A DISTRIBUTED ROLE
FOR NUCLEUS ACCUMBENS CELL TYPES AND INPUTS
IN BEHAVIOURAL INHIBITION AND COMPULSIVITY

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ABSTRACT

Adaptive behaviour relies as much on suppressing context-inappropriate behaviour as selecting the best actions. Consequently, enduring deficits in behavioural inhibition are a common feature of numerous compulsive disorders, including OCD and Tourette syndrome. Although antagonistic interactions between nucleus accumbens (NAc) cell types and inputs are thought to play a key role in adjusting behavioural output, circuit mechanisms remain unclear. Here we examine the behavioural contributions of NAc direct and indirect pathway neurons and NAc afferents originating in the thalamus (PVT), amygdala (BLA), and hippocampus (vHPC). To test the causal role of NAc afferents in behavioural inhibition, we first validated an optical approach for projection-specific silencing. In brain slice recordings, we found that archaerhodopsin (ArchT)-mediated inhibition of axon terminals in the NAc elicited asynchronous vesicle release and increased local interneuron activity, undermining the pathway-specificity of this approach. We then identified soma-targeted ArchT inhibition as a valuable alternative, demonstrating that projection-specific silencing of PVT- or BLA-NAc afferents produced distinct behavioural outcomes. Much like direct and indirect pathway output neurons, evidence has also suggested that PVT and BLA inputs to the NAc exert antagonistic behavioural control. Accordingly, we hypothesized that effective behavioural control might arise from opposing activity between these cell types and pathways. To test this hypothesis directly, we then assessed the contributions of these circuit elements to mouse operant behavior during recurring periods of reward availability and unavailability. Although stimulation of direct and indirect pathway neurons was, respectively, reinforcing and aversive, inhibition of either cell type increased unproductive reward seeking. PVT and BLA inputs were also necessary for behavioral suppression even though they both supported self-stimulation behaviour and innervated different NAc subregions. These data suggest that effective reward seeking arises from the cooperative activity of NAc cell types and inputs, rather than opponent processes between them. We then considered the preclinical implications of our work and tested the role of PVT-NAc afferents in rodent models of compulsivity. Since striatal hyperactivity is a common feature of disorders like OCD and Tourette syndrome, we targeted repeated optical stimulation to PVT- and vHPC-NAc inputs over several days. While PVT-NAc activation elicited a progressive increase in repetitive self-grooming and impairments on a reversal learning task, vHPC afferent stimulation only impinged upon reversal learning. These data suggest that NAc thalamic afferents complement fronto-

striatal pathways as a convergent circuit vulnerability to compulsivity. Taken together, this body of work highlights NAc circuits as a key regulator of behavioural inhibition and lays the foundation for novel circuit-based therapies of compulsive disorders. Future models of basal ganglia function must recognize that behavioural inhibition is a distributed property of many neural circuits that arises from cross-regional population dynamics and not the aggregate activity of one cell type or neural pathway. From this perspective, dimensionality reduction and dynamical systems approaches represent a complement to existing circuit-interrogation techniques. These computational methods identify the low-dimensional structure of neural population dynamics and ground circuit perturbations in terms of their effects on physiological dynamics. By addressing significant hurdles in circuit neuroscience, these techniques pave a critical new path in the era of big data neuroscience.

RÉSUMÉ

Le comportement adaptatif relie autant sur la sélection des meilleures actions que sur la suppression des comportements inappropriés au contexte. Par conséquent, des déficits durables dans l'inhibition comportementale sont une caractéristique commune de nombreux troubles compulsifs, y compris les TOC et le syndrome de Gilles de la Tourette. Bien que l'on pense que les interactions antagonistes entre les types de cellules du noyau accumbens (NAc) et les afférences au NAc jouent un rôle clé dans l'ajustement de la production comportementale, les mécanismes du circuit restent peu clairs. Nous examinons ici les contributions comportementales des neurones des voies directes et indirectes du NAc et des afférences au NAc provenant du thalamus (TPV), de l'amygdale (ABL) et de l'hippocampe (HPCv). Pour évaluer le rôle causal des afférences au NAc dans l'inhibition comportementale, nous avons validé une approche optique pour l'inhibition spécifique des projections. Dans les enregistrements de coupes de cerveau, nous avons constaté que l'inhibition des terminaisons axonales dans le NAc par l'archaerhodopsine (ArchT) provoquait la libération asynchrone de vésicules et augmentait l'activité locale des interneurones, ce qui mettait en doute la spécificité de cette approche. Nous avons ensuite identifié l'inhibition de l'ArchT ciblée sur le soma comme une alternative, en démontrant que l'inhibition des afférences TPV- ou ABL-NAc produisait des résultats comportementaux distincts. Ce résultat est cohérent avec la preuve que les afférences TPV et ABL au NAc exercent un contrôle comportemental antagoniste, tout comme les neurones des voies directes et indirectes. En conséquence, nous avons proposé l'hypothèse qu'un contrôle comportemental efficace pourrait résulter d'une activité opposée entre ces types de cellules et ces afférences. Pour tester directement cette hypothèse, nous avons ensuite évalué les contributions de ces éléments du circuit au comportement opérant de la souris pendant des périodes récurrentes de disponibilité et d'indisponibilité de la récompense. Bien que la stimulation des neurones directes et indirectes soit, respectivement, renforçante et aversive, l'inhibition de l'un ou l'autre type de cellule augmente la recherche improductive de récompense. Les afférences TPV et ABL étaient également nécessaires pour la suppression du comportement, même si elles soutenaient toutes deux le comportement d'autostimulation et innervaient différentes sous-régions du NAc. Ces données suggèrent que la recherche efficace de récompense résulte de l'activité coopérative des types de cellules et des afférences au NAc, plutôt que de processus opposés entre eux. Nous avons ensuite examiné les implications précliniques de nos travaux et testé le rôle des afférences

TPV-NAc dans des modèles murins de compulsivité. L'hyperactivité striatale étant une caractéristique commune de troubles tels que les TOC et le syndrome de Gilles de la Tourette, nous avons ciblé une stimulation optique répétée sur les entrées TPV- et HPCv-NAc pendant plusieurs jours. Alors que l'activation du TPV-NAc a provoqué une augmentation progressive de l'autopalpation répétitive et des déficiences dans une tâche d'apprentissage à renforcements inversés, la stimulation afférente du vHPC n'a eu d'impact que sur l'apprentissage inversé. Ces données suggèrent que les afférences thalamiques du NAc jouent un rôle complémentaire avec les voies fronto-striatales produisant un circuit convergent vulnérable à la compulsivité. Somme toute, ces travaux démontrent que les circuits du NAc sont un régulateur clé de l'inhibition comportementale et établissent les fondements de nouvelles thérapies basées sur les circuits pour les troubles compulsifs. À l'avenir, les modèles de la fonction des noyaux gris centraux doivent reconnaître que l'inhibition comportementale est une propriété distribuée de nombreux circuits neuronaux qui découle de la dynamique des populations interrégionales et pas de l'activité agrégée d'un type de cellule ou d'une afférence neuronale. De cette perspective, la réduction de la dimensionnalité et les approches des systèmes dynamiques représentent un complément aux techniques existantes d'interrogation des circuits. Ces méthodes informatiques identifient la structure à faible dimension de la dynamique de la population neuronale et situent les perturbations du circuit par rapport à leurs effets sur la dynamique physiologique. En relevant ces obstacles importants dans la neuroscience des circuits, ces techniques ouvrent une nouvelle voie critique à l'ère de la neuroscience des mégadonées.

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CONTRIBUTIONS

CHAPTER 1 – INTRODUCTION AND LITERATURE REVIEW

In chapter 1 we review the history of behavioural inhibition through the lens of learning theory and models of basal ganglia function. We then present a focused summary of techniques used throughout the thesis to record and manipulate neural circuits. Finally, we discuss the role of nucleus accumbens (NAc) cell types and afferents in behavioural inhibition and compulsivity.

This section was written by Christopher Lafferty and edited by Jonathan Britt. Parts of the introduction were adapted from (Lafferty et al., 2021).

CHAPTER 2 – OFF-TARGET INFLUENCES OF ARCH-MEDIATED AXON TERMINAL INHIBITION ON NETWORK ACTIVITY AND BEHAVIOUR.

This chapter comprises a manuscript published in *Frontiers in Neural Circuits*, validating a projection-specific silencing approach that is used throughout the thesis. Here we demonstrate that ArchT-mediated inhibition of glutamate axons in the NAc causes asynchronous glutamate release, which increases interneuron activity and causes non-specific inhibition of the NAc. We find that these off-target effects undermine pathway-specific behavioural outcomes and highlight soma-targeted inhibition as a valuable alternative.

Christopher Lafferty and Jonathan Britt conceived the study, designed the experiments, and wrote the manuscript. Christopher Lafferty conducted the experiments. Jonathan Britt supervised the research.

CHAPTER 3 – NUCLEUS ACCUMBENS CELL TYPE- AND INPUT-SPECIFIC SUPPRESSION OF UNPRODUCTIVE REWARD SEEKING.

Chapter 3 constitutes a manuscript published in *Cell Reports*. We test the hypothesis that effective behavioural inhibition emerges as a consequence of antagonistic interactions between nucleus accumbens cell types and pathways. We used calcium imaging, along with opto- and chemogenetic manipulations in behaving mice. We also compared NAc afferent innervation patterns and pathway specific inhibition strategies. We confirm that D1 neuron stimulation is reinforcing and D2 neuron stimulation is aversive, but demonstrate that activity in both cell types is critical for the suppression of unproductive reward seeking. Inputs to the NAc from the

thalamus and amygdala are also shown to be critical regulators of behavioral inhibition, even though they both encourage behavioral responding in other contexts and innervate different areas of the NAc. Overall, this work suggests the capacity to encourage and discourage behavioural responding, a core feature of NAc-mediated action selection, is distributed across NAc cell types and inputs, and efficient reward seeking behaviour arises from complementary activity across these circuit elements rather than opponent processes between them.

Christopher Lafferty and Jonathan Britt conceived the study, designed the experiments, and wrote the manuscript. Christopher Lafferty conducted the experiments. Angela Yang and Jesse Mendoza assisted with surgeries and refinement of experimental design. Christopher Lafferty analyzed the data. Jonathan Britt supervised the research.

CHAPTER 4 – HYPERACTIVITY OF PARAVENTRICULAR THALAMIC INPUTS TO THE NUCLEUS ACCUMBENS ELICITS COMPULSIVITY.

Chapter 4 has been submitted for publication to *Neuropsychopharmacology*. Here we test the effects of repeated NAc afferent stimulation on rodent measures of compulsivity. We find that hyperactivity of NAc thalamic inputs elicits excessive self-grooming and impairs performance on a reversal learning task. Repetitive grooming is blocked by a metabotropic glutamate receptor antagonist. Stimulation of NAc hippocampal afferents affects reversal learning without altering self-grooming, demonstrating a pathway-specific role for thalamic afferents in compulsivity.

Christopher Lafferty and Jonathan Britt conceived the study, designed the experiments, and wrote the manuscript. Christopher Lafferty conducted the experiments. Jonathan Britt supervised the research.

CHAPTER 5 – DISCUSSION

In chapter 5 we contextualize our work in contemporary models of basal ganglia function. We leverage an evolutionary perspective to identify the strengths and weaknesses of a circuit-based approach to the study of NAc cell types and inputs. We highlight novel behavioural analysis and computational techniques to address challenges emerging in the era of large-scale neuroscience.

This section was written by Christopher Lafferty and edited by Jonathan Britt.

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ABBREVIATIONS

AAV	adeno-associated virus
ACR	anion-conducting channelrhodopsin
ACSF	artificial cerebrospinal fluid
ANOVA	analysis of variance
AP	anterior/posterior
ArchT	archaerhodopsin
ASD	autism spectrum disorder
BG	basal ganglia
BLA	basolateral amygdala
C21	compound 21
ChR2	channelrhodopsin 2
D1	D1 dopamine receptor
D2	D2 dopamine receptor
DAPI	4′,6-diamidino-2-phenylindole
DBS	deep brain stimulation
DREADD	designer receptors exclusively activated by designer drugs
DV	dorsal/ventral
eACR	engineered anion-conducting channelrhodopsin
eEPSC	evoked excitatory postsynaptic current
EP	entopeduncular nucleus
eGFP	enhanced green fluorescent protein
eYFP	enhanced yellow fluorescent protein
FR1	fixed ratio (1)
GABA	γ-Aminobutyric acid
GECI	genetically encoded calcium indicator
GtACR	*Guillardia theta* anion-conducting channelrhodopsin
KO	knock-out

LTD	long term depression
M1	primary motor cortex
mGluR5	metabotropic glutamate receptor (5)
ML	medial/lateral
mPFC	medial prefrontal cortex
MSN	medium spiny neuron
MTEP	3-((2-Methyl-4-thiazolyl)ethynyl)pyridine
NA	numerical aperture
NAc	nucleus accumbens
NpHR	halorhodopsin
OCD	obsessive-compulsive disorder
OCSD	obsessive-compulsive spectrum disorder
OFC	orbitofrontal cortex
PBS	phosphate buffered saline
PCA	principal components analysis
PFA	paraformaldehyde
PV	parvalbumin
PVT	paraventricular thalamus
SAPAP3	SAP90/PSD95-associated protein 3
SEM	standard error of the mean
sEPSC	spontaneous excitatory postsynaptic current
sIPSC	spontaneous inhibitory postsynaptic current
SNr	substantia nigra pars reticulata
vHPC	ventral hippocampus
VMH	ventromedial hypothalamus
VMS	ventromedial striatum
VR3	variable ratio (3)

CHAPTER 1 – INTRODUCTION AND LITERATURE REVIEW

1.1. BEHAVIOURAL INHIBITION

Selecting context-appropriate actions and suppressing competing actions are critical features of adaptive behaviour. Although the study of animal behaviour often focuses on action selection and behavioural facilitation, the capacity to dynamically up- *and* downregulate behaviour is needed to meet fluctuating environmental demands (Jahanshahi et al., 2015). As such, behavioural inhibition can be viewed as a complement to behavioural facilitation. Balance between these processes supports effective executive control and is necessary for healthy behaviour. Basal ganglia circuits, including the nucleus accumbens (NAc), play a key role in behavioural inhibition, but the underlying circuit mechanisms are unknown. Since the NAc is implicated in compulsive disorders characterized by deficits in behavioural inhibition, identifying these mechanisms may provide etiological insight and motivate novel circuit-based therapies. The aim of this thesis was thus to explore the role of NAc cell types and inputs in behavioural inhibition, a function serving healthy reward seeking and a process that is disrupted in an animal model of compulsivity.

1.1.1. A HISTORY OF TIDY DICHOTOMIES

Categorical descriptions impose useful structure on complex behavioural data. In neuroscience and psychology, these categories are often dichotomous, providing a tidy framework for studying the link between brain and behaviour. This heuristic echoes throughout neuroscience; we draw conceptual boundaries between habitual and goal-directed behaviour, between egocentric and allocentric navigation, between reward seeking and avoidance, between behavioural facilitation and inhibition, etc. (Collins and Cockburn, 2020). While these binaries often reflect face valid, common-sense categories, there is ongoing debate about the extent to which they reflect the true structure of behaviour and distinctions between underlying neurobiological systems (Buzsaki, 2019). Linking the brain to behaviour may require a reassessment of terminology that has been foundational to our thinking (section 5.3). Nevertheless, it is useful to consider the origin and development of these dichotomies because it guides us to contemporary models that are more informed by neurobiology.

In the early 1960s, animal learning theory began formalizing the role of unconditioned stimuli in shaping behaviour, suggesting that the frequency and intensity of behaviours can be adjusted by the presence or absence of rewards and punishments (Skinner, 1963). Stimuli were

categorized on the basis of their valence and how they influenced behaviour. Reinforcers were stimuli that promoted behaviour either through presentation of a rewarding outcome (*positive reinforcer*) or through omission of an unpleasant outcome (*negative reinforcer*). Similarly, punishments discouraged behaviours that elicited unpleasant outcomes (*positive punishment*) or that led to omission of a reward (*negative punishment*). The capacity of these unconditioned stimuli to drive associative learning and shape behaviour was formalized mathematically by the Rescorla-Wagner model of classical conditioning in which the associative strength between conditioned and unconditioned stimuli is modified by errors in predicting the delivery of the unconditioned stimulus (Rescorla and Wagner, 1972). Although this model did not explicitly account for operant behaviour, similar algorithms would later be adopted to describe the role of dopamine signaling in representing reward prediction errors that underlie behavioural reinforcement (Hollerman and Schultz, 1998). In this view, dopaminergic inputs to the striatum respond to better-than-expected outcomes and promote the recurrence of behaviours that led to those outcomes. However, these models carried the implicit assumption that reinforcers and punishers shape behaviour via symmetric processes underpinned by the same neurobiological computation. Prediction errors associated with positive and negative outcomes were thought to be represented by increases or decreases in dopamine signaling (Mark et al., 1991; Pezze and Feldon, 2004; Shippenberg et al., 1991). Decades of research have since suggested that the relationship between these processes is not so straightforward and may be a distributed function of many brain regions (Balsam and Bondy, 1983; Bromberg-Martin et al., 2010; Hu, 2016; Thibeault et al., 2019). This behavioural dichotomy has fueled the search for antagonistic neural circuits that support reinforcement and punishment, not only in the mesolimbic dopamine system, but throughout the brain.

In the early 1990s, a highly influential model of antagonistic behavioural control emerged. The canonical model of basal ganglia function identified two classes of output neurons in the striatum that exert opposing control over behaviour. These direct and indirect pathway neurons, so named for their distinct projection targets, were thought to promote and inhibit movement, respectively (Albin et al., 1989a; DeLong, 1990). Direct pathway neurons send GABAergic projections ‘directly’ to the midbrain where they disinhibit motor programmes, while indirect pathway neurons project ‘indirectly’ to the midbrain via pallidal structures to inhibit motor programmes (Graybiel et al., 1979). These neural subpopulations can be

distinguished by their expression of dopamine receptor subtypes, as direct pathway neurons express excitatory D1 dopamine receptors and indirect pathway neurons express inhibitory D2 dopamine receptors (Gerfen et al., 1990a). This 'go/no-go' model of basal ganglia function is consistent with a role for dopamine signaling in bidirectional behavioural control since dopamine release in the striatum differently affects each output pathway (Nicola et al., 2000). In this view, enhancements in striatal dopamine signaling facilitate behaviour by exciting D1 neurons and inhibiting D2 neurons. Conversely, reductions in dopamine signaling produce the opposite effects. This dichotomy has remained highly influential and has given rise to different renditions of the striatal action selection hypothesis (Bariselli et al., 2018), which posits that antagonistic interactions between the go and no-go systems facilitate a choice between competing motor programmes generated by the cortex. A cortical motor command that successfully penetrates this action selection 'filter' is driven by focal excitation of D1 neurons that release a specific motor programme coupled with D2 neuron activation that silences competing actions.

Much of the evidence for this striatal dichotomy comes from the dorsal striatum, which strongly regulates dynamic control of movements and locomotion. The NAc, which is located in the ventral striatum, is implicated in more abstract features of behavioural control including affect, cognition and goal-selection (Mannella et al., 2013). It remains unclear how this model translates to NAc circuits where D1 and D2 neuron projection targets are less sharply segregated (Kupchik et al., 2015), and manipulations of these neural subpopulations produce mixed behavioural effects (Cole et al., 2018; Soares-Cunha et al., 2016). A central aim of this thesis was thus to identify NAc circuit elements that play an analogous role in facilitating and inhibiting behavioural output.

While most models of NAc function emphasize its role in action selection and facilitation of reward seeking (Mannella et al., 2013), the most common consequence of disturbing NAc physiology is actually an increase in uncued, unrewarded, and often unproductive actions (Ambroggi et al., 2011; Blaiss and Janak, 2009; Bowman and Brown, 1998; Dalton et al., 2014; Floresco, 2015; Floresco et al., 2008; Millan et al., 2015; Peters et al., 2008; Reading and Dunnett, 1995; Reading et al., 1991; Stopper and Floresco, 2011; Yun et al., 2004) (see section 1.3 for a detailed treatment of this topic). Because behavioural inhibition involves the omission of an action, its importance is often overlooked (Jahanshahi et al., 2015), and so we were

motivated to consider neural mechanisms of behavioural control that preferentially subserve an animal's capacity to withhold behaviour, rather than emit it. In this framework, antagonistic NAc circuit interactions may yet give rise to effective behavioural inhibition, but the balance of opponent processes may favour suppression.

1.1.2. PUNISHMENT

The study of behavioural inhibition has grown slowly over the past 40 years, often examining *positive* and *negative punishments*, i.e., the shaping of operant behaviour by unpleasant stimuli and reward omission, respectively (Piantadosi et al., 2021). While footshocks and similar punishments reduce operant responding, they often elicit a distinct repertoire of defensive and avoidant behaviours (Church, 1963; Fucich and Morilak, 2018). In contrast, reward omission following an operant response merely reduces this behaviour by signaling that it is no longer productive (Church, 1963). Although both categories of stimuli inhibit behaviour, they appear to recruit distinct psychological and neural processes (Piantadosi et al., 2021). Like reinforcers and punishments themselves, these stimuli must be considered separately. Both positive and negative punishments likely adjust the gain of multiple behaviours in tandem, across many modalities and timescales. That said, suppression of unrewarded behaviours may represent a better model to study how behaviour is dynamically regulated, since the effects of this punishment can be limited to adjustments of a single behavioural variable (e.g., frequency of lever pressing in extinction). Moreover, the absence of reward is a ubiquitous feature of the world, perhaps more so than overtly aversive stimuli (VM and Stuber, 2021). In environments where reward is sparse and animals are surrounded by motivational cues of varying importance, the capacity to suppress unproductive actions is critical for healthy behaviour. In the next section, we discuss a variety of assays that necessitate behavioural inhibition, with a special emphasis on tasks that involve reward omission and suppression of reward seeking.

1.1.3. BEHAVIOURAL INHIBITION IN REWARD SEEKING

In rodent behavioural tasks, behavioural inhibition can be defined quite broadly as a decrease in the vigour or frequency of an action. As we have seen, this decrease can be accomplished through overt punishment or by the absence of reward. In conditioned suppression, for example, an aversive stimulus like a tone previously paired with footshock reduces appetitive responding, usually in an operant task where animals are lever pressing for food (Ayres, 2012). On the other

hand, operant tasks that involve reward omission include extinction learning (Hartley and Phelps, 2012), reversal learning (Izquierdo et al., 2017), progressive ratio tasks (Stewart, 1975), tasks that reward low response rates (Reading and Dunnett, 1995) and tasks that penalize premature responding (Bowman and Brown, 1998). Many of these tasks also incorporate reinforcement of inaction or action competition (Mink, 1996), which can reduce the vigour or frequency of behaviours. In go/no-go tasks, for example, not only are rewards withheld when animals respond on no-go trials, but animals are rewarded when they refrain from responding (Donders, 1969; Roitman and Loriaux, 2014). In this assay, inaction is its own category of behaviour that can be reinforced or punished. Here we focus our attention to behavioural inhibition driven by reward omission alone. In this thesis we used a variant of an extinction learning task that comprised periods of cued, intermittent reward unavailability (Kalmbach et al., 2022) since similar tasks are known to recruit NAc circuits.

Tasks in which animals must discriminate between cued periods of reward availability and unavailability are a valuable tool for the study of behavioural inhibition. Even in Rescorla's early work, he noted that the positive value of any cue relies on negative contingencies (Balsam et al., 2010; Rescorla, 1968). In these assays, reward is withheld during the presentation or absence of some cue, providing brief, recurring extinction bouts within a session as animals repeatedly pair an operant behaviour with the absence of reward. These tasks are similar to conditioned suppression tasks, except that the cue that reduces operant responding is not associated with an overtly aversive outcome (Do-Monte et al., 2017). That said, cues signaling reward unavailability are unusually long compared to typical discriminative stimuli, likening them more to contextual occasion setters that modify CS-US associations (Holland, 1992). Recurring periods of reward omission also permit repeated temporally precise manipulations, spanning transitions from reward availability to unavailability and vice versa within the same behavioural session.

A significant challenge of measuring behavioural inhibition is that it is largely indistinguishable from a reduction in behavioural facilitation, both manifesting as reductions in behavioural output. If these processes are dissociable at the neurobiological level, and supported by antagonistic neural circuits, then perhaps we can argue that this dichotomy represents distinct behavioural processes. To address this question, we sought to identify NAc circuit elements

whose activity may be necessary for inhibitory control. In the following section, we outline recent advances in precise neural recording and manipulation techniques that will allow us to test this family of hypotheses.

