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**BEHAVIORAL AND NEURAL MECHANISMS OF SEROTONIN MODULATION OF  
IMPULSIVITY AND REWARD**

A Thesis

Submitted to the Faculty  
in partial fulfillment of the requirements for the  
degree of

Doctor of Philosophy  
in  
Psychological and Brain Sciences

by Stephanie Sara Desrochers

Guarini School of Graduate and Advanced Studies  
Dartmouth College  
Hanover, New Hampshire

April 2023

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## ABSTRACT

Despite its prevalence in many psychiatric disorders, such as attention deficit hyperactivity disorder, suicidal depression, schizophrenia, and aggression and motivational disorders, impulsivity and its biological bases remain poorly understood. Subdivisions of impulsivity, including impulsive action (reduced response inhibition) and impulsive choice (reduced delay of gratification), sometimes present in an uncorrelated manner. This complexity renders pathological impulsivity difficult to treat, as different underlying causes likely result in different phenotypic presentations, despite being placed under one umbrella term. In order to study the behavior and biology of one particular facet of impulsivity, this dissertation utilizes the serotonin 1B receptor (5-HT<sub>1B</sub>R; an inhibitory G-protein coupled receptor) knockout mouse model, which presents with a specific elevation in impulsive action but not impulsive choice.

In Chapter 1, I show that mice lacking the 5-HT<sub>1B</sub>R have increased impulsive action accompanied by enhanced motivation and responsiveness to palatable rewards, indicating that they may have dysregulation of subjective reward valuation. In Chapter 2, I then explore the 5-HT<sub>1B</sub>R knockout model from the perspective of behavioral inhibition, demonstrating that knockout mice have intact inhibitory learning despite having difficulty withhold responding for reward. Of particular interest to this particular presentation of impulsive action, therefore, is serotonin neuromodulation of reward circuitry in the brain. In Chapter 3, I first show behaviorally that normalizing reward value in 5-HT<sub>1B</sub>R knockout mice reduces impulsive action to the level of controls. Neurally, I then complete a series of experiments with targeted knockouts in reward-related brain regions, specifically projections to and from the nucleus accumbens shell, in addition to combined 5-HT<sub>1B</sub>R genetic heteroreceptor and viral autoreceptor knockout. Only combined Emx1+ heteroreceptor and autoreceptor knockout results in increased motivation and impulsivity similar to the whole brain knockout. On the other hand, combined VGAT+ heteroreceptor and autoreceptor knockout increases hedonic taste reactivity. This suggests that modified serotonin release in addition to multiple 5-HT<sub>1B</sub> heteroreceptor population losses synergistically modulate neural signaling to increase reward valuation and impulsive action. Together, these studies provide insight into the behavioral and biological bases of impulsive action and propose a framework for better understanding specific presentations of impulsivity.

## ACKNOWLEDGMENTS

So many incredible people in my life have made it possible for me to reach to this point, and I am so grateful for all the kindness and support I have received.

First, I would like to thank Dr. Katherine Nautiyal from the bottom of my heart. Kate, you have been the best mentor any Ph.D. student could possibly ask for. I can't believe how lucky I am to have been your first student, and I am so excited to see all the work the lab continues to do in the future.

Next, I would like to thank my committee, including Dr. Kyle Smith, Dr. Matthijs van der Meer, and Dr. Peter Balsam, as well as the rest of the incredible B4 group (with special thanks to Dr. Robert Leaton, Dr. Travis Todd, and Dr. Dave Bucci). I am so appreciative of all the advice, guidance, and feedback you have provided me throughout the years. This dissertation is all the better because of you.

The Nautiyal Lab has also been an amazing group of people to be part of, and I have loved all of our science together. I'm especially thankful to have been a grad student alongside Arati and Six. You are both fantastic scientists and some of the best people I know! Also thank you to Mitch for helping me troubleshoot and fix so many things, I would be so far behind without you!

I would like to thank all of my friends from grad school and beyond. There are too many to name, but to highlight a few, Mary and Megan for Star Wars nights, the Void for game nights and all the nostalgia, Mirco for being my Trekkie buddy, and Kara for being my rock and forever best friend. You have kept me social and engaged when I've needed it the most.

Finally, to my family, including a plethora of Russo cousins, aunts, and uncles, Buddy, my siblings, Gabby and Sean, and especially my Mum and Dad. Thank you so for making it possible for me to pursue my passions and make my love for science my career. I love you all so much!

## TABLE OF CONTENTS

Note that each chapter is constructed as an independent research article, so information in the introductions and discussions for each chapter may be redundant to the dissertation as a whole.

<b>Abstract.....</b>	<b>ii</b>
<b>Acknowledgments.....</b>	<b>iii</b>
<b>Table of Contents.....</b>	<b>iv</b>
<b>List of tables.....</b>	<b>v</b>
<b>List of figures.....</b>	<b>v</b>
<b>General Introduction.....</b>	<b>1</b>
<i>Introduction.....</i>	<i>2</i>
<i>The Drive: Contributions of reward processing to impulsivity.....</i>	<i>4</i>
<i>The Brake: Contributions of inhibitory control to impulsivity.....</i>	<i>7</i>
<i>The role of serotonin signaling in impulsivity.....</i>	<i>11</i>
<i>Discussion: Impulsivity as an imbalance of systems and summary of dissertation.....</i>	<i>12</i>
<i>References.....</i>	<i>13</i>
<b>Chapter 1.....</b>	<b>29</b>
<b>A role for reward valuation in the serotonergic modulation of impulsivity</b>	
<i>Introduction.....</i>	<i>30</i>
<i>Methods.....</i>	<i>32</i>
<i>Results.....</i>	<i>36</i>
<i>Discussion.....</i>	<i>41</i>
<i>References.....</i>	<i>44</i>
<i>Supplemental Figures.....</i>	<i>47</i>
<b>Chapter 2.....</b>	<b>49</b>
<b>Serotonin 1B receptor effects on response inhibition are independent of inhibitory learning</b>	
<i>Introduction.....</i>	<i>50</i>
<i>Methods.....</i>	<i>51</i>
<i>Results.....</i>	<i>54</i>
<i>Discussion.....</i>	<i>56</i>
<i>References.....</i>	<i>58</i>
<b>Chapter 3.....</b>	<b>60</b>

## **Exploring the neural mechanisms of serotonin 1B receptor knockout modulation of reward and impulsivity**

<i>Introduction</i> .....	60
<i>Methods</i> .....	63
<i>Results</i> .....	69
<i>Discussion</i> .....	78
<i>References</i> .....	81
<b>General Discussion</b> .....	<b>89</b>
<i>Overview</i> .....	89
<i>Summary of major results</i> .....	89
<i>Further behavioral considerations</i> .....	91
<i>What is the role of the 5-HT<sub>1B</sub>R in modulating reward/impulsivity based neural circuitry?</i> .....	95
<i>Significance and synthesis</i> .....	100
<i>References</i> .....	102
<b>Appendix</b> .....	<b>111</b>
<b>Mice lacking the 5-HT<sub>1B</sub>R have enhanced goaltracking behavior in a touchscreen</b>	
<b>Autoshaping paradigm</b>	
<i>Methods</i> .....	111
<i>Results</i> .....	111

## **LIST OF TABLES**

### **Chapter 1**

<i>Supplemental Table 1. Subject counts</i> .....	48
---------------------------------------------------	----

### **Chapter 3**

<i>Table 1. Subject counts for experiment 1 lickometer testing</i> .....	63
<i>Table 2. Subject counts for experiment 1 operant testing</i> .....	64
<i>Table 3. Subject counts for experiment 2</i> .....	64
<i>Table 4. Subject counts for experiment 3</i> .....	65
<i>Table 5. Subject counts for experiment 4</i> .....	66

## **LIST OF FIGURES**

### **General Introduction**

<i>Figure 1. A conceptual schematic of behavioral/cognitive processes that contribute to the control of impulsive action.....</i>	<i>3</i>
-----------------------------------------------------------------------------------------------------------------------------------	----------

## **Chapter 1**

<i>Figure 1. Lack of 5-HT<sub>1B</sub>R increases motivated responding.....</i>	<i>37</i>
<i>Figure 2. Effects of 5-HT<sub>1B</sub>R on habitual and goal-directed responding.....</i>	<i>38</i>
<i>Figure 3. Absence of 5-HT<sub>1B</sub>R increases impulsive action but not delay or effort-based discounting.....</i>	<i>39</i>
<i>Figure 4. 5-HT<sub>1B</sub>R expression influences hedonic valuation.....</i>	<i>40</i>
<i>Figure 5. Lack of 5-HT<sub>1B</sub>R expression results in increased responding in a modified Pavlovian-to-instrumental transfer test.....</i>	<i>41</i>
<i>Figure 6. Decreasing reward value ameliorates 5-HT<sub>1B</sub>R-related impulsivity.....</i>	<i>42</i>
<i>Supplemental Figure 1. Lack of 5-HT<sub>1B</sub>R increases reward consumption.....</i>	<i>47</i>
<i>Supplemental Figure 2. Lack of 5-HT<sub>1B</sub>R does not alter food consumption.....</i>	<i>47</i>
<i>Supplemental Figure 3. Reward value influences impulsive action on a trial-by-trial basis.....</i>	<i>47</i>

## **Chapter 2**

<i>Figure 1. An absence of 5-HT<sub>1B</sub>R causes impulsive responding in the 5-choice serial reaction time test.....</i>	<i>52</i>
<i>Figure 2. Mice lacking 5-HT<sub>1B</sub>R have deficits in response inhibition during training but intact inhibitory learning in tests of conditioned inhibition.....</i>	<i>53</i>
<i>Figure 3. In appetitive Pavlovian conditioning, mice lacking 5-HT<sub>1B</sub>R in a zero contingency condition have elevated responding to cue onset.....</i>	<i>55</i>
<i>Figure 4. Mice lacking 5-HT<sub>1B</sub>R do not show differences in approach behavior for 100% or 25% reinforced cues.....</i>	<i>56</i>

## **Chapter 3**

<i>Figure 1. Reducing reward outcome value decreases impulsivity in 5-HT<sub>1B</sub>R knockout mice.....</i>	<i>71</i>
<i>Figure 2. Viral knockout of the 5-HT<sub>1B</sub>R in NAc shell afferent or efferent projections does not increase reward-related or impulsive behaviors.....</i>	<i>73</i>
<i>Figure 3. Genetic knockout of the 5-HT<sub>1B</sub>R in Emx<sup>+</sup>, but not VGAT<sup>+</sup> cells, in addition to viral knockout in DRN efferent projections increases reward-related and impulsive behaviors.....</i>	<i>76</i>
<i>Figure 4. Viral knockout of the 5-HT<sub>1B</sub>R in NAc shell afferent projections in addition to DRN efferent projections does not increase reward-related or impulsive behaviors.....</i>	<i>77</i>

## General Discussion

<i>Figure 1. A schematic of the potential neural circuitry by which 5-HT<sub>1B</sub>Rs modulate reward and impulsivity .....</i>	<i>98</i>
<i>Figure 2. A schematic of the potential cellular mechanism by which 5-HT<sub>1B</sub>R expression modulates neuronal signaling.....</i>	<i>100</i>

## Appendix

<i>Figure 1. Genetic knockout of the 5-HT<sub>1B</sub>R decreases cue approach and increases goal location approach in a Pavlovian autoshaping paradigm.....</i>	<i>112</i>
------------------------------------------------------------------------------------------------------------------------------------------------------------------	------------

## GENERAL INTRODUCTION

Portions of this introduction were originally published as a review article: Desrochers, S. S., Spring, M. G., & Nautiyal, K. M. (2022). A Role for Serotonin in Modulating Opposing Drive and Brake Circuits of Impulsivity. *Frontiers in behavioral neuroscience*, 16, 791749. <https://doi.org/10.3389/fnbeh.2022.791749>. SSD, MGS, and KMN wrote and edited the original manuscript. The sections included in this introduction were originally drafted by SSD, and have been modified to introduce this dissertation's experiments. This is in compliance with all copyright requirements.

## Introduction

Impulsivity is generally conceived of as a deficit in inhibitory control, resulting in unwanted actions. However, impulsive behavior has many diverse presentations and complex neurobiological underpinnings (Dalley & Robbins, 2017; Strickland & Johnson, 2021). Many lines of work have fractionated impulsivity into a number of different subtypes and components, with dissociable biological bases (Bailey et al., 2021; Bari & Robbins, 2013; Dalley & Robbins, 2017; MacKillop et al., 2016; Nautiyal et al., 2017; Robbins et al., 2012; Winstanley et al., 2004). Impulsive choice is described as risky decision making, including discounting of delayed rewards. Alternatively, impulsive action is characterized by acting prematurely and/or the decreased ability to stop or withhold responding. This introduction focuses on the action component of impulsivity, exploring the idea that impulse control can be broadly described as competing circuits. ‘Drive’ circuitry encodes an initially learned response-reward outcome association, and the ‘brake’ circuitry subserves an opposing and subsequently learned inhibitory association. The sum of the outputs of these circuits shapes the action plan determining whether the animal will go or inhibit going.

Dysfunction in different nodes of these ‘drive’ and ‘brake’ neural circuits could result in the heterogeneity of phenotypic presentations of impulsivity. Therefore, careful dissection of the underlying behavioral and circuit level contributions to impulsivity is important for understanding the pathogenesis of increased impulsivity. This idea is highlighted in the dual systems and imbalance models of adolescent impulsivity which consider disproportionate development and changes in communication for brain areas involved in reward/motivation and inhibitory control (Casey et al., 2011; Ellingson et al., 2013; Somerville et al., 2010; Steinberg, 2010). Considering imbalance models in the context of preclinical studies aimed at understanding adult impulsivity could help elucidate different entry points to dysfunctional circuits responsible for pathological impulsivity.

This dual-systems perspective is relevant to clinical populations with disorders in which impulsivity is dysregulated. For example, attention deficit hyperactivity disorder (ADHD) is characterized by inhibitory deficits, including increased impulsive action (Grandjean et al., 2021; Nigg, 2001; Schachar & Logan, 1990; Wright et al., 2014). Impulsivity is also a key phenotype found in substance use disorder, in which both reward system and inhibitory dysfunctions are present (Jentsch et al., 2014; Weafer et al., 2014). From the perspective of reward sensitivity, genetic risk for alcoholism is associated with increased sensitivity to sweet substances (Kampov-Polevoy et al., 2001; Kampov-Polevoy et al., 2003). Poor inhibitory control is associated with sensitivity to amphetamines (Weafer et al., 2017; Weafer & De Wit, 2013) and chronic cocaine

use (Fillmore & Rush, 2002). Increased impulsive action likely reduces the ability to withhold actions to obtain or consume drugs, though it is difficult to parse out the cause versus effect, as is common generally when studying psychiatric disorders. However, it is clear that impulsivity is both a predisposing factor and a result of drug use. Several studies which supports a role for impulsivity as a causal factor shows that subjects with familial history of drug dependence have higher impulsivity across many domains, including impulsive action (Acheson et al., 2011; Ersche et al., 2012; Kumar et al., 2018). Additionally, increased impulsivity and altered reward sensitivity are also found in gambling disorder (Hodgins & Holub, 2015; Ioannidis et al., 2019; Jiménez-Murcia et al., 2017; Mestre-Bach et al., 2020; Sztainert et al., 2013; Wardell et al., 2015), which, as a behavioral addiction, is free from the confound of pharmacological effects on these phenotypes. Indeed, Brevers et al. (2012) found that the severity of problem gambling was predicted by performance on a stop-signal test of impulsive action.

Assuming the presence of competing drive and brake processes in impulsivity, we can examine the behavioral/cognitive components and the underlying neural mechanisms of each of these components. This sets up the possibility to arrive at the endpoint of increased impulsive behavior via a number of different paths and combinations of intermediate phenotypes (Fig. 1). For example, in a behavioral assay of impulsive action, increased maladaptive actions could arise from a stronger action-outcome association, an increased motivation or valuation of reward, a failure to learn the opposing behavioral response (inhibition), or even a failure to express the inhibition, despite it having been learned. Understanding which components contribute to impulsive phenotypes, can lead toward developing novel, specific treatments targeting dysfunction of neural circuitry more precisely.

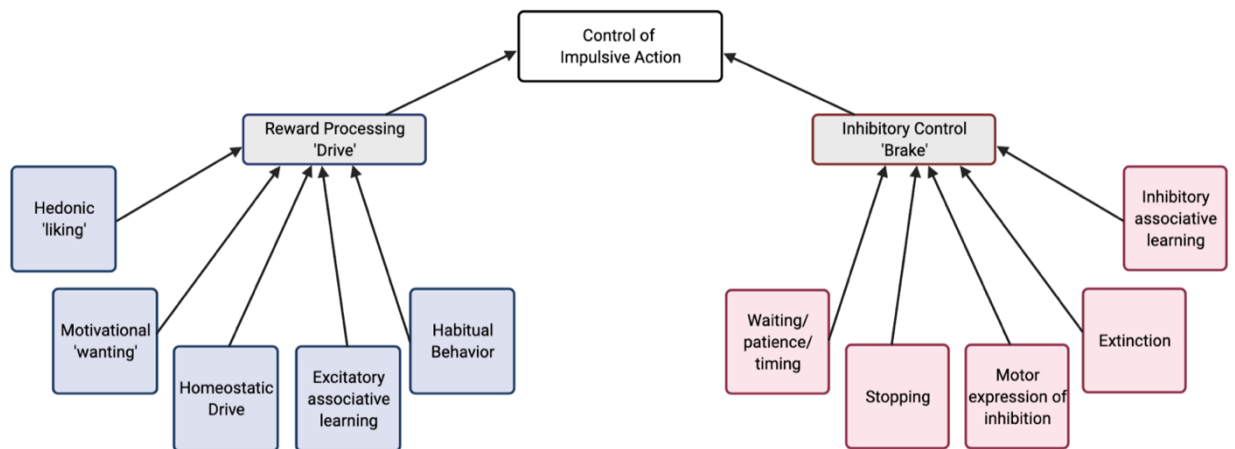


Figure 1. A conceptual schematic of behavioral/cognitive processes that contribute to the control of impulsive action. These are organized into reward ‘drive’ and inhibitory ‘brake’ processes.

## **The Drive: Contributions of reward processing to impulsivity**

Impulsivity is innately tied to reward processing, with excitatory drive being a key aspect of motivated behavior. Importantly, before being able to consider an inhibitory process, a motivated behavior needs to exist. This commonly includes a learned cue-reward or action-outcome association. In other words, we first learn to respond to obtain rewarding outcomes, prior to learning to avoid responding in certain circumstances (an innate or learned propensity to ‘go’). Alterations in appetitive associations may change the strength of the drive for reward. This could include differences in the intrinsic value/pleasurability of a reward (liking), and/or changes in the motivational value of the reward/reward paired cues (wanting). Though these are experimentally separable (Berridge & Robinson, 2016; Morales & Berridge, 2020; Peciña, 2008), they are linked together such that changes to either ‘liking’ or ‘wanting’ would likely increase actions in pursuit of reward, a characteristic of impulsive action. Therefore, superficially similar clinical presentations could actually be the result of dysfunction in different underlying neural mechanisms. Careful behavioral analysis using a variety of tests in different reward domains may allow us to identify the mechanisms contributing to pathological levels of impulsivity.

### ***Reward processing in classical conditioning***

Though impulsivity is defined in terms of operant behavior, in which impulsive behavior is characterized by actions that have unwanted consequences, the processes that underlie impulsive behavior may also be measurable at the level of Pavlovian tasks if they include changes to reward processing. In other words, if an instance of increased impulsivity was due to a change in the drive process, it may be able to be seen in altered appetitive classical conditioning, when outcomes are independent of action. For example, changes to the magnitude/value of an unconditioned stimulus influences the associative strength of conditioned cues, resulting in enhanced conditioned responding (Morris & Bouton, 2006; Pearce & Hall, 1980; Rescorla & Wagner, 1972; Van Den Bos et al., 2004). For appetitive conditioning, increased reward value due to altered hedonic pleasure or homeostatic processes could therefore increase the salience or associative strength of a cue, such that vigor of responding correlates with the perceived magnitude. If the value of a reward was subjectively increased, either due to pathological neural changes or simply everyday variations in reward preference in non-pathological cases, we would expect that subjects would form a stronger association between the cue and the reward and therefore have generally increased responding. For example, the phenomenon of signtracking, where animals may interact with a manipulable cue as if it were the reward which it has come to be associated with, shows that a classically conditioned cue can acquire increased incentive salience (Flagel et al., 2011). In fact, rats bred for a high novelty responding phenotype had

increased signtracking behaviors along with a decreased ability to withhold responding in the differential reinforcement of low-rate responding test of impulsive action (Flagel et al., 2010). This interestingly correlates incentive salience with impulsivity – either subserved by a single underlying endophenotype or possibly due to a causal link of increased incentive salience leading to increased impulsive action. Interestingly high novelty responding rats also increased preference for the large reward in a delay discounting test of impulsive choice. Overall this study supports the idea that increased reward sensitivity may underlie both the operant impulsive and Pavlovian signtracking phenotypes. Additionally, in a study of excitatory Pavlovian responding during the adolescent developmental period, which is often characterized by heightened reward reactivity and impulsivity, adolescents showed increased responding under partial reinforcement conditions compared to adults (Meyer & Bucci, 2016). This suggests that developmentally mediated impulsivity and altered classical conditioning may be modulated by similar reward-based changes. Taken together, the consideration of the processes which contribute to responding in appetitive classical conditioning may shed light on the mechanisms through which reward processing contributes to impulsive behavior.

Multiple neural substrates have been implicated in assigning value to an outcome or cue and incentive motivation. Dysregulation of any number of highly interconnected implicated brain regions could therefore result in altered reward related behavior. Several regions appear to represent or integrate reward value, including the nucleus accumbens (NAc), ventral pallidum (VP), basolateral amygdala (BLA), and regions of the prefrontal cortex including the orbitofrontal cortex (OFC) (Amiez et al., 2006; Chen et al., 2015; Howard et al., 2015; Ottenheimer et al., 2018; Wassum & Izquierdo, 2015). In particular, distinct areas of both the NAc (Castro & Berridge, 2014; Peciña, 2008; Peciña & Berridge, 2005) and the VP (Tindell et al. 2006; Ahrens et al. 2016; Richard et al. 2016; Smith et al. 2009) have been implicated in hedonic ‘liking’ of reward assessed through taste reactivity, as well as incentive motivation ‘wanting’. The NAc is poised to integrate cortical and limbic information about reward and output to the VP, the subthalamic nucleus (STN), the substantia nigra, the ventral tegmental area (VTA), and the lateral hypothalamus, providing a mechanism for translating value assessment and motivation into behavior (Mogenson et al., 1983; Robbins & Everitt, 1996). Indeed, as reviewed by Day and Carelli (2007), the NAc and its connections are critical to appetitive Pavlovian cue-outcome learning, both in association acquisition and motoric expression. In sum, changes in brain regions involved in both ‘liking’ and ‘wanting’ aspects of reward processing could contribute to increased responding to conditioned stimuli during appetitive classical conditioning by subjectively increasing the outcome value.

### ***Reward and impulsive action***

In addition to classical conditioning, reward processing is central to instrumental behavior, and increased impulsivity could result from the overvaluation or increased motivation for reward, which override the negative consequences associated with taking action. Difficulty in withholding or stopping ongoing responding for reward in tests of instrumental behavior is a defining characteristic of impulsive action. Examples of paradigms used to assess this component of impulsivity include the Go/No-go (measuring the decreased ability to withhold responding when presented with a no-go cue), 5-choice serial reaction time test (5CSRTT; assessing premature responding), stop signal reaction time test (SSRT; testing the decreased ability to halt ongoing responding), and differential reinforcement of low rate responding (DRL; measuring the decreased ability to withhold responding for a wait period).

Importantly, an increased impulsive action phenotype may influence behavioral readouts in other operant paradigms testing motivation, such as random ratio and progressive ratio. Changes in excitatory responding (actions normally taken in pursuit of reward), for example the vigor of responding, which are subserved by changes in reward circuitry (M. R. Bailey et al., 2016, 2018) may make inhibiting the response more difficult. This could drive increased/disordered responding seen in these operant tasks, as well as in clinical cases of increased impulsivity. For example, dysfunctional reward processing is frequently comorbid in psychiatric disorders characterized by levels of increased impulsivity, including substance use, gambling disorders, and schizophrenia (Billieux et al., 2012; Bjork et al., 2004; Monterosso et al., 2005; Rubio et al., 2008). Preclinically, rats that show high levels of premature responding in the 5CSRTT are also more sensitive to cue-induced reinstatement of sucrose-seeking (Diergaarde et al., 2009). The question remains if the dysregulated impulsivity is causally linked to the reward system dysfunction.

Approaches to dissect the underlying neural circuits of co-occurring reward process and inhibitory dysfunction can determine if the neural circuit dysregulation is subserved by convergent mechanisms. Many of the same brain areas noted to be involved in reward and motivation have also been implicated in impulsive action. In particular, the NAc and its core and shell subregions have been extensively studied for their individual roles in impulsive action through reaction time tests, with pharmacological manipulations and deep brain stimulation of the shell subregion causing elevated premature responding (Feja et al., 2014; Sesia et al., 2010). Optogenetic stimulation of projections from the VTA to the NAc shell also increased premature responding during a long inter-trial interval in the 5CSRTT (Flores-Dourojeanni et al., 2021).

Additionally, prefrontal cortical regions modulate impulsive action, though they are more often associated with assigning value to different decisions and choosing between actions (OFC, mPFC). Specifically, in an imaging study in humans, Mechelmens et al. (2017) found that impulsivity for high value reward cues in a 4CSRTT was accompanied by increased activity in the mOFC and in a monetary incentive delay task was associated with increased functional connectivity between the STN and left mOFC. In a rodent study of the 5CSRTT, rats that tended to respond prematurely had alterations in oscillatory patterns in the mPFC and NAc, which may cause abnormal reward encoding resulting in increased impulsive action (Donnelly et al., 2014).

The alterations in reward-related behavior in impulsivity could also be the result of impaired action selection supported by the dorsal striatum, which is important when there is an instrumental contingency between response and reward, as in many tests of impulsive action (Balleine et al., 2007; Corbit & Janak, 2007, 2010). Pharmacological manipulations of serotonin and glutamate receptors in the dorsal striatum modulate premature responding in the 5CSRTT (Agnoli & Carli, 2012). The varied regions associated with the control of impulsive action highlight the importance of considering reward processing in the study of impulsivity, as well as suggest that there may be many ways to cause an impulsive action ‘phenotype’ through modulation of different behavioral endophenotypes. Behavioral analysis which considers the learning, hedonic, and motivational contributions to pathological cases of impulsivity may help clarify and point toward more specific neural targets for treatment.

### **The Brake: Contributions of inhibitory control to impulsivity**

Alternatively to increased reward drive, disordered impulsivity can be considered as a failure of inhibitory processes, even colloquially described as a lack of ‘self-control’. In the impulsive action subtype of impulsive behavior, this presents as deficits in preventing responding or stopping ongoing responding. Withholding an action, or learning that the absence of response results in reward, is an action-outcome association that is necessary for successful performance in standard tests of impulsive action. This action-outcome association opposes the initially learned excitatory association in which the action led to reward. The ability to withhold responding, or inhibitory control, is often ascribed to higher executive functions and decision-making processes controlled by cortical areas, which act to modulate subcortical regions involved in ‘drive’ (Bari & Robbins, 2013; Dalley et al., 2011). However, deficits in response inhibition also arise locally within lower neural areas involved in the volitional process (such as the NAc, which is usually associated with the ‘drive’ component but may also have an inhibitory role). There also may be separable component processes underlying the acquisition/learning and

the expression of inhibitory control, which would require carefully designed behavioral studies to separate.

### ***Inhibition and classical conditioning***

Learning about inhibitory associations is an important component to consider in understanding response inhibition in impulsive action. This is distinct from the behavioral/motor expression. Deficits in inhibitory learning could be a cause of deficits in response inhibition, or alternatively, could be intact with the impulsivity arising at other levels of processing. While impulsivity itself is not defined in the context of classical conditioning, a behavioral output may appear impulsive if there are underlying deficits in inhibitory learning. For example, an impulsive behavior could result from the lack of learning of the response omission – reward association, or from a decreased ability to withhold a response. The acquisition of inhibitory learning has been studied extensively in the context of classical conditioning.

A primary area of inhibitory learning is extinction, where a new inhibitory memory is acquired to compete against a previously established excitatory memory. Importantly, extinction is not an erasure of a memory, but rather a competition of parallel associations, where old memories/behaviors can spontaneously renew (Bouton, 1993; Bouton et al., 2021; Todd et al., 2014). A deficit in the formation of the new inhibitory association or a failure of this association to successfully compete with the excitatory association, could result in altered impulsivity in classic tests of impulsive action. However, though there are many parallels between Pavlovian and operant extinction, there are also clear dissociations; for example, Pavlovian extinction does not usually transfer between conditioned stimuli, but operant extinction does (Bouton et al., 2021; Trask et al., 2017). Neurally, both the BLA and the infralimbic cortex, among others, are all involved in both Pavlovian and operant extinction, but the NAc shell is especially implicated in operant extinction (Millan and McNally, 2011; reviewed in Bouton et al., 2021). The hippocampus also seems to be involved in behavioral inhibition during extinction, as lesions to this region prevent extinction of a previously classically conditioned appetitive stimulus (Chan et al., 2003). All of these regions have also been implicated in the modulation of impulsive action, suggesting that deficits in extinction behavior may be involved in some presentations of impulsivity, or rely on dysfunction in similar neural mechanisms.

Another Pavlovian behavioral paradigm which could be useful in understanding the role of inhibitory learning in impulsive action is conditioned inhibition (reviewed by Sosa and dos Santos, 2018). Conditioned inhibition is a form of classical inhibitory learning where an inhibitory cue indicates the absence of an outcome when it is paired as a compound with a

normally excitatory cue (A+, AX-; Pavlov, 1927). This inhibitor cue can then ‘transfer’ and reduce responding when paired with other excitatory cues (BX-; Holland, 1989). Impulsive subjects which have diminished inhibitory control in operant paradigms may also fail to inhibit responding for the inhibitor-excitator compound cue in a test of Pavlovian conditioned inhibition, potentially suggesting common underlying mechanisms. Accordingly, He et al. (2011) found decreased expression of conditioned inhibition in a clinical population with personality disorders, often characterized by disinhibition and impulsivity. However, to dissociate the acquisition of this inhibitory learning from behavioral expression, acute time-limited experiments using optogenetic or chemogenetic inactivation of relevant neural targets during training vs. recall testing may be necessary.

Another version of Pavlovian inhibitory learning is negative occasion setting in which an inhibitory cue indicates that an outcome will not occur when presented in sequence with a normally excitatory cue (A,  $X \rightarrow A^-$ ). In this case, the conditioning is specific to the trained set of cues, and the inhibitor does not usually transfer to a different excitatory cue (Holland, 1989). Adolescent rats take longer to discriminate between reinforced and non-reinforced trials in a negative occasion setting paradigm when compared to preadolescents and adults, possibly due to the functional immaturity of the PFC during this developmental period (Meyer & Bucci, 2017). Indeed, the prelimbic region of the PFC is necessary for learning this discrimination negative occasion setting, but not expressing it following training (MacLeod & Bucci, 2010). Additionally, these findings were replicated in a conditioned inhibition paradigm, where Meyer and Bucci (Meyer & Bucci, 2014) found that lesions of the prelimbic region of the PFC decreased acquisition of conditioned inhibition learning, whereas lesions of the infralimbic cortex decreased behavioral expression following successful discrimination. Further testing inhibitory learning processes in established models for impulsive action or clinical populations are important next steps. These classical conditioning experiments could help elucidate the underlying behavioral/cognitive deficits present in specific cases of impulsivity, as well as suggesting potential shared neural substrates.

### ***Impulsive action and response inhibition***

Though disordered impulsivity could occur because of differences in inhibitory Pavlovian associations, it is defined in the context of operant conditioning requiring inhibition of an action to obtain reward. Nevertheless, similarly to classically conditioned response inhibition, the inhibitory ‘brake’ seems to rely heavily on prefrontal regions upstream of subcortical reward areas (see Bari and Robbins 2013 for an extensive review of their search). In humans, several

fMRI studies have identified neural correlates of inhibitory control during tests of impulsive action. Activity in the vIPFC was associated with successful response inhibition in no-go trials for larger monetary rewards in an incentivized inhibition task (Leong et al., 2018). Additionally, using a stop signal task, Weafer et al. (2019) found that decreased activity in the right PFC during response inhibition was associated with higher left ventral striatum activity during reward receipt, suggesting negative functional association between inhibitory control and reward drive modulated through cortico-striatal connections. The anterior cingulate cortex (ACC) has also been implicated in impulse control in subjects with ADHD (Baytunca et al., 2021), and its activity is related to error processing in a Go/No-go task (Hester et al., 2004).

There is also a large literature investigating the neural circuitry underlying cortical control of response inhibition in preclinical models. Pharmacological inactivation of various regions of the mPFC, especially the prelimbic and infralimbic regions, resulted in a loss of inhibitory control on no-go trials in a response inhibition task which included shock punishments (Verharen et al., 2019). Chemogenetic activation of the vmPFC to NAc shell pathway decreases motor impulsivity in a 1CSRTT and binge-eating in rats, suggesting that these higher order areas have inhibitory control over reward processing (Anastasio et al., 2019). Indeed, using optogenetics, Li et al. (2020) found that another cortical-subcortical connection from the dmPFC to the STN in mice was important for response inhibition in a Go/No-go task. In the ACC, inhibitory G proteins are involved in the control of premature responding in the 5CSRTT (van der Veen et al., 2021). Interestingly, these studies manipulate their pathways/regions only after subjects acquired baseline training performance, suggesting that these pathways play a role in the behavioral expression of inhibition, not necessarily the learning itself. There is also convergent human and animal evidence for a role of the OFC in response inhibition (reviewed in Winstanley et al., 2010), however, single-unit recordings by Bryden and Roesch (2015) revealed that OFC neuron activity seems to support the separation of similar actions rather than inhibition independently.

Beyond the cortex, there is also evidence for the contribution of subcortical areas to response inhibition during tests of impulsive action. Deep brain stimulation of the NAc core in rats decreased impulsivity as measured by premature responding in a reaction time test, while stimulation of the NAc shell increased impulsivity, suggesting that the different subregions of the NAc may functionally support both excitation and inhibition in pursuit of reward (Sesia et al., 2008). Also, in the NAc, local inhibitory control may occur through the activity of fast-spiking interneurons, which seem to constrain impulsive action in the 5CSRTT, likely by inhibiting signaling of medium spiny neurons (Pisansky et al., 2019). Finally, dopamine signaling in the

dorsal striatum is also important for response inhibition in a stop-signal task (Robertson et al., 2015). Together, all these studies suggest that the inhibitory control of impulsive action relies both on cortical and local sub-cortical control of reward processing areas.

