with time, as indicated by the increased consumption of 50% milkshake at test endpoint. The preference index data suggests that neurogenesis knockdown for these mice reduced the reward value of 100% milkshake, or increased the value of 50% milkshake. It may be that neurogenesis knockdown increased the tendency towards anhedonic-like behaviour with long-term exposure to reward; in other words, it could be that for this specific subset of mice, due to their previous experiences with reward on the PR task, neurogenesis knockdown may have lead to a reduction of reward

salience over time and/or an increased likelihood of anhedonic-like behaviour with increased exposure to reward. However, the reward preference test differences seen for these mice cannot be conclusively attributed to neurogenesis knockdown alone due to the lack of TK- control groups.

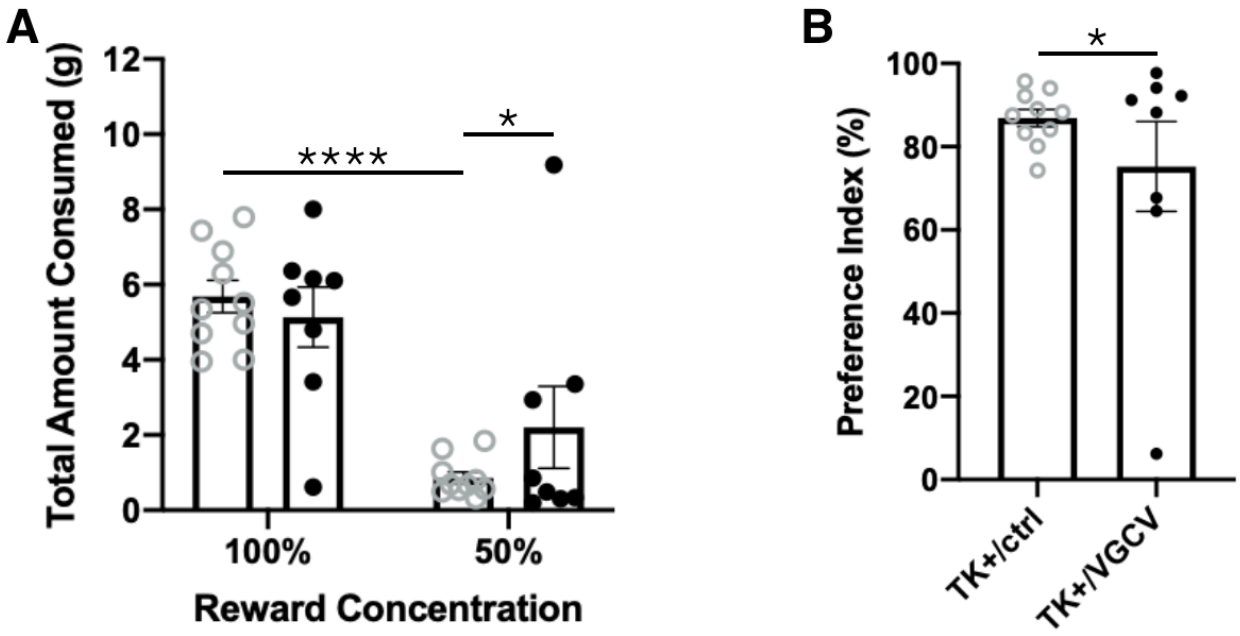

Figure B1. Results of the reward concentration preference test for mice that underwent Timeline B of milkshake concentration exposure during progressive ratio (PR) testing. (A) TK+/VGCV mice show an increased amount of 50% milkshake consumed when compared to TK+/ctrl mice. Additionally, only control mice drank more 100% milkshake than 50%; TK+/VGCV mice did not show this difference. (B) TK+/VGCV mice had a reduced preference for 100% milkshake when compared to TK+/ctrl mice. Together, these results suggest that the 100% milkshake was less rewarding for these mice with neurogenesis knockdown, or that 50% milkshake was more rewarding than 100%.

Curriculum Vitae

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Zmavc, K. D., Kramar, C. P., ... Saksida, L. M., Bussey, T. J. Neurogenesis in the Adult Hippocampus and Its Role in Mood. (varying iterations)

At:	Canadian Association for Neuroscience 13th Annual Meeting	2019
	Robarts Research Institute Research Retreat	2018, 2019
	London Health Research Day	2019, 2018
	Western Research Forum	2019
	Physiology and Pharmacology Research Day	2018
	Southern Ontario Neuroscience Association Annual Meeting	2018