1.2. CIRCUIT NEUROSCIENCE

Electrophysiological recordings, electrical stimulation, lesions studies, and behavioural pharmacology have long been primary means of observing and manipulating neural activity. The main drawback of these techniques is their lack of cell type, pathway or temporal specificity. Advancements in genetic and optical toolkits in the past two decades have altered the face of neuroscience, giving us unparalleled access to the neural circuits underlying behaviour. To study the role of NAc inputs and cell types in behavioural inhibition, we used calcium imaging and optogenetics.

1.2.1. CALCIUM IMAGING

Optical recording techniques typically employ genetically encoded calcium indicators (GECIs) (Miyawaki et al., 1997) such as the GCaMP family of proteins (Akerboom et al., 2013; Chen et al., 2013). Neuronal expression of GCaMP can be achieved using transgenic animals or viral-mediated gene delivery. Enhanced GCaMP fluorescence is evoked by rises in intracellular calcium, providing an indirect measure of neuronal spiking and bulk activity (Chen et al., 2013). To record fluorescence from deep brain structures, it is common to implant a fiber above the site of GCaMP expression to record average fluctuations in neural activity. This approach is especially useful when the activity of many neurons changes in tandem, elevating signal-to-noise ratios. For example, large amplitude, coherent fluctuations in dopamine neuron activity are detectable using this method (Mendoza et al., 2019). Although single-cell resolution calcium imaging is possible using miniature microscopes (Stamatakis et al., 2021) and 2-photon head-fixed preparations (Stosiek et al., 2003), recording from deep brain structures like the NAc can present unique challenges relating to tissue scarring and poor visualization. Although recent improvements in experimental technique (Zhang et al., 2019) and GECI kinetics (Zhang et al., 2023) have made these recordings more feasible, in this thesis we use fiber photometry to obtain bulk readouts of NAc activity in relation to reward seeking and behavioural inhibition. This methodology also complements prior work from our group recording from NAc afferents using the same technique (Reed et al., 2018).

1.2.2. OPTOGENETIC EXCITATION

The temporal, spatial and circuit precision afforded by optogenetic tools has revolutionized the field and brought us into the so-called era of light (Hausser, 2014). Channelrhodopsin-2 (ChR2) was among the first optogenetic actuators to be employed in neural circuits. Adapted from an algal protein, ChR2 is a light-sensitive cation channel that can be expressed in restricted neuronal populations using transgenic mice or viral-mediated gene delivery (Boyden et al., 2005). Pathway-specific investigations of neural activity are nearly impossible without this approach. In basal ganglia circuits, electrical stimulation can be targeted to fiber bundles, but it is difficult to know how stimulation affects nearby structures or fibers of passage, making off-target effects inevitable (Klauer et al., 1990; Schwarz et al., 2015). With optogenetics, however, it is possible to test the causal role of enhanced activity in specific projection pathways by expressing ChR2 or a variant in the afferent population and then delivering light in a downstream structure to activate axon terminals there (Tye et al., 2011). This approach has been validated *ex vivo* in brain slices (Tye et al., 2011), and used in NAc circuits to study the role of specific afferents in reward seeking (Britt et al., 2012). This technique is used throughout the thesis to study the role of specific NAc afferents in reward seeking and behavioural inhibition (chapters 3 and 4). That said, a significant challenge of optogenetic excitation approaches is that they elicit non-physiological patterns of neural of activity. Interpreting these neural circuit perturbations will require us to ground our manipulations in approximations of natural physiology, an issue which is addressed directly in section 5.4.

More recently, optical plasticity protocols have been used to normalize circuit and behavioural abnormalities in rodent models of psychiatric disease (Bagot et al., 2015; Creed et al., 2016; Hearing et al., 2016; Ma et al., 2018; Neumann et al., 2016; Pascoli et al., 2014; Zhu et al., 2016). These protocols involve repeated optical stimulation of particular cell types and pathways to induce long-lasting neural plasticity, and they have generated excitement for their potential to improve deep brain stimulation protocols in humans (opto-inspired DBS) (Creed et al., 2015; Luscher et al., 2015). This approach is applied in chapter 4 to study the role of enduring hyperactivity in NAc afferents in behavioural inhibition.

1.2.3. OPTOGENETIC INHIBITION

Shortly after the advent of *in vivo* optical stimulation techniques, tools for testing the necessity of circuit activity for behaviour emerged (Gradinaru et al., 2008; Lerchner et al., 2007). Many of these relevant opsins are light-sensitive chloride pumps (NpHR) (Gradinaru et al., 2008) or proton pumps (ArchT) (Chow et al., 2010) that induce hyperpolarization and silence neural activity. This technique can be used in a similar manner as excitatory opsins to disrupt cell type-specific signaling or impinge upon pathway-specific synaptic transmission. Unfortunately, ion transporters such as NpHR and ArchT can alter intracellular ion concentrations, shifting chloride reversal potentials or altering pH, in a manner that produces off-target physiological effects (Mahn et al., 2016; Raimondo et al., 2012). Although these effects are amplified in axon terminals on account of their small volume, many studies of neural circuits, especially in the NAc, still use these and similar opsins for long-range projection-specific silencing in behaving animals (Herrera et al., 2016; Mangieri et al., 2018; Reed et al., 2018; Stefanik et al., 2016; Trouche et al., 2019; Yamamoto and Tonegawa, 2017; Zhu et al., 2016). It remains unclear whether these off-target physiological disruptions hinder pathway- or cell type-specific interpretations. To validate the use of pathway-specific inhibition approaches in the study of NAc projections, we compare the behavioural effects of somatic and axonal ArchT silencing in chapter 2.

1.3. NUCLEUS ACCUMBENS CIRCUITS

The NAc is a forebrain structure that acts as an interface between limbic and action circuitry (Floresco, 2015; Mannella et al., 2013). Located in the ventral striatum, the NAc receives input from numerous cortical and subcortical brain structures that are thought to encode states and stimuli that shape the action selection process. Reinforcement signals emanating from midbrain dopamine neurons are thought to modulate the strength of these inputs to adjust their influence on behaviour. As a site of convergent inputs and with connectivity to structures that affect motor circuits, the NAc is well situated to adaptively control the vigour and frequency of reward seeking behaviours.

1.3.1. NUCLEUS ACCUMBENS ANATOMY

Over 90% of NAc neurons are GABAergic medium spiny projection neurons (MSNs) (Mannella et al., 2013). Like in the dorsal striatum, MSNs in the NAc can be distinguished by whether they express D1 or D2 dopamine receptors, with a small amount of overlap (Gerfen et al., 1990b; Surmeier et al., 1996). In the dorsal striatum, these populations define distinct downstream projection targets that underlie the canonical direct and indirect basal ganglia pathways that facilitate and inhibit competing behaviours, respectively (Albin et al., 1989b). Although the segregation of NAc D1 and D2 neuron projection targets is less well-defined than in the dorsal striatum (Kupchik et al., 2015), NAc D1 neurons largely target motor output structures and dopaminergic nuclei in the midbrain while D2 neurons target the ventral pallidum. These cell types have been shown to exert antagonistic control over behaviour, promoting reward and aversion in place preference assays (Cole et al., 2018) and supporting learning from rewards and aversive events, respectively (Hikida et al., 2010; Yawata et al., 2012). That said, some studies find mixed results with respect to bidirectional behavioural control by NAc D1 and D2 neurons. D1 neurons in the ventromedial NAc can drive behavioral aversion (Al-Hasani et al., 2015), and D2 neuron stimulation can increase reward seeking under certain conditions (Soares-Cunha et al., 2016). In fact, manipulations of NAc activity frequently elicit mixed behavioural responses and many studies account for this variance by looking at differences in the precise anatomical locations targeted by their manipulations. The NAc comprises several subregions, and most noisy data can be explained by one of many crisscrossing neuroanatomical partitions.

The NAc can be subdivided into core and shell subregions (Zahm and Brog, 1992). The boundary between these regions is defined by distinct expression levels of several histological markers, including acetylcholinesterase and cholecystokinin (Záborszky et al., 1985; Zahm and Brog, 1992). While the NAc core is located dorsally and contains the anterior commissure fiber bundle, the NAc shell sits ventrally, surrounding the core and bordering the septum and olfactory tubercles. Despite an even distribution of D1R- and D2R-expressing MSNs throughout the NAc core and shell, numerous studies have found distinct roles for these subregions in behavioural inhibition. Recordings from the core show enhanced responsivity to reward-associated cues that trigger reward seeking (Hollander and Carelli, 2007; Setlow et al., 2003), and disruptions of axonal input to the core impairs behavioural responding to reward-associated cues (Ciano et al.,

2001; Parkinson et al., 2000). The NAc core also has the same cytoarchitecture and afferent/efferent organization as dorsal striatal regions involved in traditional action selection processes (Mannella et al., 2013; Voorn et al., 2004). Taken together, these data situate the NAc core as a behavioural facilitator, critical for promoting appropriate behavioural responses to reward-relevant stimuli. Meanwhile, lesions and reversible inactivations of the NAc shell have been shown to disinhibit a spectrum of appetitive (Maldonado-Irizarry et al., 1995) and defensive behaviours (Reynolds and Berridge, 2002). These antagonistic contributions to behaviour are quite reminiscent of the purported role of D1 and D2 neurons in behaviour. The intermingling of these cell types and anatomical axes poses a challenge since behavioural inhibition can be attributed to multiple overlapping circuit elements.

Antagonistic anatomical dichotomies are surprisingly common in NAc literature. Researchers have uncovered contributions to reward and aversion, or behavioural facilitation and inhibition, across anatomical axes that span core and shell regions as well as D1 and D2 neurons across anterior/posterior (Faure et al., 2010; Reed et al., 2018; Reynolds and Berridge, 2008), dorsal/ventral (Al-Hasani et al., 2015; Castro and Berridge, 2014), and medial/lateral subdivisions (Chen et al., 2023; Klawonn and Malenka, 2018; Lammel et al., 2014; Yang et al., 2018).

To muddy the waters further, recordings from the NAc show multiplexed signaling patterns that are heterogeneous between conditions in which animals produce or withhold behaviour. Neural correlates of behavioural facilitation and inhibition do not appear to fall along obvious explanatory axes, anatomical or otherwise. In discriminative stimulus tasks known to recruit NAc activity, animals learn to discriminate reward-associated cues from unrewarded cues, and recordings of NAc neurons reveal a zoo of response profiles. Some neurons respond to only one task element, but many neurons respond to multiple. These responses include increases *or* decreases in firing in combination across multiple tasks elements (cues, lever presses, and food port entries under varying conditions of reward availability) (Nicola et al., 2004).

With the advent of improved recording techniques that permit spatially precise and cell type-specific recordings, traditional neurobiological divisions appear insufficient to capture this variance. On any given task involving behavioural facilitation or inhibition driven by reward or punishment, neural response patterns tend to be similar throughout the brain but locally

heterogeneous. When neural activity is analyzed in relation to a task-relevant variable, a similar fraction of cells often exhibits increases, decreases, or no change in neural activity. A striking example is the finding that D1 and D2 neurons do not naturally exhibit opposing activity profiles, but are instead highly co-active, and this activity, in aggregate, is weakly correlated with every motor behaviour that an animal emits (Klaus et al., 2017; Tecuapetla et al., 2016). Mixed encoding of multiple behavioural variables is a common feature of NAc activity (Chen et al., 2023; Pedersen et al., 2022; Reed et al., 2018) and can be observed throughout the brain (Burgos-Robles et al., 2017; de Vries et al., 2020; Kira et al., 2023; Klaus et al., 2017; Libby and Buschman, 2021; Nair et al., 2023; Otis et al., 2019). This promiscuous encoding evokes the spectre of equipotentiality (Lashley, 1929) – that is, the idea that all brain areas contribute similarly to all behavioural tasks. While these challenges may be addressed by considering coding schemes beyond simple aggregate measures of neural activity (section 5.4), circuit neuroscience may represent a step forward.

The precision with which we can now target increasingly specific cell types and pathways has led to the search for the smallest neural subpopulation that governs a behaviour. The potential value of this approach is that while we may observe heterogeneous activity within the NAc, these multiplexed neuronal response profiles could be driven in a projection-specific manner such that responses related to specific task elements are attributable to particular afferents. In this view, antagonistic processes such as behavioural facilitation and inhibition arise from distinct, projection-defined neural populations that are intermingled with cells involved in other behavioural functions.

The NAc integrates inputs from across the brain, including the basolateral amygdala (BLA), ventral hippocampus (vHPC), medial prefrontal cortex (mPFC), paraventricular thalamus (PVT), and ventral tegmental area (Mannella et al., 2013). These glutamatergic inputs have been shown to converge on single NAc projection neurons (French and Totterdell, 2002) where they functionally interact (O'Donnell and Grace, 1995), suggesting a means by which multiplexed NAc neural responses arise. However, there is also evidence that these excitatory afferents preferentially target distinct subregions since the axonal density of a given afferent tends to vary systematically across the extent of the NAc (Berendse et al., 1992; Groenewegen et al., 1999; Wright et al., 1996; Wright and Groenewegen, 1995). These afferents may thus define

functionally segregated loops that govern specific behavioural domains, as has been observed in the dorsal striatum (Lee et al., 2020). While these innervation patterns fail to demarcate sharp boundaries, they may yet account for some of the mixed behavioural consequences of direct NAc manipulations. Accordingly, distinct contributions of NAc inputs may underlie bidirectional behavioural control and drive the heterogeneous responses observed in MSNs during reward seeking tasks and behavioural inhibition (Nicola et al., 2004).

1.3.2. NUCLEUS ACCUMBENS AFFERENTS

Excitatory NAc afferents are thought to encode discrete aspects of the cues and states that facilitate and inhibit reward seeking behaviour. For example, the BLA and vHPC inputs are thought to convey information to the NAc about reward-associated cues and contexts, respectively (Mannella et al., 2013). The BLA has long been assigned a role in associative learning, linking neutral sensory stimuli to rewarding cues and outcomes (Everitt et al., 2003). BLA lesions and disconnection experiments that employ asymmetric lesions of the BLA and NAc disrupt animals' ability to adjust instrumental behaviour on the basis of a devalued outcome or a degraded action-outcome contingency (Hatfield et al., 1996; Shiflett and Balleine, 2010), suggesting a key role in guiding operant behaviour under varying motivational conditions. In particular, appetitive cues that shape reward seeking are thought to be encoded by this pathway since reversible inactivation of the BLA reduces NAc neural responses to cues that signal reward availability (Ambroggi et al., 2008), and pathway-specific optical inhibition of BLA inputs to the NAc core impairs cue-evoked sucrose seeking (Stuber et al., 2011). Conversely, the vHPC is thought to play a role in both anxiety-related behaviours (Bannerman et al., 2004) and contextual memory-retrieval (Ito et al., 2006). Disconnection lesions targeted to the vHPC and NAc shell impair appetitive spatial context conditioning (Floresco et al., 1997; Ito et al., 2008), suggesting that this pathway may link spatial-contextual information to rewarding outcomes.

While the BLA- and vHPC-NAc pathways are largely emphasized for their capacity to promote behaviour, mPFC afferents are thought to have a more mixed role in behavioural control. Like the BLA, mPFC signaling is critical for cue-evoked firing in the NAc, (Ishikawa et al., 2008) and reversible inactivation of the mPFC hinders reward seeking behaviour (Sangha et al., 2014; van Holstein and Floresco, 2020). However, the cortical origins of this afferent have long suggested a loftier function. Inhibition of the mPFC and its afferents have been shown to

disinhibit fear responses (Sangha et al., 2014), reduce conditioned suppression (Piantadosi et al., 2020), and enhance perseverative drug-seeking (Pascoli et al., 2018). This is consistent with a general role for the mPFC in executive control, an idea that is supported by evidence implicating dysregulated fronto-striatal pathways in many psychiatric diseases characterized by impaired behavioural inhibition (Harrison et al., 2013; Harrison et al., 2009; Isobe et al., 2018; Mink, 2006). These antagonistic functions of mPFC afferents have been dissociated along dichotomous anatomical lines once again; prelimbic and infralimbic cortex are thought to contribute distinctly to behavioural facilitation and suppression, respectively (Piantadosi et al., 2020; Sangha et al., 2014; van Holstein and Floresco, 2020). However, most models of NAc function emphasize the core role of the mPFC in promoting behaviour by providing several possible goals or action plans to the NAc. Cue- and context-associated values are then computed in the BLA and vHPC and transmitted to the NAc to weigh in on the action selection process (Floresco, 2015; Mannella et al., 2013). Each of these afferents supports self-stimulation behaviour (Britt et al., 2012) which further supports the notion that they have a shared role in facilitating motivated behaviour.

In contrast to other excitatory NAc afferents, the PVT-NAc pathway has recently been highlighted as a uniquely aversive circuit that promotes avoidance and underlies the aversive properties of opioid withdrawal (Keyes et al., 2020; Vollmer et al., 2022; Zhu et al., 2016). Inhibition of this pathway has been shown to disinhibit feeding and reward seeking (Do-Monte et al., 2017; Kessler et al., 2021; Zhu et al., 2018), while stimulation has the opposite effect (Gargiulo et al., 2022; Vollmer et al., 2022). As the PVT itself receives convergent input from hypothalamic and brainstem arousal regions, this pathway has been suggested as a critical suppressor of food seeking behaviours, particularly under conditions of stress, novelty, aversion or conflict (Choi et al., 2019; Choi and McNally, 2017; Vertes et al., 2015). This sets the stage for antagonistic interactions between the PVT-NAc pathway and the other excitatory afferents. Consistent with this hypothesis, PVT axons preferentially target D2 neurons (Li et al., 2018) and innervate NAc subregions that are distinct from the other afferents (Berendse et al., 1992; Groenewegen and Berendse, 1994; Wright and Groenewegen, 1995). These findings suggest that effective reward seeking may arise from opponent processes between these pathways, and that PVT afferents play a key role in behavioural inhibition.

In chapters 2 and 3 of this thesis, we examine the role of BLA- and PVT-NAc afferents as well as D1 and D2 neuron populations in opponent control of reward seeking, particularly as it pertains to behavioural inhibition. In chapter 4 we consider the role of dysregulated PVT afferents in driving long-term deficits in behavioural inhibition. Similar impairments are a common feature of compulsive disorders which we will discuss in the next section.

1.4. COMPULSIVITY

Disrupted behavioral inhibition is a core feature of numerous psychiatric and neurological disorders, including obsessive compulsive disorder (OCD), hair-pulling disorder (trichotillomania), skin-picking disorder (dermatillomania), autism spectrum disorder (ASD), and Tourette syndrome, among others (Hollander and Wong, 1995). Patient behaviour is often characterized by repetitive behaviours or rituals that persist despite negative outcomes and the fact that they are counterproductive to expressed goals (Allen et al., 2003). Because of similar compulsive symptom presentation, shared neural substrates, and comparable treatment responsivity, it has been proposed that these disorders lie on an obsessive-compulsive spectrum. Due to the chronic and severe nature of compulsivity in these disorders, they are associated with a heavy disease burden (Siddiqui et al., 2018). Compulsive symptoms of OCD are widely known, and OCD draws significant attention because it accounts for more than half of serious anxiety cases (Fornaro et al., 2009; Ruscio et al., 2010). Moreover, more than half of patients experiencing OCD fail to fully respond to first line pharmacological treatments (Erzegovesi et al., 2001), likely due to a lack of mechanistic specificity. Therapies for psychiatric disease often target entire neurotransmitter systems (e.g., selective serotonin reuptake inhibitors, SSRIs) or entire brain regions (e.g., deep brain stimulation, DBS) (Lafferty et al., 2021), and fail to account for the precise and complex mechanisms that underlie diverse disease etiologies and presentations. For example, while OCD is heritable (48%; Monzani et al., 2014), it is also polygenic, suggesting small additive contributions of many gene variants (Mahjani et al., 2021). Consequently, genome-wide association studies (GWAS) of OCD have not yet identified single loci that contribute significant genetic risk for OCD (Mattheisen et al., 2015), but larger sample sizes will be required to draw firm conclusions. Near-significant candidate gene variants identified in these studies provide little mechanistic clarity since they frequently impinge upon brain wide neurotransmitters systems, like glutamate (Arnold et al., 2018), or synaptic

scaffolding proteins, like SAPAP3 (Bienvenu et al., 2009). It is possible, however that these and other genetic factors may converge on a few mechanistically-relevant circuit disturbances. A common feature of the obsessive-compulsive spectrum disorders (OCSDs) is the dysregulation of basal ganglia circuit elements, and the circuit approaches outlined in section 1.2 represent a step forward as they can be leveraged to identify pathway and cell type-specific dysregulations that underlie discrete aspects of disease symptomology. In the following sections, we summarize what is known about the neural underpinnings of compulsive disorders, particularly the role of the ventral striatum and its afferents in both patient populations and rodent models of disease.

1.4.1. PATIENT POPULATIONS

In patient populations, the role of specific brain structures in OCSDs comes from differences in baseline brain morphology and aberrant brain activation during repetitive behaviours. For example, the caudate nucleus, which is homologous to the mouse dorsal striatum, is hyperactive in patients with OCD, particularly during symptom provocation (Breiter et al., 1996). In these and similar studies, patients with hygiene or contamination-related obsessions are usually shown a triggering stimulus such as a soiled garbage bin, and functional imaging demonstrates event-related increases in striatal activity. Numerous imaging studies also implicate aberrant anatomical and functional connectivity between prefrontal cortex and both the dorsal and ventral striatum across a variety of compulsive spectrum disorders including OCD (Beucke et al., 2013; Fitzgerald et al., 2011; Gu et al., 2008; Harrison et al., 2009; Hou et al., 2014; Page et al., 2009; van den Heuvel et al., 2005), Tourette syndrome (Makki et al., 2009; Marsh et al., 2009; Peterson et al., 1996; Plessen et al., 2009), and trichotillomania (Isobe et al., 2018; Roos et al., 2023; Stein et al., 1997; van den Heuvel et al., 2010). While most of these studies focus on the dorsal striatum, an increasing number of deep brain stimulation (DBS) studies have causally implicated ventral striatal circuits, including the NAc. In treatment-resistant OCD and other compulsive disorders, DBS targeted to the NAc has proven effective at providing short-term relief from compulsive symptomology (Flaherty et al., 2005; Huff et al., 2010; Kuhn et al., 2007; Lopez-Sosa et al., 2021; Schippers et al., 2017; Senova et al., 2019; Staudt et al., 2021; Sturm et al., 2003; Zabek et al., 2008).

Given the suggested role of accumbal circuits in dynamically regulating behavioural output, dysregulations of the NAc and its afferents may play a key role in OCSDs since domain

general deficits in behavioural inhibition can impair suppression of repetitive behaviours. Tasks that require participants to inhibit a cued or prepotent motor response are used to test behavioural inhibition in healthy controls and patient populations (Jahanshahi et al., 2015). For example, in go/no-go tasks, participants must withhold a response at cue presentation in order to receive a reward, and their errant responses are measured as commission errors (Simmonds et al., 2008). Similarly, stop-signal reaction-time tasks require participants to stop themselves from responding in a habitual manner when represented with a "stop" cue (Lappin and Eriksen, 1966). Probabilistic reversal learning tasks have participants make a series of choices between two options that give a monetary reward with high and low probability (usually 80% and 20% of the time). Once a preference for the high-reward option is established, reward contingencies are reversed and persistence on the previously high-reward option is measured as perseverative behaviour (Mehta et al., 2001). Although there are many similar tasks (Jahanshahi et al., 2015), these three are commonly modified for use in rodents (section 1.4.2). Critically, these assays span motor and cognitive domains, testing a patient's capacity to inhibit prepotent actions and thoughts, respectively. Action inhibition is thought to recruit motor cortical areas and dorsal striatum, while suppressing unproductive thoughts relies on the PFC and ventral striatum (Balleine and O'Doherty, 2010). These forms of inhibition are dysregulated across compulsive disorders. While Tourette Syndrome is largely characterized by impaired inhibition in the motor domain, OCD may be a failure of reactively inhibiting recurring actions and thoughts. Games that require behavioural suppression can reveal these latent deficits (Atkinson-Clement et al., 2021; Benzina et al., 2021; Eichele et al., 2010; Lee et al., 2009; McLaughlin et al., 2016), and often trigger dysregulated, hyperactive striatal activity (Atkinson-Clement et al., 2021; Gu et al., 2008). These data suggest that deficits in suppressing internally generated thoughts and actions may contribute to deficits in withholding externally generated thoughts and actions, providing a useful cross-species heuristic for behavioural inhibition. By adapting behavioural assays for use in both patient populations and rodent models, findings relating to their underlying neurobiology will be cross-translatable (Al Dahhan et al., 2019). In this case, we can observe how measures of behavioural inhibition are altered in animal models of compulsivity and then study the causal role of NAc circuits in these impairments.