### **The role of serotonin signaling in impulsivity**

Serotonin (5-HT) has been strongly implicated in encoding reward *and* mediating behavioral inhibition, and is poised to modulate the balance of reward-based approach and adaptive inhibition of action. Manipulation of serotonin neuron activity in preclinical models clearly show that serotonin is involved in waiting and inhibiting behavioral responses (Fletcher, 1995; Fonseca et al., 2015; Jolly et al., 1999; K. Miyazaki et al., 2018, 2020; Winstanley et al., 2004; Wogar et al., 1992). Studies using *in vivo* monitoring, through single-unit electrophysiology and photometric calcium monitoring, in the dorsal raphe (DRN) also implicate serotonin neurons in encoding both rewards and associated cues (Cohen et al., 2015; Y. Li et al., 2016; Ren et al., 2019; Zhong et al., 2017). A large number of studies have also investigated the role of serotonin signaling—through many of its 15 receptors—in both reward-related behaviors and behavioral inhibition. Though many have used pharmacological approaches with systemically administered drugs, some studies have targeted brain region specificity with local drug administration and cell- and circuit-specificity using genetic models (e.g. for 5-HT<sub>1A</sub>R Balleine et al., 1996; Fletcher et al., 1993; Fletcher et al., 1995; Groft et al., 2019; Miyazaki et al., 2012; Korte et al., 2017; for 5-HT<sub>1B</sub>R Harrison et al., 1999; Brunner & Hen, 1997; Nautiyal et al., 2015, 2017; Acosta et al., 2005; Fletcher et al., 2002; Pattij et al., 2003; for 5-HT<sub>2A</sub>R and 5-HT<sub>2C</sub>R Frick et al., 2015; Fletcher et al., 2017; Koskinen et al., 2000; Silveira et al., 2020; Talpos et al., 2006; for 5-HT<sub>2B</sub>R Doly et al., 2009; Goldman et al., 2010). Given that serotonin, as a neuromodulator, tunes synaptic signaling and guides plasticity to alter learning and motivated behaviors, it is relevant to explore the idea that serotonin acts within the neural circuits governing ‘drive’ and ‘brake’ processes in impulsivity.

Of particular relevance to this dissertation is the contributions of the serotonin 1B receptor (5-HT<sub>1B</sub>R) to impulsivity. 5-HT<sub>1B</sub>R acts as a presynaptic inhibitory G-protein coupled receptor, inhibiting neurotransmitter release in both serotonin (as an autoreceptor) and non-serotonin (as a heteroreceptor) releasing neurons (Boschert et al., 1994; Jolimay et al., 2000; Mizutani et al., 2006). Previous work in humans has implicated mutations in the gene encoding the 5-HT<sub>1B</sub>R with various disorders characterized by decreased impulse control. For example, single nucleotide polymorphisms in the 5-HT<sub>1B</sub> gene have been associated with substance use

disorders (Cao et al., 2013; Contini et al., 2012; Proudnikov et al., 2006), impulsive aggression (Conner et al., 2010; Zouk et al., 2007), and ADHD (Hawi et al., 2002).

In genetic mouse models, knockout of the 5-HT<sub>1B</sub>R in the whole brain for whole life results in similar effects on impulsive behaviors. Specifically, mice lacking this receptor have increased impulsive action as demonstrated by increased responding for cued ‘withhold responding’ trials in the Go/No-go and premature responding in the DRL tests (Nautiyal et al., 2015, 2017). The 5-HT<sub>1B</sub>R knockout, however, does not result in increased impulsive choice in tests of delay or probability discounting (Nautiyal et al., 2017), suggesting this behavioral change is specific to one domain of impulsivity. Therefore, the 5-HT<sub>1B</sub>R knockout mouse model is well poised for the study of the behavioral constructs underlying impulse action, as well as its neural basis within the serotonin system.

### **Discussion: Impulsivity as an imbalance of systems and summary of dissertation**

Dysregulations of either reward or inhibition can create an imbalance of the neural systems responsible for impulse control. Neurally, widespread DRN serotonergic projections place serotonin signaling through its various receptor types, in a prime position to modulate both the excitatory and inhibitory components of these systems. Indeed, there may be multiple serotonin subsystems which separably mediate responses to rewarding or aversive outcomes (Ren et al., 2018). Either excess excitation or decreased inhibitory control could result in increased impulsive action as observed by a decreased ability to stop or withhold responding. In this case, the initially learned ‘go’ association overrides the ‘no-go’ or stop association. Increased impulsivity could also be the result of altered activity in both drive and brake processes. Ultimately, both processes compete over controlling the same endpoint: motor output. For animals to achieve efficient, flexible behavior, the drive and brake circuitry must each be responsive to task demands in guiding action selection.

Adolescence is an interesting case which allows us to probe the role of these two processes and how serotonin influences the balance. Specifically, adolescence is a developmental period characterized by increased impulsivity, risky decision making, and hyper reward-sensitivity. In the dimension of impulsive action, compared to children and adults, teenagers have more false alarms for no-go cues in the Go/No-go test (Dreyfuss et al., 2014; Somerville et al., 2011). This heightened impulsive action is thought to be the result of the linear development of the PFC and the nonlinear development of the ventral striatum and other components of the reward system, which peak in sensitivity during adolescence (Blakemore & Robbins, 2012). This results in an imbalance between the subcortical systems which motivate behavior and the cortical

systems providing inhibitory control compared to childhood and adulthood (Casey et al., 2011). Substance use disorders have also been considered through a similar lens, with both increased appetitive drive and disordered executive control potentially resulting in impulsive behavior, though the extent to which impulsivity is causal or resultant to addiction is unclear (Bechara, 2005; Camchong et al., 2014; Jentsch et al., 2014; Kozak et al., 2019).

Importantly, the imbalance of reward and inhibitory processing could be the result of dysfunction of many different regions, cell types, and/or receptor types, which may each result in an impulsive action phenotype, albeit through different neural and behavioral processes. Careful dissections of these processes which contribute to impulsive action allows for the fractionation of different paths to an overall impulsive phenotype. In this dissertation, I therefore use non-traditional tests for the study of impulsivity in a mouse genetic model allowing for manipulations of 5-HT<sub>1B</sub>R expression, including the consideration of Pavlovian and instrumental learning processes, the expression of behavioral inhibition, and reward processes. Specifically, Chapter 1 focuses on reward ‘drive’ processes, included tests of impulsive action and choice, motivation, homeostatic feeding, and hedonic taste reactivity. Chapter 2 then takes an alternative perspective by examining ‘brake’ processes, including withholding responding in an instrumental task and inhibitory learning in a Pavlovian task. Finally, in Chapter 3, I explore the neural mechanisms of 5-HT<sub>1B</sub>R based impulsivity using combined genetic and viral knockout approaches to get regional and cell type specificity.

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## CHAPTER 1

### **A role for reward valuation in the serotonergic modulation of impulsivity**

This chapter was originally published as a research article: Desrochers, S. S., Lesko, E. K., Magalong, V. M., Balsam, P. D., & Nautiyal, K. M. (2021). A role for reward valuation in the serotonergic modulation of impulsivity. *Psychopharmacology*, 238(11), 3293–3309.

<https://doi.org/10.1007/s00213-021-05944-2>. VMM and EKL assisted in data collection for figures 1, 2, 3A/B, and 6. All remaining data was collected by SSD. All data was analyzed by SSD with assistance from KMN. SSD and KMN wrote the manuscript, with edits from PDB. This is in compliance with all copyright requirements.

Note:

Since publication, we have learned that a more common name for what we refer to as the ‘modified Pavlovian-to-instrumental transfer’ paradigm is Conditioned Reinforcement.



# A role for reward valuation in the serotonergic modulation of impulsivity

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Received: 27 April 2021 / Accepted: 22 July 2021 / Published online: 14 August 2021  
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## Abstract

**Rationale** Impulsive behavior is a deleterious component of a number of mental health disorders but has few targeted pharmacotherapies. One contributing factor to the difficulty in understanding the neural substrates of disordered impulsivity is the diverse presentations of impulsive behavior. Defining the behavioral and cognitive processes which contribute to different subtypes of impulsivity is important for understanding the neural underpinnings of dysregulated impulsive behavior.

**Methods** Using a mouse model for disordered impulsivity, our goal was to identify behavioral and cognitive processes that are associated with increased impulsivity. Specifically, we were interested in the facets of impulsivity modulated by serotonin signaling. We used mice lacking the serotonin 1B receptor (5-HT<sub>1B</sub>R) and measured different types of impulsivity as well as goal-directed responding, extinction, habitual-like behavior, cue reactivity, and reward reactivity.

**Results** Mice lacking expression of 5-HT<sub>1B</sub>R had increased levels of impulsive action, goal-directed responding, and motivation, with no differences seen in rate of extinction, development of habitual behavior, delay discounting, or effort-based discounting. Interestingly, mice lacking 5-HT<sub>1B</sub>R expression also showed an overall increase in the choice of higher value rewards, increased hedonic responses to sweet rewards, and responded more for cues that predict reward. We developed a novel paradigm to demonstrate that increasing anticipated reward value could directly increase impulsive action. Furthermore, we found that 5-HT<sub>1B</sub>R KO-induced impulsivity could be ameliorated by decreasing the reward value relative to controls, suggesting that the increased 5-HT<sub>1B</sub>R-associated impulsive action may be a result of increased reward valuation.

**Conclusions** Taken together, these data show that the effects of serotonin on impulsive action are mediated through the modulation of hedonic value, which may alter the reward representations that motivate action. Overall, this data supports a role for reward value as an important substrate in impulsive action which may drive clinically relevant increases in impulsivity.

**Keywords** Reward valuation · Serotonergic modulation · Impulsivity · 5-HT1B

## Introduction

Impulsivity is an important component of daily life, but can lead to many deleterious outcomes such as making unhealthy eating decisions or excessive online shopping. It is also a core component of a number of psychiatric disorders including attention deficit disorder, alcohol and substance use

disorders, and gambling disorder (Dalley and Robbins 2017; MacKillop et al. 2016; Robbins et al. 2012). Treatment options to decrease impulsivity are limited, and those that exist are not targeted to impulsive behavior. One underlying issue in the development of approaches to reduce impulsive behavior lies within the complexity of the broad construct of impulsivity. Individual facets of what is broadly referred to as impulsive behavior, for example, impulsive action (e.g., acting on a whim) and impulsive choice (e.g., wanting immediate gratification), are likely mediated by different behavioral/cognitive processes with different neurobiological substrates (Bari and Robbins 2013; Nautiyal et al. 2017; Robbins et al. 2012; Winstanley et al. 2004a). Though, some argue against the use of the term impulsivity at all given the divergence and independence of its latent factors, in favor of more specific labels which have internally consistent

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behavioral and neurobiological substrates (Strickland and Johnson 2020). Furthermore, processes such as reward valuation, compulsivity, motivation, attention deficits, novelty-seeking, and anxiety have all been associated with some aspects of impulsivity using trait-level behavioral measures in humans (particularly in psychiatric populations) and in preclinical models (Chamorro et al. 2012; Dalley et al. 2011; Diergaarde et al. 2008; Ferland et al. 2014; Lovic et al. 2011; Moustafa et al. 2017; Weafer et al. 2014). Understanding how these behavioral and cognitive substrates are causally associated with different components of impulsive behavior will lead to an understanding of the behavioral/cognitive scaffolding and associated underlying neural circuits which lead to dysregulated impulsivity.

Ours and others' previous work has examined how different dimensions of impulsivity can be dissociated, behaviorally and biologically (Dalley and Robbins 2017; Nautiyal et al. 2017; Winstanley et al. 2004b; Zeeb et al. 2016, 2013). Specifically, impulsive action, characterized by the reduced ability to withhold or delay responses, is independent from impulsive choice, which includes an exaggerated discounting of future or risky rewards. While many studies have focused on the role of dopamine in the modulation of impulsivity, serotonin signaling is particularly relevant when focusing on dissociation of the neurobiology of impulsive choice from impulsive action. Manipulation of serotonin signaling in humans and rodents supports its role in modulating impulsive action specifically (Fletcher et al. 2007; Higgins et al. 2016; Winstanley et al. 2004a; Worbe et al. 2014). The mechanisms of these effects are likely through a number of the 14 serotonin receptors including 5-HT<sub>1B</sub>, 5-HT<sub>2A</sub>, and 5-HT<sub>2C</sub>. We previously reported that mice lacking the 5-HT<sub>1B</sub> receptors show increased impulsive action, but not impulsive choice (Brunner and Hen 1997; Nautiyal et al. 2015, 2017; Pattij et al. 2003). Interestingly, in humans, 5-HT<sub>1B</sub>R has also been implicated in disorders which present with dysregulated impulsivity, such as substance use and gambling disorders. Cocaine-dependent individuals had reduced 5-HT<sub>1B</sub>R activation compared to controls (Matuskey et al. 2014), and single-nucleotide polymorphisms in *htr1b* were associated with cocaine, alcohol, and heroin abuse (Cao et al. 2013). In preclinical models, genetic knockout of 5-HT<sub>1B</sub>R caused increases in cocaine self-administration in mice (Rocha et al. 1998), and administration of a 5-HT<sub>1B</sub>R agonist decreased behavior motivated by both cocaine and sucrose rewards (Acosta et al. 2005).

Serotonin signaling could act through several cognitive and behavioral mechanisms which may promote difficulty withholding responses (elevated impulsive action). While it is common to attribute this type of impulsivity to a deficit in inhibitory control, behavioral action is a product of both inhibitory and excitatory processes. Given intact inhibitory control mechanisms, an exaggerated representation of

reward value may also make “holding back” difficult due to increased drive, resulting in increased impulsivity. The representation of reward value that motivates behavioral responding arises from experiences with the current hedonic value of rewards (Balleine and Dickinson 1998; Dickinson and Balleine 1994, 2002). When response-outcome contingencies are learned, the likelihood of responding is guided by the incentive value of the outcome, which can be seen in the hedonic reaction (Dickinson and Balleine 1995). For example, when subjects are hungry, food rewards have greater value than when subjects are sated, and thus hedonic reactions are diminished. When the expected reward value is lowered, motivation is also reduced. While the hedonic reactions to reward referred to as “liking” and the motivational processes that energize behavior referred to as “wanting” can be dissociated, wanting is generally directed at available outcomes that are liked (Berridge et al. 2009, 1989; Smith and Berridge 2007; Ward et al. 2012). Here we refer to the increased responding for an appetitive outcome as reward reactivity to include components of both liking and wanting.

Both increased reward reactivity and increased impulsivity are found in patients with substance use disorders, and potentially represent risk factors for the development of addictive disorders (Crane et al. 2018; Dissabandara et al. 2014; Jonker et al. 2014; Kamarajan et al. 2015; Verdejo-García et al. 2008). In this framework, substance use disorders may arise from deficits in inhibitory control, but also from exaggerated hedonic valuation and/or through increased incentive salience, as in the Incentive-Sensitization Theory (Berridge and Robinson 2016). It is possible that the contribution of the 5-HT<sub>1B</sub>R to drug-seeking behaviors is due to the modulation of hedonic reactions to drugs, at least in part. Reward reactivity has also been implicated in gambling disorder, with increased pleasure derived from winning potentially promoting the escalation of the behavioral addiction (Gaher et al. 2015; Jimenez-Murcia et al. 2017). Additionally, in gambling disorder patients, levels of 5-HT<sub>1B</sub>R in brain regions associated with reward processing, including the ventral striatum, correlated with the severity of the disorder (Potenza et al. 2013). These considerations suggest the possibility that 5-HT<sub>1B</sub>R may impact impulsivity through changes in hedonic reactions and thus alter the representation of the value of a reward that motivates behavioral action.

The goal in the present studies was first to investigate what behavioral/cognitive processes, such as reward valuation, contribute to deficits in behavioral inhibition, and second to understand which process mediates the effect of serotonin on impulsive action. Specifically, we explored the effect of 5-HT<sub>1B</sub>R on potential substrates of impulsivity including goal-directed responding, motivation, habitual-like responding, and hedonic responding. While each of these can be conceptualized as unique behavioral phenotypes with

distinct neural substrates, we focused on how alterations in impulsive action could potentially be subserved by changes in these processes. We specifically tested the hypothesis that the influence of serotonin on impulsivity is mediated by effects on the valuation of reward outcome. Additionally, by assessing these phenotypes in a mouse model for pathological impulsivity with deficits limited to impulsive action (absence of 5-HT<sub>1B</sub>R), we were also able to determine how associated behavioral mechanisms are related to different domains of impulsivity (Winstanley et al. 2004a). Coming to a better understanding of the specific neural and behavioral substrates of different dimensions of impulsivity will help us understand how these components combine to generate dysregulated impulsivity in psychiatric disorders.

## Methods

### Mice

Animals were bred in the Center for Comparative Medicine at Dartmouth College, or in the Department of Comparative Medicine at the New York State Psychiatric Institute. All mice were weaned at postnatal day (PN) 21 into cages of 2–5 same sex littermates on a 12:12 light–dark cycle, and maintained on ad lib chow until experimental operant behavioral testing began at 10–14 weeks. The floxed tetO1B mouse model was used to generate groups of mice lacking expression of 5-HT<sub>1B</sub>R through crosses to a  $\beta$ Actin-tTS mouse line (tetO1B +/+ females crossed to tetO1B +/+:: $\beta$ Actin-tTS + males), as previously reported (Nautiyal et al. 2015). In the validation of the Variable Value Go/No-Go paradigm, only tetO1B +/+ control mice were used. In all other studies, tetO1B:: $\beta$ Actin-tTS + mice and their littermate controls—tetO1B:: $\beta$ Actin-tTS – mice were used. For the adult rescue groups, tetO1B:: $\beta$ Actin-tTS + mice were fed chow with doxycycline (DOX; 40 mg/kg, BioServ) beginning at PN60 in order to rescue expression of the 5-HT<sub>1B</sub>R in the adult mouse, as previously validated and reported (Nautiyal et al. 2015). All procedures were approved by the Institutional Animal Care and Use Committees of the New York State Psychiatric Institute or Dartmouth College.

A summary table of the mice used in these experiments is provided in Table S1. One group of male ( $N=23$ ) and female ( $N=35$ ) mice were used in goal-directed behavior, extinction, concurrent choice, and satiety-induced devaluation. One mouse was excluded from progressive ratio due to technical issues, and one mouse died prior to the test, resulting in  $N=22$  males and  $N=34$  females included in the final analysis for the progressive ratio. Subsets of the total group were used in satiety-induced devaluation experiments (males  $N=23$ , females  $N=18$ ) and Go/No-Go and delay discounting (males  $N=12$ , females  $N=8$ ). One mouse

was excluded from delay discounting due to not meeting criteria (see delay discounting methods below). A separate group of mice was used to test effort-based discounting (males  $N=7$ , females  $N=14$ ). Two mice were excluded from effort-based discounting due to not meeting criteria. A group of mice ( $N=19$ , all female) were used to test free consumption of evaporated milk diluted at different concentrations. Additional groups of naïve mice were used in the lickometer (males  $N=6$ , females  $N=5$ ) and Pavlovian-to-instrumental transfer (PIT; males  $N=6$ , females  $N=9$ ) studies. One mouse was excluded from the PIT study because of a failure to lever press. An additional naïve group of 12 control mice (males  $N=7$ , females  $N=5$ ) was used for the validation study of the novel Variable Value Go/No-Go paradigm. Finally, for the study of the role of the 5-HT<sub>1B</sub>R in the Variable Value Go/No-Go paradigm, an additional group of naïve mice (males  $N=14$ , females  $N=7$ ) was used to test the effect of 5-HT<sub>1B</sub>R expression. Three mice were removed due to not meeting criteria during lever training. A subset of the animals from this experiment (males  $N=13$ , females  $N=5$ ) were used to examine the effect of 5-HT<sub>1B</sub>R expression on chow consumption.

### Operant behavioral apparatus

Operant studies were conducted in eight identical chambers (Med Associates, St. Albans, VT) individually enclosed in ventilated isolation boxes. Each operant chamber consisted of stainless steel modular walls, and stainless steel bar floors. Each chamber contained a noseport receptacle for the delivery of liquid reward by a dipper (0.02 ml cup volume), with head entry detected by an infrared beam break detector. On either side of the noseport, the chamber contained two ultra-sensitive retractable stainless steel levers placed 2.2 cm above the chamber floor. In paradigms in which only one of the two levers was used, the lever was counterbalanced across mice and remained the same throughout all paradigms. There were LEDs located above each lever, and a houselight and speaker located on the upper portion of the wall opposite the levers. A computer equipped with MED-PC IV (Med Associates Inc., St Albans, VT) computer software delivered stimuli and collected behavioral data.

### Operant behavioral training

Operant training and testing were run 5–7 days a week. Mice were maintained at approximately 90% of their free-feeding weight. Water was provided ad libitum throughout the experiment. Undiluted evaporated milk was used as the reward for all operant studies in MedAssociates chambers. All mice were first trained to retrieve an evaporated milk reward through head entry into the receptacle, and then trained to press one of the two retractable levers to receive

the evaporated milk reward on a continuous reinforcement (CRF) schedule. Daily sessions ended when mice received a maximum of 60 rewards, or after 60 min elapsed if the maximum had not been reached. Mice were trained until the criterion of 55 lever presses in a 60-min session was reached. The mice were then trained on a random ratio (RR) schedule of escalating effort requirements (3 days of RR-5, 3 days of RR-10, 3 days of RR-20). The data from the last day on each schedule was analyzed. Subsequently, they were tested in extinction trials, concurrent choice, satiety-induced devaluation (a subset), and then progressive ratio. A subset of mice were then tested in Go/No-Go and delay discounting paradigms.

### Progressive ratios of responding

Following random ratio testing, mice were tested on a progressive ratio (PR) schedule for three consecutive days. A PRx2 schedule was used in which the number of lever presses required to receive a reward doubled following each reward. The session ended following either 2 h, or a 3-min period in which no lever presses were recorded (Drew et al. 2007). The total number of lever presses were summed over the session. One mouse was excluded from analysis due to technical problems with the operant box.

### Extinction testing

Mice were exposed to an RR-20 schedule of reinforcement for 3 days, before being tested in 3 consecutive days of extinction training. Mice were placed in the operant box with the lever extended; however, rewards were not administered. Lever presses and head entries were recorded for the duration of the 60-min extinction sessions.

### Concurrent choice

Following 3 days of RR-20 schedule of reinforcement, mice were placed in the operant box on each of 2 days with either freely available chow pellets or freely available evaporated milk in a cell culture dish. The lever of the operant box was also extended and was rewarding the mice with evaporated milk on a RR-20 schedule. These chow and milk conditions were counterbalanced across mice over the 2 days separated by a no choice RR-20 schedule day. Chow pellets and the dish of evaporated milk were weighed before and after the test session. Lever presses and head entries were recorded during the 60-min session.

### Satiety-induced devaluation

Following 3 days of RR-20 schedule of reinforcement, mice were prefed either chow or evaporated milk on each of 2 days, counterbalanced across mice. Mice were placed individually in a holding cage similar to their home cage for 1 h, and were free to consume an unlimited amount of either chow or evaporated milk presented in a cell culture dish. Chow pellets, the dish of evaporated milk, and the mice were weighed before and after the hour-long prefeeding session. Mice were then placed in the operant box and allowed to lever press for a RR-20 schedule of reinforcement. Lever presses and head entries were recorded during the 60-min session.

### Food consumption

Mice were temporarily housed in individual cages for measurement of food consumption with ad lib access to water. Mice were placed on the food restriction protocol 48 h prior to testing in the “food restricted” state testing, to mimic the food restriction state of the operant paradigms which consisted of 1.5 h free access to chow daily. Twenty-four hours following the 1.5 h free access, chow was returned to mice and intake was measured at 1 h, 3 h, and 24 h time points. Following this 24 h ad lib period, mice continued to have free access to chow for an additional 48 h prior to “sated” state testing, when intake was recorded for a 24-h period.

### Go/No-Go

Mice were trained and tested as previously described (Nautiyal et al. 2015). Briefly, following training on Go Trials, mice were presented with 7 daily sessions consisting of 30 discrete Go trials and 30 No-Go trials which were pseudo-randomly presented across blocks of 10 trials with a variable ITI averaging 45 s. In No-Go trials, the lever was presented simultaneously with 2 cues (the house lights turning off, and a small LED light above the lever turning on). A lever press during the 5-s trial caused the lever to retract, the house lights to turn on, the LED light to turn off, and a new ITI to begin without any reward for that trial. A lack of presses during the 5-s trial resulted in a reward presentation. The impulsivity index was calculated by subtracting the proportion of correct No-Go trials from the proportion of correct Go trials.

### Delay discounting

Mice were trained and tested as previously described (Nautiyal et al. 2017). Briefly, following training mice were presented with two levers for which presses resulted in either small or large (3 × volume) rewards. The large reward was

assigned to the lever which was initially least preferred by the mice, and remained consistent throughout the paradigm. Each daily session began with 10 forced choice trials (five on each lever randomly distributed) to ensure a minimum experience with each lever in each session, before presentation of 20 experimental choice trials. Mice were trained in 14 sessions with no delays on either lever. One mouse was eliminated because it did not meet the criteria of greater than 25% preference for the large lever averaged over the last 3 sessions. Subsequently, a delay was introduced after the large reward lever was pressed, before the reward was presented. There was no delay for the small reward, and time delays for the large reward (0, 2, 4, 6, 8, or 10 s) were presented in separate sessions with 3 days for each time delay, in ascending delay order. Data were used from the last session of each time delay. A linear equation was fit to the preference data for each mouse over all delays, and the slope, intercept, and indifference point (imputed delay at 50% preference) were calculated from the linear regression.

### Effort discounting

Mice were initially trained as described for the delay discounting paradigm by presenting two levers for which presses resulted in either small or large ( $3 \times$  volume) rewards. The large reward was assigned to the lever which was initially least preferred by the mice, and remained consistent throughout the paradigm. Each daily session began with 10 forced choice trials (five on each lever randomly distributed) to ensure a minimum experience with each lever in each session, before presentation of 20 experimental choice trials. Mice were trained in 14 sessions, after which two mice were eliminated because they did not meet the criteria of  $> 25\%$  preference for the large lever averaged over the last 3 sessions. Subsequently, the fixed ratio (FR) schedule was increased from FR1 for the large reward lever, with 3 days at each of the follow schedules: FR2, FR4, FR8, FR16, FR24, and FR32. The small reward lever remained at the FR1 schedule throughout the paradigm. Any single press to the small reward lever resulted in presentation of the small reward and termination of the trial. Percent preference for the large reward was calculated as the percentage of choice trials in which the large reward was obtained. Data from the last session at each FR schedule are presented and used for statistical analysis.

### Consumption of varied value rewards

Prior to testing in this paradigm, mice were previously exposed to evaporated milk in both consumption tests and 13 weeks of operant behavioral testing under food restriction (as described above) rewarded with 100% evaporated milk in a variety of reinforcement paradigms (data not shown).

For the reward testing, mice were placed individually in a cage and given 5-min free access to a small cell culture dish (Falcon, 35 mm  $\times$  10 mm) with varying concentrations of evaporated milk in a separate clean cage identical to their home cage. Milk concentration was varied across 5 days of testing, with 33%, 66%, 100%, 66%, and 33% on each day respectively (data was only analyzed for first 3 days because of anchoring effects on the descending concentration presentation). Mice were weighed immediately before and after testing to determine milk consumption during the session. Because of the potential inaccuracies in weighing the dishes due to milk spillage or bedding being pushed into the dishes, change in mouse weight was used to assay consumption over this short 5-min timeframe.

### Lickometer

A Davis Rig 16-bottle Lickometer (Med Associates MED-DAV-160 M) was used to test the effect of 5-HT<sub>1B</sub>R expression on reward reactivity to various concentrations of sucrose in sated and restricted conditions as described previously (Ostlund et al. 2013). Mice were water restricted for 5 days of initial training, during which mice were placed individually in the apparatus and allowed to drink water for 30 min from the spout which recorded licks using a capacitance-based system. Subsequently, mice were maintained on ad libitum water, except for the night before exposure to a new concentration of sucrose to promote maximal consumption for habituation to the new taste. Mice were exposed daily to sucrose in the testing chamber in a number of conditions, and licking behavior was recorded for 30 min. The order of exposure was 10% sucrose with water restriction (1 day), 10% sucrose with food restriction (2 days), and 10% sucrose sated (2 days). These conditions were then repeated in the same order with 2% sucrose. For food restricted conditions, mice were food deprived from the previous days' testing, and given 1 h free access to food following testing. For sated conditions, mice had ad lib access to food and water for at least 24 h. Conditions were run for two consecutive days to measure stability of licking within each set of parameters. There were no differences between any 2 days within the same condition, so data was averaged across the 2 days for analysis. The number of licks over the whole session and lick rate for the first 2 min were analyzed. The first 2-min lick rate was used as a way to assess hedonic component of licking behavior without the influence of post-ingestion satiety-related factors (Davis 1973; Glendinning et al. 2002).

### Pavlovian-to-instrumental transfer

Mice were tested in a modified Pavlovian-to-instrumental transfer protocol aimed at assessing the extent to which a Pavlovian conditioned stimulus (CS) can support the

acquisition of a novel instrumental response, as previously described (O'Connor et al. 2010). All mice were first trained to retrieve an evaporated milk reward through head entry into the reward receptacle of the Med Associates chambers for 5 sessions. Mice were then trained for 12 sessions in a Pavlovian conditioning phase in which a cue (conditioned stimulus, CS) was paired with an evaporated milk reward. In each session, mice experienced 20 CS + presentations (10 s 10 Hz click or white noise) in which a dipper containing evaporated milk reward was presented for 5 s following the cue onset. In each session, mice also experienced 20 presentations of a CS – with which no reward was associated. CSs were presented in a pseudo-random order, with variable ITIs averaging 60 s (30–90 s range). The conditioned stimuli of either a click or white noise were counterbalanced across mice. The number of nosepokes into the reward receptacle was analyzed during CS + and CS – presentations for all sessions with the immediately preceding 10 s of ITI responding subtracted out (elevation score). There was no instrumental conditioning phase, and so the instrumental transfer test was performed on the day following the 12th Pavlovian conditioning session. In the transfer test session, mice were presented with two levers extended for 45 min. A drop of evaporated milk reward was placed on each lever to promote lever pressing. Lever presses resulted in a 3-s presentation of either the CS + or CS –, but no reward was presented. The CS paired with the left or right lever was counterbalanced across mice and CS type. The number of presses to each lever was recorded, and grouped by association with CS + or CS – across mice. The difference score (CS + minus CS – lever presses) was calculated for each mouse.

### Variable value Go/No-Go paradigm

To assess the effect of reward value on impulsive action, we developed a novel paradigm based on the Go/No-Go Test of impulsive action. Mice were trained as described in Operant Behavioral Training, except CRF training took place with both levers extended such that pressing either lever provided reward. All mice initially sampled each lever. For the validation study, training continued for 6 days, by which point all mice had formed stable and strongly biased lever preferences, which was determined based on average percentage of presses during the final three days of CRF training (range 77 to 100%). For the experimental study, training continued for 7 days, with 3 mice being excluded from future testing due to not acquiring lever pressing behavior. The remaining mice again formed biased lever preferences (range 61 to 100%). In order to cause a reversal of their preference, the less preferred lever was then rewarded with three times the amount of evaporated milk reward compared to the more preferred lever, which remained at 0.02 ml evaporated milk. In order to deliver the larger, 0.06 ml reward, the dipper

was activated three times in short succession, as previously described. In these reversal sessions, mice were presented with 10 forced choice trials (5 per lever) in which only one lever was extended until the lever was pressed (requiring them to sample each lever), followed by 20 choice trials in which both levers were presented. Mice were required to reach a criterion of 25% choice for the higher reward lever (averaged over the final 3 days of training) in order to be included in future testing. After 14 sessions, mice in the validation study were choosing the high reward lever  $69 \pm 6\%$  of the time (averaged over the final 3 days of training). In the experimental study, 3 mice failed to reach the 25% criterion and were removed. With this exclusion, mice chose the higher reward lever  $57 \pm 3\%$  of the time (averaged over the final 3 days of training). Sixty trials were presented in each session, with 30 trials presented on each of the large and small reward levers randomly in blocks of 10 trials. In all trials, the lever extended for 5 s. A press within 5 s initiated reward delivery, and lever retraction (“Successful Go Trial”). Otherwise, the lever retracted after 5 s and no reward was delivered (“Unsuccessful Go Trial”). Finally, mice were exposed to 8 sessions in which No-Go trials were added such that there were 16 Go and 16 No-Go trials on each lever (64 total trials/session). In No-Go trials, the lever was presented simultaneously with 2 cues (the house lights turning off, and a small LED light above the lever turning on). A lever press during the 5-s trial caused the lever to retract, the house lights to turn on, the LED light to turn off, and a new ITI to begin without any reward for that trial (“Unsuccessful No-Go Trial”). A lack of presses during the 5-s trial resulted in a reward presentation (“Successful No-Go Trial”). Hit rate was calculated as the proportion of Successful Go trials and the false alarm rate as the proportion of Unsuccessful No-Go trials, respectively averaged over all days. Impulsivity index was calculated for small and large reward levers by subtracting the proportion of correct No-Go trials from the proportion of correct Go trials. This composite index has a maximum score of + 1, which indicates highest impulsive responding (always responding on Go and No-Go trials). The minimum score of – 1 indicates lowest impulsive behavior, essentially never responding on either No-Go or Go trials. The latency to lever press was also recorded for each trial and averaged across days; the latency was recorded as the maximum 5 s if there was no lever press during the trial.