1.4.2. RODENT MODELS

Rodent models of compulsivity must demonstrate face, construct, and predictive validity. In relation to human disorders, a good model must have similar behavioural features, neurobiology, and responsivity to primary pharmacological therapies. Common models include whole-brain knock-outs of proteins involved in synaptic signaling. SAPAP3 is a synaptic scaffolding protein expressed richly in the dorsal striatum, and its knock-out causes an OCD-like phenotype in mice. These mutants have deficits in reversal learning and exhibit excessive self-grooming that causes facial lesions (Ade et al., 2016; Benzina et al., 2021; Welch et al., 2007). Similarly, knockout of glutamate kainate receptors results in enhanced self-grooming, along with repetitive digging and perseverative performance on a Y-maze alternation task (Xu et al., 2017a). Other models such as BTBR (Silverman et al., 2010) and Shank3b mutants (Peça et al., 2011) are used to model autism, but they also exhibit compulsive self-grooming in addition to other social behaviour impairments. Across these models, repetitive self-grooming and impaired behavioural inhibition provide face valid measures, while disruptions of striatal neurobiology and responsivity to SSRIs provide construct and predictive validity (Ade et al., 2016; Silverman et al., 2010; Welch et al., 2007). Many features of human OCSDs are recapitulated in these models, but they typically focus on the motoric aspects of compulsive repetitive behaviours and their link to dysfunctions of the dorsal striatum. Few models consider the role of ventral striatal circuits in controlling the cognitive and affective states that may elicit compulsive behaviours (Wood and Ahmari, 2015). Since the NAc is poised at the nexus between limbic reward circuits and motivated behaviour, it may process features of anxiety and reinforcement that drive compulsions. A key finding from Ahmari et al., (2013), showed that repeated stimulation of orbitofrontal cortex inputs to the ventral striatum increases repetitive self-grooming and enhances striatal neuron responsivity to afferent stimulation. This circuit model sought to simulate the altered resting state fronto-striatal connectivity observed in OCD patient populations (Nakamae et al., 2014). While many genetic models of compulsivity involve global KOs characterized by brain-wide disruption of synaptic transmission, something rarely observed in human disorders, this circuit approach suggests that the net contribution of genetic and environmental factors may lead to a few critical circuit abnormalities that map onto specific disease dimensions. Additionally, this study highlights an emerging role for NAc circuit elements in compulsivity, suggesting that it is insufficient to focus on the dorsal striatum alone (Wood and Ahmari, 2015). Compulsive phenotypes in both humans

and animal models have diverse etiologies and symptom presentations, demanding a description of the convergent circuit disruptions that give rise to behavioural deficits common across models and disorders alike. The weight of the evidence suggests that these descriptions will include the NAc and its afferents.

A common behavioural feature of these rodent models is excessive self-grooming, a highly conserved, repetitive sequence of self-directed motor behaviours (Kalueff et al., 2016). While self-grooming is upregulated to serve hygiene maintenance and dearousal functions (Spruijt et al., 1992; van Erp et al., 1994), its levels can also vary in a complex manner with stress (van Erp et al., 1994). Self-grooming comprises behavioural syllables that can be sequenced to produce stereotyped chains of grooming that proceed from head to tail, known as syntactic chains (Kalueff et al., 2007). These behavioural sequences serve as a useful model for studying the basic neural circuits that support the concatenation of discrete behavioural units (Kalueff et al., 2016). In translational neuroscience, aberrant self-grooming is a known common feature of OCSDs, particularly those involving self-directed bodily preoccupations and contamination obsessions. While the initiation and coordination of self-grooming arises from motor-associated regions in the brainstem and cerebellum (Berntson et al., 1988; Berridge and Whishaw, 1992), cerebral structures, including the cortex, amygdala, and striatum exert top-down control (Ahmari et al., 2013; Hong et al., 2014). Lesions of the dorsolateral striatum impair the completion of syntactic grooming chains, demonstrating a role for motor striatum in behavioural sequencing (Cromwell and Berridge, 1996). In contrast, circuit manipulations of the ventral striatum (Ahmari et al., 2013) and its afferents (Burguiere et al., 2013; Sun et al., 2022) can induce repetitive self-grooming without affecting sequence completion, suggesting a corticolimbic contribution to compulsivity that extends beyond motor domains.

Behavioural inhibition is globally disrupted in many of these animal models of compulsivity. SAPAP3 KO mice are the most studied mutant model of OCD and have been tested on numerous assays for disinhibited behavioural output (Hadjas et al., 2019). SAPAP3 KOs exhibit impaired performance on reversal learning tasks (van den Boom et al., 2019; Yang et al., 2021), a deficit which is accompanied by increased mPFC activity (Manning et al., 2019) and which reflects perseverative checking, a behavioural profile observed in human OCD patients performing a similar task (Benzina et al., 2021). Although other genetic models exhibit

similar patterns of impaired behavioural inhibition (Thompson et al., 2019; Xu et al., 2017b), ventral striatal circuit models of compulsivity have not yet been assessed on these tasks. If excessive self-grooming is driven by some cognitive or affective dysregulation that originates in the ventral striatum, then deficits on these tasks should be apparent following enduring disruptions of NAc afferent activity (Ahmari et al., 2013). To directly test this idea in chapter 4, we evaluate cognitive flexibility in a circuit model of excessive self-grooming elicited by repeated PVT-NAc stimulation.

1.5. RATIONALE AND OUTLINE

Altogether, this thesis investigates the role of distinct NAc afferents and cell types in behavioural inhibition and compulsivity. We test a prominent hypothesis of NAc function – that effective behavioural inhibition emerges as a consequence of pathway-specific, antagonistic interactions between opposing NAc circuit elements. We find, however, that dysregulation of multiple NAc afferents and cell types similarly impairs behavioural suppression and elicits an enduring compulsive phenotype.

The body of this dissertation begins with a validation of projection-specific optical inhibition of NAc afferents. We demonstrate that axon terminal inhibition using ArchT can cause off-target effects that undermine the pathway-specificity of this manipulation, and we then identify soma-targeted inhibition as a valuable alternative for silencing NAc afferents. Using calcium imaging and optogenetics, we then test the causal role of NAc afferents and cell types in behavioural inhibition. We find that D1 neuron stimulation is reinforcing and D2 neuron stimulation is aversive but demonstrate that activity in both cell types is critical for the suppression of unproductive reward seeking. Inputs to the NAc from the thalamus and amygdala are also demonstrated to be critical regulators of behavioral inhibition, even though they both encouraged behavioral responding in other contexts and are confirmed to innervate different NAc subregions. Overall, these data suggest the capacity to encourage and discourage behavioural responding, a core feature of NAc-mediated action selection, is distributed across NAc cell types and inputs. Efficient reward seeking behaviour arises from integrated activity across these circuit elements rather than opponent processes between them. Finally, we explored the role of enduring NAc afferent dysregulation in compulsivity. Repeated stimulation of PVT-NAc afferents elicited an enduring compulsive phenotype which comprised impaired reversal

learning and enhanced self-grooming. vHPC-NAc stimulation incompletely recapitulated this phenotype, suggesting a unique contribution of PVT afferents to compulsivity.

This body of work highlights the NAc and associated circuits as broad regulators of behavioural inhibition. Although we focused on the contributions of specific cell types and pathways to behaviour, these data suggest that activity distributed throughout ventral striatal networks subserves a delicate balance between behavioural facilitation and inhibition. We challenge the conventional view that these behavioural processes are easily segregated on the basis of anatomical divisions. Instead, we suggest that dynamic regulation of behavioural output relies on time-evolving patterns of neural activity across multiple interacting brain regions (section 5.4). Moreover, the sensitivity of NAc circuits to disruptions that impair behavioural inhibition reveals the NAc as a site of vulnerability to compulsive disorders. OCSDs likely arise from slight imbalances in the activity of the NAc or its afferents, suggesting circuit-based targets for novel therapies, particularly DBS as informed by optogenetics (Creed et al., 2015; Luscher et al., 2015).

CHAPTER 2

OFF-TARGET INFLUENCES OF ARCH-MEDIATED AXON TERMINAL INHIBITION ON NETWORK ACTIVITY AND BEHAVIOR.

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2.1. ABSTRACT

Archaerhodopsin (ArchT)-mediated photoinhibition of axon terminals is commonly used to test the involvement of specific long-range neural projections in behavior. Although sustained activation of this opsin in axon terminals has the unintended consequence of enhancing spontaneous vesicle release, it is unclear whether this desynchronized signaling is consequential for ArchT's behavioral effects. Here we compare axon terminal and cell body photoinhibition of nucleus accumbens (NAc) afferents to test the utility of these approaches for uncovering pathway-specific contributions of neural circuits to behavior. First, in brain slice recordings we confirmed that ArchT photoinhibition of glutamatergic axons reduces evoked synaptic currents and increases spontaneous transmitter release. A further consequence was increased interneuron activity, which served to broadly suppress glutamate input via presynaptic $GABA_B$ receptors. *In vivo*, axon terminal photoinhibition increased feeding and reward seeking behavior irrespective of the afferent pathway targeted. These behavioral effects are comparable to those obtained with broad inhibition of NAc neurons. In contrast, cell body inhibition of excitatory NAc afferents revealed a pathway-specific contribution of thalamic input to feeding behavior and of amygdala input to reward seeking under extinction conditions. These findings underscore the off-target behavioral consequences of ArchT-mediated axon terminal inhibition while highlighting cell body inhibition as a valuable alternative for pathway-specific optogenetic silencing.

2.2. INTRODUCTION

The nucleus accumbens (NAc) is a forebrain structure that regulates the vigor of reward seeking. Its excitatory inputs likely encode motivational states and the presence of reward associated cues (Mannella et al., 2013). For example, paraventricular thalamic (PVT) input regulates food seeking behavior under conditions of hunger and threat (Labouebe et al., 2016; Choi and McNally, 2017; Do-Monte et al., 2017; Cheng et al., 2018; Choi et al., 2019), while basolateral amygdala (BLA) input encodes the motivational value of reward-associated cues (Ambroggi et al., 2008; Stuber et al., 2011; Esber and Holland, 2014). Few studies, however, have directly compared the behavioral consequences of pathway-specific manipulations, so it remains unclear how each input distinctly contributes to effective reward seeking. Maladaptive alterations in the strength of NAc inputs are thought to underlie discrete aspects of psychopathologies, including the aversive symptoms of drug withdrawal (Neumann et al., 2016; Zhu et al., 2016) and stress susceptibility in animal models of depression (Bagot et al., 2015). Thus, pathway-specific inactivation of these inputs is critical to gaining insight into how this circuitry contributes to healthy and unhealthy behavior alike.

Archaerhodopsin (ArchT)-mediated photoinhibition of axon terminals is commonly used to test the involvement of specific long-range neural projections in behavior. Sustained activation of this outward proton pump in axon terminals effectively decreases evoked transmitter release but alkalizes affected axon terminals, which has the unintended consequence of increasing spontaneous vesicle release (El-Gaby et al., 2016; Mahn et al., 2016). It is unclear whether ArchT's off-target effects undermine interpretation of its behavioral effects or still permit assessment of pathway-specific function. If the aberrant spontaneous vesicle release recruits local circuit feedforward inhibition, as previously suggested (Mahn et al., 2016), the intended pathway-specific nature of the manipulation may be compromised.

The shortcomings of ArchT terminal inhibition have been well characterized in acute slice preparations (El-Gaby et al., 2016; Mahn et al., 2016), but *in vivo* applications of this technique are still widely used to study the circuit-level basis of specific behaviors (Herrera et al., 2016; Yamamoto and Tonegawa, 2017; Mangieri et al., 2018), particularly in relation to NAc inputs (Stefanik et al., 2016; Zhu et al., 2016; Reed et al., 2018; Trouche et al., 2019). Thus, there remains a need to validate the pathway-specific nature of this manipulation in behaving animals.

Here we compare photoinhibition targeted to the axon terminals or cell bodies of NAc inputs. We test the efficacy of these two approaches for uncovering pathway-specific contributions of the PVT-NAc and BLA-NAc pathways to behavior. We first demonstrate in brain slice recordings that ArchT photoinhibition of glutamatergic fibers effectively reduced evoked excitatory synaptic currents. We also report that it increased asynchronous transmitter release and consequently interneuron spiking, which broadly suppressed glutamate release via presynaptic $GABA_B$ receptors. *In vivo*, excitatory axon terminal photoinhibition increased feeding and effortful reward seeking irrespective of the pathway targeted. These effects are comparable to those obtained with broad inhibition (O'Connor et al., 2015) or lesions (Bowman and Brown, 1998) of NAc projection neurons. In contrast, cell body inhibition of NAc afferents from the PVT and BLA revealed pathway-specific contributions to distinct aspects of reward seeking when food was available and during extinction, respectively. These data underscore the off-target behavioral consequences of ArchT-mediated terminal inhibition while highlighting cell body inhibition as a valuable alternative for pathway-specific optogenetic silencing.

2.3. RESULTS

2.3.1 ArchT-MEDIATED AXONAL INHIBITION OF NAC AFFERENTS INCREASES REWARD SEEKING BEHAVIOR

The PVT-NAc pathway is thought to integrate hunger signals (Kelley et al., 2005; Kirouac, 2015; Labouebe et al., 2016; Meffre et al., 2019) while the BLA-NAc pathway processes reward-predictive stimuli (Ambroggi et al., 2008; Esber and Holland, 2014; Beyeler et al., 2018), so we hypothesized that inhibition of these pathways would differentially influence the vigor of food seeking and responsivity to reward associated cues, respectively. We used an axon terminal photoinhibition strategy to test this idea, bilaterally targeting light to the NAc of mice expressing ArchT in their PVT or BLA (Figure 1A). We trained the mice to lever press for food reward on a variable ratio schedule of reinforcement (VR3) and compared their lever press and food port responses within behavioral sessions, across periods with and without intracranial light delivery. We also assessed the behavioral impact of photoinhibition during interspersed extinction sessions when lever presses were not reinforced.

Photoinhibition of PVT and BLA axons in the NAc increased the frequency of active lever pressing (Figures 1B,C), whereas intracranial light delivery in GFP-only control mice did

not affect reward seeking behavior. Photoinhibition of PVT and BLA axons also increased inactive lever responding during extinction sessions (Figure 1D), consistent with a general disinhibition of behavior. These behavioral effects are comparable to those obtained with direct NAc neuron photoinhibition (O'Connor et al., 2015) and NAc lesions (Bowman and Brown, 1998), which suggests that any inhibitory influence on NAc physiology may similarly disinhibit reward seeking. Alternatively, given the known off-target effects of ArchT-mediated axonal inhibition, this approach to inhibiting specific pathways may generate broad, unintended disturbances in NAc physiology.

2.3.2. AXON TERMINAL INHIBITION OF EXCITATORY NAC AFFERENTS INCREASES LOCAL INHIBITORY SIGNALING

We examined the off-target consequences of ArchT photoinhibition of PVT and BLA axons in NAc neuron brain slice recordings (Figure 2A). We first confirmed that opsin activation in PVT and BLA axons reduced the amplitude of electrically-evoked excitatory postsynaptic currents (eEPSCs) in NAc neurons (Figure 2B,C), consistent with the intended purpose of the manipulation. We next monitored the frequency of spontaneous excitatory postsynaptic currents (sEPSCs) and found it to be clearly elevated during periods of ArchT activation (Figures 2D-F), consistent with previous results (Mahn et al., 2016). There was no change in the amplitude or decay time of spontaneous synaptic currents in response to photoinhibition (Figure S1A-D), nor was there an effect of light on eEPSCs or sEPSCs in recordings from wildtype animals (Figure S2A,B).

It is possible that asynchronous glutamate release may not directly alter the firing rate of NAc projection neurons, since they have a resting membrane potential close to -80 mV and are thought to fire action potentials upon concerted excitatory input (Goto and Grace, 2008). However, certain interneuron populations in the NAc are highly excitable and may be responsive to small changes in excitatory signaling. To evaluate whether interneuron activity is affected by ArchT-mediated increases in asynchronous glutamate release, we monitored spontaneous inhibitory postsynaptic currents (sIPSCs) in NAc neurons. The frequency but not the amplitude of sIPSCs was elevated during PVT and BLA axon photoinhibition (Figures 3A-C and S1B), consistent with the hypothesis that local inhibitory signaling is upregulated by unintended glutamate release from ArchT-expressing fibers.

To more directly evaluate this hypothesis, we recorded the spiking activity of parvalbumin-positive (PV) interneurons in the NAc of PV-tdTomato mice in response to ArchT photoinhibition of PVT and BLA axons (Figure 3D). We found a sharp increase in PV interneuron spiking following opsin activation, irrespective of its localization to PVT or BLA axons (Figures 3E,F). While it is unclear if this increase in spiking is a direct consequence of asynchronous glutamate release, it suggests that activation of ArchT in any collection of excitatory axons in the NAc may cause common disruptions in NAc physiology.

While excess PV interneuron activity directly inhibits NAc projection neurons, it may also broadly suppress glutamate release via presynaptic $GABA_B$ receptors located on excitatory afferents (Kupferschmidt and Lovinger, 2015). Indeed, bath application of the $GABA_B$ receptor antagonist CGP55984 at 2 μM partially reversed the effects of axonal ArchT activation on eEPSC amplitude and pulse paired ratios (Figures 3G-J) without affecting baseline measures of eEPSC amplitude or sEPSC frequency (Figure S3). This result raises further doubt about the pathway-specificity of the axonal inhibition approach.

2.3.3. CELL BODY INHIBITION OF NAC AFFERENTS REVEALS PATHWAY-SPECIFIC CONTRIBUTIONS OF PVT AND BLA INPUTS TO REWARD SEEKING

To re-evaluate the validity of the behavioral findings we obtained with axonal ArchT activation, we repeated the experiments described above using an alternative approach to disrupting pathway function. Capitalizing on the efficient axon terminal-infecting virus retroAAV, we drove ArchT expression in all NAc-projecting neurons and targeted light to the PVT or BLA (Figure 4A). This method provided pathway specificity, but the manipulation occurred upstream of the NAc. These animals were trained and tested in the same manner as before, and we evaluated the differential influence of PVT and BLA pathways on operant reward seeking behavior.

With light directed to the PVT and BLA in different cohorts of mice, we found that photoinhibition of the PVT-NAc pathway but not the BLA-NAc pathway increased lever press and food port responding when food was available (Figures 4B,C). This result was consistent with previous findings that implicate PVT input in the integration of hunger signals that regulate the vigor of food seeking. In contrast, photoinhibition of the BLA-NAc pathway but not the PVT-NAc pathway increased inactive lever responding during extinction when food was not available (Figure 4D). This finding is consistent with the role of BLA input in regulating cue-

reward associations. Together, these results suggest that soma-targeted photoinhibition is a valuable alternative to axon-targeted photoinhibition for assessing pathway-specific contributions to behavior, as only the former revealed dissociable influences of PVT and BLA afferents to the NAc on reward seeking.

2.4. DISCUSSION

In trying to identify dissociable influences of PVT and BLA input to the NAc on reward seeking behavior, we compared axon-targeted and soma-targeted photoinhibition strategies using the outward proton pump ArchT. We found that axon terminal inhibition increased reward seeking behavior similarly when targeted to either pathway, whereas pathway-specific inhibition of upstream cell bodies produced dissociable behavioral effects that were consistent with previous literature. NAc neuron brain slice recordings confirmed that activation of ArchT in excitatory axon terminals reduces eEPSCs yet increases sEPSCs. The pathway-specific nature of this manipulation was undermined by a concomitant increase in GABAergic interneuron activity, which was associated with a broad $GABA_B$ receptor-dependent reduction in eEPSC amplitudes. These results suggest that ArchT photoinhibition of excitatory axons has off-target consequences that generally disrupt NAc physiology, which may explain why similar changes in behavior result from the inhibition of distinct axonal inputs. Soma-targeted photoinhibition appears to be a valuable alternative for pathway-specific optogenetic silencing.

The NAc integrates excitatory input from several limbic structures (Mannella et al., 2013) and is a convergent site of dysregulation in many psychiatric disorders (Ahmari et al., 2013; Bagot et al., 2015; Francis et al., 2015; Creed et al., 2016; Neumann et al., 2016). Identifying the distinct behavioral contributions of these excitatory inputs is challenging, because they similarly engage NAc physiology (Britt et al., 2012) and originate in regions that are themselves highly interconnected (Pitkanen et al., 2000; Li and Kirouac, 2008; Do-Monte et al., 2015). The study of these circuit elements thus heavily relies on our ability to selectively disrupt pathway-specific function. Unfortunately, many of the optogenetic tools commonly used to silence long-range neural projections have unclear limitations *in vivo*, and their off-target effects may mask pathway-specific differences, particularly in highly integrative structures such as the NAc. Accordingly, there is considerable value in comparing different pathway-specific inhibition strategies for manipulating multiple, parallel long-range projections.

2.4.1. ALTERNATIVES TO TARGETING ArchT PHOTOINHIBITION TO AXON TERMINALS

Optogenetic approaches for silencing neural activity have repeatedly been found to produce off-target effects and unpredictable changes in network activity that preclude straightforward interpretations of experimental outcomes. For example, tissue heating in response to prolonged light delivery is sufficient to alter potassium conductance in striatal neurons (Owen et al., 2019). Additionally, ion transporters such as ArchT and halorhodopsin can dramatically alter the intracellular ionic environment affecting the pH level and chloride reversal potential, respectively (Raimondo et al., 2012; Mahn et al., 2016). These disruptions are most pronounced in axons on account of their relatively small intracellular volume. A separate issue is the rebound excitation that often follows any acute hyperpolarization (Arrenberg et al., 2009).

Fortunately, new tools and experimental approaches have mitigated some of these unintended effects. The development of highly effective retrograde viral vectors (Tervo et al., 2016) has facilitated projection-specific cell body photoinhibition strategies, as demonstrated here. This approach benefits from the capacity of the soma to buffer against significant changes in pH and ionic composition (Wiegert et al., 2017). Opsin expression can be further restricted with intersectional strategies involving recombinase proteins such as Cre or FLP and retrograde viral vectors. This approach may be necessary when targeting specific pathways within reciprocally connected brain regions.

Anion-conducting channelrhodopsins (ACRs) may be the best option currently available for photoinhibition experiments. Since their conductance is dependent on the membrane potential, their activation can shunt voltage fluctuations of the cell without inducing strong hyperpolarization or significantly altering the ionic environment (Berndt et al., 2016). The light-driven chloride channels that have been engineered (eACRs) (Berndt et al., 2014) or found in nature (GtACRs) (Govorunova et al., 2015) are also more light-sensitive than any presently used ion transporter opsins. Unfortunately, chloride channels appear to be excitatory in many axons, due to locally elevated chloride concentrations (Khirug et al., 2008; Malyshev et al., 2017). Soma-restricted GtACR variants have been developed to circumvent this issue, but their expression level has to be optimized in order to minimize their excitatory influence in the axon hillock (Mahn et al., 2018).

These new tools have fewer drawbacks than their predecessors, but it remains difficult to interpret the effects of inhibiting specific projections that are embedded in recurrent circuitry (Spellman et al., 2015; Do-Monte et al., 2017). It is unclear how silencing a pathway will ultimately affect downstream neurons, let alone the network as a whole. Limiting the duration of photoinhibition may mitigate some concerns, but the organization of the affected circuitry and behavioral state of the animal should be carefully considered.

2.4.2. PVT AND BLA INPUTS TO THE NAC INFLUENCE DISCRETE ASPECTS OF REWARD SEEKING

PVT and BLA projections to the NAc have been found to promote and discourage reward seeking in different contexts (Stuber et al., 2011; Millan et al., 2015; Zhu et al., 2016; Do-Monte et al., 2017; Bercovici et al., 2018; Reed et al., 2018; Shen et al., 2019). For instance, photostimulation of PVT input to the NAc can reduce or increase sucrose seeking behavior (Labouebe et al., 2016; Do-Monte et al., 2017; Cheng et al., 2018), while photostimulation of different fibers in the BLA-NAc pathway can generate place preference or aversion (Shen et al., 2019). These mixed effects likely reflect the heterogeneity of the PVT and BLA. By studying coarse disruptions of pathway activity alongside manipulations of genetically and spatially defined subpopulations in varied contexts (Labouebe et al., 2016; Shen et al., 2019), we can begin to understand the net contributions of these pathways to behavior.