### Statistical analysis

Group effects were evaluated using analysis of variance (ANOVA), with post hoc Fisher's PLSD in StatView (SAS Software, Cary, NC) or SPSS (IBM, Armonk, NY). When pairwise comparisons were made following the primary ANOVAs, one-way ANOVAs were first used to test

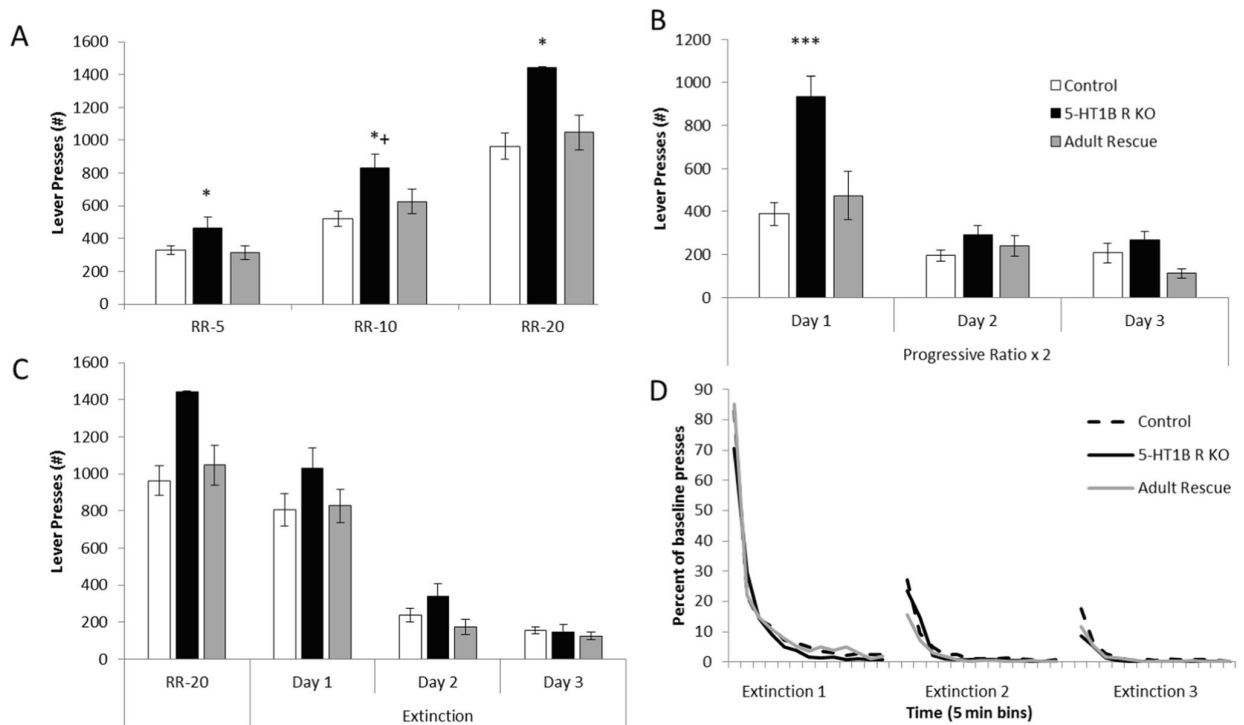
group effects within conditions when there was a significant interaction in the repeated measures ANOVAs, followed by post hoc Fisher's LSD if there was an effect of 5-HT<sub>1B</sub>R expression. Two-way repeated measures ANOVAs were used to assess the effects of 5-HT<sub>1B</sub>R (control, no expression, rescued expression) on concurrent choice and devaluation (5-HT<sub>1B</sub>R expression  $\times$  condition [evaporated milk or standard chow]), random ratio (5-HT<sub>1B</sub>R expression  $\times$  RR schedule), progressive ratio (5-HT<sub>1B</sub>R expression  $\times$  3 days), and extinction (5-HT<sub>1B</sub>R expression  $\times$  3 days). For all remaining experiments, 5-HT<sub>1B</sub>R expression levels only included whole life knockout and control. Two-way repeated measures ANOVAs were also used for the standard Go/No-Go (5-HT<sub>1B</sub>R expression  $\times$  10 days), delay discounting (5-HT<sub>1B</sub>R expression  $\times$  delay), and effort discounting (5-HT<sub>1B</sub>R expression  $\times$  FR schedule). For delay discounting, a linear equation was also fit to data from each mouse. The slope, intercept, and fit ( $r^2$ ) were compared between groups using unpaired  $t$ -tests. The indifference point, defined as the time delay when the preference was 50% was calculated for each mouse based on the linear equation, and compared between groups using an unpaired  $t$ -test. For the lickometer tests of hedonic value/reward reactivity, three-way mixed ANOVAs were used to determine the effects of 5-HT<sub>1B</sub>R expression, sucrose concentration, and fed state (restricted or sated). A two-way mixed ANOVA was used to analyze the effect of CS and genotype on nose poking during the Pavlovian training in the PIT paradigm, and an unpaired  $t$ -test was used to compare the difference score between genotypes on the instrumental transfer test. Two-way mixed ANOVAs were used for the effect of reward value on impulsivity in the Variable Value Go/No-Go paradigm validation study (reward size  $\times$  10 days for impulsivity index; reward size  $\times$  trial type for hit rate/false alarm rate and latencies). Three-way mixed ANOVAs were used to assess the effect of reward value and 5-HT<sub>1B</sub>R expression manipulation on impulsivity in the experimental Variable Value Go/No-Go paradigm (5-HT<sub>1B</sub>R expression  $\times$  reward size  $\times$  10 days for impulsivity index; 5-HT<sub>1B</sub>R expression  $\times$  reward size  $\times$  trial type for hit rate/false alarm rate and latencies). Mixed ANOVAs, as appropriate, were also used to assess the interaction of sex with these variables. For food consumption, sex was found to have a significant effect; therefore, data was analyzed with a three-way mixed ANOVA for the effects of 5-HT<sub>1B</sub>R expression, time, and sex in the restricted condition, and a two-way ANOVA for the effects of 5-HT<sub>1B</sub>R expression and sex in the sated condition. There were no significant effects of sex on the remaining behaviors measured, and therefore, the sexes are combined for all other analyses presented.

## Results

Mice lacking 5-HT<sub>1B</sub>R expression showed increased responding on operant lever pressing including on random ratio and progressive ratio schedules, which was also interestingly reversed by adult rescue of receptor expression. For random ratio schedules, 5-HT<sub>1B</sub>R influenced the number of presses—mice lacking 5-HT<sub>1B</sub>R expression pressed 40–50% more than control and adult rescue mice (Fig. 1A;  $F_{2,55}=8.0$ ,  $p=0.0009$ ). As the effort requirements increased, the effect of 5-HT<sub>1B</sub>R expression on lever pressing became larger ( $F_{4,110}=3.4$ ,  $p=0.0118$  for interaction;  $F_{2,110}=182.4$ ,  $p<0.0001$  for main effect of schedule). This suggests that the increased responding is related to goal-directed or motivated responding, rather than a general increase in activity which would likely be read out as increased responding equivalently across all schedules. Adult rescue of receptor expression rescued normal behavior which points to an online adult rather than developmental or compensatory mechanism of action (all post hoc  $p>0.05$  for all schedules for adult rescue vs. controls).

To assess motivation, we used a progressive ratio (PR $\times$ 2) schedule of responding and found that the absence of 5-HT<sub>1B</sub>R increased lever pressing, which was also reversed by adult rescue (Fig. 1B;  $F_{2,53}=7.3$ ,  $p=0.0016$ ). There were also significant effects of day ( $F_{2,106}=70.1$ ,  $p<0.0001$ ) and 5-HT<sub>1B</sub>R expression  $\times$  day interaction ( $F_{4,106}=10.2$ ,  $p<0.0001$ ). Curiously, the effect of 5-HT<sub>1B</sub>R on lever pressing was only present on Day 1 (for post hoc comparisons of genotype,  $F_{2,53}=12.0$ ,  $p<0.0001$ ;  $p<0.0001$  for control and  $p=0.0008$  for adult rescue compared to 5-HT<sub>1B</sub>R KO,  $p=0.4237$  for control vs. adult rescue), and not on Days 2 or 3 ( $F_{2,53}<2.1$ ,  $p>0.1378$ ).

One interpretation of the increased responding on the first day, but not subsequent days of testing in the PR is that 5-HT<sub>1B</sub>R KO mice show faster extinction resulting in lower responding after Day 1. To test this idea, we measured extinction of lever pressing behavior in non-rewarded sessions following RR-20 training. Over 3 days of extinction sessions, while all mice decreased lever pressing ( $F_{2,110}=153.9$ ,  $p<0.0001$ ), there were no significant effects of 5-HT<sub>1B</sub>R expression on number of lever presses (Fig. 1C;  $F_{2,55}=1.5$ ,  $p=0.2376$  for main effect of 5-HT<sub>1B</sub>R expression;  $F_{4,110}=1.3$ ,  $p=0.2705$  for interaction). The number of lever presses was also normalized to baseline lever pressing behavior to control for the higher starting point in mice lacking 5-HT<sub>1B</sub>R expression, and there were still no differences in extinction rates between groups (Fig. 1D;  $F_{2,55}=1.6$ ,  $p=0.2040$  for main effect of 5-HT<sub>1B</sub>R expression). This suggests that the behavioral pattern seen in the progressive ratio task is not due to differences in extinction rate.



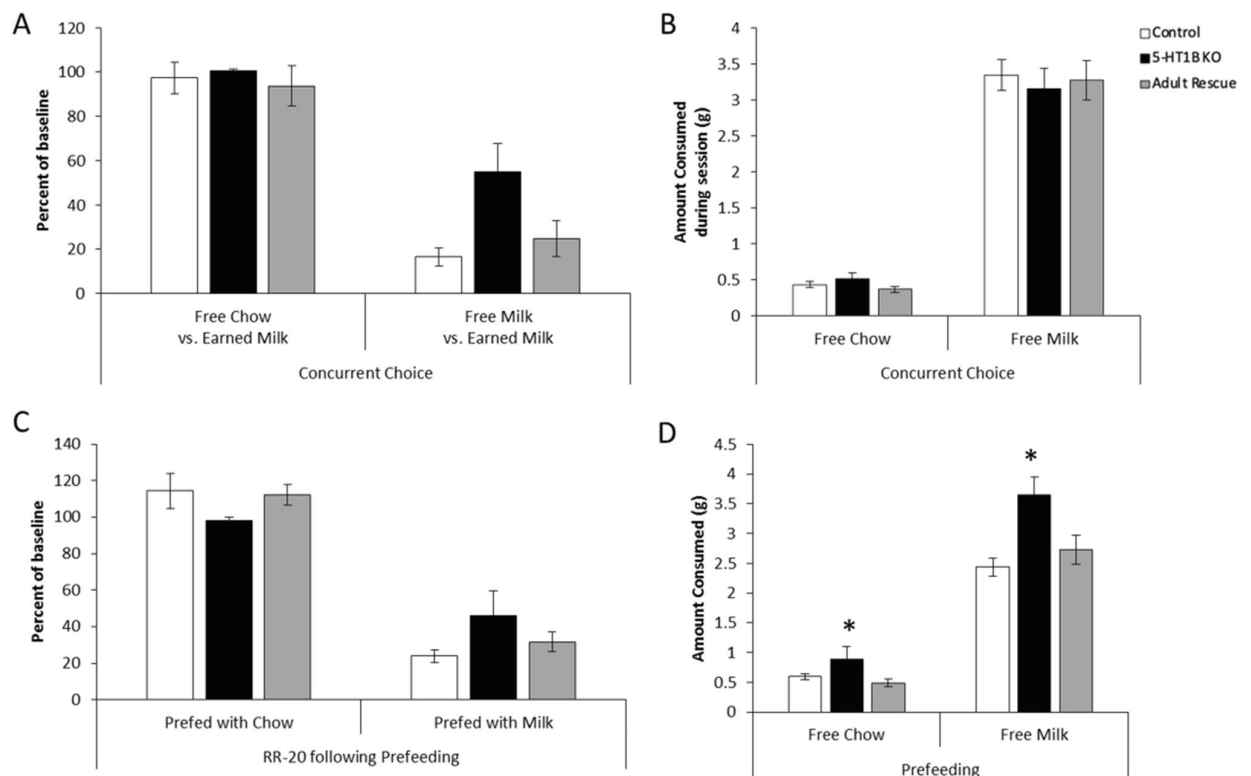
**Fig. 1** Lack of 5-HT<sub>1B</sub>R increases motivated responding. **A** Number of lever presses are shown during random ratio 5, 10, and 20 schedules of reinforcement. \* $p < 0.05$ , 5-HT<sub>1B</sub>R KO compared to control and adult rescue groups for RR-5 and RR-20. \*+ $p < 0.05$ , 5-HT<sub>1B</sub>R KO compared to control group, and  $p = 0.054$  for 5-HT<sub>1B</sub>R KO vs. adult rescue for RR-10. **B** Number of lever presses are shown for a progressive ratio  $\times 2$  schedule of reinforcement, presented over three

consecutive days. \*\*\* $p < 0.001$  and  $p = 0.0008$  for 5-HT<sub>1B</sub>R KO compared to control and adult rescue groups. **C** Lever presses shown during 3 extinction sessions, compared to the previous RR-20 session. **D** Percentage of presses from RR-20 baseline, during 3 sessions of extinction trials, binned by 5 min. All data shown are group means  $\pm$  SEM

To further investigate effort-based decision making, we used a concurrent choice task in which mice were provided with a choice between freely available food/reward in the operant chamber or lever pressing for evaporated milk (Fig. 2A). There was an effect of 5-HT<sub>1B</sub>R expression on this effort-based operant task, with mice lacking the receptor continuing to press more despite having a freely available option ( $F_{2,55} = 3.6$ ,  $p = 0.0345$ ). While all mice decreased their lever pressing behavior when the freely available option was evaporated milk compared to chow ( $F_{1,55} = 106.1$ ,  $p < 0.0001$ ), mice lacking 5-HT<sub>1B</sub>R expression continued pressing at 55% of their baseline rate despite concurrent access to freely available evaporated milk in the operant chamber, while control mice and mice with adult rescue of receptor expression reduced their pressing to 17% and 25% of their baseline rates, respectively (interaction term approaching significance  $F_{2,55} = 2.8$ ,  $p = 0.069$ ). All mice continued to lever press at high rates for evaporated milk when the freely available option was chow (average 97% of baseline). There were no group differences in the consumption of the freely available reward, though all mice consumed

more milk than food (Fig. 2B;  $F_{2,55} = 0.1$ ,  $p = 0.9187$  for main effect of 5-HT<sub>1B</sub>R expression;  $F_{1,55} = 325.8$ ,  $p < 0.0001$  for main effect of freely available option;  $F_{2,55} = 0.3$ ,  $p = 0.7535$  for interaction). Taken together, these results suggest that 5-HT<sub>1B</sub>R expression could influence either the representation of the outcome value that guides goal-directed action or habitual-like responding.

We first tested the hypothesis that mice lacking 5-HT<sub>1B</sub>R respond more habitually and are less guided by the outcome/goal of their actions. To do this, we measured goal-directed behavior following satiety-induced devaluation of the reward. There were no significant effects of 5-HT<sub>1B</sub>R expression in this test of habitual-like behavior. All mice similarly reduced responding when pre-fed with evaporated milk reward, but not when pre-fed with chow, showing that mice were responding in a similar goal-directed, rather than habitual manner on the RR-20 schedule (Fig. 2C;  $F_{2,38} = 0.1$ ,  $p = 0.8929$  for main effect of 5-HT<sub>1B</sub>R expression;  $F_{1,38} = 89.3$ ,  $p < 0.0001$  for main effect of prefed option;  $F_{2,38} = 2.1$ ,  $p = 0.1318$  for interaction). Furthermore, this suggests that the increased lever pressing behavior in



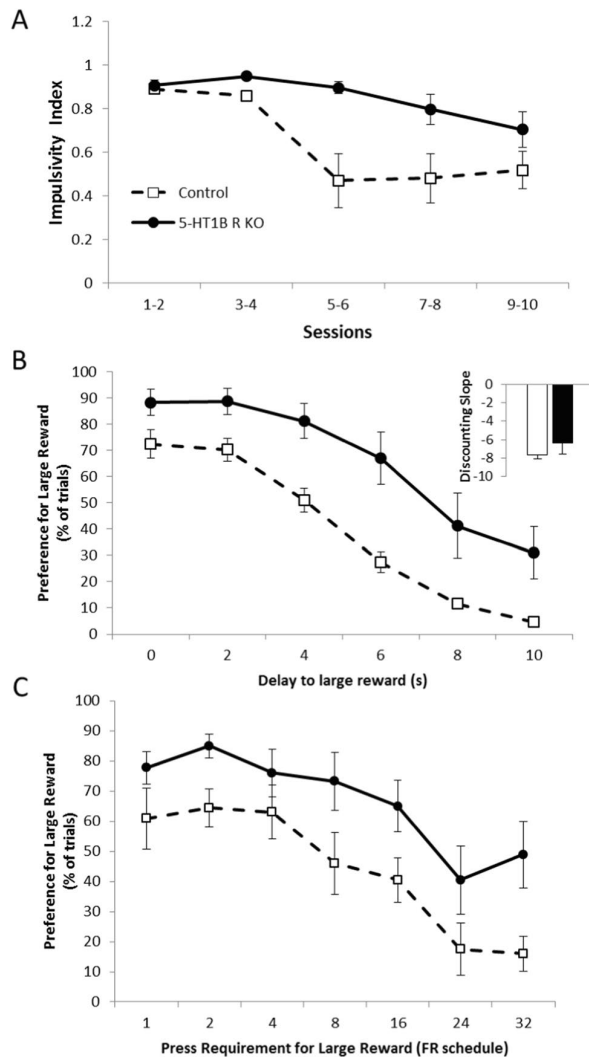
**Fig. 2** Effects of 5-HT<sub>1B</sub>R on habitual and goal-directed responding. **A** Lever presses are shown as a percentage of a total presses from a baseline RR-20 schedule in conditions in which chow or evaporated milk were presented as free alternatives to lever pressing for evaporated milk. **B** The amount of free alternative chow or evaporated milk that was consumed during the operant session is shown. **C** Lever presses are shown as a percentage of a total presses from a base-

line RR-20 schedule in conditions in which mice were prefed chow or evaporated milk before the operant test session. **D** The amount of chow or evaporated milk that was consumed during the prefeeding session prior to operant session is shown. \* $p < 0.05$ , 5-HT<sub>1B</sub>R KO compared to control and adult rescue groups. All data shown are group means  $\pm$  SEM

mice lacking 5-HT<sub>1B</sub>R in the concurrent choice paradigm is likely not a function of increased habitual-like responding, but rather potentially due to altered representations of the reward value. This is also supported by an increased intake in the pre-operant test satiety induction with mice lacking 5-HT<sub>1B</sub>R expression consuming more reward in the pre-operant feeding sessions, with the increase being larger in the milk compared to the chow condition (Fig. 2D;  $F_{2,38} = 7.5$ ,  $p = 0.0018$  for main effect of 5-HT<sub>1B</sub>R expression;  $F_{1,38} = 343.9$ ,  $p < 0.0001$  for main effect of prefed option;  $F_{2,38} = 5.1$ ,  $p = 0.0106$  for interaction; for post hoc pairwise comparisons of genotype, all  $ps > 0.05$  for control vs. adult rescue, all  $ps < 0.05$  for 5-HT<sub>1B</sub>R vs. control and adult rescue). Overall these results suggest that the behavioral differences seen in mice lacking 5-HT<sub>1B</sub>R are not likely due to increased habitual-like responding.

As previously shown, a lack of 5-HT<sub>1B</sub>R expression increases impulsive action, but not impulsive choice. Specifically, mice lacking the 5-HT<sub>1B</sub>R receptor showed increased impulsive action in the Go/No-Go task (Fig. 3A), as

measured by a reduced ability to inhibit behavioral responding on No-Go trials ( $F_{1,18} = 7.0$ ,  $p = 0.0167$ ). While they showed some improvement in their ability to inhibit level presses on No-Go trials over 10 training sessions, this was slower and reduced compared to control mice ( $F_{9,162} = 3.1$ ,  $p = 0.0017$  for interaction;  $F_{9,162} = 8.1$ ,  $p < 0.0001$  for main effect of session). We also used a delay discounting paradigm as a second test of impulsivity aimed at measuring the impulsive choice dimension (Fig. 3B). There was a significant main effect of delay ( $F_{5,85} = 62.6$ ,  $p < 0.0001$ ) as well as an effect of 5-HT<sub>1B</sub>R expression on preference for the large reward ( $F_{1,17} = 12.4$ ,  $p = 0.0026$ ), with no interaction between 5-HT<sub>1B</sub>R expression and delay ( $F_{5,85} = 1.6$ ,  $p = 0.1649$ ). Therefore, interestingly, mice lacking 5-HT<sub>1B</sub>R expression did not show increased choice impulsivity, but rather an overall increase in preference for the large reward. This is represented by an increased indifference point ( $t_{17} = 2.4$ ,  $p = 0.0281$ )—the delay length at which small immediate and large delayed rewards are chosen equally, 11.1 s ( $\pm 3.9$  s) in mice lacking 5-HT<sub>1B</sub>R, compared to 2.5 s



**Fig. 3** Absence of 5-HT<sub>1B</sub>R increases impulsive action but not delay or effort-based discounting. **A** Impulsivity index calculated as the proportion of successful Go trials minus the proportion of successful No-Go trials is shown as a measure of impulsive action (1.0 is the highest impulsivity that a mouse can display) over 10 days presented in 2-day bins. **B** Data from a delayed discounting paradigm are shown as the percentage of trials on which the large (delayed) reward was chosen, represented over delays ranging from 0 to 10 s. Inset shows discounting slope, with more negative slopes indicating a more impulsive choice behavior. **C** Performance on an effort-based discounting task is shown for mice lacking 5-HT<sub>1B</sub>R and controls as a percentage of trials in which the large reward was chosen, represented over effort requirements ranging from fixed-ratio (FR)1 to FR32 schedules. All data are shown as group means  $\pm$  SEM

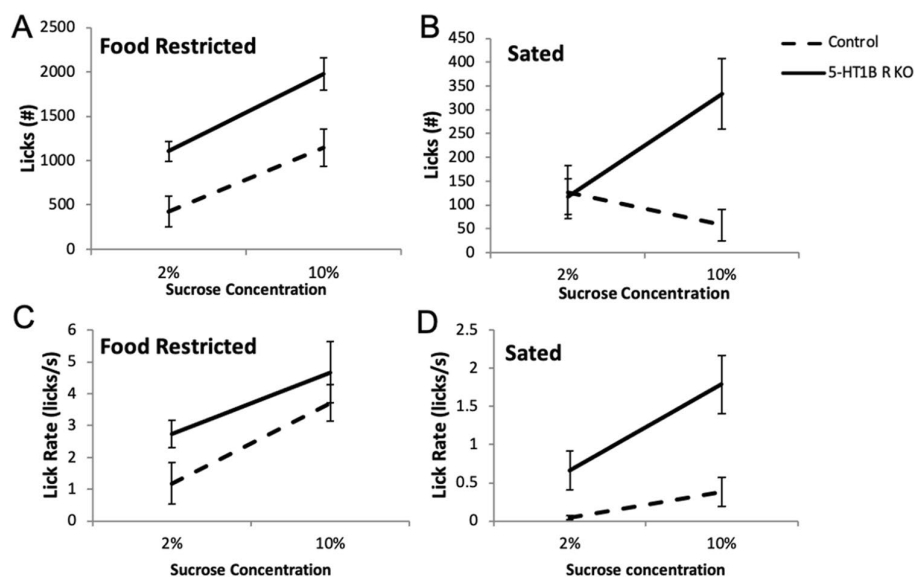
( $\pm 1.0$  s) in controls. However, the increased preference for the large reward was seen across all delays with no group differences in the slope of the discounting function ( $t_{17} = 1.1$ ,  $p = 0.2991$ ), suggesting that the effect of 5-HT<sub>1B</sub>R is not on impulsivity. Rather, the overall increase in preference for the

large reward across all delays is shown by an upward shift in the discounting curve ( $t_{17} = 2.0$ ,  $p = 0.0576$  for trend toward change in intercept) suggesting that the effect of 5-HT<sub>1B</sub>R on choice may be due to changes in valuation of the reward.

To assess if the increased preference for the large reward was unique to delays or could be seen more generally in reward value-decision making, we tested the behavior of mice lacking 5-HT<sub>1B</sub>R in an effort-based discounting task. A similar pattern to the delay discounting data emerged—namely that mice lacking 5-HT<sub>1B</sub>R expression showed increased preference for the large reward, over all effort requirements (Fig. 3C;  $F_{1,17} = 5.8$ ,  $p = 0.0273$  for main effect of 5-HT<sub>1B</sub>R expression;  $F_{6,102} = 19.9$ ,  $p < 0.0001$  for main effect of effort requirement;  $F_{6,102} = 0.6$ ,  $p = 0.6928$  for interaction). As seen in the delay discounting paradigm, there was no significant difference between groups in the slope of the discounting function ( $t_{17} = 0.9$ ,  $p = 0.3754$ ), suggesting that the 5-HT<sub>1B</sub>R does not influence effort-based discounting, but rather might alter baseline reward value scaling.

Next, we addressed the hypothesis that an exaggerated representation of outcome value could arise from a difference in hedonic reactions to the reward. First, we measured amount of consumption to varied concentrations of evaporated milk reward used in operant tests (Fig S1). We found that mice lacking 5-HT<sub>1B</sub>R consume more evaporated milk than controls and increase their consumption as the reward concentration goes up, suggesting that they scale reward value differently ( $F_{1,17} = 5.9$ ,  $p = 0.027$  for main effect of 5-HT<sub>1B</sub>R expression;  $F_{2,34} = 15.2$ ,  $p < 0.001$  for main effect of concentration;  $F_{2,34} = 4.4$ ,  $p = 0.020$  for interaction; for post hoc pairwise comparisons of genotype,  $p < 0.01$  at 66% milk concentration). Next, in order to test the effect of 5-HT<sub>1B</sub>R on hedonic value more directly, we used a standard lickometer to examine licking behavior to different concentrations of sucrose (Berridge and Robinson 2003; Dwyer 2012). The lickometer reduces some of the motivational components required for operant-based tasks, and also eliminates the contribution of post-ingestive factors found in consumption tests, therefore allowing measurement of a more immediate hedonic reaction through analysis of licking behavior. Mice lacking 5-HT<sub>1B</sub>R expression showed overall increased in hedonic reactivity, as measured by increased licking for sucrose compared to controls (Fig. 4A,B;  $F_{1,9} = 12.0$ ,  $p = 0.007$ ). Across different conditions, mice lacking 5-HT<sub>1B</sub>R also showed greater increases in total licks as the motivational state or value increased suggesting that these mice were scaling reward value differently ( $F_{1,9} = 8.6$ ,  $p = 0.016$  for interaction of 5-HT<sub>1B</sub>R expression  $\times$  fed state;  $F_{1,9} = 4.8$ ,  $p = 0.056$  for suggestive interaction of 5-HT<sub>1B</sub>R expression  $\times$  concentration). For all mice, there were main effects of deprivation state (food restricted vs. sated;  $F_{1,9} = 89.9$ ,  $p < 0.001$ ) and sucrose concentration (2% vs. 10%,  $F_{1,9} = 76.7$ ,  $p < 0.001$ ), as well as a

**Fig. 4** 5-HT<sub>1B</sub>R expression influences hedonic valuation. Total number of licks to a spout delivering sucrose, shown in food restricted (A) and sated (B) conditions to 2% and 10% sucrose. Lick rate in the first 2 min of the session, for food restricted (C) and sated (D) conditions to 2% and 10% sucrose. All data are shown as group means  $\pm$  SEM

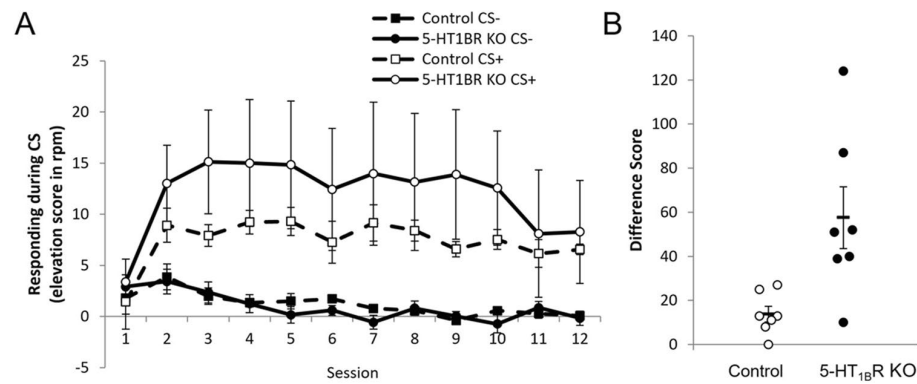


fed state  $\times$  concentration ( $F_{1,9}=85.9$ ,  $p<0.001$ ), with mice licking more in the restricted and 10% sucrose conditions. We also examined lick rate during the first 2 min of the sessions to remove any potential confound of effects of satiety on hedonic readouts since brief access durations reduce non-taste effects (Davis 1973; Glendinning et al. 2002). Again, all groups had increased lick rates toward 10% sucrose ( $F_{1,9}=35.8$ ,  $p<0.001$  for main effect) and in the restricted condition ( $F_{1,9}=41.2$ ,  $p<0.001$  for main effect), with the increase being the largest in the restricted 10% condition ( $F_{1,9}=11.5$ ,  $p=0.008$  for concentration  $\times$  fed state interaction). Mice lacking 5-HT<sub>1B</sub>R expression showed increased lick rates that approached significance (Fig. 4C,D;  $F_{1,9}=4.3$ ,  $p=0.069$ ). Together, these results show that mice lacking 5-HT<sub>1B</sub>R expression have exaggerated licking responses compared to controls across all conditions, though importantly maintain the normal relative changes based on motivational state and concentration. This suggests that the absence of the 5-HT<sub>1B</sub>R acts to shift the scale of the normal valuation of reward.

We also addressed the possibility that the effects of 5-HT<sub>1B</sub>R expression were due to increased feeding drive rather than specific to reward responsivity. There were no significant differences in chow intake between 5-HT<sub>1B</sub>R KO and littermate control mice in either restricted (Fig S2A;  $F_{1,14}=0.4$ ,  $p=0.555$  for 5-HT<sub>1B</sub>R expression;  $F_{2,28}=0.3$ ,  $p=0.758$  for 5-HT<sub>1B</sub>R expression  $\times$  time interaction) or sated conditions (Fig S2B;  $F_{1,14}=0.4$ ,  $p=0.524$ ). All mice consume more food over longer periods of time in a deprived state ( $F_{2,28}=463.8$ ,  $p<0.001$ ), with males consuming more in general and increasing with length of time ( $F_{1,14}=10.3$ ,  $p=0.006$  for sex;  $F_{2,28}=14.6$ ,  $p<0.001$  for sex  $\times$  time interaction). This effect of increased consumption in males also

occurred in the 24-h sated period ( $F_{1,14}=11.9$ ,  $p=0.004$ ). Importantly, there was no interaction of 5-HT<sub>1B</sub>R expression and sex in either experiment ( $F_{1,14}=0.1$ ,  $p=0.725$  and  $F_{1,14}=0.1$ ,  $p=0.725$ ). These results suggest that the increase in reward-motivated behaviors seen in the absence of 5-HT<sub>1B</sub>R is not due to a general increase in hunger or feeding, and thus lends support to our interpretation that 5-HT<sub>1B</sub>R influences the valuation of palatable rewards.

So far, we have shown that mice lacking 5-HT<sub>1B</sub>R respond more vigorously to palatable rewards. We suggest that the exaggerated hedonic responses may be the result of higher value representations of reward, which also serve to increase goal-directed behavior relative to controls. We performed a modified Pavlovian-to-instrumental transfer (PIT) study to assess if a reward-paired cue motivates instrumental responding differently in the absence of 5-HT<sub>1B</sub>R expression. During the initial associative learning phase, all mice learned to discriminate between cues as measured by increased head entries into reward receptacle during the CS + compared to the CS - (Fig. 5A;  $F_{11,132}=4.5$ ,  $p<0.001$  for main effect of session;  $F_{1,12}=10.6$ ,  $p=0.007$  for main effect of CS type;  $F_{11,132}=6.4$ ,  $p<0.001$  for session  $\times$  CS type interaction). There were no significant effects of genotype during the Pavlovian training phase ( $F_{1,12}=0.8$ ,  $p=0.401$  for main effect;  $F_{11,132}=0.5$ ,  $p=0.922$  and  $F_{1,12}=1.0$ ,  $p=0.343$  for interaction of genotype with session or CS type, respectively;  $F_{11,132}=0.7$ ,  $p=0.709$  for genotype  $\times$  session  $\times$  CS type interaction). However, mice lacking 5-HT<sub>1B</sub>R expression displayed higher levels of lever pressing for the CS + in the instrumental transfer test compared to controls (Fig. 5B;  $t_{12}=3.1$ ,  $p=0.0107$ ). Importantly, the transfer test was performed in the absence of any prior instrumental training, highlighting the role of



**Fig. 5** Lack of 5-HT<sub>1B</sub>R expression results in increased responding in a modified Pavlovian-to-instrumental transfer test. **A** Head entries into the reward receptacle are shown for 12 sessions of Pavlovian training represented as group means ( $\pm$  SEM) of the increase in the number responses per minute (rpm) during the CS compared to rpm during 10 s of the ITI immediately preceding the CS. **B** Performance

on the instrumental transfer test is shown as the increase in the number of lever presses for the CS+ over the number of lever presses for the CS− (difference score). Each animal is represented (open circle, controls; solid circle, 5-HT<sub>1B</sub>R KO mice), as well as the group means ( $-$ )  $\pm$  SEM

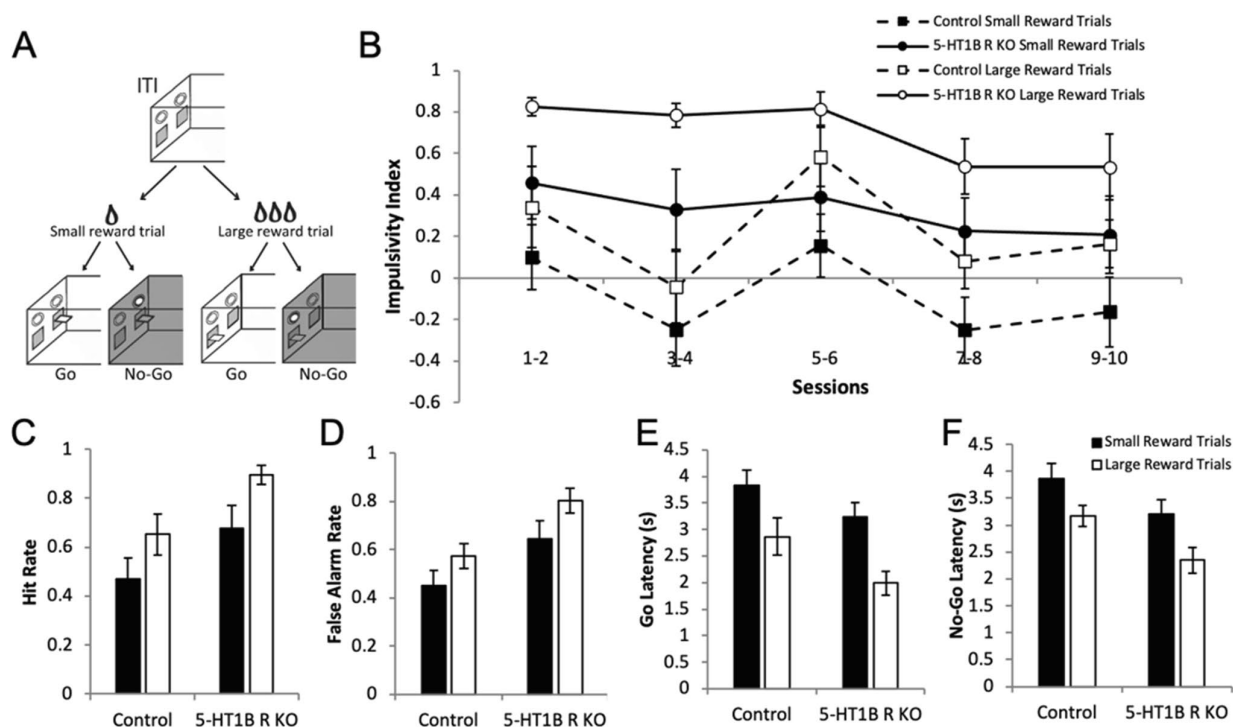
the CS+ in promoting acquisition of instrumental behavior, rather than a potentiation of a previously learned response–outcome association. This suggests that the value attributed to the CS+ motivates instrumental responding more in the absence of 5-HT<sub>1B</sub>R expression.

Given the effects of 5-HT<sub>1B</sub> on driving reward associated cue-motivated behavior, we next tested if alterations in reward valuation could also explain the increased impulsivity seen in mice lacking 5-HT<sub>1B</sub>R expression. We first developed and tested a novel paradigm—the Variable Value Go/No-Go, to directly examine how manipulating reward value could impact impulsive action on a trial-by-trial basis (Fig. 6A). By varying reward value in a Go/No-Go paradigm within a single session, we could compare impulsivity within mice between trials in which large or small rewards were expected. In control mice, we found that mice were more impulsive on large compared to small reward trials (Fig. 6B;  $F_{1,16} = 19.1$ ,  $p < 0.001$  for main effect of reward size) which decreases over days ( $F_{9,99} = 7.0$ ,  $p < 0.001$  for main effect of days;  $F_{9,99} = 2.2$ ,  $p = 0.025$  for interaction of reward  $\times$  days). The increased impulsivity index in the large reward condition was influenced by both more correct Go trials and more incorrect No-Go trials ( $F_{1,11} = 19.1$ ,  $p = 0.001$  for main effect of reward size), with faster responding on large reward trials ( $F_{1,11} = 9.5$ ,  $p = 0.010$ ). Using this novel paradigm, we were then able to investigate the role of increased reward valuation in 5-HT<sub>1B</sub>R-induced deficits in impulsive action, and test whether the increased impulsivity in mice lacking 5-HT<sub>1B</sub>R expression could be ameliorated by decreasing reward value. Our results show that the increased impulsivity in mice lacking 5-HT<sub>1B</sub>R was ameliorated, in part, by reducing the reward value by three times. Specifically, behavior on small reward trials in mice lacking 5-HT<sub>1B</sub>R

was similar to that of high reward trials in controls (Fig. 6B;  $F_{1,16} = 6.3$ ,  $p = 0.023$  for main effect of 5-HT<sub>1B</sub>R expression;  $F_{1,16} = 25.9$ ,  $p < 0.001$  for main effect of reward size). Overall, both a lack of 5-HT<sub>1B</sub>R expression and a larger reward magnitude increased impulsivity as seen in false alarm rates and hit rates (Fig. 6C,D;  $F_{1,16} = 6.3$ ,  $p = 0.023$  for main effect of 5-HT<sub>1B</sub>R expression;  $F_{1,16} = 25.9$ ,  $p < 0.001$  for main effect of reward size). These effects could also be read out by decreased response latencies in mice lacking 5-HT<sub>1B</sub>R and in controls on large reward trials (Fig. 6E,F;  $F_{1,16} = 4.7$ ,  $p = 0.046$  for main effect of 5-HT<sub>1B</sub>R expression;  $F_{1,16} = 46.7$ ,  $p < 0.001$  for main effect of reward size). Interestingly, this shows that 5-HT<sub>1B</sub>R-associated impulsivity can be reduced by decreasing the reward value and suggests that alterations in reward value alone can lead to increased impulsivity. Our data suggest that reward reactivity is an important behavioral component to measure in the study of the neural circuits underlying impulsivity, and point to a behavioral mechanism through which serotonin influences impulsive action.

## Discussion

Overall, our data points to a role for altered reward value representation in the serotonin modulation of impulsive behavior. Specifically, we show that 5-HT<sub>1B</sub>R expression influences goal-directed behavior, motivation, PIT, and hedonic valuation, along with effects on impulsive action, but not impulsive choice. We also tested a subset of these phenotypes with adult rescue of 5-HT<sub>1B</sub>R expression which rescued normal behavior, suggesting ongoing modulation of neural circuits rather than compensatory effects.



**Fig. 6** Decreasing reward value ameliorates 5-HT<sub>1B</sub>R-related impulsivity. **A** Diagram of Variable Value Go/No-Go paradigm. **B** Impulsivity index calculated as the proportion of successful Go trials minus the proportion of successful No-Go trials is shown as a measure of impulsive action (1.0 is the highest impulsivity that a mouse can dis-

play) over 10 days presented in 2-day bins. Data is shown for each small and large reward trials for controls and 5-HT<sub>1B</sub>R KOs. **C** Hit rate for Go trials and **D** false alarm rate for No-Go trials. **E** Latency to press the lever for Go trials and for **F** No-Go trials. All data are shown as group means ± SEM

While, we have previously shown that 5-HT<sub>1B</sub>R receptor expression influences impulsive action during adulthood, we now provide a behavioral mechanism of action. First, mice lacking 5-HT<sub>1B</sub>R expression show increases in hedonic responses to sucrose, compared to controls. We propose that this may be a readout of increased valuation of rewards. This interpretation is consistent with a recent study which illustrates the influence of 5-HT<sub>1B</sub>R on the representation of outcomes through changes in sensitivity to the sensory qualities of reinforcers (Corbit et al. 2019). Interestingly, in our studies, mice lacking 5-HT<sub>1B</sub>R expression also show increased goal-directed responding, which is sensitive to extinction and devaluation, and we propose that this is driven by increased valuation of the reward. This is supported by increased responding seen in the PIT study in 5-HT<sub>1B</sub>R KO mice, which suggests that a higher attribution of value to the CS+ (acquired during the Pavlovian training) motivates higher levels of instrumental responding. Taken together with the effects of 5-HT<sub>1B</sub>R on impulsive action, these data point to the possibility that the influence of 5-HT<sub>1B</sub>R on reward valuation may contribute to the effects on goal-directed behavior and motivation, as well as on impulsive action.

Previous studies in humans and animal models have examined the relationship between hedonic value and impulsivity (Anker et al. 2008; Mechelmans et al. 2017; Weafer et al. 2014). In rats, increased sucrose-seeking is associated with increased impulsive action (measured in the 5-choice serial reaction time task) (Diergaarde et al. 2009), and rats bred for high sucrose consumption displayed higher levels of impulsive action (on the Go/No-Go task) when responding for cocaine (Anker et al. 2008), and also higher levels of impulsive choice (on the delay discounting task) (Perry et al. 2007). Though in humans, one study showed that increased hedonic value measured with varying sweet concentrations is associated with increases in impulsive choice (assessed in a delay discounting task), but not impulsive action (measured in a Go/No-Go paradigm) (Weafer et al. 2014). However, a confound in the interpretation of many of these studies suggesting associations between reward value and impulsivity arises from between-subjects designs measuring more trait-like phenotypes. This leaves open the possibility for another trait-level behavioral construct to mediate the association between reward value and impulsivity (e.g., learning about appetitive goal-directed behavioral contingencies). In order to test the causal association of higher

valued incentive stimuli leading to increased impulsivity, we developed a within-subject, within-session experiment varying reward value, and could therefore directly measure the effects on impulsive action in the Go/No-Go task. The results from this Variable Value Go/No-Go paradigm show that increased reward value causes increased impulsive action as measured by a decrease in behavioral inhibition in No-Go trials. This supports a causal role for reward value in impulsive action. Furthermore, we were able to increase the impulsivity in controls to similar levels to that seen in mice lacking 5-HT<sub>1B</sub>R by tripling the reward value. This suggests that the impulsive phenotype seen in mice lacking 5-HT<sub>1B</sub>R could feasibly be derived by only changing the subjective value of the reward.

Given that past studies have implicated serotonin in the regulation of feeding and locomotion, alternative interpretations for our data include that the phenotypes are driven by an influence of 5-HT<sub>1B</sub>R on increased hunger drive or general activity. To rule out hunger, we directly measured feeding behavior, and found no effect on food intake in fed or restricted conditions. Importantly, past work implicating 5-HT<sub>1B</sub>R in body weight regulation in the original 5-HT<sub>1B</sub>R knockout mouse line (generated in the 1980s) includes a methodological limitation of not controlling for genetic background (using non-congenic, non-littermate controls) making reported effects on bodyweight difficult to interpret (Bouwknicht et al. 2001b; Lee et al. 2004). Other pharmacology work has reported that 5-HT<sub>1B</sub>R agonists decrease food consumption; however, we suggest that these effects are derived from a non-specific behavioral effect on motivation, and because of the use of large doses that may bind non-specifically (Lee et al. 2004). For example, the authors report no effect on feeding at 5 mg/kg of the 5-HT<sub>1B</sub>R agonist CP-94,253, a dose that elicits behavioral effects on impulsivity, and report that the effects on food intake were only seen at doses more than twice at high (10–20 mg/kg). These higher doses also have suggestive or significant effects on feeding in 5-HT<sub>1B</sub>R KO mice suggesting non-specific binding. Additionally, the idea that 5-HT<sub>1B</sub>R influences motivation for non-food reward is further supported by past studies showing increased motivation for cocaine in 5-HT<sub>1B</sub>R KO mice (Rocha et al. 1998). To address the possibility that the reported phenotypes are due to hyperactivity, we also referenced past work in the 5-HT<sub>1B</sub>R KO mouse. This past report of 5-HT<sub>1B</sub>R involvement in modulating a hyperactive response was specific to non-entrained stimuli (unexpected intruder in the resident-intruder task or disturbance by an experimenter) in a startle-like manner rather than a conditioned response to an entrained stimulus (Bouwknicht et al. 2001a). We would argue the unexpected and potentially stressful stimuli which induce the startle-like hyperactivity are unlike any stimuli presented in our studies. In fact, our data shows increased responding in a stimulus-free

well-learned action-outcome contingency. Additionally, our results show that extinction and pre-feeding both reduce responding to control levels which would not be expected in a model of general hyperactivity. Based on these reports and our results, we maintain our initial interpretation that the behavioral effects seen here are not likely due to a change in feeding drive or activity, but rather an exaggeration of the representation of hedonic value of rewarding stimuli.

Past work has examined the role of 5-HT<sub>1B</sub>R in the modulation of a number of models of psychiatric disorders which present with reward-related dysfunctions including addiction and depression. However, there has been limited careful exploration of the underlying behavioral mechanisms that contribute to these 5-HT<sub>1B</sub>R-associated phenotypes which is important for our understanding of complex behavioral processes found across multiple psychiatric disorders. Our data presents a basic reward reactivity-related phenotype that may serve as a framework for synthesizing these previously reported varied effects. Additionally, our work on behavioral mechanisms adds value to past and future studies investigating the neural circuit mechanisms through which 5-HT<sub>1B</sub>R exerts its effects on more complex phenotypes seen in substance use disorder and major depressive disorder.

Early studies in the original 5-HT<sub>1B</sub>R KO mouse line showed an increased motivation for (Castanon et al. 2000; Rocha et al. 1997, 1998) and decreased motivation for cocaine following 5-HT<sub>1B</sub>R agonist administration (Acosta et al. 2005), now we can investigate the circuit specific effects of 5-HT<sub>1B</sub>R on drug taking behavior. We propose a role for 5-HT<sub>1B</sub>R expressed on the terminals of nucleus accumbens shell neurons, particularly in the rewarding properties of low-doses of cocaine that do not induce reward behavior in controls (Barot et al. 2007; Hoplight et al. 2007; Pentkowski et al. 2012). Additional work also implicates 5-HT<sub>1B</sub>R expression on these accumbens projection neurons in the consumption of ethanol (Furay et al. 2011). Alterations in ventral striatal 5-HT<sub>1B</sub>R expression are also seen in major depressive disorder (Murrough et al. 2011a, 2011b), and in rodents, 5-HT<sub>1B</sub>R expression in the ventral striatum is implicated in inducing depressive states (Alexander et al. 2010; Svenningsson et al. 2006). Finally, the 5-HT<sub>1B</sub>R has also been examined in the context of social reward. Particularly, the rewarding properties of social behavior in mice requires activation of 5-HT<sub>1B</sub>R in the nucleus accumbens (Dolen et al. 2013). On one hand, these neural mechanisms point to a potential neural circuit mechanism for our results, and concurrently, our studies provide the behavioral mechanistic link between the circuit level mechanisms and the complex behavioral readouts.

It is interesting to note that the increasing reward value was associated with increased impulsive action, but not impulsive choice. Specifically, in the delay discounting task used to measure impulsive choice, mice lacking 5-HT<sub>1B</sub>R

expression chose the large delayed reward more than controls across all delays. This increased preference was seen in trials without any delay, suggesting that the differences seen in the delay discounting task are not due to changes in the tolerance to delay, but rather to some factor that is common across all delays, such as reward valuation. This interpretation is consistent with past studies which have found that rats prone to attribute incentive salience to reward cues show increased impulsive action but not impulsive choice (Lovic et al. 2011). Though there is evidence that also supports a link between the sensitivity to the hedonic valuation of sweet reward and impulsive choice, it is possible that 5-HT<sub>1B</sub>R signaling acts through striatal mechanisms to link reward value and impulsive action rather than cortical areas like the vmPFC which may mediate the link between sweet taste activated reward and delay discounting (Rudenga and Small 2013; Sellitto et al. 2010; Weafer et al. 2014). This would fit with the lower relative levels of 5-HT<sub>1B</sub>R protein expression in the cortex compared to the ventral striatum (Boschert et al. 1994; Varnas et al. 2005). Indeed, a lack of 5-HT<sub>1B</sub>R expression results in increased dopamine release in the nucleus accumbens, which is a substrate for goal-motivated behaviors and impulsive action (Pecina and Berridge 2005; Pisansky et al. 2019; Sesia et al. 2008; Taha and Fields 2006). Understanding the 5-HT<sub>1B</sub>R-induced changes in reward reactivity that correlate with behavioral inhibition in impulsive action paradigms, but not temporal discounting in impulsive choice paradigms, may shed light on the neural circuits which underlie psychopathologies that have disordered reward responsiveness and impulsivity.

Overall, we propose that a behavioral mechanism for the effect of serotonin signaling on impulsive action is alterations in reward reactivity. While prior work demonstrated a role for 5-HT<sub>1B</sub>R expression in the modulation of impulsivity as well as the rewarding properties of drugs and social stimuli, our studies provide a unifying hypothesis for all of these effects by identifying a common underlying behavioral substrate. Specifically, we show that there is a causal effect of reward value on impulsive action in our novel Variable Value Go/No-Go paradigm and that decreasing reward value alone is enough to decrease 5-HT<sub>1B</sub>R-associated impulsivity. These studies contribute to research aimed at understanding factors that contribute to increases in impulsivity seen in clinical populations. Additionally, our research points to the utility of serotonin receptor-specific treatment strategies to alter hedonic valuation for psychiatric disorders which involve dysregulated impulsivity.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s00213-021-05944-2>.

**Acknowledgements** We would like to thank Jun Ho Lee for lab management, Abigail Kalmbach for helpful assistance with the lickometer set-up, and Min Oh, Arati Sharma, and Anne George for help with data

collection. We would also like to thank Professor Robert Leaton for his helpful comments on a previous version of this manuscript.

**Author contribution** SSD, EL, VMM, and KN acquired and managed the data. SSD, EL, and KN conducted data analysis. SSD and KN wrote the first draft of the paper. SSD, KN, and PB designed the studies. All authors contributed to and approved the final version of the paper.

**Funding** Funding was provided by NIH R00 MH106731 to KMN.

## Declarations

**Conflict of interest** The authors declare no competing interests.

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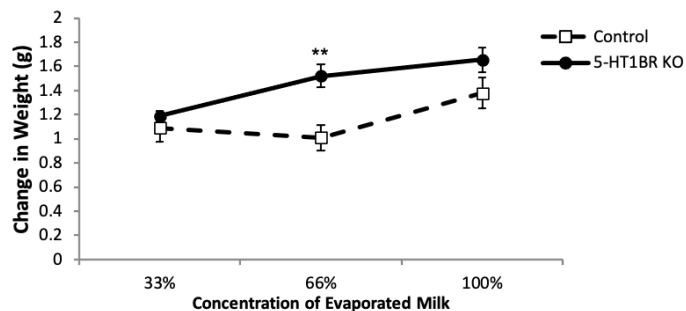
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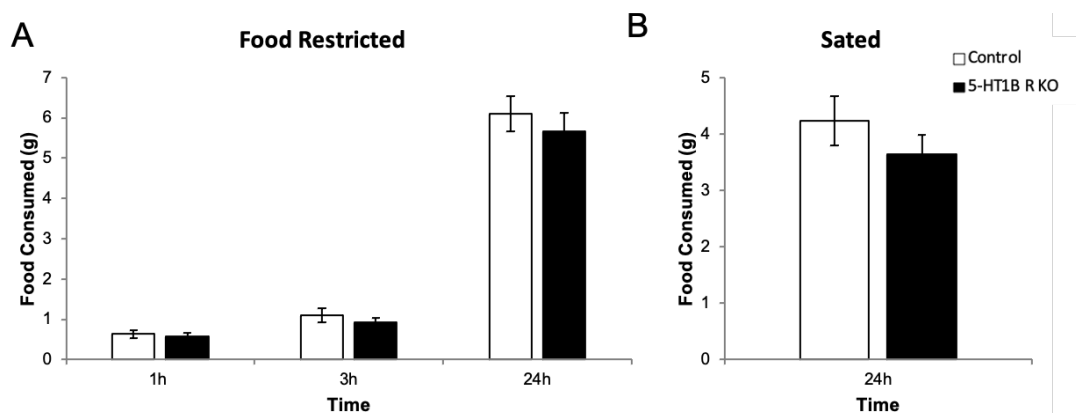
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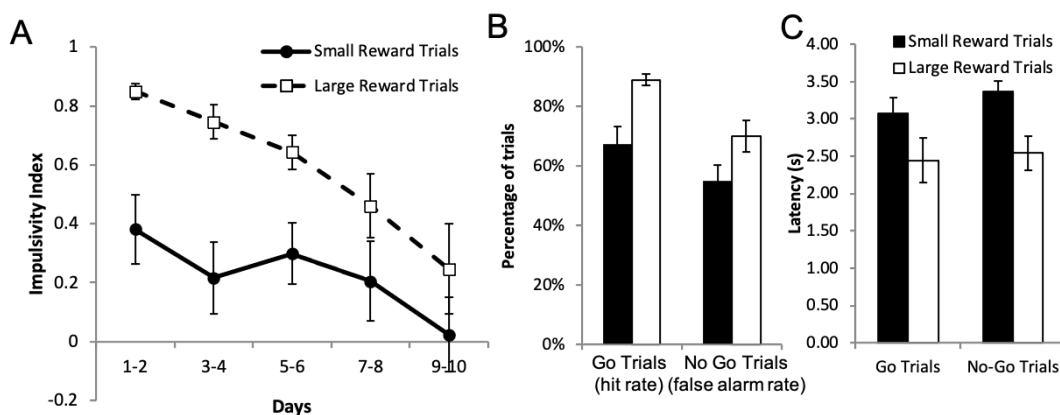
## Supplemental Figures



**Supplemental Figure 1. Lack of 5-HT<sub>1B</sub>R increases reward consumption.** Change in bodyweight following 5 minute free access to 33%, 66%, and 100% evaporated milk in Control and 5-HT<sub>1B</sub>R KO mice. \*\*, p < 0.01. All data are shown as group means +/- SEM.



**Supplemental Figure 2. Lack of 5-HT<sub>1B</sub>R does not alter food consumption.** (A) Food (standard chow) consumed in restricted (over 1, 3, and 24 hours) and (B) sated (over 24 hours) conditions, in control and 5-HT<sub>1B</sub>R KO mice. All data are shown as group means +/- SEM.



**Supplemental Figure 3. Reward value influences impulsive action on a trial-by-trial basis.** (A) Impulsivity index calculated as the proportion of successful Go trials minus the proportion of successful No-Go trials is shown as a measure of impulsive action (1.0 is the highest impulsivity that a mouse can display) over 10 days presented in 2-day bins. Data is shown for each small and large reward trials. (B) Hit rate for Go trials and false alarm rate for No-Go trials. (C) Latency to press the lever for Go trials and No-Go trials. All data are shown as group means +/- SEM.

Supplemental Table 1. Subject counts.

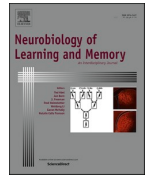
Group 1: tetO1B:: $\beta$ Actin-tTS- N= 16 tetO1B:: $\beta$ Actin-tTS+ N=13 tetO1B:: $\beta$ Actin-tTS-(with DOX) N=15 tetO1B:: $\beta$ Actin-tTS+(with DOX) N=14	female N=35 male N=23	RR, PR, Extinction (Fig 1) Concurrent choice (Fig 2)
SUBSET of Group 1: tetO1B:: $\beta$ Actin-tTS- N=12 tetO1B:: $\beta$ Actin-tTS+ N=8 tetO1B:: $\beta$ Actin-tT-(with DOX) N=11 tetO1B:: $\beta$ Actin-tTS+(with DOX) N=10	female N=18 male N=23	Satiety-induced devaluation (Fig 2)
SUBSET of Group 1: tetO1B:: $\beta$ Actin-tTS- N=12 tetO1B:: $\beta$ Actin-tTS+ N=8	female N=8 male N=12	Go/No-Go (Fig 3) Delay discounting (Fig 3)
Group 2: tetO1B:: $\beta$ Actin-tTS- N=12 tetO1B:: $\beta$ Actin-tTS+ N=9	female N=14 male N=7	Effort-based discounting (Fig 3)
Group 3: tetO1B +/- N=6 tetO1B:: $\beta$ Actin-tTS- N=4 tetO1B:: $\beta$ Actin-tTS+ N=9	female N=19	Reward consumption (Supp Fig 2)
Group 4: tetO1B+/-:: $\beta$ Actin-tTS- N=6 tetO1B+/-:: $\beta$ Actin-tTS+ N=5	female N=5 male N=6	Lickometer (Fig 4)
Group 5: tetO1B+/-:: $\beta$ Actin-tTS- N=8 tetO1B+/-:: $\beta$ Actin-tTS+ N=7	female N=9 male N=6	Pavlovian-to-instrumental transfer (Fig 5)
Group 6: tetO1B+/- N=12	female N=5 male N=7	Variable Value Go/No-Go Validation (Supp Fig 3)
Group 7: tetO1B:: $\beta$ Actin-tTS- N=12 tetO1B:: $\beta$ Actin-tTS+ N=9	female N=7 male N=14	Variable Value Go/No-Go Experiment (Fig 6)
SUBSET of Group 7: tetO1B:: $\beta$ Actin-tTS- N=9 tetO1B:: $\beta$ Actin-tTS+ N=9	female N=6 male N=12	Food consumption (Supp Fig 1)

## CHAPTER 2

### **Serotonin 1B receptor effects on response inhibition are independent of inhibitory learning**

This chapter was originally published as the research article Desrochers, S. S., & Nautiyal, K. M. (2022). Serotonin 1B receptor effects on response inhibition are independent of inhibitory learning. *Neurobiology of learning and memory*, 187, 107574.

<https://doi.org/10.1016/j.nlm.2021.107574>. All data was collected and analyzed by SSD with assistance from KMN. SSD and KMN wrote and edited the manuscript. This is in compliance with all copyright requirements.



# Serotonin 1B receptor effects on response inhibition are independent of inhibitory learning

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## ARTICLE INFO

### Keywords:

5-HT<sub>1B</sub> receptor  
Impulsive action  
Instrumental conditioning  
Classical conditioning  
Conditioned inhibition  
Pavlovian appetitive conditioning

## ABSTRACT

Impulsivity is defined in terms of deficits in instrumental response inhibition, when the inability to withhold an action produces a negative outcome. However, there are many behavioral and cognitive constructs which theoretically could contribute to disordered impulsivity, including Pavlovian responding, which few studies have considered in this context. In the present set of studies, we examine Pavlovian inhibitory learning and excitatory responding in a mouse model for dysregulated impulsivity, specifically, mice lacking the serotonin 1B receptor (5-HT<sub>1B</sub>R). Consistent with previous results, we show that these mice display increased impulsivity as measured by premature responding in the operant 5-choice serial reaction time test. In a Pavlovian conditioned inhibition paradigm, they also show a decreased ability to withhold responding, but importantly have an intact ability to learn inhibitory associations. In a Pavlovian appetitive conditioning experiment, 5-HT<sub>1B</sub>R knockout mice show normal responding under a positive contingency schedule, however, they display increased responding to cues presented on an independent schedule from reinforcement in a zero contingency schedule. Interestingly this difference does not occur when the cues are explicitly unpaired in a negative contingency schedule, nor during a 25% reinforcement schedule. Overall, while our results show that the deficits in operant response inhibition in mice lacking 5-HT<sub>1B</sub>R are likely not due to Pavlovian inhibitory or excitatory learning, it is relevant to consider associative learning in the context of dysregulated impulsive behavior.

## 1. Introduction

Impulsivity is a complex construct which is a major component of many psychiatric disorders, including attention deficit hyperactive disorder (ADHD), schizophrenia, substance use disorders, and gambling disorders (Dalley & Robbins, 2017; Mestre-Bach et al., 2020; Robbins, Gillan, Smith, de Wit, & Ersche, 2012; Ouzir, 2013). The diversity in the presentation of impulsivity across these disorders likely arises from its multiple, independent subcomponents. These different aspects of impulsivity have dissociable behavioral and biological underpinnings in humans and preclinical models (Dalley & Robbins, 2017; MacKillop et al., 2016; Nautiyal et al., 2017; Zeeb et al., 2013, 2016). One component of impulsivity is impulsive choice, which includes decreased tolerance for delays and risky decision making, as famously measured in the Marshmallow Test (Mischel et al., 1972). Another distinct category of impulsive behavior is impulsive action, which is characterized by deficits in response inhibition, including difficulty stopping, omitting, or delaying responding. Each of these components of impulsivity are also themselves complex phenotypes with a number of contributing factors,

including components of learning and memory. For example, elevated impulsive action could be the result of deficits in learning inhibitory associations rather than the inability to inhibit an action. A better understanding of how differences in associative learning could support alterations in impulsive behavior may be helpful in delineating neural circuits which underlie pathological levels of impulsivity.

Limited research has considered how deficits in Pavlovian responding may contribute to standard assays of impulse control (Sosa & dos Santos 2018). Of particular interest for impulsive action is inhibitory learning, given that exhibiting behavioral inhibition first requires an understanding of the inhibitory association. Inhibitory learning is commonly assessed by Pavlovian conditioned inhibition, which develops when a cue predicts the absence of reinforcement that would otherwise be expected (Pavlov, 1927; Rescorla, 1969b). In clinical populations, lower conditioned inhibition is associated with schizotypy (Migo et al., 2006), and violent offenders with personality disorders (often characterized by high levels of impulsive behavior) have deficits in conditioned inhibition such that an inhibitory stimulus had little effect on decreasing excitatory responding (He et al., 2011). Additionally,

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<https://doi.org/10.1016/j.nlm.2021.107574>

Received 14 June 2021; Received in revised form 7 November 2021; Accepted 3 December 2021

Available online 12 December 2021

1074-7427/© 2021 Published by Elsevier Inc.

normal adolescent development is associated with increased impulsivity and reward sensitivity (Casey & Jones, 2010; Somerville et al., 2011), and interestingly, adolescent rats take longer to discriminate between trial types in negative occasion setting, another form of inhibitory learning (Meyer & Bucci, 2014b, 2017b). However, a direct examination of conditioned inhibition in an animal model for increased impulsive action may clarify relevant underlying behavioral mechanisms of the disordered impulsivity.

Another aspect of classical conditioning which could be affected in subjects predisposed to impulsivity is excitatory responding, when cues predict reinforcement independent of action. Thus, there are conceivably several behavioral mechanisms which could affect appetitive responding in both operant tests of impulsivity and Pavlovian appetitive conditioning behavior. For example, increasing the subjective value of reward could enhance responding during the presentation of a predictive cue. Associative learning theories suggest that reinforcer magnitude influences the rate of learning and level of responding in classical conditioning (Rescorla & Wagner, 1972), and increased reward sensitivity is prevalent in populations with increased impulsivity (Dissabandara et al., 2014; Jonker et al., 2014; Kamarajan et al., 2015). Thus, changes in reward processing could support changes in both operant and classical conditioning in individuals with pathological levels of impulsivity.

A wealth of preclinical and clinical studies have identified varied neural mechanisms underlying impulsivity, and have more recently dissociated their contributions to different components of impulsivity. In the present study, we focus on the role of serotonin signaling, which has emerged as a candidate for the modulation of the impulsive action component, particularly. For example, global reductions of serotonin levels generally increase impulsive action (Winstanley et al., 2004; Worbe et al., 2014), and several serotonin receptors, including serotonin 1B, 2A and 2C regulate impulsive action (Fink et al., 2015; Higgins et al., 2017; Nautiyal et al., 2017). In particular, strong translational evidence points to the role of the serotonin 1B receptor (5-HT<sub>1B</sub>R) in a number of phenotypes associated with increased impulsivity. For example, single nucleotide polymorphisms in the gene encoding this receptor are associated with cocaine, alcohol, and heroin abuse, and impulsive-aggressive behaviors (Cao et al. 2013; Contini et al. 2012; Zouk et al. 2007; Proudnikov et al. 2006). Additionally, mice lacking the 5-HT<sub>1B</sub>R show increased impulsive action, increased cocaine self-administration, and deficits in response inhibition in instrumental tests of impulsive action (Nautiyal et al., 2015, 2017; Rocha et al., 1998).

The goal of the present experiments was to determine whether impulsive action modulated by serotonin may be subserved by deficits in classical conditioning. We examined Pavlovian inhibitory learning and excitatory responding for appetitive cues in mice with a global knockout of the 5-HT<sub>1B</sub>R, which produces deficits in assays aimed at measuring impulsive action. First, we used the operant 5-choice serial reaction time test (5CSRTT) to show that mice lacking the 5-HT<sub>1B</sub>R have increased impulsive action as measured by premature responding. Next, we examined changes in responding during classical conditioning for inhibitory associations in a Pavlovian conditioned inhibition test and excitatory associations in Pavlovian appetitive conditioning experiments, with various schedules of reinforcement. We find that, in addition to elevated impulsive action, mice lacking the 5-HT<sub>1B</sub>R show deficits in inhibitory responding and changes in excitatory responding under certain conditions in tests of classical conditioning. Overall, this work demonstrates the importance of examining how differences in learning Pavlovian associations may be important for the interpretation of measured deficits in impulsivity.

## 2. Materials and methods

### 2.1. Mice

Animals were bred in the vivarium at Dartmouth College and were

All mice were maintained on a 12:12 light-dark cycle and on *ad libitum* chow and water until experimental operant behavioral testing began at 10–14 weeks. Groups of mice lacking expression of 5-HT<sub>1B</sub>R and littermate genetic controls were generated by crossing the floxed tetO1B mouse model to a  $\beta$ Actin-tTS mouse line (tetO1B+/+ females crossed to tetO1B/+:: $\beta$ Actin-tTS + males), as previously reported (Nautiyal et al., 2015). All procedures were approved by the Dartmouth College Institutional Animal Care and Use Committee.

### 2.2. 5-Choice serial reaction time test (5CSRTT)

#### 2.2.1. Mouse touchscreen operant chambers

Behavioral training and testing for the 5CSRTT was conducted in four identical mouse Bussey-Saksida touchscreen operant chambers (Lafayette Instruments Co., Lafayette, IN). Each apparatus consisted of a sound attenuating chamber with a fan for ventilation/ background noise reduction enclosing a trapezoidal operant area with black plastic walls, a perforated stainless-steel floor, and a clear plexiglass roof. A speaker and LED houselight were attached to the ceiling of the sound attenuation chamber, directly above the operant arena (the houselight was off unless otherwise specified). A touchscreen (30.7 cm, 800 × 600 resolution) located at the front of the arena was covered by a black plastic mask with 5 square openings (4 × 4 cm each, spaced 1 cm apart, 1.5 cm above floor; '5 choice' mask; Lafayette Instruments Co., Lafayette, IN) to define response areas and reduce accidental background touches. A feeder with an LED light was located at the back end of the chamber, with undiluted evaporated milk (Nestle Carnation) reward delivered by a liquid pump. Infrared beams were positioned at the front and back of the chamber, as well as in the feeder. Stock behavioral programs (5-Choice Serial Reaction Time Task for Mouse Touch Screen Systems and ABET II) were executed by the ABET II software (Lafayette Instruments Co., Lafayette, IN) and Whisker Server (Cardinal & Aitken, 2010).

#### 2.2.2. Initial touchscreen chamber training

5CSRTT training and testing were run 5 days a week, with procedures modified from Fletcher et al. 2013. Mice lacking 5-HT<sub>1B</sub>R expression (males = 4, females = 5) and genetic controls (males = 3, females = 4) were maintained at approximately 90% of their free-feeding weight, with *ad lib* water provided throughout the experiment. For initial touch training (5-choice Mouse Initial Touch Training), a 4 × 4 cm white stimulus appeared for 30 s randomly at one of the 5 response windows on the touchscreen. At stimulus offset, the LED in the feeder turned on and reward was delivered (280 ms pump time, 7  $\mu$ l). The light turned off after reward retrieval and the next stimulus was presented. If the mouse touched the correct stimulus window during the 30 s presentation, 3x reward (840 ms pump time, 21  $\mu$ l) was delivered in the lit feeder. After all mice reached a criterion of 30 trials in 30 min (2 days), they moved on to must touch training (5-choice Mouse Must Touch Training). In these sessions, the stimulus was presented randomly at one of the 5 response windows, and remained present until the mouse responded in the correct window. Reward (840 ms pump time, 21  $\mu$ l) was delivered in the lit feeder, and the LED turned off after retrieval. After an ITI of 5 s, the next stimulus was presented. After all mice reach a criterion of 20 trials in 30 min (2 days), they moved on to 5CSRTT training.

#### 2.2.3. Training to baseline for 5CSRTT

For training in the 5CSRTT (5-choice Mouse Touch basic), each session began with a priming reward delivery (840 ms pump time, 21  $\mu$ l) delivered in a lit feeder. Following reward retrieval, the LED turned off and a 5 s ITI began. Then, the white light stimulus was randomly presented at one of the 5 touchscreen windows. The stimulus durations during 5CSRTT training were 32 s (4 days), 16 s (2 days), 8 s (2 days), 4 s (2 days), 2 s (2 days), 1.8 s (2 days), 1.6 s (2 days), 1.4 s (2 days), 1.2 s (3 days), and 1 s (3 days; baseline stimulus). The stimulus duration was reduced over training when all mice achieved at least > 65% accuracy

trials/presented trials). A nosepoke response to the correct stimulus window within the time of presentation plus a 5 s limited hold after the stimulus presentation ended resulted in immediate reward delivery in the feeder (as well as the removal of the stimulus if it was still present). Reward retrieval triggered the start of the ITI. Nose poke responses to any window during the ITI were considered premature responses, the houselight turned on and there was a 5 s timeout. After the timeout was over, a response in the feeder initiated the next trial's ITI. If a mouse responded in the incorrect window, or if no responses were made during the stimulus presentation and limited hold, a 5 s timeout was initiated (see Fig. 1A for trial structure). The session ended after 100 trials or 30 min. Data for 5CSRTT training were averaged for each stimulus duration, with days following a break in training (i.e. following a weekend) excluded.

#### 2.2.4. 5CSRTT tests: Long ITI and short variable stimulus test

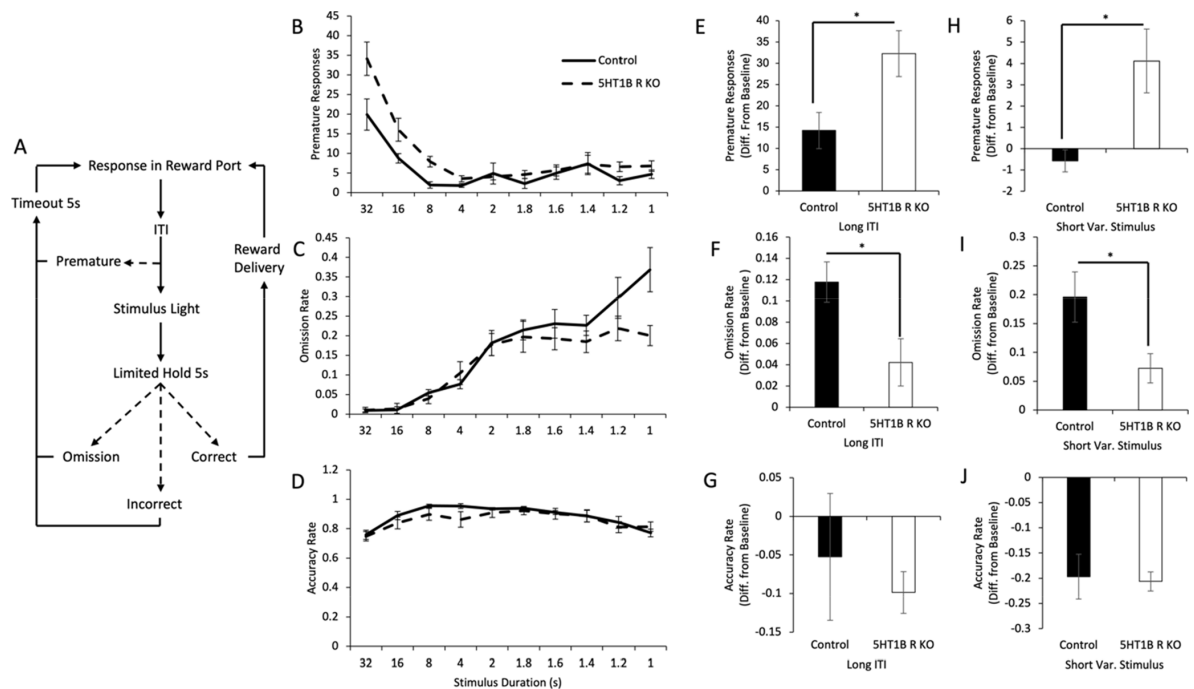
The same cohort of mice described in 5CSRTT training were then tested in two manipulations of the 5CSRTT paradigm. Mice were trained with a 1 s stimulus for at least 3 days before each test session. For the Long ITI manipulation, the procedure was the same as the baseline 5CSRTT training except the ITI was extended to 9 s. For the Short Variable Stimulus manipulation (5-choice Mouse Var2) the procedure was the same as the baseline 5CSRTT training except the stimulus duration for each trial was shortened to a variable 0.8 s, 0.6 s, 0.4 s, or 0.2 s duration. Data for each test was analyzed as a difference from the previous day's baseline performance and averaged over 2 separate test sessions.