Here we identified a unique role of PVT inputs in modulating the vigor of food seeking behavior, which is consistent with a wealth of literature implicating this structure in integrating hunger signals and regulating feeding (Kelley et al., 2005; Stratford and Wirtshafter, 2013; Kirouac, 2015; Labouebe et al., 2016; Choi and McNally, 2017; Do-Monte et al., 2017; Cheng et al., 2018; Meffre et al., 2019). While stimulation of a smaller glucose-sensing subset of PVT-NAc projectors can promote consumptive behavior (Labouebe et al., 2016), we find that the net effect of bulk PVT-NAc inhibition is an increase in food seeking, consistent with the net reductions in PVT-NAc pathway activity that have been observed in the rostral NAc during feeding (Reed et al., 2018). Gross excitatory drive from this input may therefore gate food seeking behaviors by downregulating NAc activity overall, while smaller, genetically defined populations – like those that sense low levels of interstitial glucose (Labouebe et al., 2016) – may

target relatively circumscribed areas of the NAc which have opposing influences on feeding behavior.

Our soma-targeted photoinhibition of the BLA-NAc pathway did not affect lever pressing behavior when food was available but increased it under extinction conditions, consistent with the role of this pathway in regulating operant responding following changes in outcome (Shiflett and Balleine, 2010). BLA-NAc activity may thus have a pronounced role in behavioral suppression under varied conditions. Overall, the use of increasingly refined tools to inhibit projection-specific activity will aid efforts to dissect neural circuit function in relation to behavior.

2.5. ACKNOWLEDGMENTS AND DISCLOSURES

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The authors report no biomedical financial interests or potential conflicts of interest.

2.6. METHODS

2.6.1. EXPERIMENTAL MODEL AND SUBJECT DETAILS

Adult wild-type and transgenic C57BL/6J mice were used, including tdTomato Cre-reporter mice (JAX#007914) and parvalbumin-Cre mice (JAX#008069, Jackson Laboratory, Sacramento, CA). All animals were bred in-house and kept on a reverse light cycle with a 12 h photoperiod. Animals underwent surgery at approximately three months of age (25-30 g). Six weeks later they were placed on a restricted feeding schedule and maintained at 85-90% of their pre-surgery body weight. The number of male and female mice were balanced within groups. All experiments were conducted in accordance with the Canadian Council of Animal Care and the McGill Animal Care *Committee*.

2.6.2. VIRAL CONSTRUCTS AND SURGERY

Prior to surgery, animals were anesthetized using a ketamine (Ventoquinol, 100mg/kg) and xylazine (Bayer, 10mg/kg) cocktail. The skull of the animal was then secured to a stereotaxic frame (Kopf Instruments) and prepared for intracranial virus injections according to standard

stereotaxic procedure. 700 nL of virus (5.0 x 10^{12} GC/mL) was injected bilaterally over a ten-minute period using a Nanoject II Injector with an oil-filled glass micropipette pulled to a tip diameter of 10 µm (Drummond Scientific, 3-000-203-G/X).

For axonal photoinhibition experiments, rAAV5-CaMKIIα-eArchT3.0-eYFP (UNC Vector Core) was delivered into the BLA (AP -1.8 mm, ML ±2.8 mm, DV -5.15 mm) and PVT (AP -1.1 mm, ML ±0.35 mm, DV -3.3 mm) of different cohorts of wildtype mice. Optical fibers with a 200 µm core were implanted in the NAc ten minutes later (10° angle, AP 1.5 mm, ML ±1.35 mm, DV -4.6 mm). Animals used for brain slice electrophysiology experiments included PV reporter mice and were prepared in the same manner, but optical fibers were not implanted.

For afferent-specific cell body photoinhibition experiments, retroAAV2-CAG-ArchT-GFP (Neurophotonics Centre at Université Laval) was delivered to the NAc (AP 1.5 mm, ML ±0.62 mm, DV -4.7 to -4.2) and an optical fiber was placed above the BLA (AP -2.06 mm, ML ±3 mm, DV -4.02 mm) or PVT (10° angle, AP -1.20 or -0.95 mm, ML ±0.56 mm, DV -2.82 mm) of different cohorts of wildtype mice.

2.6.3. BEHAVIORAL TESTING

Mice were trained in sound attenuating chambers (Med Associates), in which levers were extended on either side of a centrally located food receptacle. A houselight and speaker were located on the opposite side of the chamber. All behavioral data were collected using Med Associates software.

Food-restricted mice were tethered to optical fiber and placed in these operant chambers daily for 40-minute sessions. One lever was randomly designated the active lever. Initially each press on this lever was reinforced with delivery of 30 µL of a 15% sucrose solution (m/v) to the food receptacle and a tone presentation (4.8 kHz, 80dB, 5 s duration). After mice earned 40 rewards in a single session, we switched them to a variable ratio (VR3) reinforcement schedule. The number of active lever presses required for reward delivery and tone presentation then varied randomly between 1 and 5. Inactive lever presses were always inconsequential. Both levers remained extended throughout each session.

Photoinhibition experiments were carried out after animals consistently attained 20 rewards per daily session. Photoinhibition involved bilateral intracranial light delivery (532 nm,

10 mW) for two 8-minute periods within the 40-minute session. The timing of the photoinhibition periods was counterbalanced across two days of testing. Animals subsequently experienced two sessions under extinction conditions, in which presses on the active lever were no longer reinforced. Both extinction sessions were preceded by three daily sessions on the VR3 reinforcement schedule.

2.6.4. BRAIN SLICE ELECTROPHYSIOLOGY

Mice were anesthetized and perfused with a modified artificial cerebrospinal fluid that contained, in mM, 92 NMDG, 20 HEPES, 25 glucose, 30 $NaHCO_3$, 1.25 NaH_2PO_4, 2.5 KCL, 5 sodium ascorbate, 3 sodium pyruvate, 2 thiourea, 10 $MgSO_4$, 0.5 $CaCl_2$ (pH 7.35). 200 μm thick coronal slices containing the NAc were prepared using a VT-1200 vibratome (Leica) and held at 34°C for 10 min in this same solution. Slices were then transferred to a ‘‘holding ACSF’’ at room temperature, which was identical except that NaCl (92 mM) was included instead of NMDG and the $MgSO_4$ and $CaCl_2$ concentrations were 1 and 2 mM, respectively. The ACSF used on the patch rig was maintained at 31°C and contained, in mM, 119 NaCl, 2.5 KCL, 1.25 NaH_2PO_4, 2 $MgSO_4$, 2 $CaCl_2$, 24 $NaHCO_3$, and 12.5 glucose. All ACSF preparations were saturated with 95% O_2 and 5% CO_2. Cells were visualized on an upright microscope with infrared differential interference contrast and fluorescence microscopy. Whole-cell patch-clamp recordings were made using a MultiClamp 700B amplifier using 2 kHz lowpass Bessel filter and 10 kHz digitization with pClamp 11 software (Molecular Devices). Recordings were made using glass pipets with resistance 4.0-6.0MΩ, filled with internal solution containing, in mM, 117 cesium methanesulfonate, 20 HEPES, 0.4 EGTA, 2.8 NaCl, 5 TEA-Cl, 4 Mg-ATP and 0.4 Na-GTP (pH 7.3). Projection neurons identified by morphology, membrane resistance, and hyperpolarized resting membrane potential were patched in the NAc shell in areas with bright eYFP fluorescence. Patched cells were held at -70 mV to record evoked and spontaneous EPSCs or at 0 mV to record spontaneous IPSCs. sEPSCs were recorded over 10 mins (5 mins per condition). sIPSCs were recorded over 30 mins. EPSCs were evoked in pairs (50 ms interval) once per minute for 30 mins with a single stimulating electrode positioned 100-200 mm dorsal to the recorded neuron. Recordings that included photoinhibition and CGP55984 lasted 15 mins (5 mins per condition). Series resistance (10-25 MΩ) remained stable over the period of data collection. To record interneuron spiking, cell attached recordings were carried out on tdTomato$^+$

neurons of PV-reporter mice in the NAc shell in areas with bright eYFP fluorescence. Recordings of spiking activity took place over 10 mins (5 mins per condition). Opsin activation was achieved with 590 nm light (2 mw, ThorLabs, DC4104) directed through the microscope objective.

2.6.5. HISTOLOGY

At the end of each behavioral experiment, animals were anesthetized with 270 mg/kg Euthansol (Merck) and transcardially perfused with 4% paraformaldehyde (PFA, Sigma-Aldrich). Brains were removed, post-fixed in PFA for 24 h, and then transferred to PBS for 48 h. Tissue was then sliced into 60 μm sections on a vibratome (Leica VT1000s) and mounted on microscope slides with a MOWIOL plus DAPI (Sigma-Aldrich) solution.

2.6.6. QUANTIFICATION AND STATISTICAL ANALYSIS

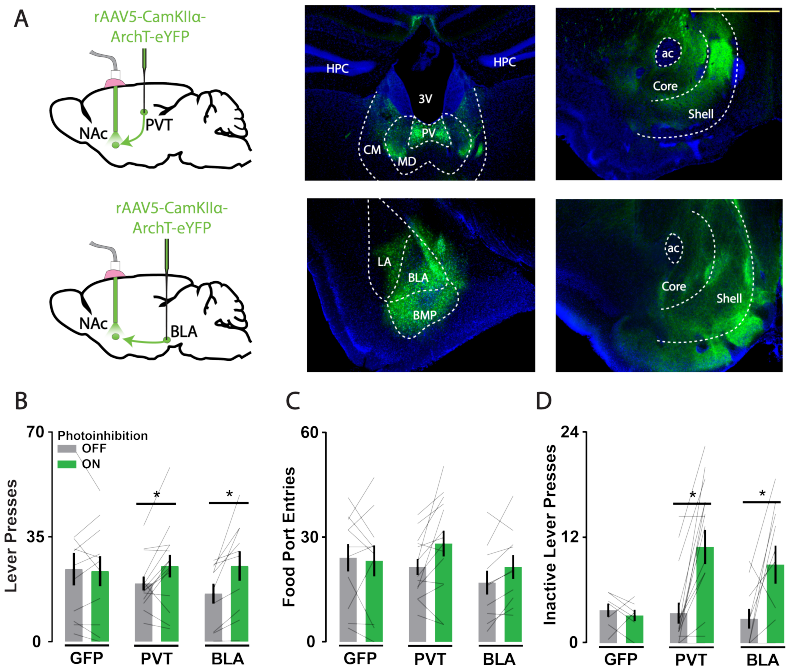
For electrophysiological recordings, all data were normalized to the last 3 mins of the baseline condition of a given recording. The frequency of sEPSCs, sIPSCs, and spiking was calculated by counting the number of events that occurred in one-minute bins across each recording. Once normalized, these data were used for all time-course graphs. For summary graphs, the mean normalized frequency was calculated for each experimental condition.

Two-tailed paired t-tests and two-way ANOVAs were used for statistical comparisons of behavior across photoinhibition conditions and across pathways.

Sidak's multiple comparisons tests were conducted for all ANOVA *post hoc* tests. The significance of all statistical tests was determined using $\alpha = 0.05$. All data are reported as the mean ± SEM.

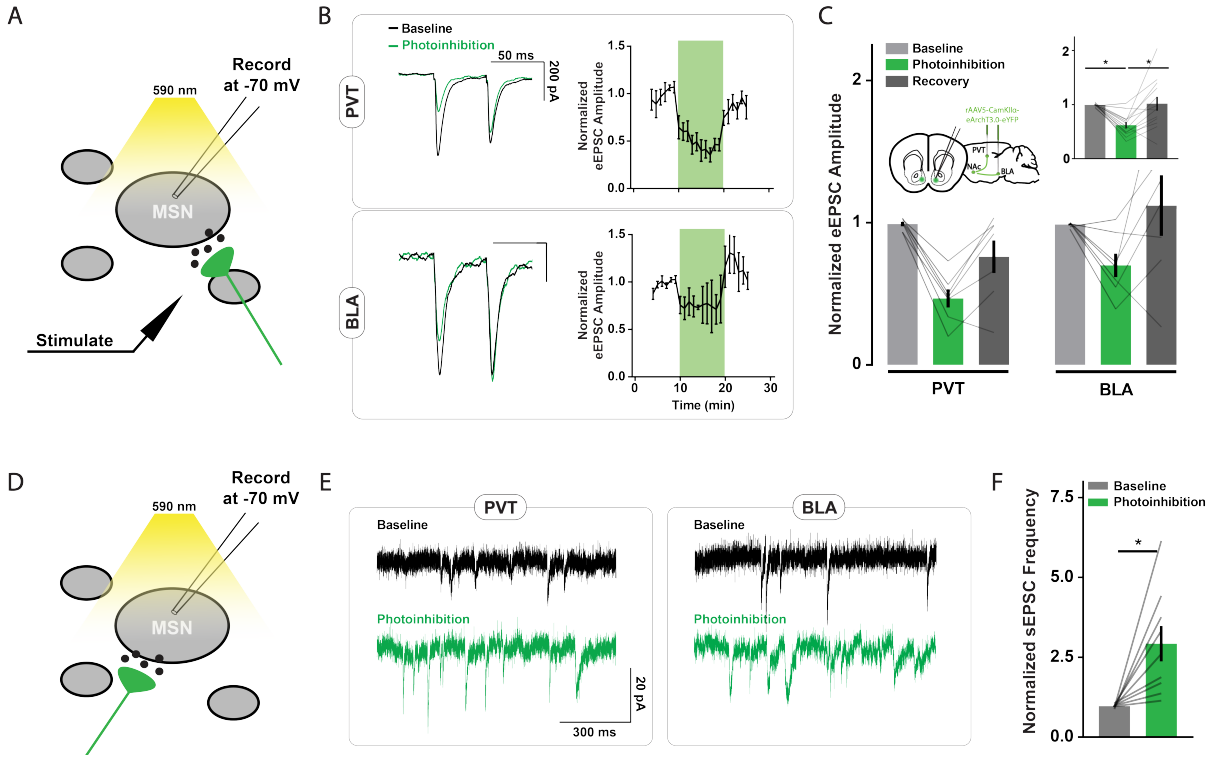
2.7. FIGURES

FIGURE 1. ArchT-MEDIATED AXON TERMINAL INHIBITION OF GLUTAMATE AFFERENTS IN THE NAC INCREASES REWARD SEEKING BEHAVIOR.



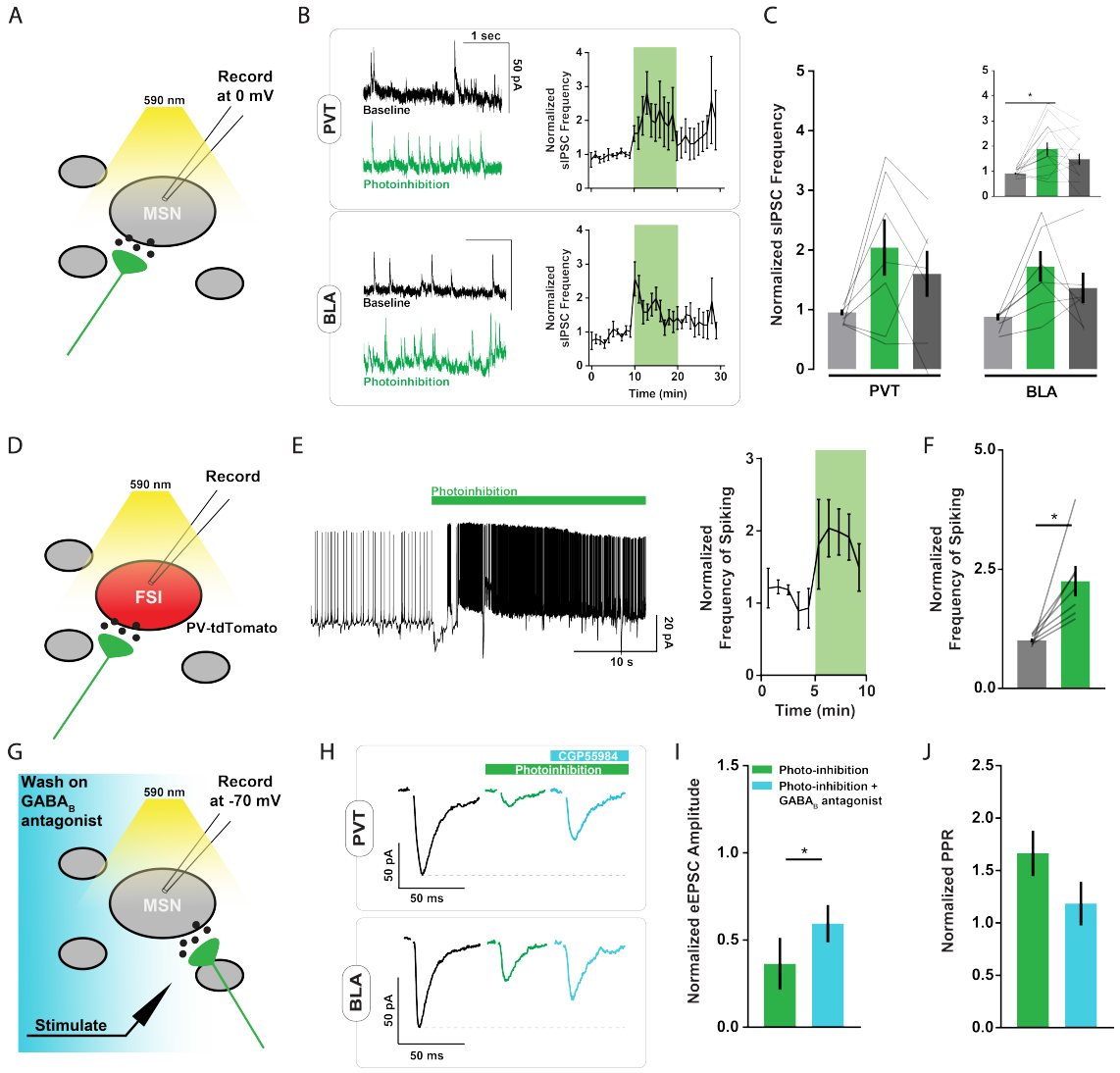
A) Schematic of viral injections and optic probe placements (*left*). Representative coronal brain slices showing ArchT-eYFP expression in PVT and BLA neurons (*middle*) and their associated axons in the NAc (right). Scale bar, 500 µm. **B-C)** Photoinhibition of PVT and BLA axons increased active lever responses (n_{GFP} = 11; n_{PVT} = 14; n_{BLA} = 10; $F_{(2,32)} = 3.96$, $p < 0.05$; for significant post hoc tests $t_{(32)} > 2.63$) but not food port entries when reward was available ($F_{(2,32)} = 2.52$, $p = 0.10$). **D)** During extinction, photoinhibition of PVT and BLA axons increased inactive lever responses (n_{GFP} = 7; n_{PVT} = 15; n_{BLA} = 10; $F_{(2,29)} = 4.70$, $p < 0.05$; for significant post-hoc tests $t_{(29)} > 3.31$). Error bars represent SEM. *signifies $p < 0.05$. 3V, third ventricle; ac, anterior commissure; BLA, basolateral amygdala; BMP, posterior basomedial amygdaloid nucleus; CM, central medial thalamic nucleus; HPC, hippocampus; LA, lateral amygdaloid nucleus; MD, mediodorsal thalamic nucleus; PV, paraventricular thalamic nucleus.

FIGURE 2. ArchT-MEDIATED INHIBITION OF EXCITATORY AXONS IN THE NAC INCREASES sEPSC FREQUENCY.



A) Schematic of brain slice recording conditions where electrically-evoked-EPSCs were recorded from NAc spiny neurons before, during, and after Arch-mediated photoinhibition of excitatory axon terminals. **B)** Example recordings from NAc neurons showing changes in the amplitude of electrically-evoked EPSCs during photoinhibition of PVT (*top left*) and BLA (*bottom left*) axons in the NAc. Summary of relative change in EPSC amplitudes (*right*) over time in response to photoinhibition during minutes 10-20. **C)** Summary of effect of photoinhibition on EPSC amplitudes. Inset shows data collapsed across pathways, highlighting main effect of photoinhibition (n_{PVT} = 7(3 animals); n_{BLA} = 7(3); $F_{(2,24)} = 10.40$, $p < 0.001$; $t_{\text{baseline vs inhibition}(24)} = 4.18$*; $t_{\text{inhibition vs recovery}(24)} = 3.68$*). **D)** Schematic of brain slice recording conditions where spontaneous EPSCs were recorded from NAc spiny neurons during Arch-mediated photoinhibition of excitatory axon terminals. **E)** Example NAc neuron recordings highlighting the increase in spontaneous EPSCs that accompanied Arch-mediated inhibition of PVT and BLA axons. **F)** Summary of effect of photoinhibition on normalized sEPSC frequency (n = 9(3); $t_{(8)} = 3.48$, $p < 0.01$). Error bars represent SEM. *signifies $p < 0.01$. MSN, medium spiny neuron.

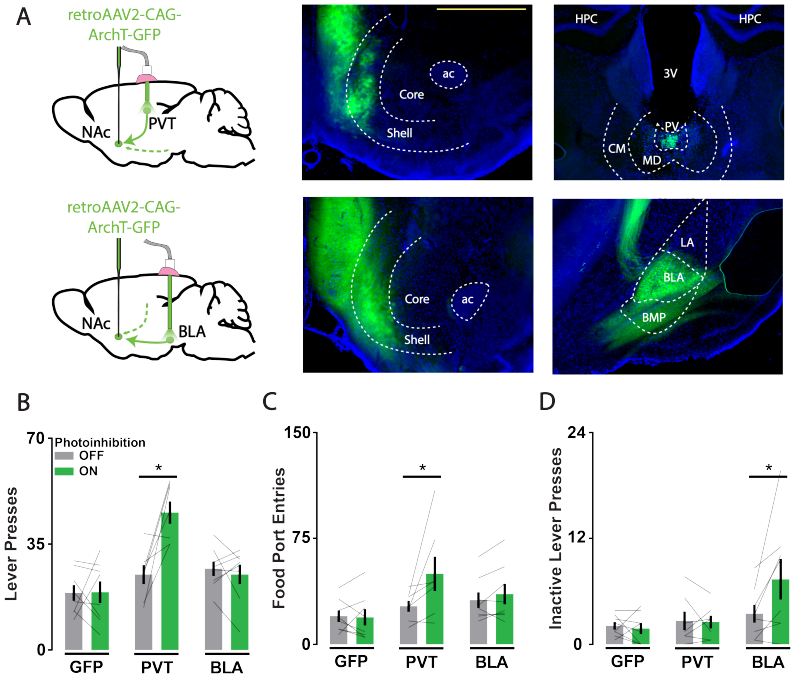
FIGURE 3. ArchT-MEDIATED INHIBITION OF EXCITATORY AXONS IN THE NAC INCREASES SPINY NEURON sIPSC FREQUENCY AND PV+ INTERNEURON SPIKING.



A) Schematic of brain slice recording conditions where spontaneous IPSCs were recorded from NAc spiny neurons before, during, and after Arch-mediated photoinhibition of excitatory axon terminals. **B)** Example NAc neuron recordings showing changes in sIPSC frequency during photoinhibition of PVT (*top left*) and BLA (*bottom left*) axons in the NAc. Summary of relative change in sIPSC frequency over time in response to photoinhibition (*right*). **C)** Summary of effect of photoinhibition on sIPSC frequency. Inset shows data collapsed across pathways, highlighting main effect of photoinhibition (n_{PVT} = 7(3 animals); n_{BLA} = 7(3); $F_{(2,24)} = 7.26$, $p < 0.01$; $t_{baseline\ vs\ inhibition(24)} = 3.79$*). **D)** Schematic of brain slice recording conditions where spiking activity was recorded in tdTomato-labeled PV+ fast spiking interneurons (FSIs) during Arch-

mediated photoinhibition of excitatory afferent inputs. **E)** Example recording from a PV+ interneuron in the NAc showing elevated spiking activity coincident with Arch-mediated axon terminal photoinhibition (*left*). Summary of relative change in FSI spiking frequency over time in response to photoinhibition (*right*). **F)** Summary of effect of photoinhibition on normalized interneuron spiking (n = 7(2); $t_{(6)} = 3.64$, $p < 0.05$). **G)** Schematic of brain slice recording conditions, where electrically-evoked EPSCs were recorded from NAc spiny neurons during Arch-mediated photoinhibition of excitatory axon terminals in the presence of a GABAB antagonist (CGP55984). **H)** Example NAc neuron recordings showing the effects of a GABAB antagonist on evoked EPSC amplitudes during photoinhibition of PVT (*top*) and BLA axons (*bottom*). **I-J)** Summary of effect of GABAB antagonist on evoked EPSC amplitude (n = 3(2); $t_{(2)} = 5.51$, $p < 0.05$) and normalized pulse-paired ratio (PPR) ($t_{(2)} = 2.90$, $p = 0.10$) during photoinhibition of excitatory axon terminals. Baseline data not shown. Error bars represent SEM. *signifies $p < 0.05$. FSI, fast spiking interneuron; MSN, medium spiny neuron.