#### 2.3. Behavioral apparatus and initial training for classical conditioning tests

Classical conditioning studies (Conditioned Inhibition, Pavlovian Appetitive Conditioning, and 100% versus 25% Reinforcement experiments) were conducted in eight identical operant chambers individually enclosed in ventilated, sound attenuating isolation boxes (Med Associates, St. Albans, VT). Each operant chamber consisted of stainless-steel modular walls, stainless-steel bar floors, and a noseport receptacle for the delivery of liquid reward by a dipper (undiluted evaporated milk; 0.02 ml cup volume). Head entry into the reward port was detected by an infrared beam break. The chamber also contained two stainless steel levers placed 2.2 cm above the chamber floor on either side of the reward port, though these were not used in the present study. A houselight and speaker were located on the upper portion of the wall opposite the reward port. A computer equipped with MED-PC IV (Med Associates Inc., St Albans, VT) computer software delivered stimuli and collected behavioral data. Training and testing were run 5–7 days a week, and mice were maintained at approximately 90% of their free-feeding weight, with ad lib water provided throughout the experiment. Before classical conditioning testing, all mice were trained to retrieve an evaporated milk reward through head entry into the reward port.

#### 2.4. Conditioned inhibition

Mice lacking 5-HT<sub>1B</sub>R expression and genetic controls were split into experimental (5-HT<sub>1B</sub>R KO: males = 3, females = 9; genetic control: males = 5, females = 8) and procedural control (5-HT<sub>1B</sub>R KO: males = 3, females = 8; genetic control: males = 5, females = 8) conditions for a



**Fig. 1.** An absence of 5-HT<sub>1B</sub>R causes impulsive responding in the 5-choice serial reaction time test. A) A diagram of the 5CSRTT trial structure. During training, the ITI period was 5 s and the stimulus began at 32 s and decreased based on group performance until a baseline of 1 s was achieved. The Long ITI test increased in the ITI to 9 s, and the Short Variable Stimulus test decreased the stimulus duration (0.2 s, 0.4 s, 0.6 s, 0.8 s, randomly ordered), with all other parameters the same as the baseline procedure. The total premature responses for B) training and difference from baseline premature responses for E) Long ITI and H) Short Variable Stimulus tests are shown in the top row. Proportion of total trials in which the mouse did not respond for C) training and difference from baseline omission rate for F) Long ITI and I) Short Variable Stimulus tests in the center row. Proportion of correct non-omission trials in which the mouse for D) training and difference from baseline accuracy rate for G) Long ITI and J) Short Variable Stimulus tests in the bottom row. \*  $p < 0.05$ . All data are groups means  $\pm$  SE.

conditioned inhibition experiment, with procedures modified from Bonardi et al. 2010. Each session for this experiment was about 70 min long, and the houselight was off unless otherwise specified.

#### 2.4.1. Preexposure

All mice were preexposed to the inhibitor stimulus (X; houselight), the experimental excitator inhibitor compound stimulus (AX; 75 dB 10 Hz click and houselight), and the control excitator inhibitor compound stimulus (BX; 75 dB white noise and houselight) in the absence of reward over 2 sessions. Each session had 10 trials of each trial type presented for 20 s, randomly ordered with an average 120 s variable ITI (range 69.8–206 s).

#### 2.4.2. Excitor training

To increase excitator trial responding, all mice were trained with the experimental excitator stimulus (A; 75 dB 10 Hz click) and the control excitator stimulus (B; 75 dB white noise) with 5 s reward delivery at offset over 4 sessions. Each session had 15 trials of each trial type presented for 20 s, randomly ordered with an average 120 s variable ITI (range 69.8–206 s). Note that stimuli were not counterbalanced between mice, but both excitator cues were of the same sensory modality.

#### 2.4.3. Conditioned inhibition training

Next, mice were trained for 15 sessions on conditioned inhibition. All mice were presented with rewarded trials for both excitator cues (A+, B+). Mice in the experimental condition also had inhibitor trials with an excitator-inhibitor compound stimulus (AX–). A Pavlovian differential conditioning procedure was used as a conservative procedural control condition where the excitator trials were interspersed with inhibitor trials with no reward (X–) (see Fig. 2A, Conditioned Inhibition Training). Each session had 10 trials of each the 3 trial types presented for 20 s, randomly ordered with an average 120 s variable ITI (range 69.8–206 s). Data was recorded as the total duration of responding in reward port

during the cue presentation minus duration of responding during the immediately preceding 20 s of ITI (elevation score; there were no significant differences between groups during this pre-trial period). Results for the B + trials are not shown, as they are used as a control only for the summation test and were not statistically different from A + trial results in the procedural controls.

#### 2.4.4. Summation test

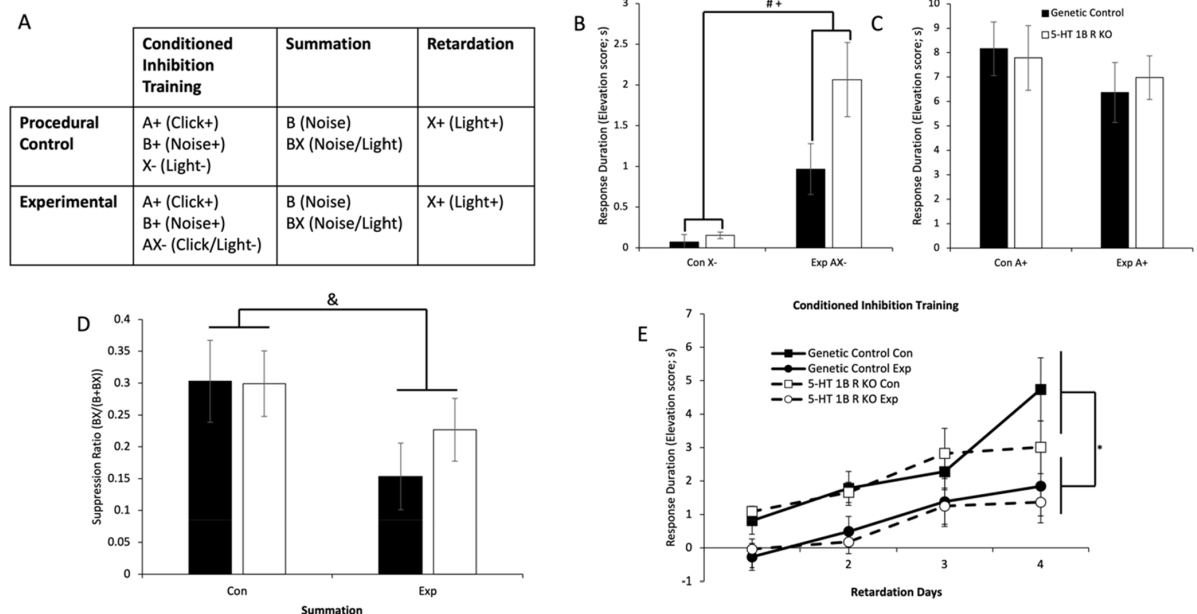
Following 15 sessions of conditioned inhibition training, behavior was assessed in summation tests over 2 sessions. These tests were completed in the absence of reward delivery. Each session had 15 trials each of 20 s presentations of the control excitator (B) and the control excitator inhibitor compound (BX), with an average 120 s variable ITI (range 69.8–206 s; see Fig. 2A, Summation). Data from the summation test was averaged over the 2 sessions and recorded as a suppression ratio: duration of response during BX/(B + BX) responding. A suppression ratio of 0.5 would indicate equal responding to B and BX, whereas 0 would indicate no responding to BX.

#### 2.4.5. Retardation of acquisition test

Finally, mice were tested for retardation of acquisition over 4 sessions. Each session had 30 trials which consisted of a 20 s presentation of the inhibitor cue immediately followed by a 5 s reward presentation (X+; houselight) (see Fig. 2A, Retardation). The ITI was a variable average 120 s (range 69.8–206 s). Data was recorded as the total duration of responding in reward port during the cue presentation minus duration of responding during the immediately preceding 20 s of ITI (elevation score; there were no significant differences between groups during this pre-trial period).

#### 2.5. Pavlovian appetitive conditioning

Separate groups of mice were tested in positive contingency (5-



**Fig. 2.** Mice lacking 5-HT<sub>1BR</sub> have deficits in response inhibition during training but intact inhibitory learning in tests of conditioned inhibition. A) Trial types presented to the conditioned inhibition procedural control and experimental groups during training (note B + trial data not shown), summation, and retardation. Elevation score (average response duration during cue-average preceding ITI responding) averaged over days for B) inhibitor and C) excitator A trials during conditioned inhibition training. D) Suppression ratio (duration of responding during BX/(B + BX)) for the summation test. E) Response duration elevation score over days for X + trials for the retardation of acquisition test. & p of main effect of condition = 0.051; \* p of main effect of condition < 0.05; # p of main effect of genotype < 0.05; + p of interaction = 0.080. All data are groups means ± SE.

HT<sub>1B</sub>R KO: males = 2, females = 6; genetic control: males = 4, females = 5), zero contingency (5-HT<sub>1B</sub>R KO: males = 6, females = 9; genetic control: males = 11, females = 5), and negative contingency (5-HT<sub>1B</sub>R KO: males = 4, females = 5; genetic control: males = 5, females = 5) conditions for a Pavlovian appetitive conditioning experiment over 9 sessions, with procedures modified from Ward et al. 2012. The house-light was on for the duration of each session. For mice in the positive contingency condition, each trial consisted of an 8 s conditioned stimulus (CS; 75 db white noise) followed by a 5 s reward delivery, with an average 80 s variable ITI (range 4.4–201 s). For the zero contingency condition, mice had the 8 s CS and the 5 s reward delivered on separate schedules each with an average 80 s variable ITI (range 4.4–201 s), such that the CS-CS and US-US intervals were completely independent of one another. For mice in the negative contingency condition, each trial consisted of a randomly selected 8 s CS or 5 s reward presented after an average 40 s variable ITI (range 2.2–100.5 s). For every condition, there were 40 CS presentations and 40 reward presentations, with each session lasting around 62 min. Data was recorded as the duration of responding in the reward port over the duration of the cue minus duration of responding over the immediately 8 s of ITI (elevation score; there were no significant differences between groups during this pre-trial period).

### 2.6. 100% versus 25% reinforcement

Naive mice were tested either in 100% reinforcement (5-HT<sub>1B</sub>R KO: males = 3, females = 5; genetic control: males = 4, females = 4) or 25% reinforcement (5-HT<sub>1B</sub>R KO: males = 4, females = 4; genetic control: males = 4, females = 4) conditions over 9 sessions. The house-light was on for the duration of each session. For the mice in the 100% reinforcement condition, each of 20 trials consisted of an 8 s CS (75 db white noise) followed by a 5 s reward delivery, with an average 180 s variable ITI (inclusive of cue; range 177.8–182.8 s). For the 25% reinforcement condition, mice had the 8 s CS presented with an average 45 s variable ITI (inclusive of cue; range 42.8–46.8 s). 80 total CS presentations occurred, with one out of every 4 CS presentations randomly reinforced at offset by a 5 s reward delivery. For both conditions, there were 20 rewards in total delivered in each session, with each session lasting around 64 min. Data was recorded as duration of responding in the reward port minus duration of responding over the immediately 8 s of ITI (elevation score; there were no significant differences between groups during this pre-trial period).

### 2.7. Statistical analysis

Data was analyzed using the car and ez packages in the R statistical software (Fox et al., 2016; Lawrence, 2016; R Core Team, 2019). For the 5CSRTT, premature responses, omission rate, and accuracy rate were analyzed with a two-way mixed ANOVA for training (10 stimulus durations  $\times$  2 genotypes) and with two-tailed, unpaired ttests comparing genotypes for the tests. For conditioned inhibition training, response duration was analyzed with a four-way mixed ANOVA (15 sessions  $\times$  2 experimental conditions  $\times$  2 trial types  $\times$  2 genotypes). Because we expected genotype differences to present in responding during inhibitor trials (X- for procedural control, AX- for experimental group), a three-way mixed ANOVA was also performed as a planned comparison of just the inhibitor trials during training (15 sessions  $\times$  2 conditioned inhibition conditions  $\times$  2 genotypes). The summation test suppression ratios were analyzed with a two-way independent measures ANOVA (2 experimental conditions  $\times$  2 genotypes) while retardation response duration was analyzed with a three-way mixed ANOVA (4 sessions  $\times$  2 experimental conditions  $\times$  2 genotypes). Two-way mixed ANOVAs were also used for response duration elevation score for each of the three Pavlovian Appetitive Conditioning experiment contingency conditions (9 sessions  $\times$  2 genotypes). Response duration across cue presentation was also analyzed in two-way mixed ANOVAs with data collapsed across

session for each of the three contingency conditions (8 s  $\times$  2 genotypes). A three-way mixed ANOVA was used to analyze response duration elevation score in the 25% versus 100% Reinforcement experiment (9 sessions  $\times$  2 experimental conditions  $\times$  2 genotypes). Response duration across cue presentation was also analyzed for this experiment in a three-way mixed with data collapsed across session (8 s  $\times$  2 experimental conditions  $\times$  2 genotypes). All data was first analyzed with sex included as a factor, but there were no significant fully powered effects found so data was collapsed across sex for all reported statistics.

## 3. Results

Mice lacking the 5-HT<sub>1B</sub>R are more impulsive than controls. Specifically, in the 5CSRTT measuring impulsive action, they showed increased premature responding during the training sessions (Fig. 1B;  $F_{1,14} = 5.79$ ,  $p = 0.031$  for main effect of genotype). However, this effect decreased over training, ( $F_{9,126} = 31.39$ ,  $p < 0.001$  for main effect of stimulus length;  $F_{9,126} = 3.03$ ,  $p = 0.003$  for interaction), as all mice improved task performance by reducing premature responses. Additionally, omission rate for all mice increased as the stimulus length decreased and the task became more difficult, however, mice lacking the 5-HT<sub>1B</sub>R omitted less than controls toward the end of training (Fig. 1C;  $F_{9,126} = 36.35$ ,  $p < 0.001$  for main effect of stimulus length;  $F_{9,126} = 2.72$ ,  $p = 0.006$  for interaction;  $F_{1,14} = 1.77$ ,  $p = 0.204$  for main effect of genotype). Finally, accuracy rate increased overall across training, (Fig. 1D;  $F_{9,126} = 18.29$ ,  $p < 0.001$ ), and importantly there were no observed genotype differences ( $F_{1,14} = 0.39$ ,  $p = 0.545$  for main effect;  $F_{9,126} = 1.58$ ,  $p = 0.128$  for interaction), which is commonly interpreted to rule out attention deficits (Robbins, 2002; Turner et al., 2016). These results suggest that mice lacking 5-HT<sub>1B</sub>R show increased impulsivity which can be ameliorated to control levels with extended training.

We next performed two tests to determine if the differences in premature responding would reemerge in different manipulations of the 5CSRTT. First, we extended the ITI from 5 s to 9 s to make the waiting period longer, and therefore more difficult for mice to withhold responding. Under these conditions, 5-HT<sub>1B</sub>R knockout mice had higher premature responding compared to control mice (Fig. 1E;  $t_{13,9} = -2.63$ ,  $p = 0.020$ ), indicating that they were less able to withhold responding under the pressure of a longer wait period. They also had fewer omission trials (Fig. 1F;  $t_{14,0} = 2.58$ ,  $p = 0.022$ ), as seen during the training sessions, but had similar accuracy (Fig. 1G;  $t_{7,3} = 0.53$ ,  $p = 0.609$ ). Next, we varied the stimulus duration to make it shorter and unpredictable, increasing the attention requirement by requiring faster responding. Importantly, there were no genotype differences in accuracy (Fig. 1J;  $t_{8,1} = 0.20$ ,  $p = 0.849$ ). Consistent with the behavior in the extended ITI test, mice lacking the 5-HT<sub>1B</sub>R had increased premature responses compared to controls (Fig. 1H;  $t_{9,8} = -2.96$ ,  $p = 0.015$ ), with decreased omission rate (Fig. 1I;  $t_{9,9} = 2.45$ ,  $p = 0.035$ ). These data suggest that mice susceptible to impulsive action show increased premature responding when pressured to respond quickly in response to an increased demand on attentional resources.

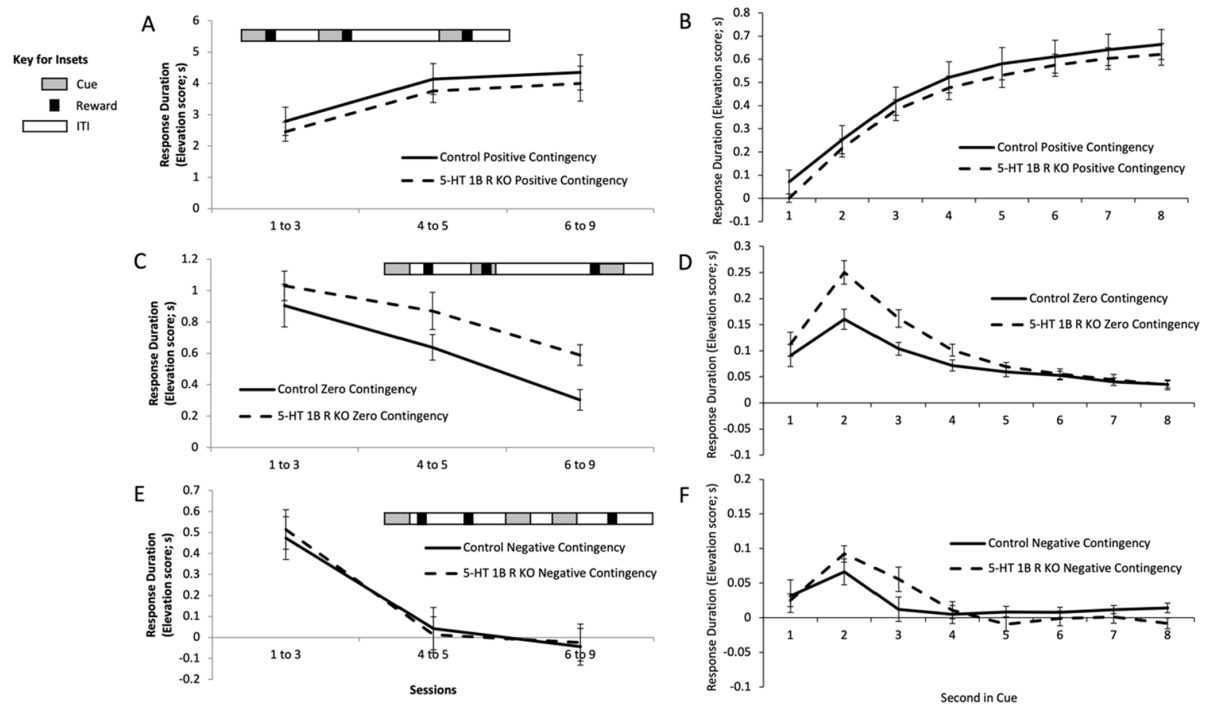
To assess whether the increases in impulsive action seen in these mice could be influenced by an inability to learn inhibitory associations and/or general increased activity in response to excitatory cues, we used appetitive classical conditioning experiments. First, we used a Pavlovian conditioned inhibition paradigm to determine if mice lacking 5-HT<sub>1B</sub>R show differences in inhibitory learning or in responding to inhibitory cues. We found that they showed deficits in response inhibition, rather than inhibitory learning during training for conditioned inhibition. A planned comparison of inhibitor trials (X- for procedural control, AX- for experimental group; Fig. 2B) revealed significant main effects of genotype ( $F_{1,45} = 4.32$ ,  $p = 0.043$ ) and condition ( $F_{1,45} = 24.43$ ,  $p < 0.001$ ), and a trend toward a genotype by condition interaction ( $F_{1,45} = 3.21$ ,  $p = 0.080$ ). Since the conditioned inhibition procedural control can sometimes generate inhibition due to the negative contingency between the X- cue and reward (Rescorla, 1969b, 1969a), the main effect of

genotype suggests that mice lacking the 5-HT<sub>1B</sub>R have deficits in response inhibition, in both the procedural control and experimental conditions. Overall during training, all mice increased responding in the goal location during excitator (A+) trials and decreased responding during nonrewarded (X-, AX-) trials (Fig. 2B,C, data shown averaged over days, note different scales;  $F_{1,45} = 159.97$ ,  $p < 0.001$  for main effect of trial type;  $F_{14,630} = 11.39$ ,  $p < 0.001$  for main effect of day;  $F_{14,630} = 12.21$ ,  $p < 0.001$  for trial type  $\times$  day interaction). This indicates that all mice were able to discriminate between trial types, with mice in the experimental condition able to learn that the conditioned inhibitor indicated the reward was not coming. There was also a significant interaction between experimental condition and trial type such that mice in the experimental group had increased responding for the excitator-inhibitor (AX-) compound compared to the procedural control group's response to the inhibitor (X-) cue alone, suggesting that they had some remaining excitation to the excitator (A) cue despite the pairing ( $F_{1,45} = 6.88$ ,  $p = 0.012$ ). All other effects were nonsignificant ( $ps > 0.05$ ).

Increased responding to inhibitory compounds during training (i.e. Fig. 2B) could reflect either a deficit in learning of inhibitory associations or in the expression of that learning. Given that mice lacking 5-HT<sub>1B</sub>R had reduced response inhibition to the inhibitor trials, we performed two tests to directly assess their learning of the inhibitory association. In a summation test, we examined whether the inhibitor (X) could transfer to another excitator cue (B), that was not previously presented in conjunction with the inhibitor during training. Interestingly, despite the genotype differences in inhibitor responding during training, we found no significant effects of genotype on suppression ratio during summation (Fig. 2D;  $F_{1,45} = 0.39$ ,  $p = 0.533$  for main effect;  $F_{1,45} = 0.48$ ,  $p = 0.491$  for interaction). Mice in the experimental condition had lower responding to the excitator-inhibitor (BX) compound, though this effect did not reach statistical significance ( $F_{1,45} = 4.01$ ,  $p = 0.051$ ),

potentially because of a reduced suppression ratio in the procedural controls due to an acquired latent inhibition to X- during training. Finally, in a retardation of acquisition test, we tested for differences in responding when the previously inhibitory cue was then rewarded, making it positively associated with reward (X+). There were no differences in learning rate between the conditions (Fig. 2E;  $F_{3,135} = 1.30$ ,  $p = 0.276$  for session  $\times$  condition interaction), however, mice in the conditioned inhibition experimental condition had overall lower responding indicating that the previously inhibitory cue acquired less excitatory meaning, suggesting successful conditioned inhibition learning ( $F_{1,45} = 11.64$ ,  $p = 0.001$ ). All mice increased responding to the previously inhibitory cue (X+) over retardation sessions ( $F_{3,135} = 19.76$ ,  $p < 0.001$ ), and, as in the summation test, there were no significant main effects or interactions with genotype (all  $ps > 0.05$ ). The collective data from this conditioned inhibition experiment indicate that despite decreased response inhibition during training, 5-HT<sub>1B</sub>R knockout mice display evidence of learned inhibitory associative relationships as demonstrated by normal performance in the summation and retardation of acquisition tests.

Given that the deficits in response inhibition are unlikely due to deficits in inhibitory learning, we next examined the role of the 5-HT<sub>1B</sub>R in modulating responding to appetitive cues. We hypothesized that increased cue reactivity could contribute to the increased impulsive action in the 5CSRTT as well as the deficits in withholding responding shown in the conditioned inhibition test. Even though we did not see genotype effects in the excitator trials in conditioned inhibition, we reasoned that this could have been due to a ceiling effect or an inability to capture the subtleties of initial approach behavior. Therefore, we conducted an experiment to explore differences in Pavlovian appetitive conditioning by measuring responding to cue presentation. First, in a positive contingency condition, we examined responding at the reward receptacle over the duration of a cue that always predicted reward at



**Fig. 3.** In appetitive Pavlovian conditioning, mice lacking 5-HT<sub>1B</sub>R in a zero contingency condition have elevated responding to cue onset. Total cue response duration elevation score over sessions for A) positive, C) zero, and E) negative contingency conditions. Response duration elevation score across seconds in the cue for B) positive, D) zero, and F) negative contingency conditions (averaged over training days), with insets showing the general trial structure for the conditions. All data are groups means  $\pm$  SE.

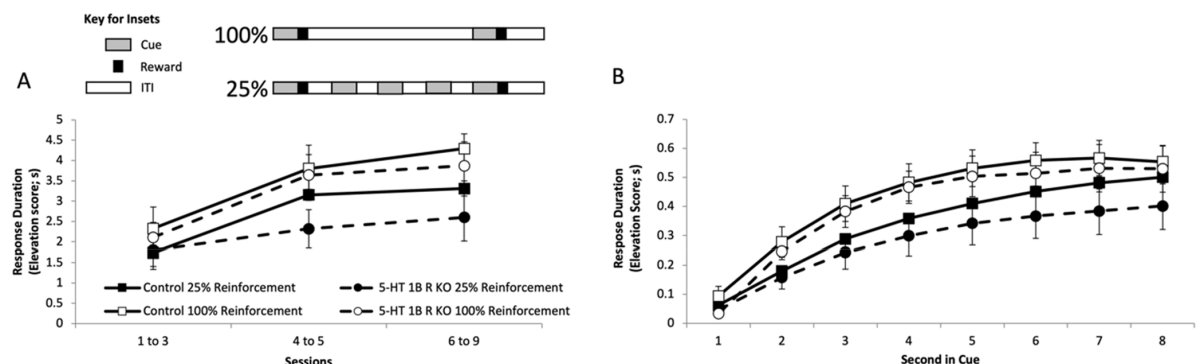
offset (Fig. 3A inset). We found that all mice tended to increase responding to the paired cue over training days (Fig. 3A;  $F_{8,120} = 15.72$ ,  $p < 0.001$ ), with no genotype difference ( $F_{1,15} = 0.39$ ,  $p = 0.542$  for main effect;  $F_{8,120} = 0.50$ ,  $p = 0.857$  for interaction). When we examined responding in a second by second analysis across the duration of the cue, as expected mice increased responding as reward approaches toward offset (Fig. 3B;  $F_{7,105} = 121.10$ ,  $p < 0.001$  for main effect of second in cue), again, with no group differences ( $F_{1,15} = 0.39$ ,  $p = 0.542$  for main effect of genotype;  $F_{7,105} = 0.09$ ,  $p = 0.999$  for interaction). Next, in a zero contingency condition, with the cue and the reward on independent interval schedules (Fig. 3C inset), mice decreased responding to cue over training (Fig. 3C;  $F_{8,232} = 7.88$ ,  $p < 0.001$ ). Mice lacking the 5-HT<sub>1B</sub>R, however, showed increased responding compared to controls, which was maintained across days of training ( $F_{1,29} = 4.66$ ,  $p = 0.039$  for main effect;  $F_{8,232} = 1.05$ ,  $p = 0.396$  for interaction). Analysis across the duration of the cue revealed that the increased responding occurs at cue onset (Fig. 3D;  $F_{7,203} = 48.01$ ,  $p < 0.001$  for main effect of second in cue;  $F_{1,29} = 4.66$ ,  $p = 0.039$  for main effect of genotype;  $F_{7,203} = 3.81$ ,  $p < 0.001$  for interaction). While this supports the idea that mice lacking 5-HT<sub>1B</sub>R are more reactive to cues alone, it is also possible that the increased responding in 5-HT<sub>1B</sub>R knockout mice in the zero contingency condition was due to the low probability of the cue and the reward overlapping or happening in sequence. To address this possibility, we next presented the cue and reward as explicitly unpaired in a negative contingency design, such that there was always an ITI between any cue or reward presentation (Fig. 3E inset). Again, cue responding decreased over days (Fig. 3E;  $F_{8,136} = 14.31$ ,  $p < 0.001$ ), but there were no differences between the two genotype groups ( $F_{1,17} = 0.01$ ,  $p = 0.912$  for main effect;  $F_{8,136} = 0.92$ ,  $p = 0.505$  for interaction). Analyzed across seconds in the cue, we found a main effect of second and interaction such that there was still a small effect of genotype at cue onset (Fig. 3F;  $F_{7,119} = 12.09$ ,  $p < 0.001$  for main effect of second in cue;  $F_{7,119} = 2.13$ ,  $p = 0.045$  for interaction), but no overall main effect of genotype ( $F_{1,17} = 0.01$ ,  $p = 0.912$ ). Given the diminished effect of 5-HT<sub>1B</sub>R on responding to cues when they were always separated from reward, it is possible that the increased responding under a zero contingency schedule is instead due to differential responding for weakly predictive pairings, where there is a low probability of the cue predicting the outcome.

Therefore, we lastly tested whether the genotype difference in cue reactivity in the Pavlovian appetitive conditioning experiment was due to differences in responding to a weakly predictive cue by systematically controlling the probability of the cue predicting reward. We reduced the predictive strength of the cue by making the probability of the cue predicting the reward 25%, and compared this to a control 100% reinforced condition (Fig. 4A insets). Overall responding during the cue increased over training in both conditions, but was lower in the 25% compared to the 100% reinforced condition as expected (Fig. 4A;  $F_{8,224}$

$= 22.99$ ,  $p < 0.001$  for main effect of session;  $F_{1,28} = 4.94$ ,  $p = 0.035$  for main effect of condition), with no significant main effects of genotype or other interaction (all  $p$ s  $> 0.05$ ). When analyzed over the seconds of the cue, we similarly found that mice in both reinforcement conditions increase responding toward the end of the cue (Fig. 4B;  $F_{7,196} = 145.83$ ,  $p < 0.001$  for main effect of second in cue), but the rate of this elevation differed such that mice in the 100% condition reach maximum responding earlier in the cue than those in the 25% condition ( $F_{1,28} = 4.94$ ,  $p = 0.035$  for main effect of condition;  $F_{7,196} = 2.93$ ,  $p = 0.006$  for second  $\times$  condition interaction). Again, there were no main effects or interactions with genotype (all  $p$ s  $> 0.05$ ). This suggests that the difference between genotypes in the independent interval Pavlovian appetitive conditioning condition was not due to altered responding for fully or partially predictive cues.

#### 4. Discussion

Overall, our data suggest a role for the 5-HT<sub>1B</sub>R in operant responding in tests of impulsivity as well as responding in classical conditioning paradigms. Specifically, mice lacking the 5-HT<sub>1B</sub>R demonstrate elevated impulsive action in the 5CSRTT, as measured by premature responding, which is consistent with our previous studies of impulsive action in this model (Nautiyal et al., 2017). Additionally, these mice do not show deficits in performance accuracy in the 5CSRTT, which is often interpreted as normal attention (Robbins, 2002; Turner et al., 2016). Inattention and impulsivity are key characteristics of ADHD (Nigg, 2016), and it is possible that in some cases, impulsive action may arise through a decreased ability to attend properly to cues and respond at the correct time. Our data suggests that this is not the case in these mice, however, it is still possible that attentional changes such as sensitivity for cue detection could contribute to these results. Next, we used a Pavlovian conditioned inhibition test to show that all mice were able to learn inhibitory associations, and during training discriminated between excitatory versus inhibitory trials. However, mice lacking the 5-HT<sub>1B</sub>R show increased responding to inhibitor trials during training compared to controls, suggesting a deficit in response inhibition consistent with the impulsive action phenotype. Interestingly, this difference in responding was not seen in the summation test when the control excitator-inhibitor (BX-) compound was presented in extinction conditions with no rewards presented during the session. This is consistent with our prior findings that 5-HT<sub>1B</sub>R knockout mice have increased instrumental responding in tests of motivation, but show normal extinction of responding in the absence of reward, which is also dependent on intact inhibitory learning (Bouton et al., 2021; Desrochers et al., 2021). Therefore, we suggest that the increased responding during conditioned inhibition training is reflective of differences in responding when there is the potential for rewarded trials.



**Fig. 4.** Mice lacking 5-HT<sub>1B</sub>R do not show differences in approach behavior for 100% or 25% reinforced cues. A) Total cue response duration elevation score over sessions for 100% reinforcement and 25% reinforcement conditions. B) Response duration elevation score across seconds in the cue for 100% reinforcement and 25% reinforcement conditions (averaged over training days), with insets showing the general trial structure for the conditions. All data are groups means  $\pm$  SE.

Beyond conditioned inhibition, negative occasion setting could also be an important inhibitory classical conditioning consideration in the context of impulsivity. The trial structure of this procedure may more closely mimic the 5CSRTT given that the inhibitory cue precedes the normally excitatory cue rather than being simultaneously presented, as in conditioned inhibition. The negative occasion setting procedure has been used in preclinical work, including in adolescent rats (a developmental period characterized by increased impulsivity; Meyer & Bucci, 2014, 2017a, 2017b), adult rats with decreased prefrontal cortex activity and increased nucleus accumbens activity to mimic the imbalance present during adolescence (Meyer & Bucci, 2016), as well as spontaneously hypertensive rat model for ADHD (Bucci et al., 2008). Conditioned inhibition, on the other hand, is more similar to the Go/No-go test of impulsive action in which mice withhold responding during no-go cues presented simultaneously with a lever operand (in the absence of the no-go cue, presses the lever gives reward). Interestingly we have also previously reported deficits in the Go/No-go task in mice lacking the 5-HT<sub>1B</sub>R (Nautiyal et al., 2017). It could be useful to study multiple different kinds of inhibition in the same preclinical model for impulsivity, as negative occasion setting and conditioned inhibition can be biologically dissociated (MacLeod & Bucci, 2010; Meyer & Bucci, 2014a). Whether performance in either, or both, of these procedures is impacted in a model could suggest which brain regions and circuits could be driving impulsivity as well.

To consider the role of the 5-HT<sub>1B</sub>R in responding to classically conditioned excitatory cues, we measured Pavlovian appetitive conditioning behavior under various contingencies, as well as responding in 100% versus 25% reinforcement schedules. We found that mice lacking the 5-HT<sub>1B</sub>R show no differences in responding to cues in a positive contingency reinforcement schedule, but did have increased responding to cues that were presented in the context of reward, but were not explicitly predictive of reward (a zero contingency condition). Interestingly, the increased responding to cues did not occur when the cues and rewards were separated by an ITI in a negative contingency condition or when the mice were on a 25% reinforcement schedule. There were some procedural differences between the contingency experiments and the 100% versus 25% reinforcement to maintain session length; there were fewer US presentations and a longer US-US interval in the 100% versus 25% reinforcement experiment. It is possible that a difference in partial reinforcement would only emerge with shorter interval timings, so future experiments could explore the effects of ITI length on partial reinforcement in this model. If there is no difference in partial reinforcement as our data suggest, then the increased responding in the 5-HT<sub>1B</sub>R may be something else unique to the zero contingency condition, such as the variable orientations of the CS and US presentations, including the presence of trials similar to backwards or trace conditioning. Future studies could explore the effect of 5-HT<sub>1B</sub>R on trace conditioning, where there is a delay between cue offset and the onset of reinforcement. We would expect mice lacking the 5-HT<sub>1B</sub>R to have increased responding to the cue over longer delay periods compared to controls. Interestingly, in support of this hypothesis, in adolescence, rats have enhanced trace conditioning compared to preadolescent and adult controls (Hunt et al., 2016).

One potential unifying explanation for the increased responding to cues in the zero contingency condition and the increased operant responding in tests of impulsive action is that the absence of the 5-HT<sub>1B</sub>R receptor could alter perception of timing. If the ITI of the 5CSRTT is perceived as being shorter than it actually is, animals may respond prematurely. Similarly, in the conditioning paradigms, if the time between cue and reinforcement was subjectively reduced, the associative strength of that cue may be increased, resulting in the increased responding to cues when there is a weak temporal relationship with reward. This would also be consistent with previous findings that mice lacking the 5-HT<sub>1B</sub>R maladaptively respond early in an instrumental differential reinforcement of low-rate responding paradigm, resulting in a left shifted response distribution, i.e. earlier time of peak responding

(Nautiyal et al., 2017). Additionally, there are previously reports suggesting a role for serotonin signaling in modulating temporal perception and discrimination (Asgari et al., 2006; Halberstadt et al., 2016). Interestingly, this explanation would conform with our previous results showing the effects of 5-HT<sub>1B</sub>R in a delay discounting test, in which we report that mice lacking the 5-HT<sub>1B</sub>R actually have no differences in rate of delay discounting, but in fact have a higher preference for a larger reward regardless of delay (Nautiyal et al., 2017). This could feasibly be due to a subjective shortening of time perception such that delays for seems shorter and are therefore more tolerated (Paasche et al., 2019; Wittmann & Paulus, 2008).

An alternative explanation for the effect of 5-HT<sub>1B</sub>R on behavioral responding is that the phenotype is the result of generalized hyperactivity. However, we find that our data do not support this interpretation. First, in the 5CSRTT training, mice lacking the 5-HT<sub>1B</sub>R learned to inhibit premature responding over time. If impulsive action were the result of general increased activity, we would expect to see this behavior persist over all sessions. Additionally, in the classical conditioning paradigms, all responding was measured as an increase from ITI responding to control for potential differences in baseline responding, so it is unlikely hyperactivity contributed to these results. Finally, we have previously reported no effect of 5-HT<sub>1B</sub>R knockout on open field activity (Nautiyal et al., 2017). More plausibly, changes in reward processing, as we have previously reported (Desrochers et al., 2021), could alter responding in both operant and classical conditioning experiments. If mice lacking the 5-HT<sub>1B</sub>R have increased subjective valuation of reward or increased motivation for reward, this could enhance the salience of excitatory cues (Rescorla & Wagner, 1972), potentially enhancing the relative associative strength over the temporal separation of the cue and reward in the independent interval condition. In this interpretation, it is possible that the deficits in response inhibition seen in the 5CSRTT and conditioned inhibition experiments could occur without changes in inhibitory processing and could alternatively be the result of increased reward drive (Desrochers et al., 2021).

## 5. Conclusion

Overall, these studies show that serotonin signaling through the 5-HT<sub>1B</sub>R influences cue reactivity in both excitatory and inhibitory contexts, despite intact inhibitory learning. Additionally, the conditioned inhibition and Pavlovian appetitive conditioning experiments demonstrate that increased impulsivity may be seen in differences in responding in classical conditioning, in the absence of action-based consequences. However, the extent to which the operant and Pavlovian effects seen in mice lacking the 5-HT<sub>1B</sub>R have similar underlying behavioral and neural mechanisms remains unclear. It is possible that 5-HT<sub>1B</sub>R plays a role in distinct systems supporting these different behaviors, so subsequent experiments could examine the potential convergence of neural circuits using tissue-specific manipulations of serotonin signaling. More broadly, we suggest that careful designed and analyzed behavioral testing could contribute to a better understanding of the underlying cognitive and neural mechanisms of impulsivity, as well as characterization of clinical presentation and preclinical models. Specifically, combining tests of classical conditioning, especially Pavlovian conditioned inhibition, with traditional operant-based tests of impulsivity may be important to gain insight into the learning processes which contribute to deficits in response inhibition.

## CRedit authorship contribution statement

**Stephanie S. Desrochers:** Conceptualization, Methodology, Software, Formal analysis, Investigation, Writing – original draft. **Katherine M. Nautiyal:** Conceptualization, Methodology, Resources, Funding acquisition, Project administration, Supervision, Writing – review & editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

Funding was provided by NIH R00 MH106731 and R01 MH126178 to KMN. We would like to thank Jamie Dinulos for help with data collection in early pilot work. We would also like to thank Professors Robert Leaton and Travis Todd for their insight and guidance, as well as comments on a previous version of this manuscript. Finally, we are incredibly grateful to have received the wonderful mentorship of Dave Bucci. His previous work on inhibitory learning inspired some of the present experiments and our thinking about impulsivity in the context of conditioned inhibition, and his early guidance on this project was much appreciated. Beyond his wonderful scientific advice, Dave's kindness and support were invaluable to us as a junior faculty member and a new graduate student.

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## CHAPTER 3

### **Exploring the neural mechanisms of serotonin 1B receptor knockout modulation of reward and impulsivity**

#### **Abstract**

Impulsivity is a complex construct which can be conceptualized as either a deficit in response inhibition or an increase in reward drive, resulting in a deficit in behavioral control. Observing which specific behaviors occur in a given presentation of impulsivity can guide hypotheses and provide a better understanding of the underlying neural circuitry. In the current study we focus on a preclinical model for impulsivity using manipulations of the serotonin 1B receptor (5-HT<sub>1B</sub>R) in mice. As previously shown, we report that mice lacking the 5-HT<sub>1B</sub>R in a whole-brain, whole-life genetic knockout have increased hedonic taste reactivity and increased impulsive action in a Go/No-go test compared to controls. In the first experiment of this study, we were also able to reduce the impulsivity in 5-HT<sub>1B</sub>R mice by using a lower reward outcome value equivalent to the maximum reward value of controls. This suggested there may be a relationship between reward valuation and impulsivity in these mice. Therefore, we began to target the 5-HT<sub>1B</sub>R knockout to reward-related brain regions, namely the nucleus accumbens shell (NAc shell). Manipulations of the NAc shell in isolation did not cause any changes in the behaviors measured, suggesting that receptors in this region are not solely responsible for the behavioral phenotype of the whole brain knockout. Therefore, we expanded our targeting to simultaneously eliminate multiple populations of 5-HT<sub>1B</sub>Rs, including both heteroreceptor (localized to non-serotonergic cells) and autoreceptor (localized to serotonergic cells) receptors. Through a series of viral and genetic, region and cell type specific knockouts, we discovered that both 5-HT<sub>1B</sub> autoreceptor (through viral knockout of receptors on DRN cells) and Emx1+ heteroreceptor knockout are likely involved in the increased reward-motivated behaviors and impulsive action present in this model. Additionally, combined autoreceptor and VGAT+ heteroreceptor knockout increased hedonic taste reactivity. These experiments demonstrate a dissociation between populations of 5-HT<sub>1B</sub>Rs that modulate the motivation/impulsivity and hedonic phenotypes and support the hypothesis that multiple populations of 5-HT<sub>1B</sub>Rs are together involved in reward and impulse control.

#### **Introduction**

Impulsivity is a primary characteristic of many mental health disorders but is rarely a target phenotype for treatment. Increased impulsive behavior is prevalent in attention deficit hyperactive disorder (ADHD), conduct disorders, substance use disorders, and gambling disorders (Dalley & Robbins, 2017; MacKillop et al., 2016; Robbins et al., 2012). In all of these disorders, the pathological impulsive behavior may vary greatly, and impulsivity may even play different roles as either a causal or emergent property. The lack of specific treatment options for impulsivity in these different presentations is likely due to its complexity in pathology, with many distinct, non-overlapping components with dissociable biological bases (Bari & Robbins, 2013; Nautiyal et al., 2017; Winstanley et al., 2004). Therefore, parsing the individual components that contribute to distinct impulsive presentations can inform the etiology of disordered impulse control and guide hypotheses about specific neural mechanisms. Of significance to the present study, impulsivity could be the ultimate effect of increased reward drive resulting in an enhance ‘go’ response in the presence of an appetitive outcome (Desrochers et al., 2022).

One neural mechanism of particular interest in the study of impulsivity and reward is the expression of the serotonin 1B receptor (5-HT<sub>1B</sub>R). 5-HT<sub>1B</sub>R is an inhibitory G-protein coupled receptor expressed on axon terminals where it inhibits neurotransmitter release in both serotonin and non-serotonin neurons (Boschert et al., 1994; Jolima et al., 2000; Mizutani et al., 2006). In humans, single nucleotide polymorphisms in the gene encoding 5-HT<sub>1B</sub>R are associated with various disorders with dysregulated impulse control (Cao et al., 2013; Contini et al., 2012; Proudnikov et al., 2006; Zouk et al., 2007). In mice, global knockout of 5-HT<sub>1B</sub>Rs causes elevated impulsive action, but not impulsive choice, in standard instrumental tests of impulsivity, making it an ideal model for studying the substrates of one specific kind of impulsivity (Brunner & Hen, 1997; Nautiyal et al., 2017; Pattij et al., 2003). Using a transgenic line (tetO1B<sup>fl/fl</sup>) which allows conditional and tissue-specific knockout of 5-HT<sub>1B</sub>R, our research has previously shown that adult absence of the 5-HT<sub>1B</sub>R increases impulsive action as measured by a decreased ability to withhold responding in the Go/No-go test (Desrochers et al., 2021; Nautiyal et al., 2015). Interestingly, we found that this impulsivity can be ameliorated by decreasing the amount of reward. While there is no effect on impulsive choice as measured by discounting rate in a delay discounting task, mice lacking 5-HT<sub>1B</sub>R have increased preference for a larger reward, which has also been seen in high novelty responsive rats (Flagel et al., 2010). This suggests that they may ‘like’ the reward more (Ostlund et al., 2013). Taken together, these data suggest that expected reward value could be one behavioral substrate that contributes to impulsive action in mice lacking 5-HT<sub>1B</sub>R.

Given that impulsivity is behaviorally complex, it is not surprising that many different brain systems and regions have been implicated in its control. Specific to reward-based impulsive action, the NAc shell subregion in particular is critical for hedonics, valence coding, and motivational value, contributing to the subjective valuation of outcomes (Castro & Berridge, 2014; Mannella et al., 2013). Alternatively, the core subregion appears to be more involved in the modulation of impulsive choice, while manipulations of the shell subregion impact inhibitory control and impulsive action (Ghods-Sharifi & Floresco, 2010; Pothuizen et al., 2005; Sesia et al., 2008; Wiskerke et al., 2011). Specifically, prior research has shown that inactivation of the NAc shell increases premature responding on the 5CSRTT (Feja et al., 2014). Additionally, behavioral inhibition of reward motivated behaviors seems to be mediated by intact afferent signaling, especially in the vmPFC, which expresses 5-HT<sub>1B</sub>R mRNA (Anastasio et al., 2019; Bruinvels et al., 1994; Chudasama et al., 2003; Ghazizadeh et al., 2012). Importantly, both 5-HT<sub>1B</sub>R mRNA and protein are expressed in the NAc (Bruinvels et al., 1993, 1994). Additionally, global knockout of the 5-HT<sub>1B</sub>R results in increased dopamine release in the NAc, but not other regions like the dorsal striatum (Nautiyal et al., 2015). Based on the role of the NAc shell circuitry in reward behavior and impulsivity, along with the localization of 5-HT<sub>1B</sub>Rs in the relevant nodes, we hypothesized that 5-HT<sub>1B</sub>R expression on projections to the NAc shell modulates impulsive action via enhanced reward valuation.

An alternative hypothesis to NAc shell 5-HT<sub>1B</sub>R expression being solely required for impulse control is that there are multiple populations of 5-HT<sub>1B</sub>Rs which act in concert to modulate impulsive behavior. This is supported by prior evidence demonstrating that neither forebrain heteroreceptor nor autoreceptor knockout result in impulsive action in a differential reinforcement of low rate responding test (Nautiyal et al., 2015). However, mice lacking 5-HT<sub>1B</sub> only on serotonergic neurons have some increased sucrose preference compared to controls (Nautiyal et al., 2016). This suggests that to present with increased impulsivity, both an absence of a 5-HT<sub>1B</sub> autoreceptor population and a heteroreceptor population may be required, which can be tested using a combined genetic and viral knockout approach.

In the present set of experiments, we first establish a relationship between impulsivity and reward value in 5-HT<sub>1B</sub>R knockout mice by normalizing outcome value (based on measured taste reactivity in a lickometer) in operant tests of motivation and a Go/No-go test of impulsive action. We then target neural systems associated with hedonic reward valuation, hypothesizing that changes in signaling due to 5-HT<sub>1B</sub>R loss increase reward-related behaviors as well as impulsivity. Specifically, we knockout 5-HT<sub>1B</sub>Rs on neurons projecting to and from the NAc shell, as well as autoreceptor populations of 5-HT<sub>1B</sub>R on serotonergic neurons from the DRN.

We additionally explore the role of broader populations of 5-HT<sub>1B</sub>Rs in the behavioral phenotypes by combining genetic knockouts of Emx1+ or VGAT+ localized 5-HT<sub>1B</sub>Rs with viral autoreceptor knockout. Ultimately, we find that combined 5-HT<sub>1B</sub> Emx1+ heteroreceptor and autoreceptor knockout produces increased reward motivation and impulsivity, while VGAT+ and autoreceptor knockout enhances taste reactivity, suggesting that serotonin modulation of multiple populations of neurons together mediates these behaviors.

## Methods

### *Mice*

All animals were bred in the Center for Comparative Medicine at Dartmouth College, and were weaned at postnatal day (PN) 21 into same sex littermate cages of 2-4 mice in ventilated racks. Mice were housed on a 12:12 light-dark cycle, and fed *ad lib* chow until behavioral testing at >10 weeks, at which time they were food restricted to maintain 85-90% baseline bodyweight for the duration of the experiment. Water was *ad lib* for the duration of all experiments. All procedures were approved by the Institutional Animal Care and Use Committee of Dartmouth College.

### *Experiment 1: Reward normalization effects on serotonin 1B receptor knockout-based impulsivity*

The floxed tetO1B mouse model was used to generate groups of mice lacking expression of 5-HT<sub>1B</sub>R through crosses to a  $\beta$ Actin-tTS mouse line (tetO1B +/+ females crossed to tetO1B +/+ :: $\beta$ Actin-tTS + males), as previously reported (Nautiyal et al., 2015). TetO1B:: $\beta$ Actin-tTS + mice and their littermate tetO1B:: $\beta$ Actin-tTS – mice were used as experimental knockout (5-HT<sub>1B</sub>R knockout) and control (control) groups, respectively.

Table 1. Subject counts for Experiment 1 lickometer testing. Final counts are listed in parentheses following exclusion criteria as listed in Lickometer methods below.

	5-HT <sub>1B</sub> R knockout	Control
Male	16 (14)	18 (17)
Female	10 (10)	18 (14)

Table 2. Subject counts for Experiment 1 operant testing (subset of lickometer group above). Final counts are listed in parentheses following exclusion criteria as listed in operant testing methods below.

	5-HT <sub>1B</sub> R knockout/ 100% milk	5-HT <sub>1B</sub> R knockout/ 40% milk	Control/ 100% milk	Control/ 40% milk
Male	5 (5)	6 (5)	8 (6)	4 (4)
Female	7 (7)	7 (7)	6 (6)	5 (5)

### ***Experiment 2: Viral nucleus accumbens serotonin 1B receptor knockout***

The floxed tetO1B mouse model was combined with viral targeting of the nucleus accumbens medial shell using Cre-based viruses to knockout the serotonin 1B receptor. Mice were anesthetized with 5% isoflurane, then maintained at 1-2% during stereotaxic surgery. Skull was exposed and 0.5mm diameter holes were drilled at the appropriate coordinates. For the present experiment, 500nL of a Cre virus (pAAV-EF1a-mCherry-IRES-Cre (AAV8); Addgene 55632-AAV8;  $2.1 \times 10^{12}$  GC/mL), retrograde Cre virus (pAAV-EF1a-mCherry-IRES-Cre (AAVr); Addgene 55632-AAVrg;  $4.3 \times 10^{12}$  GC/mL), control GFP virus (pEEN-AAV-EF1a-eGFP-wPRE-rBG (AAV9); Addgene 105547-AAV9;  $2.7 \times 10^{12}$  GC/mL), or retrograde control GFP virus (pAAV-hSyn-eGFP (AAVr); Addgene 50465-AAVrg;  $7.4 \times 10^{12}$  GC/mL) was injected into the nucleus accumbens medial shell (mm coordinates relative to bregma: AP +1.34, DV - 4.7, ML +/- 0.75) of each hemisphere at a rate of 100nL/m. Following surgery, mice were group housed and administered 0.1mL ketoprofen (1mg/mL) subcutaneously for 3 days. At least 5 weeks were allowed for viral expression and receptor knockout prior to behavioral testing. Note that control groups were combined for analysis.

Table 3. Subject counts for Experiment 2. Final counts are listed in first parentheses following exclusion criteria for lickometer, and second parentheses following exclusion criteria for operant testing.

	NAc Cre	NAc retro Cre	NAc Control	NAc retro Control
Male	9 (9) (9)	6 (6) (6)	6 (5) (6)	3 (3) (3)
Female	5 (4) (5)	3 (3) (3)	3 (3) (3)	2 (1) (1)

### ***Experiment 3: Genetic cell-type and viral dorsal raphe nucleus serotonin 1B receptor knockout***

The floxed tetO1B mouse model was used to generate groups of mice lacking expression of 5-HT<sub>1B</sub>R in the forebrain through crosses to a Emx1-cre mouse line (tetO1B + / + females crossed

to tetO1B +/+ ::Emx1-cre + males), and in GABAergic cells through crosses to a VGAT-cre mouse line (tetO1B +/+ females crossed to tetO1B +/+ ::VGAT-cre + males), as previously reported (Nautiyal et al., 2015). TetO1B:: Cre + mice and their littermate tetO1B:: Cre – mice were used as experimental knockout and control groups, respectively. General surgical procedures were performed as described for Experiment 2. For the present experiment, 500nL of virus was injected into the dorsal raphe nucleus at a 30° angle from vertical (mm coordinates relative to bregma: AP -4.6, DV -3.0, ML +/- 0) at a rate of 100nL/m. TetO1B:: Emx1-cre+ and tetO1B:: VGAT-cre+ were injected with a cre virus (pAAV-EF1a-mCherry-IRES-Cre (AAVr); Addgene 55632-AAVrg;  $4.3 \times 10^{12}$  GC/mL), while tetO1B:: Emx1-cre- and tetO1B:: VGAT-cre- were injected with a GFP virus (pEEN-AAV-EF1a-eGFP-wPRE-rBG (AAV9); Addgene 105547-AAV9;  $2.7 \times 10^{12}$  GC/mL). Note that control groups were combined for analysis.

Table 4. Subject counts for Experiment 3. Final counts are listed in first parentheses following exclusion criteria for lickometer, and second parentheses following exclusion criteria for operant testing. \*One mouse died after 7 sessions of Go/No-go testing.

	Emx1-cre+/DRN Cre	VGAT-cre+/DRN Cre	Emx1-cre-/DRN Control	VGAT-cre-/DRN Control
Male	7 (6) (7)*	4 (3) (4)	4 (4) (4)	2 (2) (2)
Female	0 (0) (0)	4 (3) (3)	0 (0) (0)	2 (2) (2)

#### ***Experiment 4: Viral nucleus accumbens and dorsal raphe nucleus serotonin 1B receptor knockout***

The floxed tetO1B mouse model was combined with viral targeting of the nucleus accumbens medial shell and dorsal raphe nucleus using Cre-based viruses to knockout the serotonin 1B receptor. General surgical procedures were as described in Experiment 2. For the present experiment, 500nL of a retrograde Cre virus (pAAV-EF1a-mCherry-IRES-Cre (AAVr); Addgene 55632-AAVrg;  $4.3 \times 10^{12}$  GC/mL) or retrograde control GFP virus (pAAV-hSyn-eGFP (AAVr); Addgene 50465-AAVrg;  $7.4 \times 10^{12}$  GC/mL) was injected into the nucleus accumbens medial shell (mm coordinates relative to bregma: AP +1.34, DV -4.7, ML +/- 0.75) of each hemisphere at a rate of 100nL/m. Additionally, 500nL of a Cre virus (pAAV-EF1a-mCherry-IRES-Cre (AAV8); Addgene 55632-AAV8;  $2.1 \times 10^{12}$  GC/mL) or control GFP virus (pEEN-AAV-EF1a-eGFP-wPRE-rBG (AAV9); Addgene 105547-AAV9;  $2.7 \times 10^{12}$  GC/mL) was injected into the dorsal raphe nucleus at a 30° angle from vertical (mm coordinates relative to bregma: AP -4.6, DV -3.0, ML +/- 0).

Table 5. Subject counts for Experiment 4. Final counts are listed in first parentheses following exclusion criteria for lickometer, and second parentheses following exclusion criteria for operant testing.

	NAc rCre/DRN Cre	NAc rControl/DRN Cre	NAc rControl/DRN Control
Male	3 (3) (3)	4 (4) (4)	4 (4) (4)
Female	4 (3) (4)	4 (4) (4)	4 (4) (4)

### ***RNAscope and Viral Targeting Confirmation***

Viral targeting and receptor knockout were confirmed using RNAscope multiplex fluorescent assays v2 (ACDbio 323100) for Experiment 2. Animals were excluded from data if correct targeting to the bilateral NAc medial shell was unable to be confirmed for this experiment. Targeting has not yet been confirmed for Experiment 3-4. Following completion of *in vivo* testing, mice were administered a lethal dose of ketamine (0.1mL) intraperitoneally. Brains were extracted and fresh frozen on dry ice prior to being stored at -80°C. Brains were then sectioned at 20µm in a cryostat and thaw mounted onto Superfrost Plus slides (Fisher Scientific 12-550-15) prior to RNAscope protocol. Tissue was fixed for 15m with 4% paraformaldehyde in phosphate buffered saline at 4°C followed by ethanol dehydration. RNAscope assays were performed according to the manufacturer's instructions for fresh frozen sections, including pre-hybridization, hybridization, washing, and fluorescent tagging (Akoya Biosciences Opal Fluorophores FP1488001KT, FP1497001KT) for RNA transcripts and DAPI nuclear staining before coverslipping. The RNAscope probes used were 5-HT<sub>1B</sub> (ACDbio 315861), and mCherry (ACDbio 431201-C3) or eGFP (ACDbio 400281-C2) as appropriate for the expressed viruses.

Images for each brain were taken with a 4x objective on a Keyence Fluorescent Microscope (Keyence Co.). Viral spread was aligned and traced onto appropriate plates of the Mouse Brain in Stereotaxic Coordinates (Franklin & Paxinos, 2019) using Adobe Photoshop (Adobe Inc.).

### ***Behavioral Testing***

#### ***Lickometer***

Taste reactivity to evaporated milk reward was tested in 2 identical 16 bottle Davis Rig Lickometers (MEDAssociates, MED-DAV-160 M), which measure licking to bottle spouts using a capacitance-based system. Spouts are gated by a stainless steel door, which remains closed

before/after sessions and during ITI periods. Mice were initially trained to lick to a single bottle of 100% evaporated milk for 15m constant-access sessions in cage mate groups, followed by solo training. These group and solo sessions were repeated until mice achieved >200 licks/session. Subjects were excluded from further lickometer testing if this criteria was not achieved after 3 sessions. Subjects were then trained in a 30 minute session of a 2 bottle task, with both bottles containing 100% evaporated milk, to habituate them to shorter access times and a moving door. Trials were terminated after 5s after the first lick or after 60s if no licks occurred, at which point the apparatus door closed and there was a 7.5s ITI before alternating to the other bottle. Finally, mice were tested in a 30m session of a 6 bottle task, with 0%, 20%, 40%, 60%, and 100% evaporated milk diluted in drinking water. Trials terminated 60s after the first lick or after 120s if no licks occurred, at which point the apparatus door closed and there was a 7.5s ITI before alternating to the next bottle. Sequence of bottle presentations were randomized without replacement. The 6 bottle task was repeated for a second day with a new sequence to prevent order effects, and data was collected as average licks per 60s trial for each concentration, averaged over the 2 sessions, and normalized by bodyweight. For experiment 3, lickometer testing was repeated following completion of the Go/No-go, and consisted of a 15m constant-access reminder session with a 30m 6 bottle task the following day.

### ***Operant behavioral apparatus***

All remaining behavioral tests were conducted in 8 identical modular operant chambers (Med Associates), each in an individual, ventilated isolation chamber. Operant chambers consisted of stainless steel walls and bar floor, with one side of the chamber having a reward noseport opening where liquid reward was delivered by a dipper of volume 0.02mL. Entries to this noseport were detected by an infrared beam break detector. There was an ultra-sensitive stainless steel retractable lever 2.2cm above the floor on either side of the reward port with an LED light positioned above. The house light was positioned on the upper center of the wall opposite the reward port, with a speaker on the outer side of the same wall. This house light remained on for the duration of operant training/testing sessions. Stimuli were delivered and data were collected through a computer running MED-PC IV software (Med Associates).

### ***Operant Training***

All operant training and subsequent testing was conducted in 1 session/day, 4-6 days/per week. For Experiments 2-4, the liquid reward was 100% evaporated milk (Nestle Carnation). In Experiment 1, mice were given either 100% milk or 40% milk diluted in drinking water, which

remained consistent through all operant training and testing. Mice were first trained to retrieve the reward from the dipper in the reward noseport. They were then randomly assigned one lever which was maintained throughout testing (only one lever is ever presented to each mouse). During training, lever presses were rewarded on a continuous reinforcement schedule (CRF) until mice received 55 of 60 possible rewards in a 60m session. Mice were excluded from further testing if they did not meet this criteria (mice were also excluded if performance dropped below 10 rewards per session for >2 operant sessions during subsequent testing).

### ***Random and Progressive Ratios of Responding***

Following CRF lever training, mice proceeded through random ratios of responding (RR) schedules, where the lever was present for a 60m session and the mice had to press an average number of times to receive a reward. Mice began at a RR5 schedule, followed by RR10, then RR20, each for 3 sessions (though note that one mouse in experiment 4 only had 2 days of random ratio 5 data included due to being behind for extra CRF training sessions; in experiment 3 due to a computer error causing lost data/stopping in the middle of a random ratio 5 session, 7 mice were given a reminder CRF and random ratio 5 session to combat extinction and therefore only had 2 days of random ratio 10). Data was recorded as rewards earned during the session, which was averaged over all sessions of each schedule for each mouse. After RR 20 was complete, mice proceeded to a progressive ratio schedule. For experiment 1, this schedule was x2 where the lever press requirement to get a reward doubled with each trial. As this schedule was determined to be too difficult for this current set of mice to see group differences, for experiments 2-4 this schedule was +8 where the lever press requirement to get a reward increased by 8 with each trial. The progressive ratio session timed out after 3m of no lever presses or maximum 2h. Again, data was recorded as rewards earned during the session.

### ***Go/No-go***

Next, mice were moved onto Go trial training (60 trials per session) for 5 sessions prior to the Go/No-go test of impulsive action (as previously described in (Nautiyal et al., 2015)). During the main test, mice had 9 sessions which each included 30 discrete Go trials and 30 No-Go trials which were pseudo-randomly presented across blocks of 10 trials with a variable ITI averaging 45s. Go trials consisted of a lever presentation, and mice had to press this lever within 5s to get a reward. No-go trials consisted of a lever presentation in addition to 2 cues (housetlight turning off and LED above the lever turning on), and mice had to withhold pressing for 5s to get a reward. If a press occurred before 5s elapsed, no reward was presented, the lever retracted, the cues

switched back to standard, and the next ITI began. The impulsivity index was calculated by subtracting the proportion of correct No-Go trials from the proportion of correct Go trials (a higher impulsivity index indicates more impulsive responding). For experiment 1, impulsivity index was averaged over the first 2 days of testing for group difference analysis, as there was a rapid drop off of impulsivity in the entire experiment after this, suggesting all mice learned this task very quickly. For experiment 4, mice were also tested for 3 days with long (10s) No-go trials following standard testing. Also in experiment 4, one mouse died after 7 sessions of testing, so data were analyzed only for these sessions for the first phase. This animal was then not included in the second, long ITI phase.

### ***Statistics***

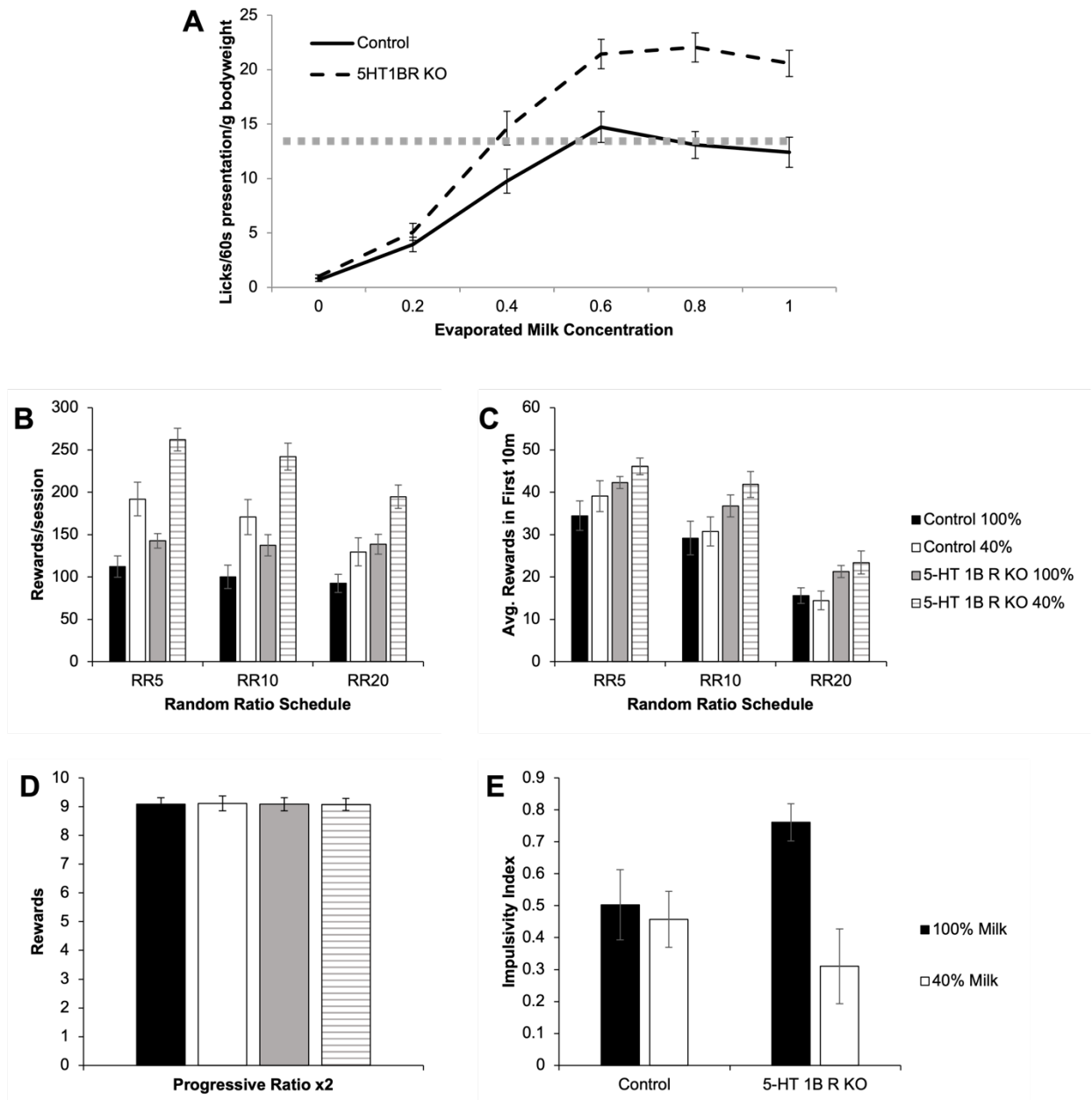
Statistics were calculated in SPSS (IBM). Mixed repeated and independent measures ANOVAs were used as appropriate. For initial analysis, sex was included as an additional factor where possible, but no measures included significant sex interactions with variables of interest, so this factor was excluded from the final analysis.

## **Results**

### ***Experiment 1: Reward normalization effects on serotonin 1B receptor knockout-based impulsivity***

In this first experiment, we aimed to probe the relationship between reward value and impulsivity in whole brain, whole life 5-HT<sub>1B</sub>R knockout mice. First, we measured hedonic taste reactivity in a lickometer test for different concentrations of evaporated milk reward (Fig. 1A). All mice increase licking as concentration increases ( $F_{5,265}=194.486$ ,  $p<0.001$ ), with 5-HT<sub>1B</sub>R knockout mice licking at a higher rate compared to controls, especially at higher concentrations ( $F_{1,53}=15.244$ ,  $p<0.001$  for main effect of genotype;  $F_{5,265}=176.193$ ,  $p<0.001$  for interaction). We used these lickometer results to determine the concentration for the 5-HT<sub>1B</sub>R knockouts where their licking rate was similar to the 100% evaporated milk licking rate in the controls (indicated by the horizontal dotted line on Fig. 1A). In this experiment, that concentration was 40%, which represents the point at which taste reactivity in the 5-HT<sub>1B</sub>R knockouts would be approximately normalized to the level of controls. We then used this normalized reward value as the reward for a group of mice in operant motivational and impulsivity tests, to determine if reducing reward value also reduces the enhanced reward-related behaviors previously observed in 5-HT<sub>1B</sub>R knockout mice.

In a random ratio test for operant motivation, we replicated the effect that mice lacking 5-HT<sub>1B</sub>R work to get more rewards than controls across effort schedules (Fig. 1B;  $F_{1,42}=15.806$ ,  $p<0.001$  for main effect of genotype). However, reducing reward value actually increased rewards obtained by both groups ( $F_{1,42}=33.677$ ,  $p<0.001$  for main effect of concentration condition), likely due to decreased satiety with the lower caloric content of each individual reward. To suggest this is indeed the case, looking at performance in the first 10m of the test shows a genotype effect but no significant effect of reward reduction before satiety has been reached in any group (Fig. 1C;  $F_{1,42}=7.076$ ,  $p=0.011$  for main effect of genotype;  $F_{1,42}=3.015$ ,  $p=0.09$  for main effect of concentration condition). We also performed a progressive ratio x2 task, however, this schedule did not produce any group differences likely because the effort cost was too high and all mice stopped responding at a similar time (Fig. 1D; all  $ps>0.9$ ). All subsequent experiments using this test therefore used a progressive ratio +8 schedule. Finally, we used a Go/No-go test and replicated the effect that mice lacking the 5-HT<sub>1B</sub>R have increased impulsive action or difficulty withholding responding for 100% milk reward as measured by impulsivity index (Fig. 1E). Interestingly, reducing the reward value to 40% did reduce the impulsivity in the 5-HT<sub>1B</sub>R knockout mice to the level of controls ( $F_{1,42}=4.105$ ,  $p=0.049$  for interaction of concentration condition x genotype;  $F_{1,42}=6.130$ ,  $p=0.017$  for main effect of concentration condition).