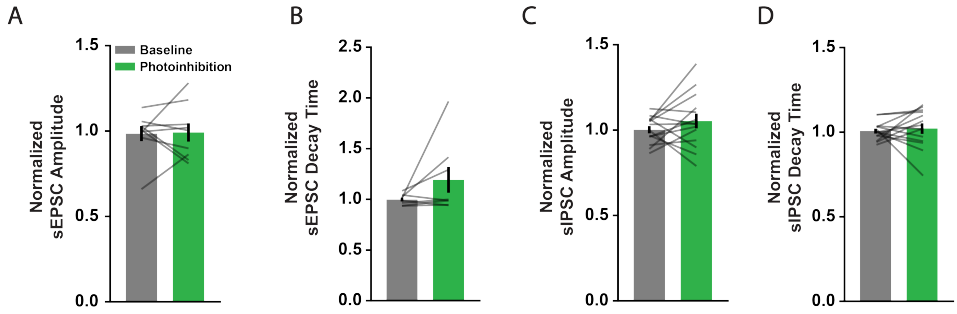
FIGURE 4. CELL BODY INHIBITION OF NAC AFFERENTS REVEALS PATHWAY-SPECIFIC CONTRIBUTIONS OF PVT AND BLA INPUTS TO REWARD SEEKING.



A) Schematic of viral injections and optic probe placements (*left*). Representative coronal brain slices showing ArchT-GFP expression in axon terminals in the NAc (*middle*) and in soma that project to the NAc in the PVT and BLA (*right*). Scale bar, 500 µm. **B-C)** Photoinhibition of NAc-projecting PVT neurons increases active lever responses ($n_{GFP} = 8$; $n_{PVT} = 7$; $n_{BLA} = 8$; $F_{(2,20)} = 8.31$, $p < 0.01$, $t_{PVT(20)} = 4.68$*) and food port entries ($F_{(2,20)} = 3.76$, $p < 0.05$; $t_{PVT(20)} = 3.47$*). **D)** During extinction, photoinhibition of NAc-projecting BLA neurons increases inactive lever responses ($F_{(2,20)} = 4.32$, $p < 0.05$; $t_{BLA(20)} = 3.44$*). Error bars represent SEM. *signifies $p < 0.05$. 3V, third ventricle; ac, anterior commissure; BLA, basolateral amygdala; BMP, posterior basomedial amygdaloid nucleus; CM, central medial thalamic nucleus; HPC, hippocampus; LA, lateral amygdaloid nucleus; MD, mediodorsal thalamic nucleus; PV, paraventricular thalamic nucleus.

FIGURE S1. ArchT-MEDIATED PHOTOINHIBITION OF EXCITATORY AXONS IN THE NAC DOES NOT AFFECT THE AMPLITUDE OR THE DECAY RATE OF SPONTANEOUS SYNAPTIC CURRENTS.

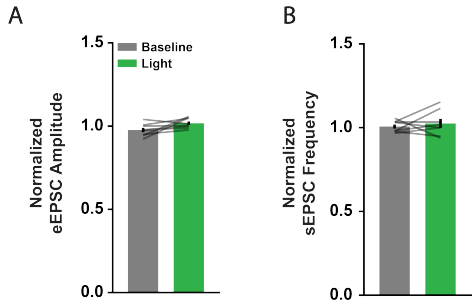
Related to Figures 2 and 3.



Summary of effect of ArchT axon terminal inhibition on **A)** the amplitude of spontaneous EPSCs (n = 9(3 animals); $t_{(8)} = 0.13$, p = 0.90), **B)** the decay rate of spontaneous EPSCs (n = 8(3); $t_{(7)} = 1.61$, p = 0.15) **C)** the amplitude of spontaneous IPSCs (n = 14(6); $t_{(13)} = 1.22$, p = 0.25), and **D)** the decay rate of spontaneous IPSCs (n = 14(6); $t_{(13)} = 0.46$, p = 0.65).

FIGURE S2. LIGHT ALONE HAS NO EFFECT ON THE AMPLITUDE OF eEPSCS OR THE FREQUENCY OF sEPSCS IN NAC CELL RECORDINGS FROM WILDTYPE ANIMALS.

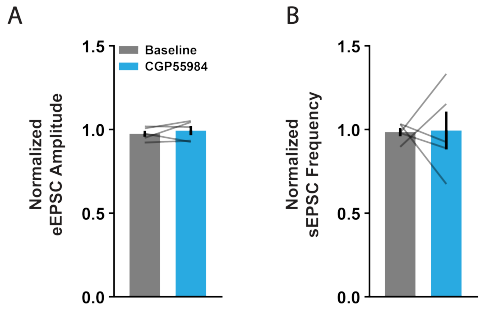
Related to Figure 2.



Summary of the effect of light on **A)** the amplitude of evoked EPSCs (n = 8(3 animals); $t_{(7)}$ = 2.11, p = 0.07) and **B)** the frequency of spontaneous EPSCs ($t_{(7)}$ = 0.63, p = 0.55).

FIGURE S3. IN THE ABSENCE OF PHOTOINHIBITION, A GABA B ANTAGONIST HAS NO EFFECT ON THE AMPLITUDE OF eEPSCS OR THE FREQUENCY OF sEPSCS RECORDED FROM NAC NEURONS OF WILDTYPE ANIMALS.

Related to Figure 3.



Summary of the effect of light on **A)** the amplitude of evoked EPSCs (n = 5(3 animals); $t_{(4)}$ = 0.80, p = 0.47) and **B)** the frequency of spontaneous EPSCs ($t_{(4)}$ = 0.08, p = 0.94).

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2.9. CONNECTING THE TEXT: CHAPTERS 2 – 3

The previous chapter tested the efficacy of ArchT axon terminal inhibition for projection-specific silencing of NAc afferents originating in the PVT and BLA. We demonstrated that terminal inhibition has off-target effects on NAc physiology, undermining the pathway-specificity of the approach. We then highlighted soma-targeted ArchT inhibition as a viable alternative, as it revealed dissociable contributions of each pathway to reward seeking.

PVT and BLA afferent inhibition were shown to enhance lever pressing for food during a VR3 task and in extinction, respectively, suggesting that they may not exert antagonistic control over behaviour. Having validated a technique for projection-specific silencing, we sought to test this hypothesis directly by inhibiting these circuit elements during a mouse operant task that included recurring periods of reward availability and unavailability.

In the next chapter, we combine afferent-specific inhibition and excitation with recordings and manipulations of direct and indirect pathway neurons of the NAc. By broadly surveying opponent NAc circuit elements, we tested whether efficient reward seeking arises as a consequence of antagonistic interactions between them.

CHAPTER 3

NUCLEUS ACCUMBENS CELL TYPE- AND INPUT-SPECIFIC SUPPRESSION OF UNPRODUCTIVE REWARD SEEKING.

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3.1. ABSTRACT

The nucleus accumbens (NAc) contributes to behavioral inhibition and compulsions, but circuit mechanisms are unclear. Recent evidence suggests that amygdala and thalamic inputs exert opposing control over behavior, much like direct and indirect pathway output neurons. Accordingly, opponent processes between these NAc inputs or cell types may underlie efficient reward seeking. We assess the contributions of these circuit elements to mouse operant behavior during recurring conditions when reward is and is not available. Although direct pathway stimulation is rewarding and indirect pathway stimulation aversive, the activity of both cell types is elevated during periods of behavioral suppression, and the inhibition of either cell-type selectively increases unproductive reward seeking. Amygdala and thalamic inputs are also necessary for behavioral suppression, even though they both support self-stimulation and innervate different NAc subregions. These data suggest that efficient reward seeking relies on complementary activity across NAc cell types and inputs rather than opponent processes between them.

3.2. INTRODUCTION

The ability to recognize and suppress unrewarding actions is a critical aspect of healthy behavior. Deficits in the ability to suppress unproductive behavior - actions that are patently inconsequential - can give rise to compulsions, which are prominent features of several psychopathologies, including obsessive compulsive disorder (OCD), autism spectrum disorder, and Tourette syndrome. Aberrant corticostriatal activity has been implicated in compulsions (Ahmari et al., 2013; Beucke et al., 2013; Burguière et al., 2015; Nakamae et al., 2014; Wood and Ahmari, 2015), but the underlying neural mechanisms are unclear and current treatments are inadequate.

Much evidence supports a role for the nucleus accumbens (NAc) in behavioral inhibition (Ambroggi et al., 2011; Floresco, 2015; Piantadosi et al., 2018; Yun et al., 2004). NAc lesions hinder performance on tasks that reinforce low response rates (Reading and Dunnett, 1995) or penalize premature responding (Bowman and Brown, 1998). They also increase resistance to extinction learning (Reading and Dunnett, 1995; Reading et al., 1991), perseverance under reversal conditions (Reading et al., 1991), and breakpoints on progressive ratio schedules of reinforcement (Bowman and Brown, 1998). In addition, pharmacological inactivations that specifically target the NAc shell tend to disinhibit uncued, unreinforced, and previously extinguished behavioral responding (Ambroggi et al., 2011; Blaiss and Janak, 2009; Floresco et al., 2008; Ghazizadeh et al., 2012; Millan et al., 2015; Peters et al., 2008; Yun et al., 2004). Across studies, an absence or reduction of NAc shell activity is associated with impulsive, perseverative, and generally unproductive reward seeking (Basar et al., 2010; Floresco, 2015). Moreover, NAc spiking activity increases when animals actively refrain from responding on a go/no-go task (Roitman and Loriaux, 2014) or perceive non-rewarded cues when performing a discriminative-stimulus task (Ambroggi et al., 2011). These data suggest that NAc shell activity is important for behavioral inhibition, but the role of specific NAc cell types and afferent pathways is unclear.

Some evidence indicates the two populations of NAc projection neurons (often defined by dopamine D1 or D2 receptor expression) exert opposing control over behavior (Cole et al., 2018; Hikida et al., 2010; Lobo et al., 2010). For example, D1 neuron activity is important for reward learning and supports a real time place preference, while D2 neuron activity elicits

behavioral aversion, supports aversive learning, and suppresses perseverative behavior under reversal conditions (Cole et al., 2018; Hikida et al., 2010; Yawata et al., 2012). Accordingly, behavioral inhibition may arise from the competition between these cell populations, whereby D2 neurons compete with D1 neurons to reduce the frequency of specific actions (Bock et al., 2013; LeBlanc et al., 2020). However, other data suggest a more complex relationship between NAc D1 and D2 neurons, since putative D1 (dynorphin-containing) neurons in the ventral NAc shell can drive behavioral aversion (Al-Hasani et al., 2015), and D2 neuron stimulation can increase reward seeking in certain conditions (Soares-Cunha et al., 2016).

It is also likely that distinct excitatory afferent pathways to the NAc differentially encourage or suppress behavior, as antagonistic relationships have been found between them with respect to NAc spiking activity (Calhoon and O'Donnell, 2013) and behavior (Bagot et al., 2015). For example, input from the paraventricular nucleus of the thalamus (PVT) reduces reward seeking and drives aversive behavior (Do-Monte et al., 2017; Zhu et al., 2016), while basolateral amygdala (BLA) input motivates reward seeking and supports self-stimulation (Britt et al., 2012; Stuber et al., 2011). Other data, however, suggest that this competitive model of pathway function may be incomplete, since both PVT and BLA inputs positively respond to non-rewarding distractor cues (Reed et al., 2018). Additional direct comparisons are needed between PVT and BLA inputs and between D1 and D2 neurons to better understand how the interplay of these pathways and cell types regulates behavioral inhibition.

Here, we assess the role of these distinct NAc circuit elements on the suppression of unproductive behavior by selectively recording and disrupting cell type and afferent pathway activity as mice perform an operant task that includes cued periods of reward unavailability. We first show that D1 and D2 neuron activity is tonically elevated during periods of behavioral suppression and then demonstrate that unproductive reward seeking readily arises from inhibition of D1 and D2 neurons as well as PVT and BLA inputs. Overall, the data suggest that efficient reward seeking depends on complementary activity across NAc cell types and inputs, rather than opponent processes between them. These results emphasize the importance of studying circuit elements in parallel, especially in integrative structures, such as the NAc, as different circuit disturbances often produce similar behavioral outcomes (Britt et al., 2012; Reed et al., 2018).

3.3. RESULTS

3.3.1. NAC SHELL D1 AND D2 NEURONS EXHIBIT ELEVATED ACTIVITY DURING PERIODS OF BEHAVIORAL SUPPRESSION

To probe the neural underpinnings of behavioral suppression, we trained mice to press an intermittently available lever for food on a variable ratio schedule of reinforcement (VR3), and then introduced 4-min-long, cued periods of reward unavailability (**Figure 1A**). Across daily sessions, rates of operant responding diverged between periods when reward was and was not available, as mice learned to suppress unproductive responses (**Figure 1B**).

To assess whether NAc shell D1 and D2 neurons have opposing activity in relation to behavioral suppression, we used fiber photometry to record calcium-dependent GCaMP7s fluorescence from these cell populations in D1 receptor-Cre (D1) and preproenkephalin-Cre (D2) mice (**Figure 1C**). Following behavioral training, the gross activity of D1 and D2 neurons tracked the availability of food, as the GCaMP fluorescence measured from each of these cell populations was tonically elevated during periods of behavioral suppression relative to periods with high rates of operant responding (**Figure 1D**). This pattern of neural activity only emerged late in training, when animals were successfully suppressing unproductive lever responses (**Figure 1E**), which suggests that it correlates with the animals' behavioral modes rather than the act of consumption.

D1 and D2 neurons also exhibited event-related activity to certain components of the behavioral task (**Figure S1**), but changes in neural activity coincident with the act of lever pressing did not vary in relation to reward availability (**Figure S1C**). D1 neurons did show increased activity in response to light transitions that signaled reward availability and to lever extensions that occurred when food was available (**Figures S1A** and **B**), suggesting that this cell population may encode cues that signal the presence of reward in the environment. In contrast, transient event-related changes in D2 neuron activity did not differ significantly between reward availability conditions, which suggests this event-related activity may not be consequential for behavioral inhibition.

These findings demonstrate that NAc shell D1 and D2 neurons do not have opposing activity profiles with respect to behavioral suppression, as both cell populations had higher mean

activity levels when animals were withholding operant responses. We next tested whether this tonic elevation in D1 and D2 neuron activity was important for behavioral suppression.

3.3.2. PHOTO-INHIBITION OF NAC D1 OR D2 NEURONS INCREASES UNPRODUCTIVE REWARD SEEKING

We hypothesized that inhibition of D1 or D2 neuron activity would interfere with behavioral suppression, so we targeted Cre-dependent expression of eArchT-eGFP to D1 and D2 neuron populations in the NAc shell in different transgenic mice (**Figures 2A** and **S2A**). Animals were trained to lever press for food on a VR3 schedule that, again, included 4-min-long cued periods of reward unavailability (**Figures S2B** and **S2C**). Within-session comparisons of reward seeking behavior were made across periods with and without intracranial light delivery, spanning periods when reward was and was not available (**Figure 2B**). While intracranial light did not affect the behavior of GFP-only control mice, D1 and D2 neuron photo-inhibition selectively increased behavioral responding when reward was unavailable (**Figures 2C-2E**). No behavioral effects of D1 or D2 neuron photo-inhibition were evident when reward was available, which suggests these neuronal populations in the NAc shell are more consequential for suppressing ineffective behaviors during this task than promoting effective ones.

To compare unproductive response rates across groups, we calculated an efficiency error score by dividing behavioral response rates when reward was unavailable by response rates when reward was available. Error scores for lever press and food port responses were elevated during photo-inhibition epochs relative to intervening periods for both D1 and D2 mice, but not GFP-only controls (**Figure 2F**), suggesting that D1 and D2 neuron activity mutually discourages reward seeking in the absence of reward.

Other behavioral measures were differentially affected by D1 and D2 neuron photo-inhibition. Inactive lever responses, which were never reinforced, increased only in response to D2 neuron photo-inhibition (**Figure 2G**). Furthermore, in a subsequent assay of feeding behavior, only D1 neuron photo-inhibition increased the consumption of freely available food (**Figures S3A-S3C**). However, this effect on consumption did not translate into higher rates of operant responding when reward was available during the initial operant task or when the mice were later evaluated on a standard VR3 schedule of reinforcement that did not include periods of reward unavailability (**Figures S3D** and **S3E**).

The most prominent behavioral effect of D1 and D2 neuron inhibition in the NAc shell was an increase in unproductive reward seeking. This result is inconsistent with the hypothesis that behavioral inhibition depends on D2 neurons outcompeting D1 neurons, but it is in line with our recording data showing concurrent activation of both cell types during behavioral inhibition. While the importance of NAc activity for motivating behavior is often emphasized, this result underscores its notable contribution to behavioral inhibition. We next sought to determine whether the suppression of unproductive reward seeking arises from antagonistic interactions between distinct excitatory inputs to the NAc from the PVT and BLA, as these pathways have been implicated in divergent behavioral functions.

3.3.3. PHOTO-INHIBITION OF NAC AFFERENTS INCREASES UNPRODUCTIVE REWARD SEEKING

Considerable evidence suggests that PVT and BLA afferents to the NAc exert opposing control on behavior (Cheng et al., 2018; Choi and McNally, 2017; Correia et al., 2016; Do-Monte et al., 2017; Stuber et al., 2011; Zhu et al., 2016), and there is even some indication that these pathways target different cell populations, at least in the NAc core (Groenewegen et al., 1999; Wright and Groenewegen, 1996). However, few experiments have directly compared the behavioral effects of pathway-specific inhibitions or the innervation patterns of these inputs to the NAc shell.

We evaluated the extent to which PVT and BLA axons innervate the same areas of the NAc shell by examining brain slices from mice that expressed different fluorescent proteins in PVT and BLA neurons (**Figure 3**). With these fluorescent labels, it was evident that these pathways preferentially innervated distinct areas of the NAc. Regions with a high density of axons from one pathway contained relatively few axons from the other, such that there was a significant negative correlation in the intensity of the two fluorescent signals in a pixel-by-pixel analysis of the medial NAc shell (**Figures 3C** and **3D**).

We next evaluated whether these PVT and BLA afferents that innervate distinct areas of the NAc shell differentially encourage or suppress reward seeking. To suppress activity in these PVT and BLA neurons, we targeted retrograde adeno-associated virus (AAV2-retro)-Cre to the NAc shell (to drive Cre-recombinase expression in NAc afferents) and transduced either PVT or BLA neurons with a Cre-dependent ArchT viral construct (**Figure 4A**). Animals were trained on the same operant task as before in which periods of reward availability alternated with periods of

reward unavailability (**Figure 4B**). Once response rates clearly diverged between these conditions, we targeted light intracranially to either the PVT or BLA using a within-session experimental design.

In the absence of any photo-manipulation, animals' rates of lever pressing and food port entries reflected the availability of reward. However, when NAc-projecting PVT and BLA cell bodies were photo-inhibited, animals exhibited high response rates, irrespective of reward availability (**Figures 4C** and **4D**). As a result, lever press and food port entry error scores were higher during photo-inhibition epochs relative to intervening periods for PVT and BLA ArchT mice, but not for GFP controls (**Figure 4E**). This result was unexpected for the BLA input but consistent with reports that BLA neuron activity is sensitive to the absence of reward and correlates with extinction learning (Tye et al., 2010). We observed a similar pattern of results in a separate experiment in which we targeted eArchT-mediated photo-inhibition to axon terminals in the NAc (**Figure S4**). Although it has been demonstrated that this methodological approach has mixed effects on transmitter release and may disrupt NAc physiology in unintended ways (Mahn et al., 2016), these results nevertheless bolster the conclusion that both of these afferent pathways contribute to behavioural suppression.

In subsequent behavioral tests, we found that soma-targeted inhibition of these afferent pathways did not increase free food consumption (**Figure 4F**) or lever responding on an operant task that did not incorporate periods of reward unavailability (**Figure 4G**), suggesting that the observed increases in unproductive reward seeking are not the result of changes in hunger or motivation. The only pathway-specific effect that we observed was an increase in inactive lever presses during BLA neuron inhibition (**Figure S5**).

These data are inconsistent with the hypothesis that one NAc cell type or one afferent pathway promotes behavior while another suppresses it. Rather, efficient reward seeking behavior likely arises from complementary activity among the different cell types and afferent pathways such that disturbances in any circuit element can increase the tendency of an animal to exhibit unproductive behavior. Accordingly, we next assessed the consequence of increased input activity on unproductive reward seeking.

3.3.4. CHEMOGENETIC ACTIVATION OF PVT BUT NOT BLA INPUT TO THE NAC DECREASES INSTRUMENTAL REWARD SEEKING

Photo-stimulation of PVT input, unlike BLA input, has been shown to elicit avoidance, so we hypothesized that there would be differential effects of pathway activation on reward seeking behavior. We chose to upregulate pathway activity with a chemogenetic approach to avoid the non-physiological synchronous firing patterns that are associated with optogenetic stimulation (Hausser, 2014).

We drove expression of the excitatory hM3D designer receptors exclusively activated by designer drugs (DREADD) receptor selectively in PVT or BLA neurons that innervate the NAc by targeting AAV2-retro-Cre to axon terminals in the NAc and a Cre-dependent hM3D(Gq) viral construct to the PVT or BLA (**Figure 5A**). Intraperitoneal injections of saline or the hM3D ligand Compound 21 (C21) (Chen et al., 2015; Thompson et al., 2018) were administered to mice once they exhibited divergent response patterns across periods of reward availability and unavailability (**Figure 5B**). Compared to saline injections, C21 drug administration did not affect reward seeking efficiency on this task in any cohort of mice (**Figure 5C**).

On a separate VR3 operant task that did not include periods of reward unavailability, DREADD activation of the PVT, but not BLA, pathway reduced operant responding and food port entries (**Figure 5D**). In contrast, DREADD activation of the BLA, but not PVT, pathway increased the frequency of unrewarded lever presses that occurred after the reward was delivered (**Figure 5E**). These data suggest that the pathways contribute to varied aspects of reward seeking, but they do not support the hypothesis that PVT and BLA inputs to the NAc exert opposing control on behavior.

3.3.5. PVT INPUT SUPPORTS SELF-STIMULATION BEHAVIOR, WHILE D1 AND D2 NEURONS PROMOTE REWARD AND AVERSION, RESPECTIVELY

Considering that BLA input to the NAc can reinforce behavioral responding in some conditions (Britt et al., 2012; Stuber et al., 2011) yet suppress it in others (**Figures 4C-4E**) (Millan et al., 2017), we wondered whether PVT input could also drive reinforcement in certain conditions, even though it has been implicated in behavioral aversion (Zhu et al., 2016). We used assays of self-stimulation and place preference behavior to probe the reinforcing properties of PVT input

with mice that had channelrhodopsin 2 expressed in PVT neurons and fiber optic implants in the NAc shell (**Figure 6A**).

PVT input readily supported self-stimulation behavior when photo-stimulation was made contingent on active lever presses (**Figure 6B**), indicating that acute PVT-NAc pathway stimulation can reinforce operant behavior. However, these mice did not develop a clear real-time place preference when photo-stimulation of PVT input was coupled to one side of a place preference chamber (**Figure 6C**). Some mice avoided the side of the chamber that elicited continuous PVT pathway stimulation, while other mice sought it out or transitioned repeatedly between rooms in a manner that resembled self-stimulation (**Figure 6C**). This individual variability was not well explained by differences in self-stimulation behavior (**Figure 6B**) or by differences in fiber placement (**Figure S6**), since these variables were relatively similar across mice. Thus, although brief stimulation of the PVT input to the NAc can clearly reinforce behavior, prolonged stimulation has mixed behavioral effects.

To validate our place preference assay and to confirm that NAc D1 and D2 neurons can have opposite influences on reinforcement, we coupled photo-stimulation of these cell populations to one room of a place preference chamber. We observed that NAc D1 and D2 neuron photo-stimulation elicited clear place preference and place avoidance behavior, respectively, as previously demonstrated (**Figures 6D** and **6E**) (Cole et al., 2018). These data show that synchronous activity in these respective cell types can elicit opposing behavioral responses, even though both cell populations contribute to the suppression of unproductive reward seeking.