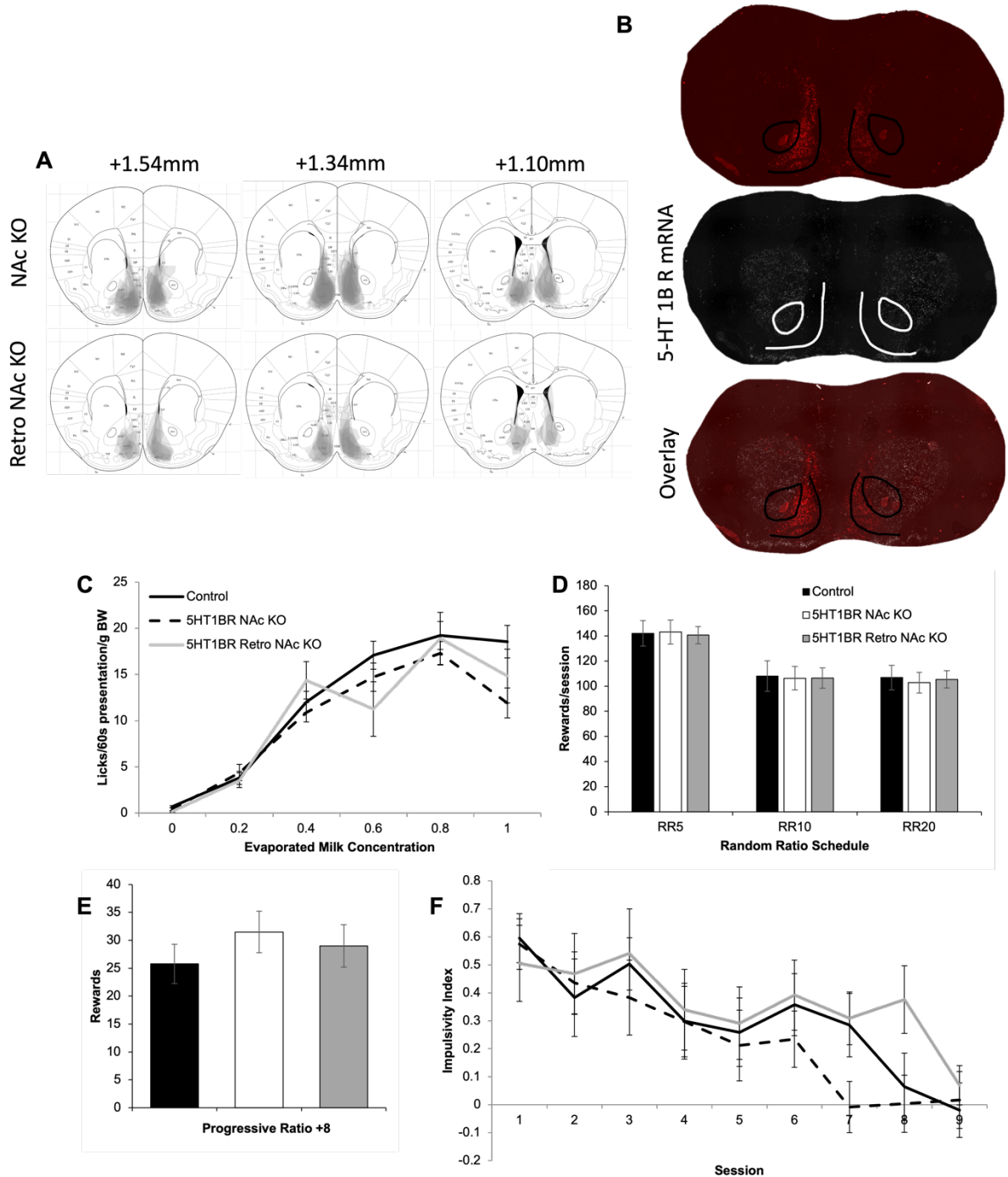


**Figure 1. Reducing reward outcome value decreases impulsivity in 5-HT<sub>1B</sub>R knockout mice.** A) Lickometer 6 bottle test measuring lick rate (normalized by bodyweight) for controls and 5-HT<sub>1B</sub>R knockout mice. Dotted grey line indicates lick rate equivalent to 100% lick rate of the controls. B) Rewards earned per session and C) rewards earned in first ten minutes of session across increasing random ratio schedules in controls and 5-HT<sub>1B</sub>R knockout mice, under normal 100% milk and reduced 40% milk conditions. D) Rewards earned in one session of a progressive ratio x2 schedule. E) Impulsivity index (proportion correct Go trials-proportion correct No-go trials) averaged over the first 2 sessions in a Go/No-go test of impulsive action. Error bars are +/- 1SE.

### *Experiment 2: Viral nucleus accumbens serotonin 1B receptor knockout*

Based on the results of Experiment 1, we first hypothesized that the NAc medial shell, a region implicated in hedonic pleasure and impulsive action, is involved in the 5-HT<sub>1B</sub>R mediated behaviors seen in the whole brain knockout model. We therefore used viral Cre-mediated

knockout of the 5-HT<sub>1B</sub>R in afferent (5-HT<sub>1B</sub>R NAc KO; Cre virus) or efferent (5-HT<sub>1B</sub>R Retro NAc KO; retro Cre virus) projections in this region. This viral manipulation was localized to the correct region (Fig. 2A) and successfully knocked out 5-HT<sub>1B</sub>R mRNA (example image of 5-HT<sub>1B</sub>R NAc KO in Fig. 2B), however no significant differences in behavioral measures were seen. Specifically, we tested mice in a series of reward and impulsivity-based behaviors. There were no overall significant differences between groups in the lickometer (Fig. 2C;  $F_{2,31}=1.007$ ,  $p=.377$  for main effect of condition;  $F_{5,155}=103.652$ ,  $p<0.001$  for main effect of concentration; though note significant interaction effect due to drop in lick rate of NAc KO at 100% milk,  $F_{10,155}=2.509$ ,  $p=0.008$ ), in random ratio increase effort schedules (Fig. 2D; all  $ps>0.9$  except  $F_{2,66}=54.692$ ,  $p<0.001$  for main effect of schedule), in a progressive ratio +8 schedule (Fig. 2E;  $p>0.5$ ), or in a Go/No-go test for impulsive action (Fig. 2F; all  $ps>0.5$  except  $F_{8,264}=11.134$ ,  $p<0.001$  for main effect of session). As we were able to confirm expected knockout of the receptor, these results seem to suggest that loss of 5-HT<sub>1B</sub>Rs in the NAc shell projections are not independently responsible for the increased reward reactivity and impulsivity present in the whole brain genetic knockout.



**Figure 2. Viral knockout of the 5-HT<sub>1B</sub>R in NAc shell afferent or efferent projections does not increase reward-related or impulsive behaviors.** A) Viral expression patterns in mice with NAc (Cre; efferent) and Retro NAc (retro Cre; afferent) 5-HT<sub>1B</sub>R knockouts. B) Example of 5-HT<sub>1B</sub>R NAc KO mCherry mRNA viral expression (red) and 5-HT<sub>1B</sub>R mRNA expression (white). C) Lickometer 6 bottle test measuring lick rate (normalized by bodyweight) for controls, 5-HT<sub>1B</sub>R NAc KO, and 5-HT<sub>1B</sub>R Retro NAc KO mice. D) Rewards earned per session across increasing random ratio schedules. E) Rewards earned in one session of a progressive ratio +8 schedule. F) Impulsivity index (proportion correct Go trials-proportion correct No-go trials) over sessions in a Go/No-go test of impulsive action. Error bars are  $\pm$  1SE.

### *Experiment 3: Genetic cell-type and viral dorsal raphe nucleus serotonin 1B receptor knockout*

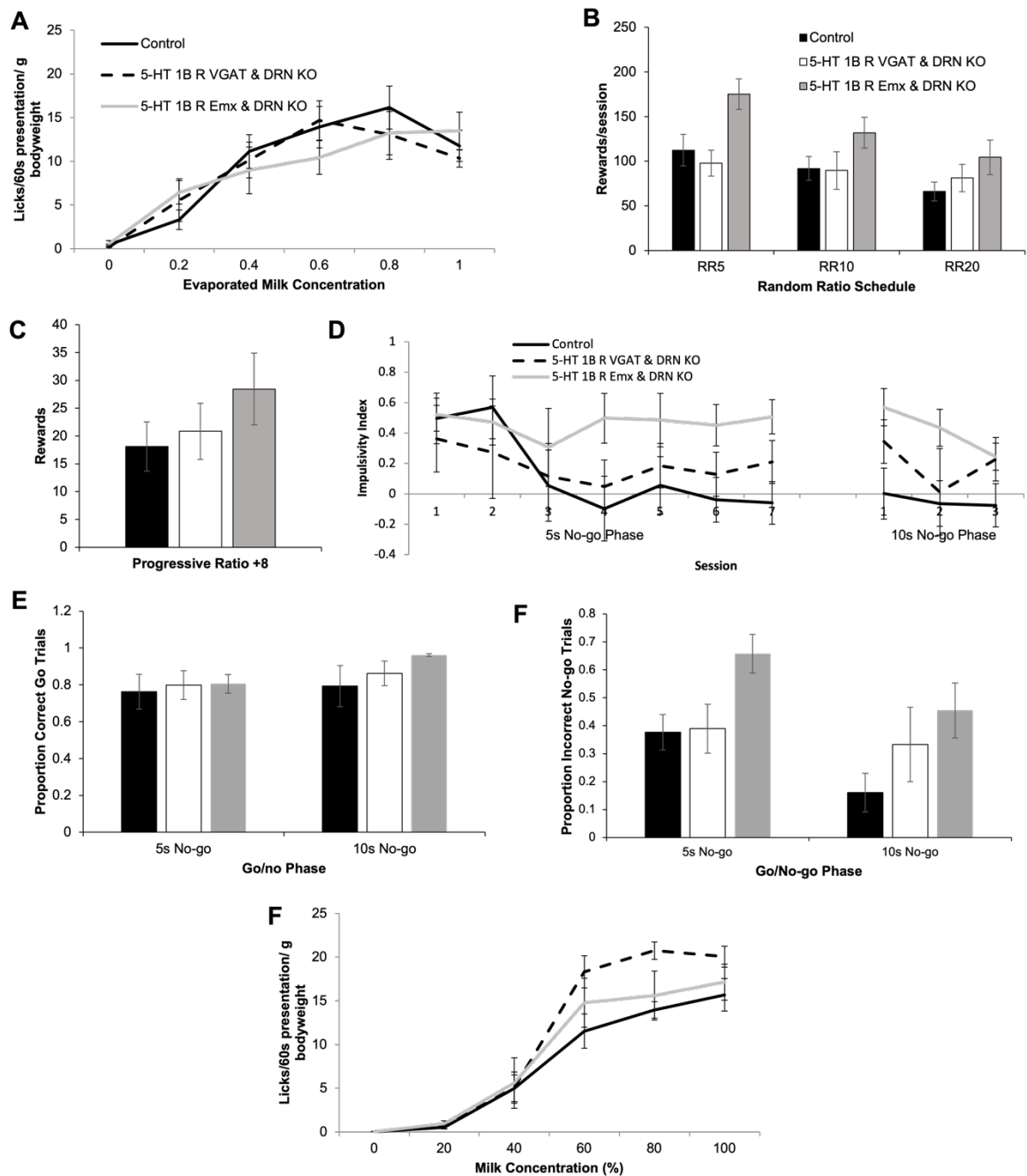
Since region specific knockouts did not yield the hypothesized behavioral phenotype, we took a broader approach by eliminating larger populations of 5-HT<sub>1B</sub> heteroreceptors in addition to autoreceptors. To accomplish this we used combined genetic cell type and viral approaches, with genetic crosses producing mice lacking 5-HT<sub>1B</sub>Rs in either Emx1 expressing or VGAT expressing cells in addition to a viral Cre-based knockout of autoreceptors by targeting the DRN. Viral expression and successful knockout have not yet been confirmed postmortem.

There were no significant differences between groups in the initial lickometer testing (Fig. 3A; all  $p$ s > 0.2 except  $F_{5,85}=42.401$ ,  $p < 0.001$  for main effect of concentration). However, under random ratio schedules of reinforcement, 5-HT<sub>1B</sub>R Emx1 & DRN knockout mice demonstrated increased motivation for reward, though this effect became smaller as effort increased (Fig. 3B;  $F_{4,38}=3.248$ ,  $p=0.022$  for interaction of viral manipulation and random ratio schedule;  $F_{2,19}=3.179$ ,  $p=0.064$  for main effect of viral manipulation; posthoc LSD test  $p$ s < 0.05 for 5-HT<sub>1B</sub>R Emx1 & DRN knockout vs. control and 5-HT<sub>1B</sub>R VGAT & DRN knockout). This significant effect did not continue in a progressive ratio +8 schedule (Fig. 3C; all  $p$ s > 0.3). Then, in the Go/No-go test, there was a non-significant increase in impulsivity index in the 5-HT<sub>1B</sub>R Emx1 & DRN knockout (Fig. 3D;  $F_{6,114}=2.595$ ,  $p=0.021$  for main effect of session;  $F_{2,19}=1.535$ ,  $p=0.208$  for main effect of condition;  $F_{12,114}=1.065$ ,  $p=0.396$  for interaction). Given the small sample size and variability in the composite measure of impulsivity index, we also separately analyzed correct Go trials and incorrect No-go trials. The impulsivity effect was stronger when specifically looking at performance on No-go trials, where 5-HT<sub>1B</sub>R Emx1 & DRN knockout mice had a decreased ability to withhold responding (Fig. 3E;  $F_{6,114}=7.428$ ,  $p < 0.001$  for main effect of session;  $F_{2,19}=4.576$ ,  $p=0.024$  for main effect of condition;  $F_{12,114}=1.232$ ,  $p=0.314$  for interaction; posthoc LSD test  $p$ s < 0.05 for 5-HT<sub>1B</sub>R Emx1 & DRN knockout vs. control and 5-HT<sub>1B</sub>R VGAT & DRN knockout). There were no significant differences between groups in performance on Go trials (Fig. 3F; all  $p$ s > 0.3 except  $F_{6,114}=2.784$ ,  $p=0.015$  for main effect of session).

In order to increase the difficulty of the task, we also increased the No-go trial length from 5s to 10s for 3 sessions. Again, for 5-HT<sub>1B</sub>R Emx1 & DRN knockout mice, there were non-significant increases in impulsivity index (Fig. 3D;  $F_{2,36}=5.309$ ,  $p=0.012$  for main effect of session;  $F_{2,18}=2.311$ ,  $p=0.128$  for main effect of condition;  $F_{4,36}=2.065$ ,  $p=0.106$  for interaction) and incorrect No-go trials (Fig. 3E;  $F_{2,36}=5.775$ ,  $p=0.007$  for main effect of session;  $F_{2,18}=2.116$ ,  $p=0.149$  for main effect of condition). However, there was a significant interaction effect such that 5-HT<sub>1B</sub>R Emx1 & DRN knockout mice performed less well on No-go trials during the

earlier sessions of this phase ( $F_{4,36}=2.698$ ,  $p=0.046$ ). There was no effect on Go trial performance (Fig. 3F; all  $ps>0.2$ ).

Finally, we repeated the lickometer testing following completion of operant tasks in case the viral knockout was not fully complete at initial lickometer testing. Interestingly, there was a trend toward 5-HT<sub>1B</sub>R VGAT & DRN knockout having increased licking for higher concentrations of evaporated milk reward (Fig. 3G;  $F_{2,16}=2.778$ ,  $p=.092$  for main effect of condition;  $F_{5,80}=109.423$ ,  $p<0.001$  for main effect of concentration;  $F_{10,80}=20.261$ ,  $p=0.070$  for interaction). Together, the data from this experiment data suggest that 5-HT<sub>1B</sub>R Emx1 & DRN knockout may increase reward-motivated behaviors and impulsivity, while VGAT & DRN knockout increase hedonic taste reactivity.

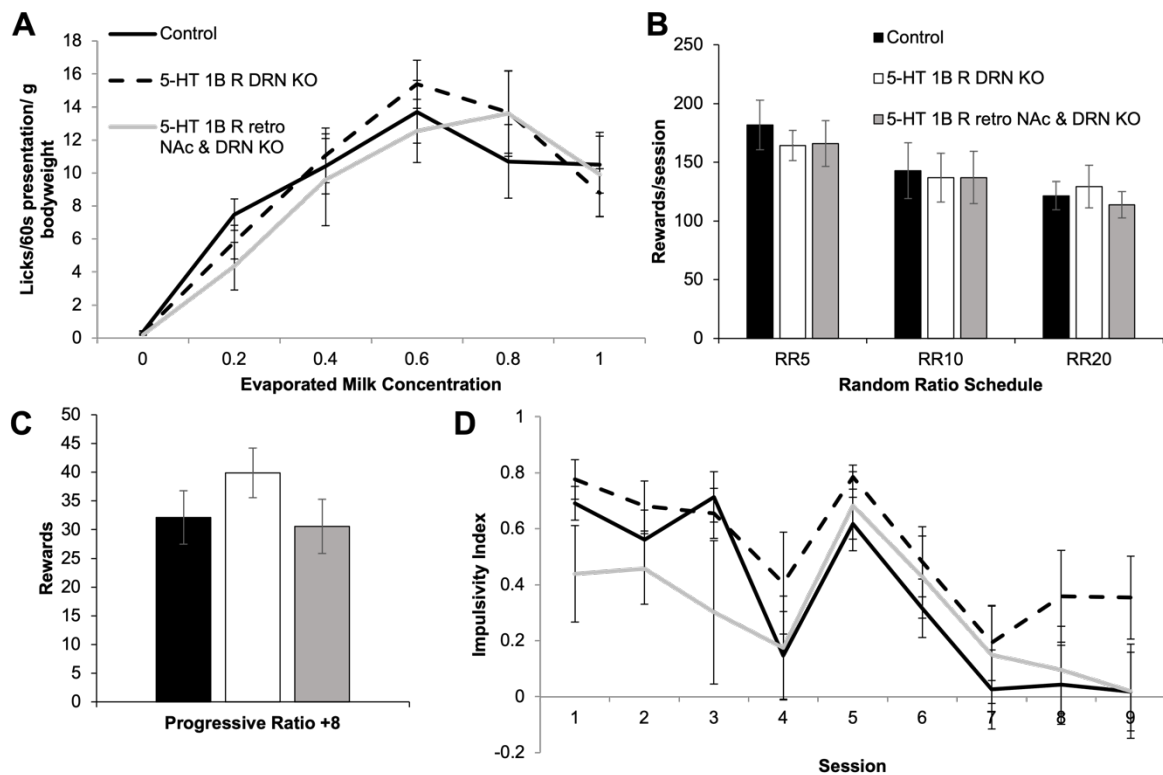


**Figure 3. Genetic knockout of the 5-HT<sub>1B</sub>R in Emx1+ in addition to viral knockout in DRN efferent projections increases reward-motivated and impulsive behaviors while knockout in VGAT+ cells increases taste reactivity.** A) Lickometer 6 bottle test measuring lick rate (normalized by bodyweight) for controls, VGAT+ & DRN, and Emx1+ & DRN 5-HT<sub>1B</sub>R knockout mice. B) Rewards earned per session across increasing random ratio schedules. C) Rewards earned in one session of a progressive ratio +8 schedule. D) Impulsivity index (proportion correct Go trials-proportion correct No-go trials) over sessions in a Go/No-go test of impulsive action. Data shown includes the first 7 sessions with 5s No-go trials (days 8-9 were excluded due to loss of a mouse), and 3 days with 10s No-go trials. Average E) proportion correct Go trials and F) proportion incorrect No-go trials for each phase of the Go/No-go. Note that though data is shown collapse over days, statistics included all sessions. G) Lickometer 6 bottle choice test repeated after operant testing was completed. Error bars are +/- 1 SE.

#### *Experiment 4: Viral nucleus accumbens and dorsal raphe nucleus serotonin 1B receptor knockout*

Based on the results of Experiment 2 that solely knocking out 5-HT<sub>1B</sub>Rs in NAc projections did not result in the expected behavioral outcomes, and evidence from Experiment 3 that there need to be multiple populations of receptor loss to produce impulsive behaviors, we next chose to knockout 5-HT<sub>1B</sub>Rs on projections to the NAc shell in addition to the autoreceptor population on projections from the DRN. Viral expression and successful knockout have not yet been confirmed postmortem.

After allowing 5 weeks for viral expression and knockout, we tested mice in a series of reward and impulsivity-based behaviors. No groups showed significant differences from controls in the lickometer (Fig. 4A; all  $p$ s > 0.4 except  $F_{5,95}=40.135$ ,  $p < 0.001$  for main effect of concentration), in random ratio increasing effort schedules (Fig. 4B; all  $p$ s > 0.5 except  $F_{2,40}=23.076$ ,  $p < 0.001$  for main effect of schedule), in a progressive ratio +8 schedule (Fig. 4C;  $p > 0.3$ ), or in a Go/No-go test for impulsive action (Fig. 4D; all  $p$ s > 0.2 except  $F_{8,160}=14.289$ ,  $p < 0.001$  for main effect of session).



**Figure 4. Viral knockout of the 5-HT<sub>1B</sub>R in NAc shell afferent projections in addition to DRN efferent projections does not increase reward-related or impulsive behaviors.** A) Lickometer 6 bottle test measuring lick rate (normalized by bodyweight) for controls, DRN, and Retro NAc & DRN 5-HT<sub>1B</sub>R knockout mice. B) Rewards earned per session across increasing random ratio schedules. C) Rewards earned in one session of a progressive ratio +8 schedule. D) Impulsivity index (proportion correct Go trials-proportion correct No-go trials) over sessions in a Go/No-go test of impulsive action. Error bars are  $\pm$  1SE.

## Discussion

In this set of experiments, we demonstrate a relationship between increased reward valuation and impulsivity in 5-HT<sub>1B</sub>R knockout mice, in addition to exploring which brain regions and cell types may be responsible for these behavioral effects. First, in Experiment 1, in a lickometer test of hedonic taste reactivity (Gaillard & Stratford, 2016; Ostlund et al., 2013), 5-HT<sub>1B</sub>R knockouts have higher lick rates across concentrations of evaporated milk reward than controls. We therefore use a lower concentration of evaporated milk in attempt to normalize disordered motivational and impulsive behaviors in the 5-HT<sub>1B</sub>R knockout mice. Interestingly, though 5-HT<sub>1B</sub>R knockout mice obtain more rewards on random ratio schedules (as previously seen), reducing the reward concentration actually increases motivation in both controls and knockouts, likely due to decreased satiation of each individual outcome. We have also shown in prior work that 5-HT<sub>1B</sub>R knockout itself does not affect hunger or satiety mechanisms, so this is unlikely to be the main cause of behavioral effects of the knockout in standard reward conditions (Desrochers et al., 2021). Alternatively, the lower reward value does decrease the heightened impulsive action of 5-HT<sub>1B</sub>R knockout mice in the Go/No-go test. Therefore, impulsivity is perhaps less affected by satiety mechanisms compared to purely motivational tasks, like the random ratio. More importantly, these results suggest that there is a relationship between reward value/motivation and impulsivity in 5-HT<sub>1B</sub>R knockout mice, and that the behavioral phenotypes present in these mice might be driven by changes to signaling in neural systems involved in reward processing.

For the next series of experiments, we hypothesized that 5-HT<sub>1B</sub>Rs modulate signaling in the NAc shell to alter reward and impulsivity related behaviors. We tested hedonic taste reactivity, motivation, and impulsive action in a brain region specific viral-mediated 5-HT<sub>1B</sub>R knockout. Unexpectedly, targeting receptor knockout to efferent or afferent NAc shell projections, even with retrograde NAc knockout in combination with a DRN knockout to target autoreceptors on serotonergic neurons, does not result in any increased reward-related or impulsive behaviors. Previous work has shown that the hedonic hotspot involved in the pleasurable experience of taste in this region is very small, and specific to one quadrant of the NAc shell (Castro & Berridge, 2014; Pecina & Berridge, 2000, 2005). It is possible that in the present experiment, the viral targeting may be missing this region or targeting too broad an area with spread, potentially masking results that may be very specific to the hotspot. Alternatively, 5-HT<sub>1B</sub>Rs in the NAc (even with modified serotonin release) might not be solely responsible for the behaviors studied in this experiment.

In our broader approach to investigate the populations of 5-HT<sub>1B</sub>Rs responsible for the whole brain knockout behavioral phenotype, we hypothesized that multiple cell-type populations of receptors may work synergistically to modulate behavior. Specifically, increased serotonin release due to autoreceptor loss may interact with other serotonin receptors on neurons where 5-HT<sub>1B</sub>Rs are also absent as heteroreceptors. Therefore, we use a combined genetic and viral approach to knockout heteroreceptor and autoreceptor populations simultaneously. Combined Emx1+ and autoreceptor (DRN) knockout does somewhat recapitulate the whole brain 5-HT<sub>1B</sub>R knockout, increasing motivation in random ratio schedules and impulsive action in the Go/No-go but with no change in taste reactivity. These effects do not appear to be as strong as those present in the whole brain knockout in prior studies (Desrochers et al., 2021), perhaps due to different genetic backgrounds of the lines, or again, an additional receptor population loss may be necessary to produce a complete behavioral outcome.

On the other hand, eliminating 5-HT<sub>1B</sub>Rs on VGAT+ cells and serotonergic cells (via DRN viral knockout) does not produce any significant differences in motivation and impulsivity (though there is perhaps an intermediate effect between controls and Emx1 & DRN knockout groups), but a repeated lickometer session at the end of behavioral testing results in increased hedonic taste reactivity. Therefore, it is possible that the viral receptor knockout was not complete at the time of initial lickometer testing, and future experiments should wait longer than 5 weeks to begin behavioral training. Together, these data indicate that multiple populations of 5-HT<sub>1B</sub> heteroreceptor and autoreceptor loss may be necessary to fully produce the behavioral effects in the whole brain knockout of interest in the present study. These results do support our prior findings that these behaviors are mediated by adult receptor expression and do not depend on development changes due to receptor loss (Desrochers et al., 2021; Nautiyal et al., 2015). Additionally, the subject counts were relatively small/not fully balanced for sex and not all controls were present, so in the future this experiment should be repeated more robustly to confirm results. Another modification that could be made for future versions of this experiment includes using a Pet-cre genetic knockout instead of a DRN region-based knockout to very specifically target autoreceptors on serotonergic neurons, as the DRN also contains other cell types (Huang et al., 2019).

In the line of Emx1-IRES-Cre mice, Cre is primarily expressed in non-GABAergic cortical and hippocampal cells (Gorski et al., 2002), suggesting that the loss of 5-HT<sub>1B</sub>R potentially changes excitatory signaling in these regions (though note there are other small subpopulations of noncortical Emx1 expressing cells which may also lack 5-HT<sub>1B</sub>Rs in this model; Willaime-Morawek et al., 2006). A strong candidate for the region responsible for the

increase in impulsivity is the vmPFC, whose dysfunction has been implicated in impulse control (Anastasio et al., 2019; Chudasama et al., 2003; Feja & Koch, 2014, 2015; Ghazizadeh et al., 2012). Both the vmPFC and the orbitofrontal cortex also have hedonic hotspots, though the extent to which these particular areas are actually causal to hedonic pleasure is unclear (Berta et al., 2019; Castro & Berridge, 2017; Peciña et al., 2006; Souther et al., 2022).

Within these cortical regions, 5-HT<sub>1B</sub>R heteroreceptors, along with autoreceptors on serotonergic projections from the DRN, could modulate local cortico-cortical signaling or could be localized to the terminals of cortico-subcortical projections (5-HT<sub>1B</sub> mRNA is present in cortical layers I-III,V; (Bruinvels et al., 1994). Experiments 2 and 4 of this study suggest that the direct subcortical target of these potential projections is not the NAc shell (though the NAc could be downstream of an intermediate target). There are many other subcortical regions highly implicated in reward or impulsivity which could be involved in this 5-HT<sub>1B</sub>R related circuitry. The ventral pallidum (VP) for example, also contains a hedonic hotspot, however, it does not have strong direct cortical input (Peciña et al., 2006; Tindell et al., 2006). Instead, the VP might be the target of NAc GABAergic MSNs (which would be impacted by the VGAT & DRN knockout) involved in hedonic signaling. The NAc core is also a possible target though, it has been implicated more strongly in impulsive choice than impulsive action, making it a less likely option given the lack of effect of 5-HT<sub>1B</sub>R expression on impulsive choice (Cardinal et al., 2001; Desrochers et al., 2021; Nautiyal et al., 2017; Wang et al., 2019). Alternative potential subcortical regions of interest, which express 5-HT<sub>1B</sub>R protein (Bruinvels et al., 1993) and receive direct cortical input, include the ventral tegmental area (Faget et al., 2016; Morales & Margolis, 2017), the subthalamic nucleus (Canteras et al., 1990; Uslaner & Robinson, 2006), and the paraventricular thalamus (Choi et al., 2012; Otis et al., 2019).

There are many potential reasons why loss of 5-HT<sub>1B</sub>Rs may cause changes in behavior due to alterations in the functioning of the aforementioned neural circuitry, including activity, plasticity, or simply broadly perturbing any piece of the circuit may cause imbalance resulting in behavioral changes, rather than any special, specific role for signaling directly at the 5-HT<sub>1B</sub>R. For example, global knockout of the 5-HT<sub>1B</sub>R causes compensatory changes in neuromodulator release and receptor expression, not limited just to serotonin receptors (Ase et al., 2008; Hagan et al., 2012; Searce-Levie et al., 1999). The extent to which these compensations are developmentally mediated to cause permanent changes or involved in the adult expression-specific behaviors examined in this study remain unclear. To test whether the behavioral effects of the 5-HT<sub>1B</sub>R are acute and activity-based (rather than longer-term compensatory), future

experiments could use optogenetic manipulations at terminals with opsins expressed under the control of a 5-HT<sub>1B</sub>R promotor, allowing for fine temporal control.

An additional, behavioral limitation of the approach taken in this study is the use of a lickometer 6 bottle choice test to measure tastant hedonic curves. Experimentally, the lickometer is a good measure of hedonic reactivity or reward ‘liking’, but it still requires *some* motivation for consumption. The brief access strategy used in this experiment is used to mitigate the effects of consumption/satiety and hunger/thirst motivation, in order to obtain a less-confounded measure of liking or pleasure (Gaillard & Stratford, 2016; Ostlund et al., 2013). Follow-up work could use orofacial expression analysis of pleasure, which has recently been used in mice to rule out motivational effects on hedonic responding (Dolensek et al., 2020). Based on the lickometer results interpreted as increased ‘liking’, this work highlights that multiple aspects of reward processing might together contribute to impulsive behavior.

Broadly, the set of experiments conducted in this study reiterate the relationship between reward value and impulsivity in mice lacking the 5-HT<sub>1B</sub>R, suggesting there may be some causality between the two behaviors. Additionally, we find evidence that these behaviors may be modulated by excitatory cortical, inhibitory GABAergic, and neuromodulatory serotonergic activity working synergistically. Future work will examine specific cortico-subcortical connections to better understand the role of serotonin signaling in the control of impulsivity.

## Acknowledgments

We would like to express thanks to Dr. Mitchell Spring for his instruction on conducting viral surgeries in mice. Additionally, we would like to thank Elaine (Yihan) Pu, a Dartmouth College undergraduate member of the WISP program for assisting in some data collection for experiment 1. SSD designed the experiments, collected and analyzed data, and wrote the manuscript. KMN advised on experimental design and provided comments for the final version of the manuscript.

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## GENERAL DISCUSSION

### Overview

This dissertation examines the behavioral and neural mechanisms underlying impulsive behavior, from both the perspectives of increased reward drive and decreased inhibitory control. Specifically, this set of experiments uses the genetic serotonin 1B receptor (5-HT<sub>1B</sub>R) knockout mouse model for impulsivity, as well as viral knockout approaches, to highlight a particular role for serotonin signaling in the control of impulsive behavior.

### Summary of major results

#### *Chapter 1*

Chapter 1 takes the perspective of enhanced ‘drive’ contributing to increased impulsivity, focusing on the presence of altered reward-related behaviors in the whole brain 5-HT<sub>1B</sub>R knockout mouse model for impulsivity. We first replicated the finding that mice lacking the 5-HT<sub>1B</sub>R have increased impulsive action in a Go/No-go test with no differences in impulsive choice in delay or effort discounting tests. However, we did find that these mice also showed an increase in preference for larger reward choices compared to controls, over all delays or effort requirements. This suggests the presence of a general change in reward valuation. Therefore, we next explored operant behaviors that do not explicitly measure impulsivity, but would instead be indicative of altered reward drive. 5-HT<sub>1B</sub>R knockout mice demonstrated increased motivation for reward under increasing effort random and progressive ratio schedules. This was not due to differences in habitual responding as measured in a satiety induced devaluation test, general hunger as measured in consumption tests, or decreased ability to extinguish a learned operant response. Additionally, these behaviors were returned to the level of controls with adult rescue of receptor expression, suggesting that they are not mediated by permanent developmental changes with 5-HT<sub>1B</sub>R absence. Instead, we discovered that mice lacking the 5-HT<sub>1B</sub>R lick more for sucrose across satiety states, and consume more evaporated milk, suggesting that enhanced impulsivity and motivation in this model may be due to increased valuation of specifically palatable outcomes. Interestingly, these mice also increased operant responding for a reward associated cue in a Conditioned Reinforcement test, where potentially the cue has acquired more predictive strength in the 5-HT<sub>1B</sub>R knockout mice. Finally, we found that impulsive action in the Go/No-go test can be modulated by reward size in all mice, with a smaller reward size ameliorating the increased impulsivity of the knockouts down to level of controls. These findings support the hypothesis that there may be a causal relationship between reward drive and

impulsivity in the 5-HT<sub>1B</sub>R knockout mouse model, and that the loss of this receptor may be modulating signaling in reward-related neural circuitry.

## *Chapter 2*

Alternatively to Chapter 1, Chapter 2 instead focuses on the perspective of decreased ‘brake’ contributing to impulsive outcomes, exploring inhibitory behaviors and Pavlovian learning in the whole brain 5-HT<sub>1B</sub>R knockout mouse model for impulsivity. First, we find again that mice lacking the 5-HT<sub>1B</sub>R have increased impulsive action measured by premature responding in the 5 choice serial reaction time test (5CSRTT), that decreases over training but can reemerge with higher task cognitive demands using either a long ITI or short variable stimulus length. This decreased ability to withhold responding through the ITI period was not related to attentional ability or generally poor performance on the task, as 5-HT<sub>1B</sub>R knockout mice did not have more incorrect or omission trials than controls at any stage of the task. Next, we were interested in whether the increased impulsive action could be due to a decreased ability to form inhibitory associations. In a Pavlovian Conditioned Inhibition test, mice lacking the 5-HT<sub>1B</sub>R were able to learn an inhibitory cue relationship and passed the summation and retardation of responding tests, but did have slightly more responding to an inhibitor-excitor compound cue compared to controls. Rather than a difference in learning, this could represent an increase in excitatory responding for the normally reward-paired excitor cue. Next, we looked more closely at how these mice perform in other kinds of Pavlovian training. We found no group differences under standard excitatory conditions (though measuring reward port entries might not be sensitive to subtle differences in reward valuation between groups) or when cue and reward were explicitly unpaired from each other. However, we did find that 5-HT<sub>1B</sub>R knockouts did have increased responding under zero contingency conditions when the cue and reward could randomly cooccur. We found that this change in Pavlovian behavior was not the result of differences in responding for low cue-reward probability. Instead, the increase in responding potentially resulted from cue-reward pairings having greater salience than when they were unpaired in the 5-HT<sub>1B</sub>R knockouts. Overall, this study highlights that the impulsive action present in mice lacking the 5-HT<sub>1B</sub>R is not due to deficits in inhibitory learning, or overall changes in Pavlovian responding, but instead is more likely related to changes in reward drive as examined in Chapter 1.