3.4. DISCUSSION

Behavioral inhibition can be driven by the reinforcement of inaction, overt punishment, or merely the absence of reward. Disruptions of NAc physiology reduce behavioral suppression in each of these conditions, as demonstrated in a myriad of behavioral assays (Ambroggi et al., 2011; Basar et al., 2010; Blaiss and Janak, 2009; Bowman and Brown, 1998; Dalton et al., 2014; Feja et al., 2014; Floresco, 2015; Floresco et al., 2008; Ghazizadeh et al., 2012; Millan et al., 2015; Peters et al., 2008; Piantadosi et al., 2018; Reading and Dunnett, 1995; Reading et al., 1991; Stopper and Floresco, 2011; Yun et al., 2004), including extinction (Reading and Dunnett, 1995; Reading et al., 1991), go/no-go tasks (Schoenbaum and Setlow, 2003), reaction time tasks

(Christakou et al., 2004; Sesia et al., 2008), and passive avoidance tasks (Bracs et al., 1984). The absence of reward is a ubiquitous motivator, so its ability to reduce unproductive actions is critical for healthy behavior, particularly when there are no compelling means to obtain reward. We tested the hypothesis that unproductive behavior is minimized by neural activity in NAc D2 neurons and PVT-NAc projections. More generally, we assessed whether efficient reward seeking behavior arises from antagonistic interactions between D1 and D2 NAc neurons and between PVT and BLA afferents. We anticipated that D1 and D2 neurons would have opposing activity profiles with respect to behavioral suppression, but found that they exhibit similar elevations in activity when animals were withholding behavior. We also expected reductions in D1 neuron activity and BLA input to reduce reward seeking behavior and for reductions in D2 neuron activity and PVT input to promote it. Instead, each of these manipulations selectively increased unproductive operant responding when reward was unavailable. There was no effect of these manipulations when reward was available. These results are consistent with many lesion and inactivation experiments targeting the NAc shell that show that inefficient reward seeking behavior is the most common consequence of disturbances in NAc physiology (Ambroggi et al., 2011; Blaiss and Janak, 2009; Bowman and Brown, 1998; Dalton et al., 2014; Floresco, 2015; Floresco et al., 2008; Millan et al., 2015; Peters et al., 2008; Reading and Dunnett, 1995; Reading et al., 1991; Stopper and Floresco, 2011; Yun et al., 2004).

3.4.1. COORDINATION OF D1 AND D2 NAC NEURON ACTIVITY

Much evidence suggests that D1 and D2 neurons throughout the striatum and NAc exert opposing control over behavior and reward learning (Bariselli et al., 2019; Cole et al., 2018; Hikida et al., 2010; Lobo et al., 2010; Yttri and Dudman, 2016), but our findings and other recent evidence suggest that this dichotomy is an oversimplification (Calabresi et al., 2014; Tecuapetla et al., 2016; Vicente et al., 2016). D1 and D2 neurons in the NAc have been shown to exhibit concurrent increases in activity during task-relevant events, and similar behavioral outcomes often result from the inhibition of either cell type (Natsubori et al., 2017; Soares-Cunha et al., 2016).

The similarity of NAc shell D1 and D2 neuron activity profiles that we observed during behavioral suppression points to the possibility that integrated activity within the NAc shell, rather than a particular imbalance or competition between output pathway activities, is necessary

for suppressing behavioral responding (Ambroggi et al., 2011; Blaiss and Janak, 2009; Feja et al., 2014; Floresco, 2015; Stopper and Floresco, 2011). This notion is consistent with our findings that demonstrate that numerous disruptions of NAc shell physiology produce similar deficits in behavioral inhibition.

While the present data provide further evidence that there is no clear functional opposition between NAc D1 and D2 neurons, we did find differences in how these cell populations regulate behavior. For example, D1 and D2 neuron photo-stimulation produced divergent behavioral outcomes on a real-time place preference assay. D1 neuron inhibition also promoted free food consumption and unproductive active lever responses, while D2 neuron inhibition increased ineffectual active and inactive lever responses without affecting consumption. It is possible that D2 neurons play a broader role in suppressing competing action plans and exploratory behavior, while D1 neurons, with their projection to lateral hypothalamic feeding circuitry, may have a more specific role in the regulation of food seeking and consumption (O'Connor et al., 2015).

There is variability and complexity in the behavioral responses obtained with D1 and D2 neuron manipulations in the NAc across studies that cannot be easily explained by the D1/D2 distinction alone (Al-Hasani et al., 2015; Natsubori et al., 2017; Soares-Cunha et al., 2016). It is likely that other subdivisions of the NAc, including core/shell (Ambroggi et al., 2011; Blaiss and Janak, 2009; Dalton et al., 2014; Floresco et al., 2008; Maldonado-Irizarry et al., 1995; Millan et al., 2015; Peters et al., 2008; Stopper and Floresco, 2011; Yun et al., 2004), striosome/matrix (Berendse et al., 1992; Crittenden and Graybiel, 2011; Graybiel and Ragsdale, 1978; Watabe-Uchida et al., 2012), rostral/caudal (Castro and Berridge, 2014; Reed et al., 2018; Reynolds and Berridge, 2001), and dorsal/ventral (Al-Hasani et al., 2015), each of which are associated with different behavioral functions, may complement the D1/D2 grouping in accounting for the behavioral variance that arises from NAc manipulations (Tye, 2018). Nevertheless, concomitant activity of both D1 and D2 neurons appears to be critical for NAc-mediated suppression of unproductive reward seeking behavior.

3.4.2. COORDINATION OF ACTIVITY AMONG PVT AND BLA INPUT TO THE NAC

As with the D1/D2 neuron distinction, recent evidence has suggested that different excitatory inputs to the NAc may have an opposing influence on behavior. PVT input was recently shown

to drive aversive behaviors, suppress reward seeking, and mediate the aversive symptoms of opiate withdrawal (Cheng et al., 2018; Choi and McNally, 2017; Do-Monte et al., 2017; Zhu et al., 2016). In contrast, BLA input to the NAc can motivate and reinforce reward seeking (Britt et al., 2012; Stuber et al., 2011) while decreasing behavioral aversion (Correia et al., 2016). These findings indicate a degree of antagonistic behavioral control, but other results suggest that there is much more complexity and in some cases similarity in how these pathways shape behavior (Reed et al., 2018; Zhu et al., 2018; Zhu et al., 2016).

PVT and BLA neurons are both recruited by positive and negative stimuli (Beyeler et al., 2018; Beyeler et al., 2016; Flagel et al., 2011; Igelstrom et al., 2010; Matzeu et al., 2014; Shabel and Janak, 2009; Zhu et al., 2018), and their projections to the NAc have both been found to promote and discourage reward seeking in different contexts (Bercovici et al., 2018; Do-Monte et al., 2017; Millan et al., 2017; Reed et al., 2018; Shen et al., 2019; Stuber et al., 2011; Zhu et al., 2018). For instance, photo-stimulation of PVT input to the NAc can reduce or increase sucrose seeking behavior (Cheng et al., 2018; Do-Monte et al., 2017; Labouèbe et al., 2016), and photo-stimulation of different components of the BLA-NAc pathway can generate place preference or aversion (Shen et al., 2019). Moreover, both PVT and BLA inputs are also necessary for certain forms of appetitive and aversive associative learning (Do-Monte et al., 2017; Zhu et al., 2018).

We sought to identify antagonistic interactions between these inputs, but instead found that disrupting either pathway caused deficits in the suppression of unproductive behavior. Notably, other NAc afferents, including projections from the infralimbic (IL) cortex and ventral hippocampus (vHPC), have been implicated in behavioral inhibition (Barker et al., 2014; Yoshida et al., 2019). For instance, disrupting the IL-NAc shell projection induces drug-seeking behavior in rats that have undergone extinction (Peters et al., 2008), while disrupting vHPC-NAc interactions results in inefficient reward seeking due to the prioritization of immediate rewards (Abela et al., 2015). These results suggest that the capacity to encourage and discourage certain behaviors, a core feature of NAc-mediated action selection (Floresco, 2015; Mannella et al., 2013), is distributed across input pathways. Each pathway likely encodes unique reasons to select or reject certain behaviors and subserves the broader NAc function of promoting efficient

reward seeking. Disruptions in any NAc circuit element could undermine the balance of activity needed to support this role.

The ability of PVT input to support self-stimulation is surprising in light of recent literature, but PVT activation has been shown to elicit dopamine release in the NAc (Parsons et al., 2007). It is likely that PVT input has mixed reinforcing and aversive properties, but the former is most prominent with brief synchronous stimulation of the pathway. Nevertheless, the capability of any glutamatergic input to evoke motivated behavior suggests that this is a property of the network (Britt et al., 2012) and that these inputs encode motivationally relevant information that can promote appetitive behavior.

While PVT and BLA inputs drive similar spiking activity in NAc neurons, they innervate complementary subregions and undoubtedly encode unique information about the states and stimuli that shape volitional behavior (Hearing et al., 2018; Mannella et al., 2013; Nicola, 2007). It remains important to identify behavioral tasks that sufficiently bias activity to particular pathways to disentangle their specific contributions, but some differences between afferents are evident in the present data. For example, weak activation of PVT, but not BLA, input with DREADD manipulations proved capable of suppressing sucrose seeking, which is consistent with a wealth of literature showing that PVT input is a critical regulator of food-seeking behavior (Cheng et al., 2018; Choi and McNally, 2017; Do-Monte et al., 2017; Kirouac, 2015; Labouèbe et al., 2016; Stratford and Wirtshafter, 2013), likely mediated through D1 neuron projections to lateral hypothalamus-feeding circuits (O'Connor et al., 2015). Stronger stimulation of the PVT input robustly supported self-stimulation but had mixed effects on a real-time place preference test. The variability in behavior that results from the activation of this input across assays suggests that a greater proportion of PVT-NAc neurons (as compared to BLA-NAc projectors; Beyeler et al., 2016) contribute to the capacity of negatively arousing stimuli to shape motivated behavior, which could explain the variable effects of activating this pathway observed in the literature (Do-Monte et al., 2017; Labouèbe et al., 2016; Zhu et al., 2018; Zhu et al., 2016). It can also be difficult to evaluate the reinforcing properties of a pathway with real-time place preference assays (Yoo et al., 2016). It will be interesting to assess the role of this input on go/no-go behavioral paradigms, since the PVT-NAc pathway appears to be recruited during conflicts between reward and potential threat (Cheng et al., 2018; Choi and McNally, 2017).

Photo-inhibition of the BLA input to the NAc distinctively affected behavior, as it increased unrewarded active and inactive lever responses. The BLA-NAc input may contribute to encoding and disambiguating the meaning of cues that signal reward availability (Ambroggi et al., 2008), making cue-driven suppression of unproductive reward seeking especially sensitive to disturbances in pathway function. This idea is further supported by our finding that chemogenetic activation of the BLA input elicited perseverative, unrewarded lever responding. Although the differences between pathways suggest they make distinct contributions to reward seeking, we propose that the combined activity of PVT and BLA inputs is needed for the NAc to suppress unproductive behavior.

3.5. CONCLUSIONS

Overall, our findings support the idea that numerous inputs to and outputs from the NAc are important for behavioral suppression. The data are inconsistent with the hypothesis that NAc regulation of behavior is mediated by simple opponent processes between cell types or pathways. There appears to be many routes by which disruptions of NAc activity can produce unproductive behavior, so it is unsurprising that aberrations in this circuitry are associated with compulsive behavior (Ahmari et al., 2013; Basar et al., 2010; Burguière et al., 2015; Welch et al., 2007; Wood and Ahmari, 2015). Appreciating that the suppression of unproductive behavior is supported by the balanced activity of D1 and D2 NAc neurons as well as PVT and BLA inputs may have important implications for understanding the neural underpinnings of compulsions and disorders, such as OCD, autism spectrum disorder, and Tourette syndrome.

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3.6.1. AUTHOR CONTRIBUTIONS

CKL and JPB conceived the study, designed the experiments, and wrote the manuscript. CKL conducted the experiments with the help of AKY and JAM. CKL analyzed the data. JPB supervised the research.

3.6.2. DECLARATION OF INTERESTS

The authors declare no competing interests.

3.7. METHODS

3.7.1. LEAD CONTACT AND MATERIALS AVAILABILITY

This study did not generate unique reagents. Further information and requests for resources should be directed to the Lead Contact, Jonathan Britt (jonathan.britt@mcgill.ca).

3.7.2. EXPERIMENTAL MODEL AND SUBJECT DETAILS

Dopamine D1 receptor-Cre mice (Jackson Laboratory stock #037156), preproenkephalin-IRES2-Cre mice (Jackson Laboratory stock #025112), and C57BL/6 wildtype mice were used, both male and female. All animals were bred in-house. Transgenic animals were back crossed with C57BL/6. Mice were housed on a reverse light cycle 12 h photoperiod. At approximately three months of age (25-30 g), animals underwent surgery. Six weeks following surgery, animals were placed on a restricted feeding schedule to maintain 85-90% of pre-surgery body weight. A daily weight and feeding log was maintained for the duration of all experiments. All experiments were conducted in accordance with the Canadian Council of Animal Care and the McGill Animal Care Committee.

3.7.3. VIRAL CONSTRUCTS AND SURGERY

Prior to surgery, animals were anesthetized using a ketamine (Ventoquinol, 100mg/kg) and xylazine (Bayer, 10mg/kg) cocktail. The skull of the animal was then secured to a stereotaxic frame (Kopf Instruments) and prepared for intracranial virus injections according to standard stereotaxic procedure. 700 nL of virus was injected over a ten-minute period using a Nanoject II Injector with an oil-filled glass micropipette (Drummond Scientific, 3-000-203-G/X) pulled to a tip diameter of 10 µm. All injections were targeted bilaterally. Viruses were obtained from the

Canadian Neurophotonics Platform (CNP) and used at 5.0 x 10^{12} GC/mL unless otherwise stated below.

For D1 and D2 neuron fiber photometry experiments, rAAV9-CAG-FLEX-jGCaMP7s-WPRE (plasmid courtesy of Douglas Kim & GENIE Project; Dana et al., 2019) was delivered to the NAc (distance from Bregma: AP 1.5 mm, ML -0.65 mm, DV -4.6 mm) of Drd1-Cre or preproenkephalin-Cre mice. Ten minutes later, one 300 µm optical fiber (0.39 NA) was implanted 200 µm above the injection site in the NAc.

For D1 and D2 neuron photo-inhibition experiments, rAAV8-CAG-FLEX-eArchT3.0-eGFP (from CNP, plasmid courtesy of Edward Boyden; Chow et al., 2010) was delivered to the NAc (10° angle, AP 1.5 mm, ML ±1.35 mm, DV -4.6 mm) of Drd1-Cre or preproenkephalin-Cre mice. Ten minutes later, two 200 µm optical fibers (0.37 NA) were implanted 300 µm above the injection site in the NAc.

For afferent-specific cell body photo-inhibition experiments, retroAAV-Syn1-BFP-Cre (from Addgene, plasmid #51507 courtesy of Hongkui Zeng; Madisen et al., 2015) was delivered to the NAc (AP 1.5 mm, ML ±0.6 mm, DV -4.5) and rAAV8-CAG-FLEX-eArchT3.0-eGFP was delivered to the BLA (AP -2 mm, ML ±3 mm, DV -5 mm) or the PVT (10° angle, AP -1.1 mm, ML ±0.56 mm, DV -3.3 mm). Optical fibers were implanted 300 µm above the BLA/PVT injection site.

For axonal photomanipulation experiments, either rAAV5-CaMKIIα-hChR2-eYFP or rAAV5-CaMKIIα-eArchT3.0-eYFP (from CNP, plasmids courtesy of Karl Deisseroth; Lee et al., 2010) was delivered into the BLA (AP -1.8 mm, ML ±2.8 mm, DV -5.15 mm) or the PVT (AP -1.1 mm, ML ±0.35 mm, DV -3.3 mm) of wildtype mice. Optical fibers were then implanted in the NAc (10° angle, AP 1.5 mm, ML ±1.35 mm, DV -4.6 mm) ten minutes later.

For chemogenetic excitation experiments, retroAAV-Syn1-BFP-Cre was delivered to the NAc (AP 1.5 mm, ML ±0.6 mm, DV -4.5 mm) and rAAV8-hSyn-DIO-hM3D(G_q)-mCherry (from CNP, plasmid courtesy of Bryan Roth; Krashes et al., 2011) was delivered to the BLA (AP -2 mm, ML ±3 mm, DV -5 mm) or PVT (AP -1.1 mm, ±0.35 mm, DV -3.3 mm).

Tracing experiments were carried out by targeting AAV9-CamKIIα-eYFP (from CNP, plasmid courtesy of Karl Deisseroth) to the BLA (AP -1.8 mm, ±2.8 mm, DV -5.15 mm) and

AAV1-CAG-tdTomato (from UNC VectorCore, plasmid courtesy of Edward Boyden) to the PVT (AP -1.1 mm, ±0.35 mm, DV -3.3 mm).

3.7.4. GCAMP PHOTOMETRY RECORDINGS

A 1-site, 2-color fiber photometry system from Doric Lenses was used to assess changes in GCaMP7s fluorescence from NAc shell D1 and D2 neurons. Calcium-insensitive fluorescence was assessed with 405 nm light that oscillated in intensity from 10 to 100 µW at 450 Hz. Calcium-sensitive fluorescence was assessed with 472 nm light that oscillated in intensity from 10 to 200 µW at 205 Hz. A 300 um core optical fiber (0.39NA, FT300-UMT, ThorLabs) was used to deliver light to and collect light from the brain. Emitted light in the 500-550 nm band was measured with a 2151 Femtowatt Photoreceiver and digitized at 6kHz with an RZ5P signal processor (Tucker-Davis Technologies). This signal was demodulated according to the two carrier frequencies and low-pass filtered at 4 Hz. Patch cord autofluorescence resulting from the oscillating 405 nm and 490 nm excitation wavelengths was measured separately for each wavelength from the untethered patch cord at the end of each testing session and subtracted from the demodulated signals. The 405 nm control signal was then scaled using a least-square linear regression to best fit the 490 nm calcium sensitive signal and then subtracted from the 490 nm signal to yield a movement-corrected calcium-sensitive signal. The resultant signal for a given session was divided by its mean value to obtain dF/F.

Task and behavioral event timestamps associated with light transitions, lever extensions, and lever presses were recorded by Med Associates and integrated with the fiber photometry signals through the RZ5P signal processor. Data were extracted, processed, and analyzed using custom MATLAB (Mathworks) scripts. Photometry signals were aligned to the timestamp of interest and averaged across each event type for each mouse. Group-average signals were obtained by averaging signals across mice for a given event. Our analysis of the photometry signals associated with lever extensions was restricted to instances when the animal pressed the lever within 10 s to ensure the animal was engaged in the task. Our analysis of the photometry signals associated with lever presses excluded instances when a food port entry occurred within 5 s to avoid potential overlap with feeding related neural activity. For illustration purposes, the photometry signals displayed in **Figure 1** were downsampled by a factor of 10 and smoothed

with a moving average filter of 500 ms. However, the statistical analyses were performed on the complete, unsmoothed datasets.

3.7.5. BEHAVIORAL TESTING

For all behavioral tasks, mice were trained in sound attenuating chambers that had levers available on either side of a centrally located food receptacle (Med Associates). A houselight and speaker were located on the opposite side of the chamber. All behavioral data were collected using Med Associates software.

Food seeking and consumption assays. For the VR3 operant task, food deprived mice were placed in an operant chamber with access to an active and inactive lever for 40 min sessions. Training consisted of three phases: FR1, VR3, and VR3 with intermittent reward availability. Rewarded lever presses always resulted in delivery of 30 µL of a 15% sucrose solution (m/v) and a tone presentation (4.8 kHz, 80dB, 5 s duration). Inactive lever presses were always inconsequential. The levers remained extended throughout each session; except for the fiber photometry experiments where they were extended for 10 s every 30 s. Mice underwent FR1 training until they attained 40 rewards in a single session. They then underwent VR3 training, which meant the number of active lever presses required for reward delivery varied randomly between 1 and 5. VR3 training continued until mice attained 20 rewards in a single session. For the last phase of training, the VR3 task consisted of alternating periods of reward availability and unavailability that were each 4 minutes long, beginning 10 minutes after the start of the session. Reward unavailability was signalled by dimming the houselight, and no lever presses were reinforced during this time. After 4-8 days on this phase of training, mice typically made twice as many active lever presses when reward was available versus when it was not. Mice tethered to the 200 um optical fiber during training (for the optogenetic experiments), as opposed to the 300 um fiber used in the photometry experiments, were generally faster to learn the task contingencies. Experimental manipulations were carried out after animals completed the final phase of VR3 training. Reward seeking behavior was compared across photo-inhibition epochs during periods when reward was and was not available. Response efficiency *error scores* were calculated by dividing the number of responses made when reward was unavailable (active lever presses or food port entries) by the number of responses made when reward was available:

$$Error\ Score = \frac{Responses\ during\ reward\ unavailability}{Responses\ during\ reward\ availability}$$

Photo-inhibition involved bilateral intracranial light delivery (532 nm, 10 mW) for two 8-minute periods within the 40 min session. These mice were optically tethered during all training and testing sessions. The timing of the photo-inhibition periods were counterbalanced across two days of testing and spanned periods when reward was and was not available. For the DREADD excitation experiments, saline or compound 21 (C21, 3 mg/kg) was injected intraperitoneally 30 minutes prior to testing. Injections occurred in a counterbalanced manner over two days.

All mice that underwent circuit manipulations subsequently experienced several additional VR3 training sessions in which reward was always available (phase 2 training). Optogenetic and chemogenetic manipulations were then performed in this condition, as described above, for the purpose of comparison. Lastly, the experimental manipulations were repeated during an assay of free food consumption in an activity box (6 x 10 inches).

Intracranial self-stimulation assay. Mice were tethered to an optical fiber and placed in an operant chamber with access to an active and inactive lever for 60 min sessions. Active lever presses resulted in bilateral intracranial light delivery (473 nm, 10 mW, 15-40 Hz, 5 ms pulse duration) for 1-2 s. Pulse frequency and train duration varied between mice since stimulation parameters were adjusted in animals that had low response rates during initial training.

Real Time Place Preference Assay: Mice were tethered to an optical fiber and placed in a 2-room real time place preference chamber (8.5 x 17 inches). Mice were given 10 minutes to habituate to the chamber. After that, light (473 nm, 10 mW, 5 ms pulse duration) was intracranially delivered whenever the mice were physically located in one particular side of the chamber (counterbalanced across mice). A frequency of 4 Hz was used for D1 and D2 neuron stimulations, and 40 Hz was used for afferent pathway stimulation. The proportion of time spent in the laser-paired room was compared between baseline and the photo-stimulation periods. Behavior was evaluated in real time and coupled to laser activation with Ethovision software.

3.7.6. HISTOLOGY

At the end of each behavioral experiment, animals were anesthetized with 270 mg/kg Euthansol (Merck) and transcardially perfused with 4% paraformaldehyde (PFA, Sigma-Aldrich). Brains

were removed, post-fixed in PFA for 24 h, and then transferred to PBS for 48 h. Tissue was then sliced into 60 μm sections on a vibratome (Leica VT1000s) and mounted on microscope slides with a MOWIOL plus DAPI (Sigma-Aldrich) solution.

3.7.7. QUANTIFICATION AND STATISTICAL ANALYSIS

Two-tailed paired t-tests and two-way repeated measures ANOVAs were used for statistical comparisons of the mean fiber photometry signals across food availability conditions and across early and late training. The amplitude of transient event-related signals was defined as difference in mean fluorescence between the periods indicated by the gray (baseline) and black (event) line segments on each of the figure panels (**Figure S1**).

Two-tailed paired t-tests, one-way ANOVAs, and two-way ANOVAs were used for statistical comparisons of behavior across photo- and chemogenetic manipulation conditions and across pathways.

Sidak's multiple comparisons tests were conducted for all ANOVA *post hoc* tests. The significance of all statistical tests was determined using $\alpha = 0.05$. All data are reported as the mean ± SEM.