## *Chapter 3*

Finally, Chapter 3 synthesizes the behavioral results from Chapters 1 and 2 to examine the behavioral connection between measured differences in reward and impulsivity, and then

investigates the neural mechanisms of 5-HT<sub>1B</sub>R modulation of these behaviors. First, we show that whole brain 5-HT<sub>1B</sub>R knockout mice have increased hedonic taste reactivity, as measured by increased lick rate for a palatable reward across increasing concentrations in a brief access lickometer test. This is strong supporting evidence for an enhanced ‘liking’ response, which results in relatively increased reward valuation compared to controls. We therefore decided to directly test this hypothesis by using the reward concentration for the knockout mice which had equivalent responding as the maximum value in the controls as the outcome in operant behavior testing. Lowering the outcome value actually increased motivation in random ratio schedules, likely due to decreased satiation of each individual reward. However, lowering the reward concentration decreased the impulsive action of the 5-HT<sub>1B</sub>R knockouts to the level of controls in a Go/No-go test. Therefore, we began targeting specific populations of neurons, including those in the nucleus accumbens (NAc) shell, a region highly implicated in reward-related behaviors and also shows high expression of 5-HT<sub>1B</sub>R protein and mRNA. Knockout of 5-HT<sub>1B</sub>Rs in this region, even when combining retrograde NAc knockout with autoreceptor knockout in the DRN, did not cause any changes in the measured behaviors. We next combined genetic and viral approaches to discover that combined cortical heteroreceptor (Emx1+; not GABAergic cell localized) and autoreceptor knockout increased motivation and impulsivity. On the other hand, combined GABAergic cell localized heteroreceptor (VGAT+) and autoreceptor knockout increased hedonic taste reactivity in a lickometer test. This data demonstrates that 5-HT<sub>1B</sub>R’s modulation of reward and impulsivity occurs through multiple receptor populations, and will guide future experiments toward more projection specific targeting.

### **Further behavioral considerations**

The behaviors tested in this dissertation have been selected carefully to narrow down the basis of 5-HT<sub>1B</sub>R-based impulsivity, but have by no means been completely exhaustive. There are many other interesting behaviors to consider in future experiments in this model, and in the study of impulsivity in general.

### ***How does timing impact impulsivity?***

This set of experiments does not directly address whether 5-HT<sub>1B</sub>R knockout mice have differences in time perception which may contribute to many impulsive behaviors. For example, perceiving time as going by faster than in reality could cause animals to complete an action prematurely, as in the 5CSRTT or differential reinforcement of low-rate responding tests of impulsive action (Desrochers et al., 2021; Nautiyal et al., 2015). Additionally, in a delay

discounting test of impulsive choice, impaired timing could change how animals change preferences for the larger delayed reward over the small. In this case, if the delay is perceived as shorter, it may be discounted less, resulting in decreased impulsive choice. However, this change in perception would likely change the discounting such that the slope over delays would be flatter. Instead, in Chapter 1, we find that 5-HT<sub>1B</sub>R knockout mice have the same discounting slope as controls (Desrochers et al., 2021). Indeed, only the intercept is different, resulting in an overall stronger preference for the larger reward even when there is no delay, suggesting this difference cannot be solely attributed to differences in timing capabilities (Brunner & Hen, 1997).

In operant tests of motivation and effort, timing could also play a role, as increasing the number of lever presses required to obtain a reward simultaneously involves increasing the time occurring in between reinforcements. If mice perceive this inter-reward time as shorter, they might persist in responding longer than others that correctly interpret the timing. Of the experiments in this dissertation, this could impact random and progressive ratio reinforcement schedules, as well as effort discounting. However, once again, in Chapter 1, 5-HT<sub>1B</sub>R knockout mice do not have a different effort discounting slope as controls, but overall shifted curve (Desrochers et al., 2021). Additionally, the motivational changes we see are accompanied by changes in hedonic taste reactivity (Chapter 1, 3) which do not have a timing component.

Finally, differences in time perception could also cause changes in learning and expression of Pavlovian conditioning. In the case of Chapter 3, perceiving the time between events as shorter could potentially result in stronger associations between non-contingent cues and rewards, such that 5-HT<sub>1B</sub>R knockout mice have increased responding at cue onset compared to controls (Desrochers & Nautiyal, 2022; though note if learning is dependent on the ratio between ITI and cue length, we would not expect to see differences in these behaviors with timing changes as the ratio would remain the same, e.g. Gibbon & Balsam, 1981). We would, then, expect changes in timing to generally affect approach behavior in other contingency situations as well. For example, if time was perceived as passing faster, we could expect 5-HT<sub>1B</sub>R knockout mice to respond earlier during cue presentation, which does not occur. However,

In sum, although not directly tested in the present set of experiments, it seems unlikely that the 5-HT<sub>1B</sub>R knockout behavioral phenotype explored in this dissertation can be attributed to altered timing capabilities. But, this is a feasible underlying change that could ultimately result in increased impulsivity, and should be considered to better understand the cause of particular impulsive behaviors. Intact timing could perhaps be tested in a non-reward-based setting to avoid confounds, like in aversive fear conditioning.

### ***Are there other Pavlovian behaviors that could be impacted along with increased impulsivity?***

Though impulsivity is generally studied under operant training conditions where actions have consequences, it is possible that changes in more basic behavioral constructs like reward and inhibition could impact both operant and Pavlovian behaviors. Chapter 2 examines the behavior of 5-HT<sub>1B</sub>R knockout mice in Pavlovian conditioning, including both excitatory conditioning under different contingencies and inhibitory learning in a test of Conditioned Inhibition. However, there are several other kinds of Pavlovian conditioning not included in this set of experiments which may be relevant to the study of impulsivity.

In the case of behavioral inhibition, other examples of Pavlovian tests of interest include latent inhibition and negative occasion setting. In latent inhibition, a preexposed cue (nonreinforced) slows acquisition of conditioning compared to a novel cue (Lubow & Moore, 1959). The rate of acquisition may be higher in animals with impulsivity driven by enhanced reward value, as there would be a stronger reward prediction error between the preexposed cue's prior inhibitory identity and its current excitatory identity. This test might therefore be able to detect more subtle changes in excitatory responding compared to simple delay conditioning alone. On the hand, inhibitory negative occasion setting would be similar to Conditioned Inhibition, but more analogous to behavior in the 5CSRTT rather than the Go/No-go. In negative occasion setting, an inhibitory cue is presented prior to a normally excitatory cue ( $X \rightarrow A^-$ , as opposed to  $AX^-$  in Conditioned Inhibition). This is similar to the 5CSRTT in that mice have a set of cues that indicate a response should be withheld prior to the presentation of an excitatory operand, as opposed to the simultaneous presentation in Conditioned Inhibition and the Go/No-go. There are deficits in both of these inhibitory phenomena in adolescent rats (Meyer & Bucci, 2014), and it would be very interesting to test if this is related to the increased impulsivity present during this developmental period, as well as more generally in other preclinical models for impulsivity. Indeed, Roman high-avoidance rats have both increased impulsive action and deficits in latent inhibition (Esnaol et al., 2016; Flagel et al., 2010).

Finally, another phenomenon termed 'signtracking' sometimes occurs in excitatory Pavlovian autoshaping and is of particular interest to the study of impulsivity, which will be discussed in more detail in the following section.

### ***How is impulsivity related to liking versus wanting?***

Chapters 1 and 3 of this thesis demonstrated that 5-HT<sub>1B</sub>R knockout mice have increased hedonic taste reactivity as well as enhanced operant motivation for reward. Though 'liking' and

‘wanting’ reward typically go together, they are behaviorally and biologically separable. Hedonic pleasure can be measured in rodents using orofacial expression analysis (Peciña & Berridge, 2005). Hedonic responses have been found using pharmacological manipulations in particular ‘hotspots’ in the brain, including, but not limited to, the NAc shell and ventral pallidum (Peciña et al., 2006; Smith & Berridge, 2007; Tindell et al., 2006). These ‘hotspots’ have been found in opioid, orexin, and cannabinoid systems (Castro & Berridge, 2014, 2017; Mahler et al., 2007; Mitchell et al., 2018; Smith & Berridge, 2007). ‘Wanting’ can be measured using operant tests of motivation, with larger ‘hotspots’ than those for ‘liking, and is sensitive to dopamine neuromodulation (Castro & Berridge, 2014; Flagel et al., 2011).

Another way of examining ‘wanting’ specifically is by measuring signtracking behavior in a Pavlovian autoshaping procedure, where an interactable cue, like a lever, precedes the presentation of a reward. Some subjects, called signtrackers, preferentially apply incentive salience to the reward-predictive cue and interact with it as if it has its own value. On the other hand, goaltrackers treat the cue simply as a predictor of reward, and therefore interact more with the reward location. Interestingly, signtracking and impulsivity have frequently been linked together, with premature responding in SRTTs and differential reinforcement of low rate responding being higher in animals characterized as signtrackers (Flagel et al., 2010; King et al., 2016; Lovic et al., 2011). However, it is possible that not all presentations of impulsivity will be accompanied by increased signtracking. If a given impulsive phenotype is driven by enhanced motivation, or ‘wanting’, we would expect more signtracking behavior. Alternatively, if the impulsivity is modulated changes in hedonic reward valuation, or ‘liking’, we might instead expect more goaltracking behavior with more salience being applied to the reward itself rather than the cue. There is some evidence that reward outcome value can modulate the magnitude of both signtracking and goaltracking behavior (Amaya et al., 2020; M. J. F. Robinson & Berridge, 2013), but goaltracking is specifically associated with enhanced hedonic taste reactivity in the lickometer (Patitucci et al., 2016). Though we do see changes in both motivational and hedonic behavior in 5-HT<sub>1B</sub>R knockout mice, they interestingly show more goaltracking rather than signtracking behavior when compared to control mice in an autoshaping paradigm (Appendix Fig. 1). In the future, it would be interesting to determine how serotonin, and its many different receptors, interacts with other neuromodulatory systems to produce these behaviors.

### ***How can we measure impulsivity separately from palatable food reward drive?***

Evidence presented across the chapters of this dissertation strongly supports that 5-HT<sub>1B</sub>R knockout-based impulsivity occurs through enhanced reward drive, but all the studied behaviors

occur in the context of food reward outcomes. We do not know how generalizable these findings are to other kinds of reward, such as novelty or social reward. If 5-HT<sub>1B</sub>R very specifically modulates the reward processing of taste, 5-HT<sub>1B</sub>R knockout mice may not show any differences in impulsivity when the reinforcement is not a palatable food. This could be explicitly tested using an operant novel sensation seeking paradigm (Olsen & Winder, 2010) or social conditioned place preference. Additionally, manipulations of 5-HT<sub>1B</sub>Rs have complex effects on drug reward behavior, which could be further explored in the present model and would be useful for better understanding the behavioral and neural differences between drug and non-drug reward (Acosta et al., 2005; Barot et al., 2007; Fletcher et al., 2002; Fletcher & Korth, 1999; Neumaier et al., 2002; Rocha et al., 1998).

Currently, we also do not address how reduced behavioral inhibition may appear in non-reward contexts. Impulsivity could be the result of either increased reward drive or decreased inhibitory brake, or a composite of both. Therefore, it would be prudent to be able to test impulsivity without always have the involvement of reward drive. To do this we could use a test like an active avoidance Go/No-go, where there are a set of cues that indicate an animal either needs to press a lever (or move to a new place) or withhold responding to avoid an aversive outcome, like a footshock. If impulsivity is generalizable to this non-reward-based behavior, we would expect impulsive animals to have difficulty withholding responding to avoid the outcome.

### **What is the role of the 5-HT<sub>1B</sub>R in modulating reward/impulsivity based neural circuitry?**

The work completed in Chapter 3 of this dissertation has suggested an important role for adult expression of 5-HT<sub>1B</sub> cortical heteroreceptors, GABAergic-localized heteroreceptors, and autoreceptors in reward valuation and impulse control. However, there are still many unanswered questions and details remaining to fully understand the details of the mechanism of action of 5-HT<sub>1B</sub>Rs in these neural systems.

#### ***On the systems level:***

Previous approaches to determining the celltype population of 5-HT<sub>1B</sub>Rs involved in impulsive behavior have been unsuccessful, likely because they target a single heteroreceptor or autoreceptor population at time (Nautiyal et al., 2015). Chapter 3 suggests that multiple populations of 5-HT<sub>1B</sub>R loss in conjunction seem to be involved in fully producing the impulsive phenotype of the whole brain knockout. In both cases of Emx1 (excitatory cortical) knockout (Gorski et al., 2002) and VGAT (GABAergic) knockout, the addition of an autoreceptor knockout (through viral DRN targeting) is necessary to produce changes in behavior. This

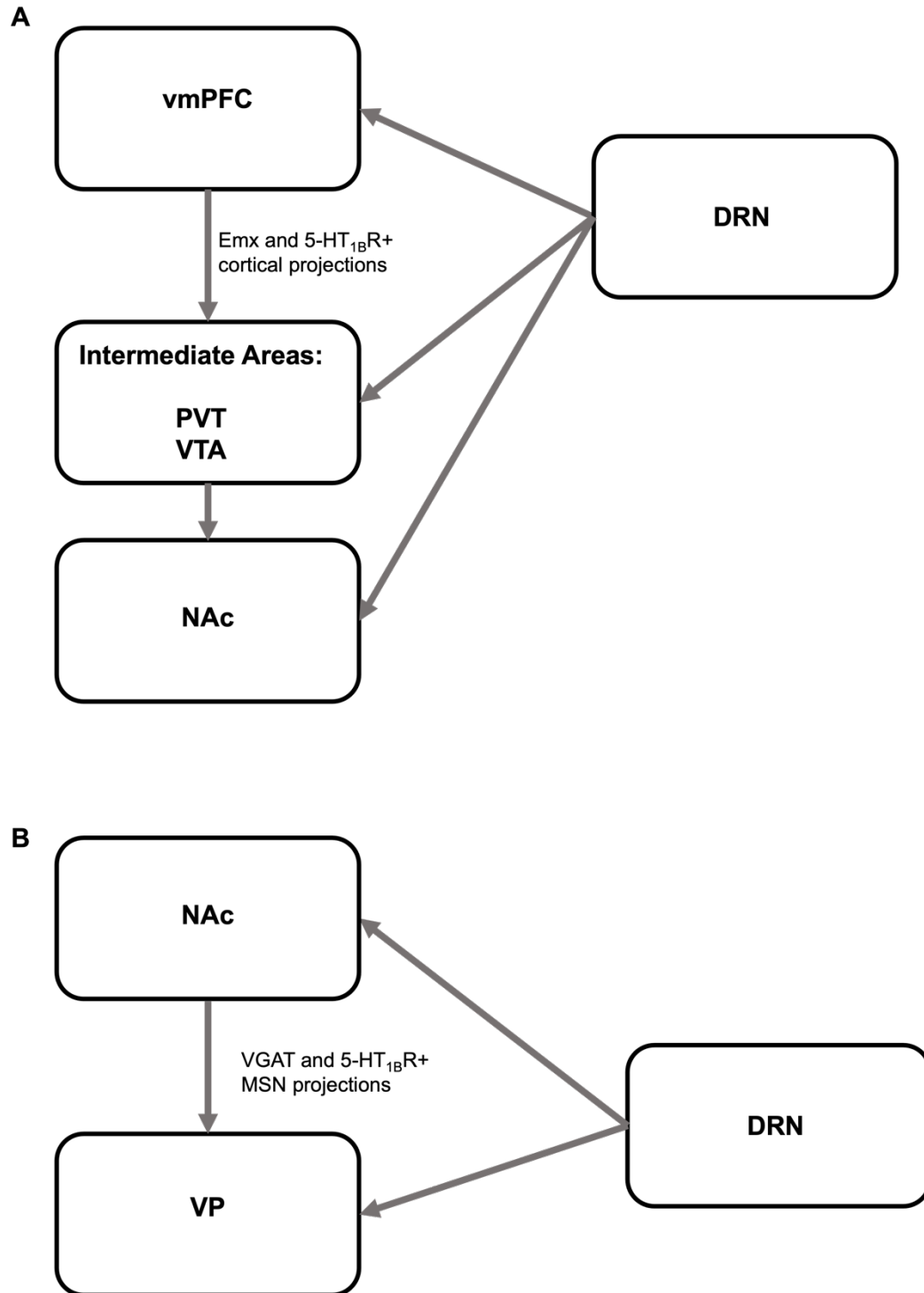
suggests that changes in serotonin release in combination with heteroreceptor loss are key to the effects observed in Chapters 1 and 2. It seems that 5-HT<sub>1B</sub>R Emx1 & DRN knockout at least partially produces enhanced reward drive and impulsive action in operant tests, though not to the full extent of complete receptor loss. So, cortical signaling may be involved in more specifically changes in ‘wanting’ or motivation. As shown in a hypothesized circuit in Figure 1A, 5-HT<sub>1B</sub>R could modulate excitatory signaling of cortico-subcortical projections (or alternatively modulate local cortico-cortical signaling; 5-HT<sub>1B</sub> mRNA is present in cortical layers I-III,V; Bruinvels et al., 1994). A specific cortical region of interest is the ventromedial prefrontal cortex (vmPFC), where manipulations cause changes in impulse control and motivation for palatable food (Anastasio et al., 2019; Chudasama et al., 2003; Feja & Koch, 2014, 2015; Ghazizadeh et al., 2012; Selleck et al., 2018).

As retrograde knockout of 5-HT<sub>1B</sub>R in the NAc, even in combination with DRN knockout, did not result in behavioral changes, it is unlikely this region is the direct target of 5-HT<sub>1B</sub>R+ cortical projections. However, 5-HT<sub>1B</sub>R whole brain knockout causes increased dopamine release in the NAc, but not the dorsal striatum (Nautiyal et al., 2015). This suggests that 5-HT<sub>1B</sub> protein may be localized to intermediate regions that project to the NAc and modulate release of dopamine there. Indeed, impulsive action and reward motivation are both enhanced with increased dopaminergic signaling in the NAc (Cole & Robbins, 1987; Pattij et al., 2007; Sesia et al., 2010; Soares-Cunha et al., 2016; Zhang et al., 2003). A potential candidate for this intermediate region is the ventral tegmental area (Fig. 1A, VTA) which receives direct cortical input and has 5-HT<sub>1B</sub>R protein (Bruinvels et al., 1993; Faget et al., 2016; Morales & Margolis, 2017). The VTA releases dopamine in the NAc, where it plays a role in the control of impulsive action and motivation. For example, optogenetic activation of the VTA to NAc shell pathway increases impulsivity in the 5CSRTT (Flores-Dourojeanni et al., 2021), while decreasing dopamine release in the pathway decreases it (Toschi et al., 2023). Alternatively, the paraventricular thalamic nucleus (PVT) also receives cortical input and contains 5-HT<sub>1B</sub>Rs (Bruinvels et al., 1993; Choi et al., 2012; Otis et al., 2019). Like the VTA, this region has been implicated in reward-motivated behaviors, as well as modulating dopamine release in the NAc (Campus et al., 2019; Cheng et al., 2018; Li & Kirouac, 2012; Otis et al., 2019; Parsons et al., 2007; Pinto et al., 2003).

Chapter 3 also revealed that the 5-HT<sub>1B</sub>R VGAT (GABAergic) & DRN (autoreceptor) knockout impacts hedonic taste reactivity, but does not have a strong effect on motivation or impulsivity. This indicates that there may need to be a combined increase in motivation/‘wanting’ through the Emx & DRN knockout alongside the increase in ‘liking’

through VGAT & DRN knockout to fully reproduce the strength of the increased reward and impulsivity behaviors in the whole brain knockout. A region that contains a lot of 5-HT<sub>1B</sub>Rs and also receives GABAergic input from medium spiny neurons in the NAc is the ventral pallidum (VP; Fig. 1B; Bruinvels et al., 1993). The VP contains a hedonic hotspot, where neuromodulatory manipulations causally affect taste responses and neural activity reflects hedonic value (Peciña et al., 2006; Tindell et al., 2006). Therefore, the loss of 5-HT<sub>1B</sub>Rs on MSNs projecting to this region, combined with increased serotonin release, may increase hedonic ‘liking’ as measured by taste reactivity in the lickometer. However, it is important to note that there is also a body of work showing that overexpression of 5-HT<sub>1B</sub>Rs (in theory having the opposite effect of the knockout) in NAc projections to the VTA, which are also MSNs, tends to increase the rewarding effects of drugs including cocaine and amphetamines (Barot et al., 2007; Ferguson et al., 2009; Neumaier et al., 2002). It is possible that the mechanisms underlying food and drug reward are reliant on different populations of 5-HT<sub>1B</sub>Rs, which could be explicitly tested in future viral knockout experiments in the present model.

Together, the 5-HT<sub>1B</sub>R populations targeted through these experiments, and serotonin signaling in general throughout the brain, could play a larger role in modulating different parts of a cortical-striatal circuit that broadly plays a role in various aspects of reward and impulse control.



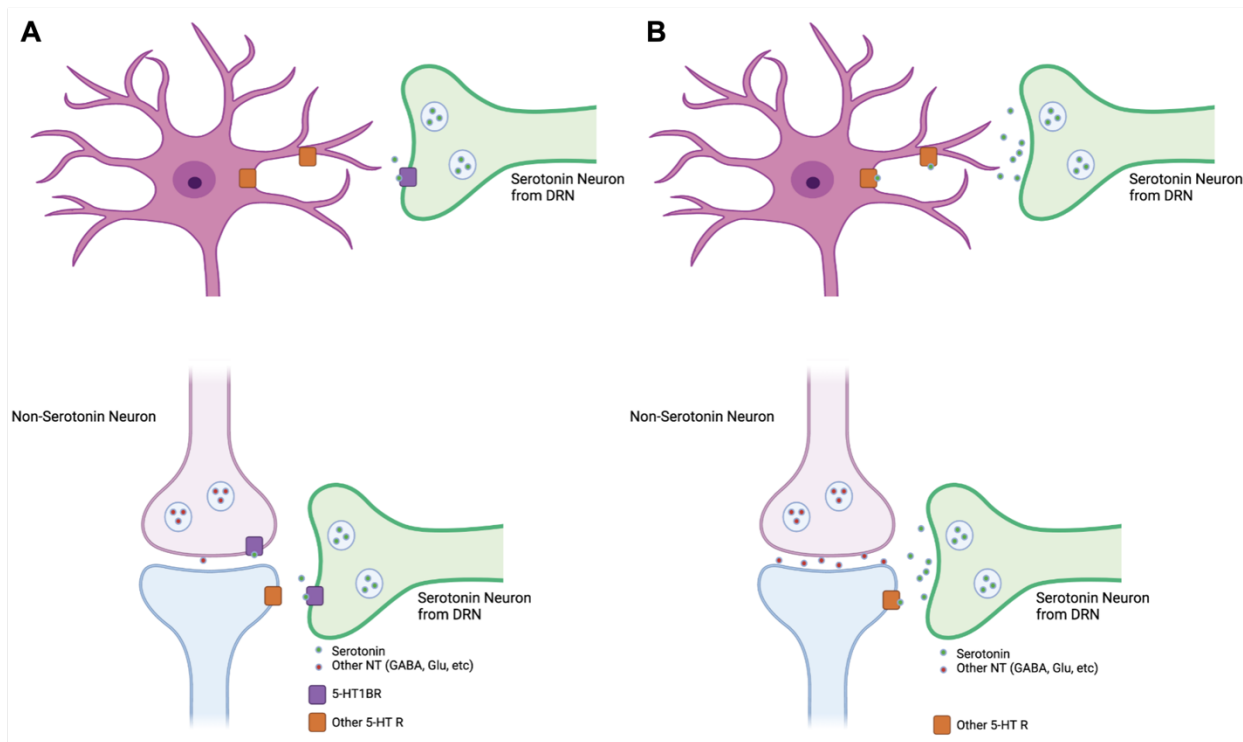
**Figure 1. A schematic of the potential neural circuitry by which 5-HT<sub>1B</sub>Rs modulate A) reward motivation and impulsivity and B) hedonic reactivity.** Abbreviations: ventral medial prefrontal cortex = vmPFC, paraventricular thalamus = PVT, nucleus accumbens = NAc, ventral pallidum = VP, dorsal raphe nucleus = DRN, medium spiny neuron = MSN

*On the cellular level:*

Though this dissertation provides evidence regarding which populations of 5-HT<sub>1B</sub>Rs contribute to reward drive and impulse control, how the loss of these receptors changes neuromodulatory signaling is unclear. Experiments from Chapter 3 suggest that autoreceptor loss is necessary for the behavioral phenotypes present in the whole brain knockout. Since 5-HT<sub>1B</sub>R is a presynaptic inhibitory G-protein coupled receptor (GPCR; Boschert et al., 1994; Jolima et al., 2000; Mizutani et al., 2006), its loss on serotonergic neurons increases the release of serotonin (Knobelman et al., 2001; Malagi et al., 2001). Coupled with the absence of heteroreceptors on other populations (e.g. cortical and GABAergic), it is possible there is relatively more signaling occurring through other serotonin receptors on the same cells where 5-HT<sub>1B</sub>Rs are absent or on the postsynaptic neuron (Fig. 2), thus contributing to the observed changes in behavior. Together, an increase in serotonin release and the loss of 5-HT<sub>1B</sub>Rs as a heteroreceptor may change the activity of serotonin responsive neurons.

Several other serotonin receptors have been implicated in reward and impulsivity, and may be involved in how loss of the 5-HT<sub>1B</sub>R on particular neurons impacts neurotransmitter release. In particular, agonists of the serotonin 1A and 2A receptors tend to increase, while antagonists decrease, impulsive action in tests like the SRTT and DRL (Anastasio et al., 2011; Carli & Samanin, 2000; Fletcher et al., 2007; Koskinen et al., 2000; E. S. J. Robinson et al., 2008; Winstanley et al., 2004). As we expect increases in serotonin release with 5-HT<sub>1B</sub>R loss, these receptors are good candidates for how changes in neuromodulatory dynamics may be contributing to the behaviors observed in this dissertation. Other receptors are also involved in impulse control, though increased serotonin release would likely have an opposing effect, as agonists decrease and antagonists increase impulsivity and reward-related behaviors (e.g 5-HT<sub>2C</sub>R; Bailey et al., 2016; Fletcher et al., 2007; Robinson et al., 2008; Winstanley et al., 2004), though the signaling cascades may be more complex than general pharmacology implies.

Additionally, beyond changing acute neural activity and release of neurotransmitters, loss of 5-HT<sub>1B</sub>Rs may alter synaptic plasticity in a manner that changes the longer-term communication pattern between regions. Indeed, 5-HT<sub>1B</sub>R plays a role in presynaptically-mediated long-term depression (LTD) on glutamatergic cortical projections to striatal regions (Atwood et al., 2014; Huang et al., 2013; Mathur et al., 2011). Loss of this form of plasticity is associated with parts of the drug addiction cycle, and may, in combination with enhanced serotonin, result in decreased impulse control and increased reward drive (Brown et al., 2011; Fasano & Brambilla, 2005; Huang et al., 2013; Kasanetz et al., 2010).



**Figure 2. A schematic of the potential cellular mechanism by which 5-HT<sub>1B</sub>R expression modulates neuronal signaling with A) 5-HT<sub>1B</sub>Rs intact and B) 5-HT<sub>1B</sub>Rs absent. Figure created in Biorender.**

## Significance and synthesis

### ***Potential for clinical research: Serotonin signaling as a target for treatment of pathological impulsivity***

Current treatments for reducing impulsivity in clinical populations include mostly dopamine and norepinephrine acting drugs such as methylphenidate and atomoxetine (Kollins & March, 2007; Swanson & Volkow, 2009). Targeting serotonin signaling as a treatment approach for pathological impulsivity has been less supported possibly due to the inconsistency and small effects seen on impulsive behavior following SSRI treatment. However, this is likely due to the diverse role of many of the 14 different serotonin receptors in modulating impulsivity, sometimes in opposing directions (Blanco et al., 2009; Coccaro et al., 2009). Therefore, targeting specific receptors may be a better treatment method; for example, serotonin 1B, 2A, and 2C receptors all influence impulsive action in preclinical studies and/or clinical trials (Fink et al., 2015; Higgins et al., 2017; Nautiyal et al., 2015, 2017). Importantly, serotonin-acting drugs can provide safe options with a low-side effect profile. In particular, the triptan class of drugs, agonists of the 5-HT<sub>1B</sub>R, are used safely in the treatment of migraines. One has been shown to cross the blood-brain barrier and could be a treatment option to reduce impulsivity (Muzzi et al., 2020). Targeting specific receptor systems could also be an important approach for the treatment

of specific impulsive symptoms. In the case of 5-HT<sub>1B</sub>Rs, treatment could potentially aim to alleviate maladaptive reward drive and impulsive action.

### ***General future directions***

There are many interesting directions and follow-up experiments to further understand the results from the set of studies presented here. Primarily, genetic and viral manipulations of 5-HT<sub>1B</sub>R can be used to explore specific and combined components of the neural circuitry proposed in Figure 1. First, this would consist of knocking out 5-HT<sub>1B</sub> autoreceptors and specific nodes of the circuit, including projections from the vmPFC for motivation/impulsivity and from the NAc for hedonics. Then, we could use a dual virus approach to get projection specificity (i.e. vmPFC to PVT/MTA and NAc to VP). Once a complete pathway has been determined, we would want to know how the loss of 5-HT<sub>1B</sub>Rs is actually changing neural communication in this circuit. To do this, we can use calcium imaging to assess the activity of neurons in different nodes of the circuit during reward and impulsive behavior, and in the absence of 5-HT<sub>1B</sub>R, potentially opening new windows into the individual neuron properties and contribution to impulse control. We would expect that the loss of 5-HT<sub>1B</sub>Rs would increase neurotransmission, and either increase (if localized to excitatory neurons; Emx+) or decrease (if localized to inhibitory neurons; VGAT+) activity of postsynaptic neurons. Calcium imaging can also be combined with fiber photometry to simultaneously assess changes in cellular activity and neuromodulator release, with serotonin and dopamine being of most interest to the present work. This line of research would help us better understand the dynamics of signaling in this circuit and the timing of when particular components are involved in the control of behaviors of interest (i.e. encoding value, reward approach/receipt, during an impulsive action, etc.). Additionally, we can explore using drugs targeting these neurons to restore the functionality of this circuit in the absence of 5-HT<sub>1B</sub>R. An ultimate direction of this work would then be to use time-specific causal manipulations with optogenetics or chemogenetics to better understand how this circuit contributes to reward drive and impulse control, beyond isolated manipulations of 5-HT<sub>1B</sub>Rs. Altogether, future work developed based on the experiments from this dissertation will contribute to our understanding of the biology of impulsive behavior and will identify a serotonin-responsive circuit which can be targeted for the treatment of dysregulated impulsivity.

### ***Conclusions***

Collectively, the work completed in this thesis uses detailed dissection of behavioral phenotype alongside innovative genetic and viral strategies to understand the role of 5-HT<sub>1B</sub>Rs in the

control of impulsive behavior. We find that enhanced reward drive, but not deficits in inhibitory learning, contribute to increased impulsivity in 5-HT<sub>1B</sub>R knockout mice, through multiple neural populations working synergistically. Importantly, this work highlights key considerations for how we test, analyze, and interpret diverse presentations of impulsivity, both in preclinical models and clinical populations, and points toward serotonergic pharmacology as a potential route for the treatment of impulse control disorders.

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## APPENDIX

### **Mice lacking the 5-HT<sub>1B</sub>R have enhanced goaltracking behavior in a touchscreen Autoshaping paradigm**

#### **Methods**

General mouse information and care was as indicated in Chapter 3, with all mice food restricted to 90% bodyweight for the duration of the experiment. Subjects included 5-HT<sub>1B</sub>R knockout mice (n=4 female; n=7 male) and their littermate controls (n=4 female, n=3 male).

Behavioral apparatuses (Bussey-Saksida Touchscreen Operant Chambers; Lafayette Instruments Co., Lafayette, IN) were as described in Chapter 2. For the Autoshaping set-up, the touchscreen was divided in half with a black mask into two cue areas, with the reward port/goal location in the center. An IR beam ran across both sides immediately in front of each side of the touchscreen, as well as in the reward port. All procedures were run using programs from the 'Mouse Touch Autoshaping v2' package (Lafayette Instruments Co., Lafayette, IN).

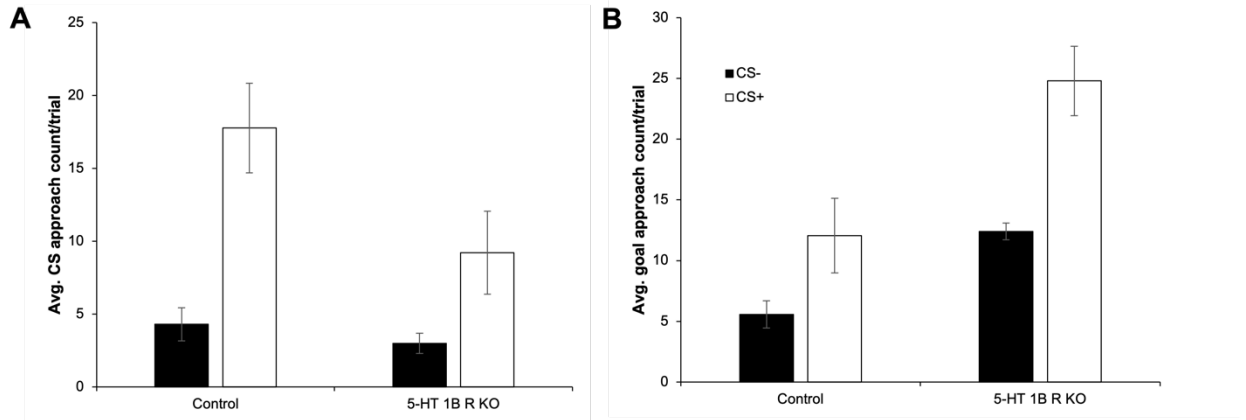
For habituation, trials consisted of reward (7uL of evaporated milk) delivered in the reward port after a variable interval of 0-30s, accompanied by a 1s tone and the port light turning on. The interval restarts once the mouse has retrieved the reward. To reach criterion, mice must have achieved 40 trials in 30m after at least 2 sessions.

For the main autoshaping procedure, each 10s trial consisted of either a CS- or CS+ cue (whole side of screen lit) presented on either the right or left side of the screen, with side counterbalanced across mice. Reward was delivered in the reward port upon termination of each CS+ cue. To initiate each trial, mice had to break an IR at the back of the chamber, followed by a variable interval of 10-40s before cue presentation. Sessions lasted 1hr or until 40 trials were completed. Approaches to the cues and the goal location were measured as IR beam break counts per trial, averaged over days 7-9 of the paradigm.

#### **Results**

In a touchscreen autoshaping paradigm, mice lacking the 5-HT<sub>1B</sub>R show a pattern of cue and goal approach behavior distinct from that present in controls. 5-HT<sub>1B</sub>R knockouts show decreased signtracking behavior compared to controls, though they do properly distinguish between CS- and CS+ trials (Fig.1A;  $F_{1,16}=4.887$ ,  $p=0.042$  for main effect of genotype;  $F_{1,16}=18.455$ ,  $p<0.001$  for main effect of CS type). On the other hand, 5-HT<sub>1B</sub>R knockouts have

enhanced responding in the reward port during CS+ trials compared to controls (Fig. 1B;  $F_{1,16}=7.428$ ,  $p=0.015$  for main effect of genotype;  $F_{1,16}=15.129$ ,  $p=0.001$  for main effect of CS type). This suggests that in addition to enhanced reward valuation and impulsivity, mice lacking the 5-HT<sub>1B</sub>R have a stronger bias toward goaltracking rather than signtracking.



**Figure 1. Genetic knockout of the 5-HT<sub>1B</sub>R decreases cue approach and increases goal location approach in a Pavlovian autoshaping paradigm.** A) Average cue approaches and B) average goal location approaches per trial for CS- and CS+ trials in control and 5-HT<sub>1B</sub>R knockout mice (averaged over days 7-9 of autoshaping). Error bars are +/- 1SE.