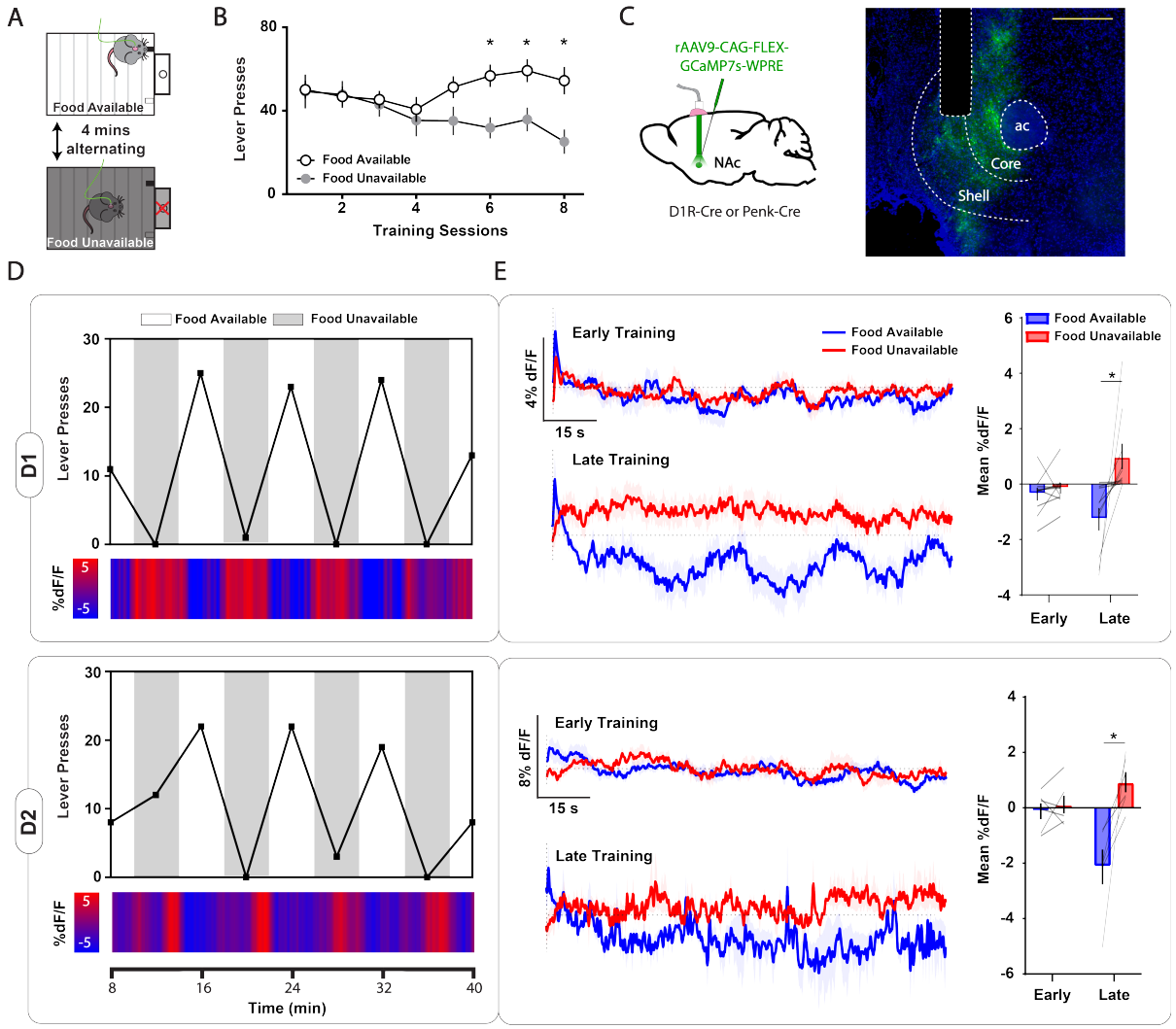
For the PVT-NAc and BLA-NAc tracing experiment, regions of interest were manually identified and the average brightness of this region in red and green channels, normalized to the brightness of a same-sized area containing no fluorescent axons, was measured in Adobe Photoshop. In order to assess the relationship between PVT and BLA axonal fluorescence in an unbiased manner, a Pearson correlation coefficient was calculated for each NAc section using red and green channel light intensity in each image pixel within the NAc. For averaging and statistical comparison, correlation coefficients were transformed using a Fisher's Z-transformation. A one-sample t-test was used to evaluate whether the correlation significantly differed from zero on average. The average z score was then back-transformed to obtain an average Pearson's r value.

3.7.8. DATA AND CODE AVAILABILITY

All software used in this study is available and listed in the Key Resources Table. This study did not generate any unique datasets or code.

3.8. FIGURES

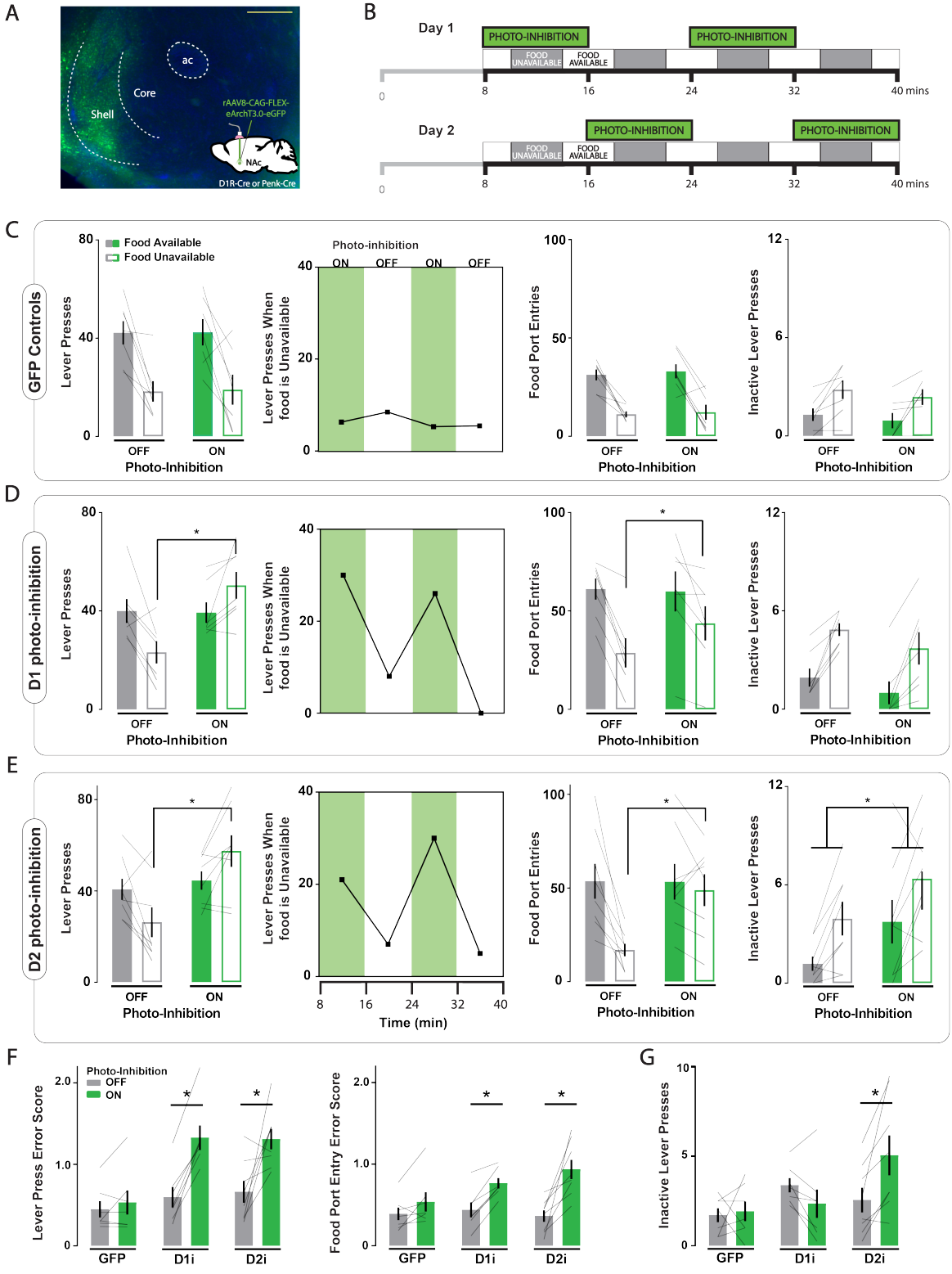
FIGURE 1. D1 AND D2 NAC NEURONS EXHIBIT ELEVATED ACTIVITY DURING PERIODS OF BEHAVIOURAL SUPPRESSION.



A) Schematic of VR3 operant task. Reward availability and house light illumination both alternated in 4-minute long intervals. **B)** Summary of task acquisition. During training, rates of operant responding diverged across periods when reward was and was not available (n = 16; $F_{(7,105)} = 2.945$, $p < 0.01$; *post hoc* $p < 0.05$ on days 6, 7, and 8, $t_{(105)} = 3.52$, 3.31, and 4.13, respectively). **C)** Schematic of viral injection and fiber optic implant alongside a representative coronal brain slice showing GCaMP7s expression in the NAc shell. Scale bar, 500 µm. **D)** Representative operant response data from two well-trained mice above color plots showing the

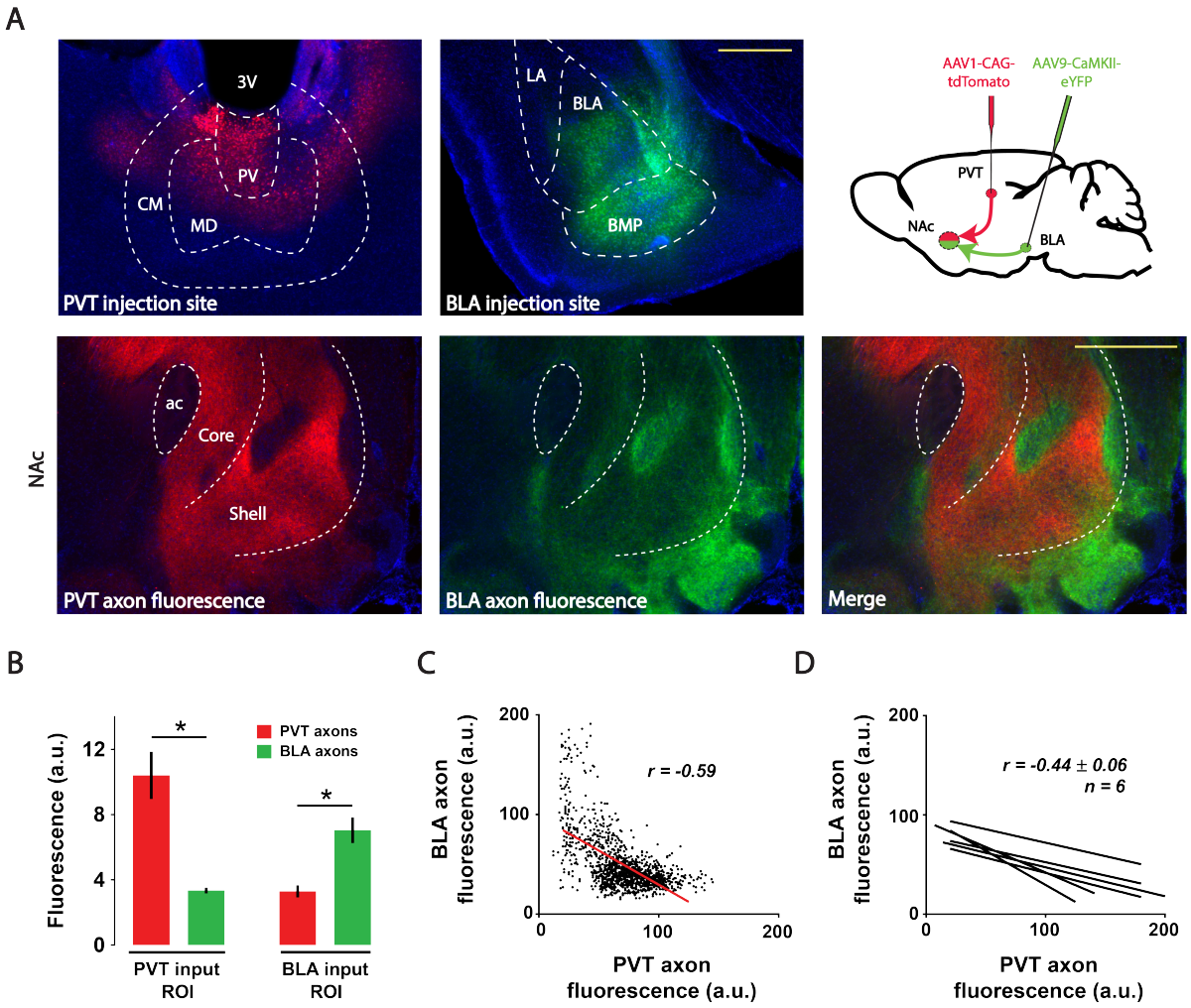
concomitant changes in GCaMP7s fluorescence from D1 (*top*) and D2 (*bottom*) neurons. **E)** Summary of D1 and D2 GCaMP7s fluorescence during food available and unavailable periods in early and late training sessions. Differences in mean GCaMP7s fluorescence across periods of reward availability and unavailability are only evident in the late training sessions when mice abstain from unproductive operant responding (D1 neuron recordings, $F_{(1,9)} = 9.409$, $p < 0.05$, effect of reward availability in late training, $t_{(19)} = 4.781$, $^*p < 0.01$; D2 neuron recordings, $F_{(1.,5)} = 13.34$, $p < 0.05$, effect of reward availability in late training, $t_{(5)} = 5.586$, $^*p < 0.01$). Error bars indicate SEM. See also Figure S1.

FIGURE 2. PHOTO-INHIBITION OF D1 AND D2 NAC NEURONS INCREASES UNPRODUCTIVE REWARD SEEKING.



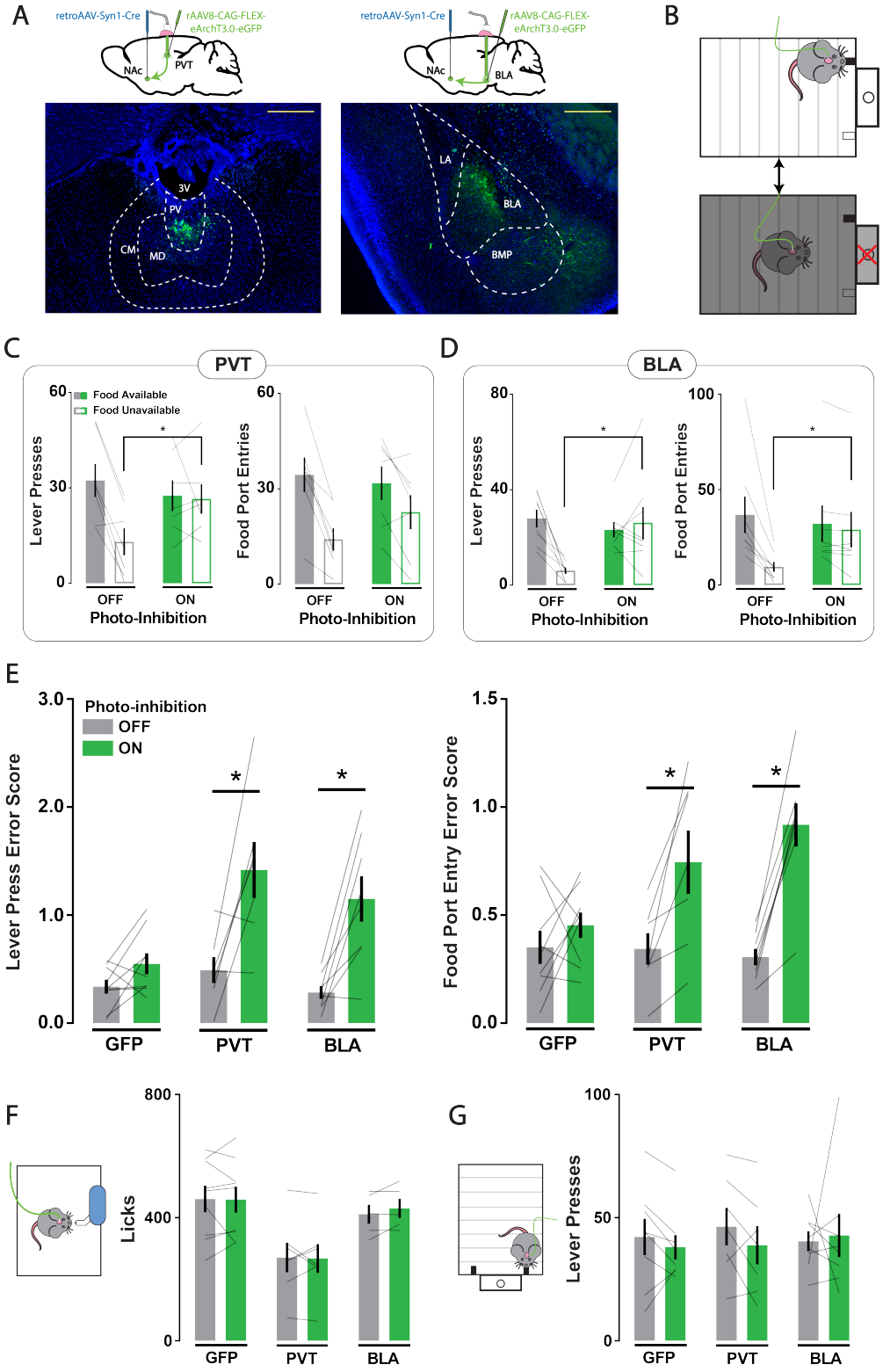
A) Representative coronal brain slice showing eArchT3.0-eGFP expression in the NAc shell. Scale bar, 500 μm. **B)** Experimental design. **C)** Summary of GFP control animals' reward seeking behavior in relation to photo-inhibition and reward availability. Control mice made fewer active lever presses (n = 7; $F_{(1,6)} = 16.87$, $p < 0.01$) and food port entries ($F_{(1,6)} = 30.46$, $p < 0.01$), but more inactive lever presses ($F_{(1,6)} = 13.94$, $p < 0.01$), when food was unavailable, irrespective of intracranial light delivery. The second panel shows data from a representative animal. **D)** Summary of change in behavioral responding on account of D1 neuron photo-inhibition. In the absence of photo-inhibition, behavioral responding was contingent on reward availability (n = 7; $F_{\text{lever }(1.6)} = 136.5$, $p < 0.001$; $F_{\text{food port}(1,6)} = 12.24$, $p < 0.05$; effect of food availability, $t_{\text{lever }(6)} = 9.95$, $p < 0.05$; $t_{\text{food port }(6)} = 9.90$, $p < 0.05$). D1 neuron inhibition increased active lever responding and food port entries specifically when reward was unavailable (effect of photo-inhibition on lever presses, $t_{(6)} = 16.14$*, and food port entries, $t_{(6)} = 4.57$*). D1 neuron inhibition did not affect inactive lever responses ($F_{(1,6)} = 4.32$, $p = 0.0829$). **E)** D2 neuron photo-inhibition also increased active lever responding (n = 8; $F_{(1,7)} = 9.16$, $p < 0.05$) and food port entries ($F_{(1,7)} = 11.97$, $p < 0.05$) specifically when reward was unavailable (effect of photo-inhibition on lever presses, $t_{(7)} = 4.89$*, and food port entries, $t_{(7)} = 4.86$*), while in the absence of photo-inhibition behavioral responding was contingent on reward availability ($t_{\text{lever }(7)} = 5.58$, $p < 0.05$). D2 neuron inhibition also increased inactive lever responding (main effect of photo-inhibition, $F_{(1,7)} = 11.91$*). **F)** Comparisons across groups. *Error scores* indicate the reward unavailable response rate divided by the reward available response rate. D1 and D2 neuron inhibition increased lever press ($F_{(2,19)} = 5.239$, $p < 0.05$) and food port entry *error scores* ($F_{(2,19)} = 4.46$, $p < 0.05$). For the significant post hoc comparisons (*), $t_{(19)} > 3.11$. **G)** Summary of inactive lever responding in relation to photo-inhibition, irrespective of reward availability, highlighting the effect of D2 neuron photo-inhibition ($F_{(2,19)} = 7.54$, $p < 0.01$; D2 photo-inhibition effect: $t_{(19)} = 3.94$*). Error bars represent SEM. *signifies $p < 0.05$. See also Figures S2-4.

FIGURE 3. PVT AND BLA NEURONS INNERVATE DISTINCT AREAS OF THE NAC.



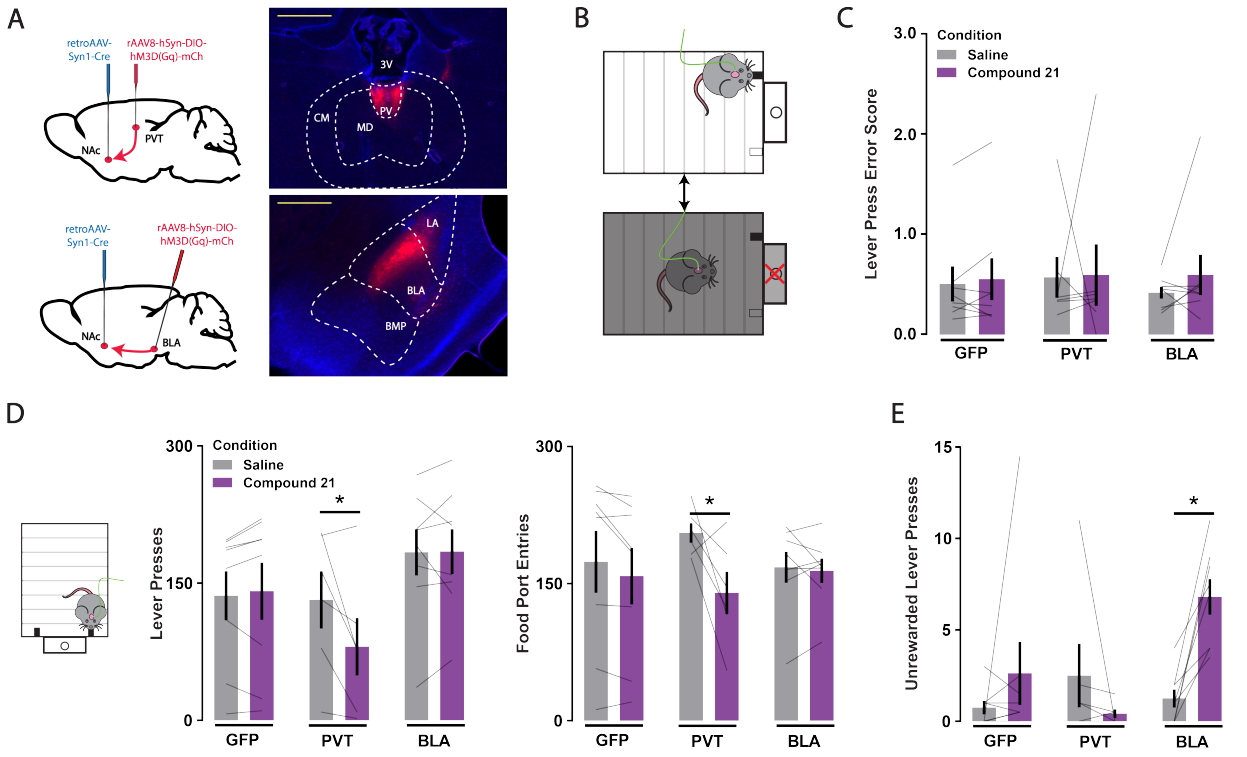
A) Schematic of virus injections (*top right*) alongside representative coronal brain slices showing site of virus delivery in PVT and BLA (*top*) and inputs from these regions in the NAc (*bottom*). Scale bars, 500 µm. **B)** Summary of PVT and BLA axonal fluorescence in regions of interest within the NAc. There are relatively few BLA axons in areas of the NAc with dense PVT input, and vice versa (n = 6; $F_{(1,10)} = 52.21$, $p < 0.001$; $t_{BLA\ (10)} = 3.54$, *$p < 0.05$; $t_{PVT\ (10)} = 6.68$, *$p < 0.001$). Error bars represent SEM. **C)** Pixel-wise analysis of PVT and BLA axonal fluorescence in a representative NAc brain slice highlighting the negative correlation (i.e., non-overlapping innervation patterns; r = -0.59, $p < 0.001$). **D)** Summary of pixel-wise correlational analysis of PVT and BLA axonal fluorescence across all examined NAc slices, indicating a significant negative correlation between PVT and BLA axonal fluorescence (n = 6; r = -0.44 ± 0.06; one sample t test of Fisher's z: $t_{(5)} = 7.94$, $p < 0.001$).

FIGURE 4. PHOTO-INHIBITION OF PVT AND BLA CELL BODIES THAT INNERVATE THE NAC INCREASES UNPRODUCTIVE REWARD SEEKING BEHAVIOR.



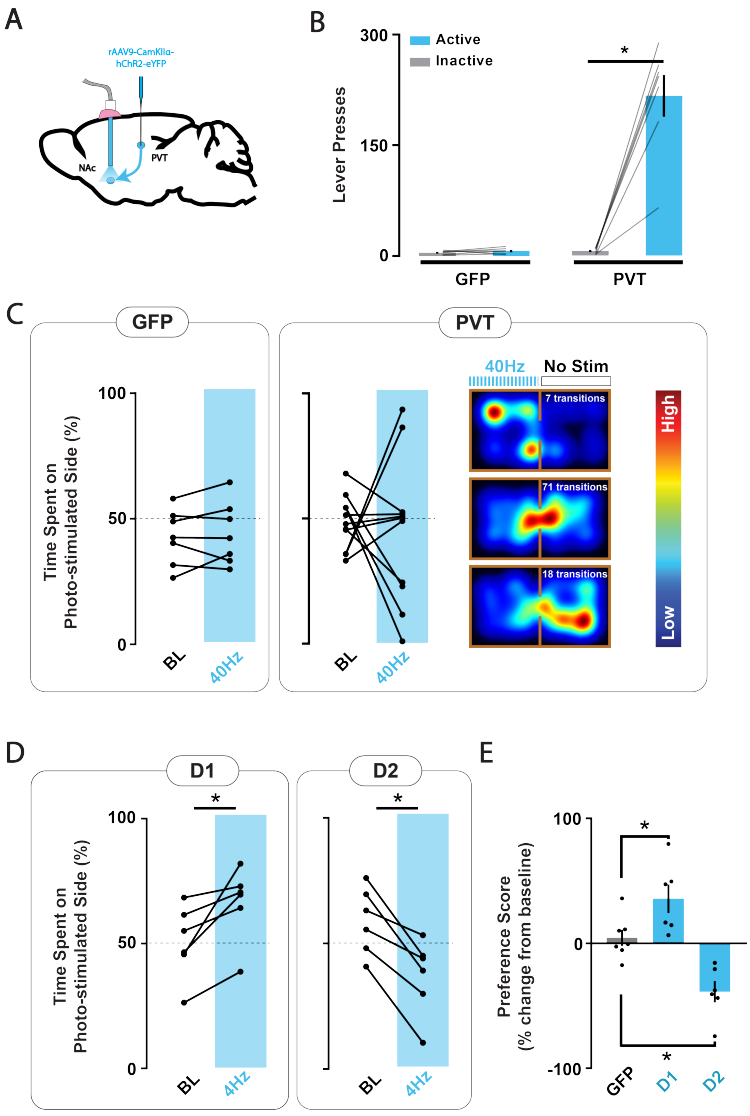
A) Schematic of experimental manipulations (*top*). Representative coronal brain slices showing eArchT3.0-eYFP expression in PVT and BLA neurons that project to the NAc (*bottom*). Scale bars, 500 μm. **B)** Schematic of VR3 operant task. **C)** Summary of behavioral responding in relation to reward availability and photo-inhibition of PVT neurons that project to the NAc . In the absence of photo-inhibition, behavioral responding was contingent on reward availability (n = 7; $F_{lever\ (1,6)} = 14.74$, $p < 0.01$, $F_{food\ port\ (1,6)} = 6.84$, $p < 0.01$, effect of food availability, $t_{lever\ (6)} = 5.73$, $p < 0.01$; $t_{food\ port\ (6)} = 6.70$, $p < 0.01$). PVT neuron photo-inhibition increased active lever responding and food port entries specifically when reward was unavailable (effect of photo-inhibition on lever presses, $t_{(6)} = 4.01$*, and food port entries, $t_{(6)} = 2.83$*). **D)** Summary of behavioral responding in relation to reward availability and photo-inhibition of BLA neurons that project to the NAc . In the absence of photo-inhibition, behavioral responding was contingent on reward availability (n = 8; $F_{lever\ (1,7)} = 14.27$, $p < 0.01$, $F_{food\ port\ (1,7)} = 12.14$, $p < 0.05$, effect of food availability, $t_{lever\ (7)} = 4.69$, $p < 0.01$; $t_{food\ port\ (7)} = 5.55$, $p < 0.01$). BLA neuron photo-inhibition increased active lever responding and food port entries specifically when reward was unavailable (effect of photo-inhibition on lever presses, $t_{(7)} = 4.32$*, and food port entries, $t_{(7)} = 3.97$*). **E)** Comparisons across groups showing PVT-NAc and BLA-NAc neuron photo-inhibition increased lever press ($F_{(2,21)} = 4.36$, $p < 0.05$) and food port entry ($F_{(2,21)} = 7.19$, $p < 0.01$) error scores. For the significant post hoc comparisons (*), $t_{(21)} > 3.80$. **F)** Photo-inhibition of PVT-NAc and BLA-NAc neurons did not affect consumption of freely available food ($F_{(2,18)} = 0.32$, $p = 0.74$). **G)** Photo-inhibition of PVT-NAc and BLA-NAc neurons did not affect active lever responding during a task in which reward was always available ($F_{(2,20)} = 0.46$, $p = 0.64$). Error bars represent SEM. *signifies $p < 0.05$. See also Figure S5.

FIGURE 5. CHEMOGENETIC EXCITATION OF PVT-NAC PATHWAY REDUCES OPERANT RESPONDING FOR FOOD REWARD.



A) Schematic of experimental manipulations (*left*). Representative coronal brain slices showing hM3D(G_q)-mCherry expression in PVT and BLA neurons that project to the NAc shell (*right*). Scale bars, 500 µm. **B)** Schematic of VR3 operant task. **C)** Summary data highlighting the lack of a behavioral effect with DREADD excitation of PVT and BLA inputs to the NAc on this task (n_{GFP} = 8, n_{PVT} = 7, n_{BLA} = 8; no main effect of drug, $F_{(1,20)} = 0.34$, $p = 0.56$, or interaction, $F_{(2,20)} = 0.11$, $p = 0.89$). **D)** Chemogenetic excitation of PVT (n = 6), but not BLA, neurons that innervate the NAc reduced active lever responding (*left*; $F_{(2,19)} = 5.36$, $p < 0.05$; PVT effect, $t_{(19)} = 3.63$, $p < 0.01$) and food port entries (*right*; $F_{(2,19)} = 5.17$, $p < 0.05$; PVT effect, $t_{(19)} = 4.34$, $p < 0.01$) during a task in which reward was always available. **E)** Chemogenetic excitation of BLA, but not PVT, neurons increased perseverative, unrewarded active lever responding ($F_{(2,19)} = 5.48$, $p < 0.05$; BLA effect, $t_{(19)} = 3.69$, $p < 0.01$). Error bars represent SEM. *signifies $p < 0.01$.

FIGURE 6. PVT INPUTS TO THE NAC SUPPORT SELF-STIMULATION, WHILE D1 AND D2 NEURONS PROMOTE REWARD AND AVERSION, RESPECTIVELY.

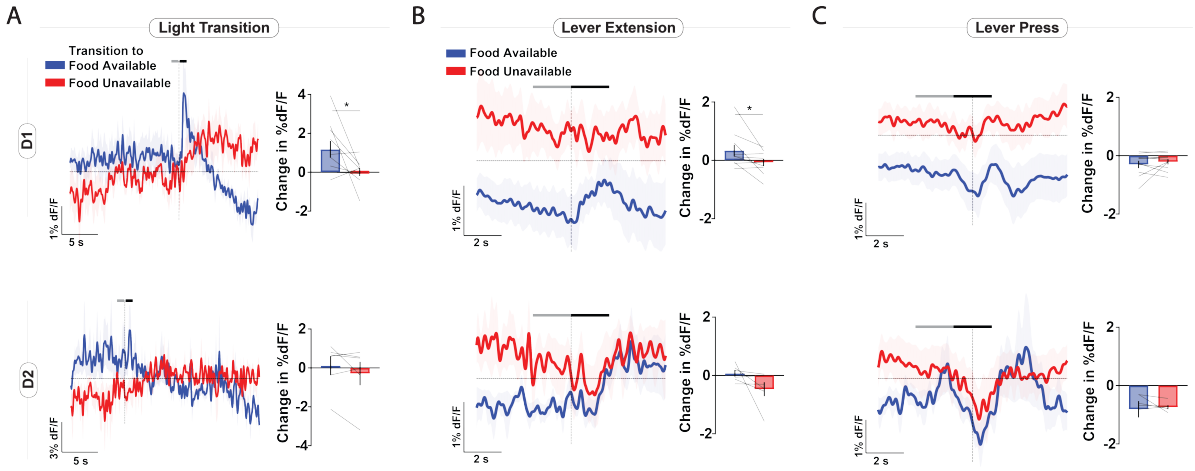


A) Schematic of experimental manipulations. **B)** Summary of self-stimulation behavior driven by PVT axon stimulation in the NAc shell (n_{GFP} = 6, n_{PVT} = 7; $F_{(1,11)}$ = 49.01 $t_{PVT(6)}$=10.42*). **C)** In a real time place preference assay, intracranial light delivery had no effect in GFP-only controls (n = 7; *left*), while photo-stimulation of PVT input (n = 8; *right*) elicited variable behavioral responses. Heatmaps from 3 representative mice showing time spent in different areas of the chamber and number of transitions between rooms. **D)** Summary of real time place preference/avoidance behavior driven by photo-stimulation of NAc D1 neurons (n = 6; $t_{(5)}$ = 3.25, *p < 0.05) and D2 neurons (n = 6; $t_{(5)}$ = 5.386, *p < 0.01). **E)** Comparisons across groups

showing D1 and D2 neuron photo-stimulation produced opposing behavioral effects in real time place preference assay ($n_{GFP} = 7$; $F_{(2,16)} = 24.89$, $p < 0.001$; $t_{D1\ vs\ GFP\ (16)} = 2.74$*; $t_{D2\ vs\ GFP\ (16)} = 4.50$*). Error bars represent SEM. *signifies $p < 0.05$. See also Figure S6.

FIGURE S1. DISCRETE CUES THAT SIGNAL REWARD AVAILABILITY TRANSIENTLY INCREASE NAC SHELL D1 NEURON ACTIVITY.

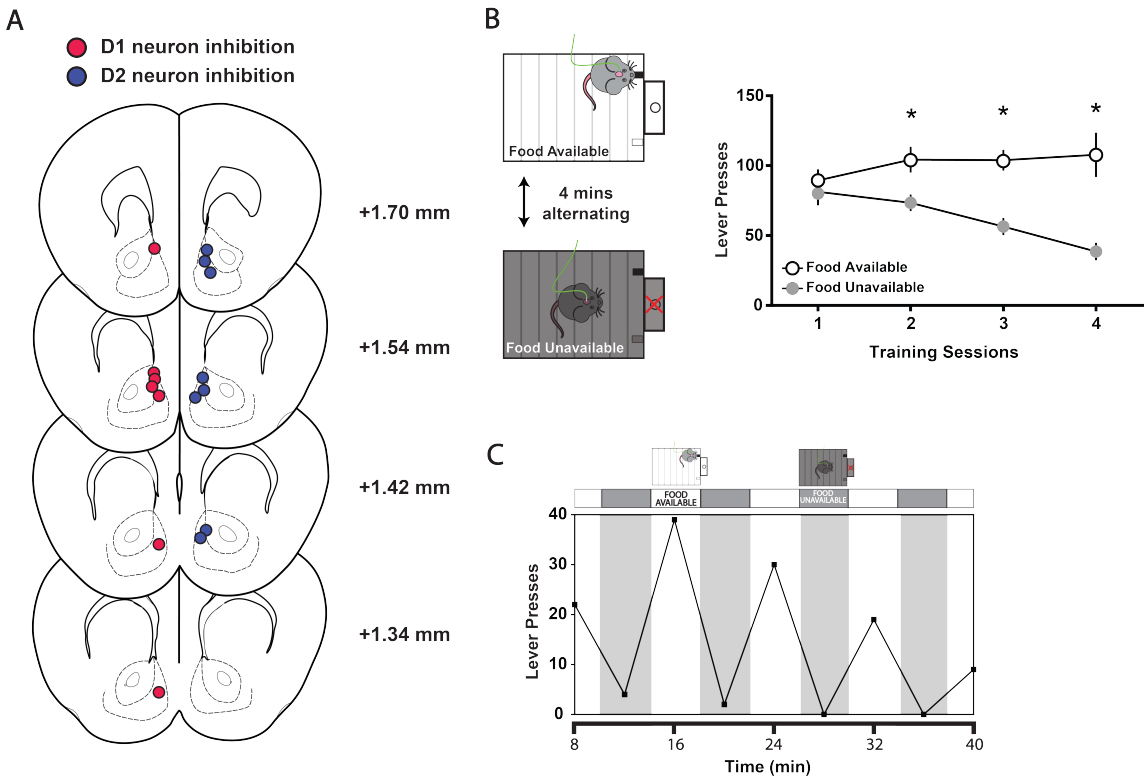
Related to Figure 1.



Summary of event-related changes in GCaMP7s fluoresence from D1 (n = 10) and D2 (n = 6) neuron populations in relation to different components of the operant task and availability of reward. Bar graphs depict the difference in the mean signals calculated beneath the grey (baseline) and black (event) line segments. **(A)** Only D1 neuron population responses differed between the two types of light transitions that signaled a change in reward availability ($t_{(9)}$ = 2.363, *p < 0.05), and **(B)** between lever extensions that occurred when reward was and was not available ($t_{(9)}$ = 2.409, *p < 0.05). **(C)** The changes in D1 and D2 neuron population activities that coincided with the act of lever pressing did not differ significantly between reward available and unvailable periods (D1, $t_{(9)}$ = 0.799, p = 0.445; D2, $t_{(5)}$ = 0.268, p = 0.799). Error bars represent SEM. * signifies p < 0.05.

FIGURE S2. OPTIC FIBER PLACEMENTS AND TRAINING SESSION OPERANT RESPONSE DATA FOR D1 AND D2 NEURON EARCHT MICE.

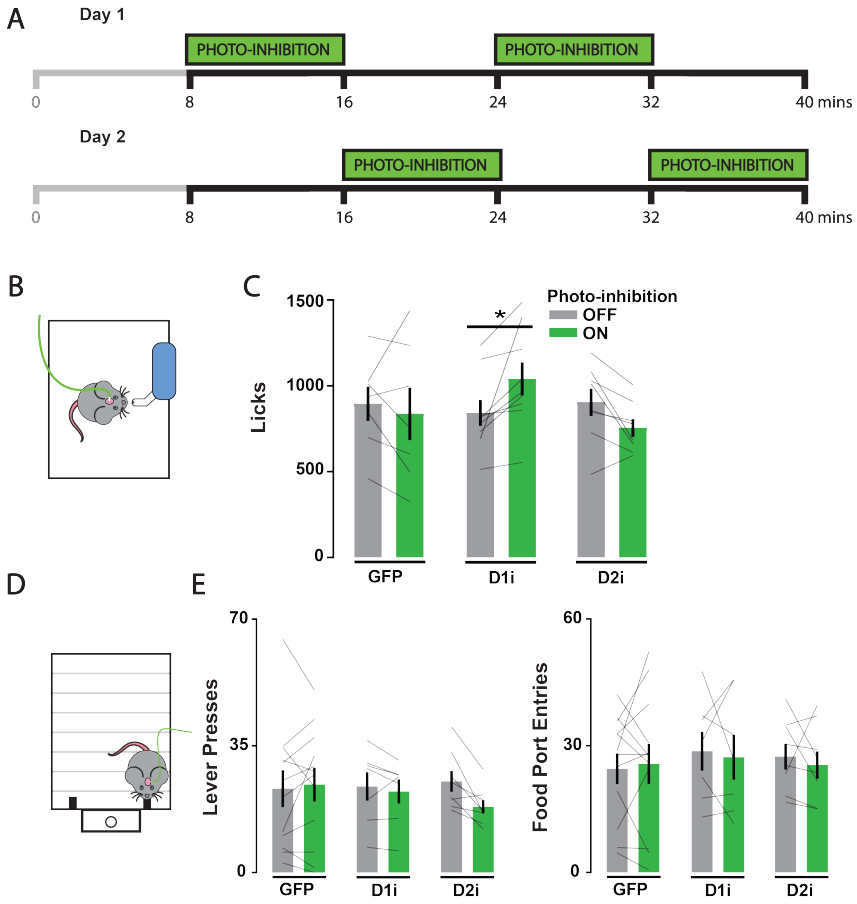
Related to Figure 2.



(A) Each circle represents the bilateral locations of the two fiber optic probes in an individual mouse. **(B)** Schematic of VR3 operant task (*left*) and summary of operant response data relative to reward availability across training sessions (*right*). During training, rates of operant responding diverged across periods when reward was and was not available (n = 20; $F_{(3,57)}$ = 13.55, $p < 0.001$; *post hoc* $p < 0.05$ on days 2, 3, and 4, $t_{(57)}$ = 4.39, 6.76, and 9.85 respectively). Error bars represent SEM. * signifies $p < 0.05$. **(C)** Representative operant response pattern from the last training session for one mouse.

FIGURE S3. PHOTO-INHIBITION OF NAC SHELL D1 NEURONS INCREASES CONSUMPTION OF FREELY AVAILABLE FOOD.

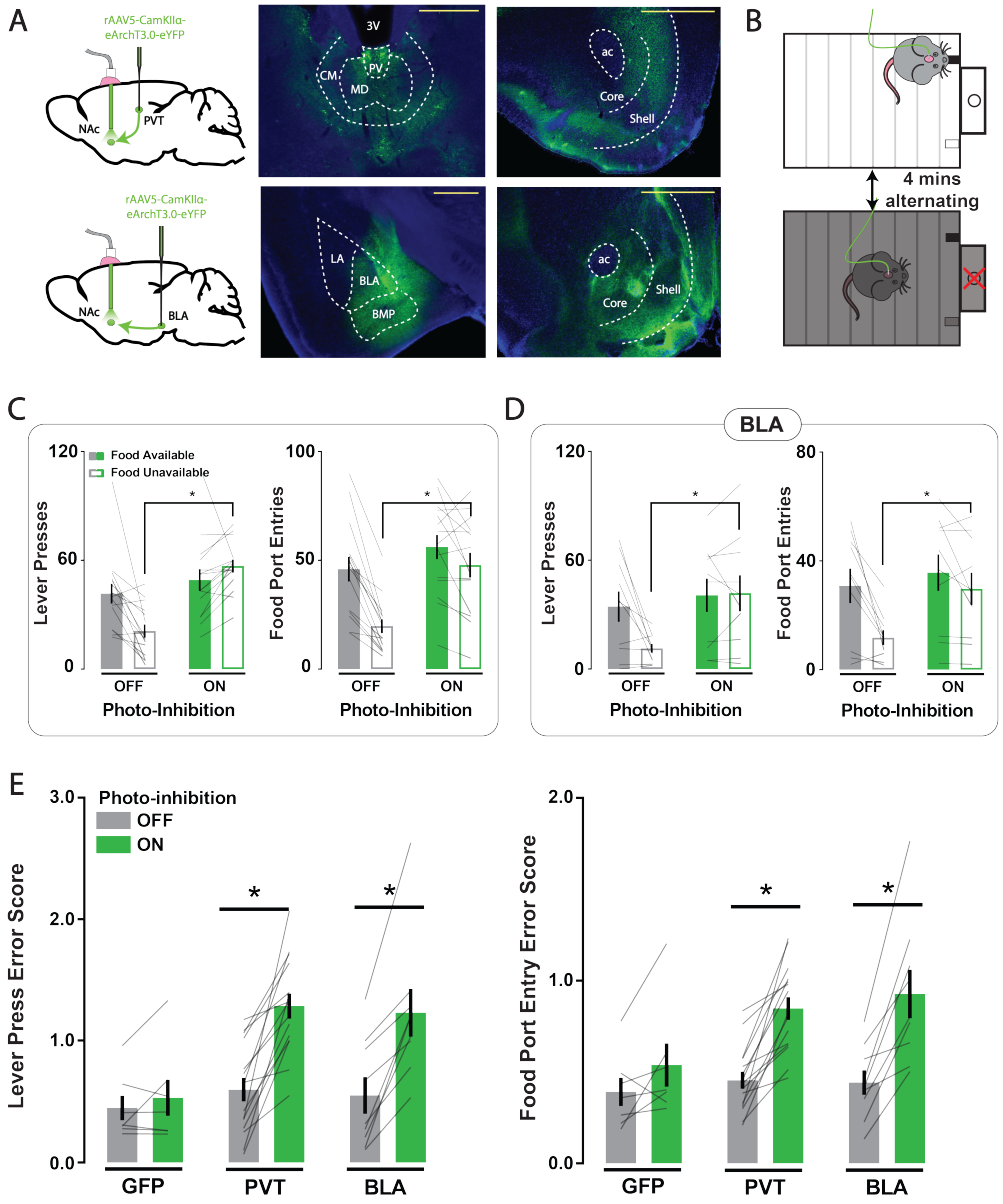
Related to Figure 2.



Experimental design. **(B)** Schematic of free feeding task. **(C)** Photo-inhibition of D1, but not D2, neurons increased animals consumption of freely available food ($n_{GFP} = 7$, $n_{D1} = 9$, $n_{D2} = 8$; $F_{(2,21)} = 6.38$, $p < 0.05$; effect of D1 photo-inhibition: $t_{(21)} = 2.85$, *$p < 0.05$). **(D)** Schematic of VR3 operant task with reward always available. **(E)** Neither D1 nor D2 neuron photo-inhibition influenced active lever responding (*left*; no main effect of photo-inhibition, $F_{(1,24)} = 2.75$, $p = 0.11$, or interaction, $F_{(2,24)} = 2.96$, $p = 0.071$) or food port entries (*right*; no main effect of photo-inhibition, $F_{(1,24)} = 0.12$, $p = 0.73$, or interaction, $F_{(2,24)} = 0.232$, $p = 0.79$) during a task in which reward was always available. Error bars represent SEM. * signifies $p < 0.05$.

FIGURE S4. PHOTO-INHIBITION OF PVT AND BLA AXONS IN THE NAC INCREASES UNPRODUCTIVE REWARD SEEKING.

Related to Figure 2.

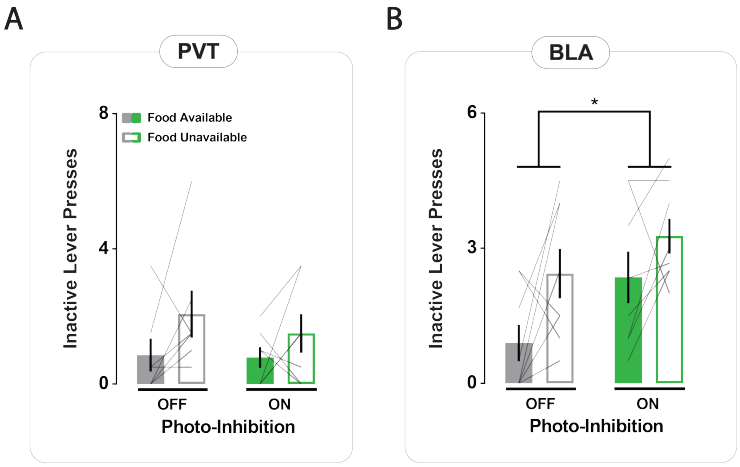


(A) Schematic of experimental manipulations (*left*) alongside representative coronal brain slices showing eArchT3.0-eYFP expression in PVT and BLA neurons (*middle*) and their associated axons in the NAc (*right*). Scale bars, 500 µm. **(B)** Schematic of VR3 operant task. **(C)** Summary of behavioural responding in relation to photo-inhibition of PVT axons in the NAc and reward availability. In the absence of photo-inhibition, behavioural responding was contingent on reward

availability (n = 15; $F_{lever\ (1,14)} = 57.57$, $p < 0.001$, $F_{food\ port\ (1,14)} = 13.63$, $p < 0.01$, effect of food availability, $t_{lever\ (14)} = 7.80$, $p < 0.05$; $t_{food\ port\ (14)} = 7.64$, $p < 0.05$). PVT axon photo-inhibition increased active lever responding and food port entries specifically when reward was unavailable (effect of photo-inhibition on lever presses, $t_{(14)} = 13.56$*, and food port entries, $t_{(14)} = 8.20$*). **(D)** Summary of behavioural responding in relation to photo-inhibition of BLA axons in the NAc and reward availability. In the absence of photo-inhibition, behavioural responding was contingent on reward availability (n = 10; $F_{lever\ (1,9)} = 7.20$, $p < 0.05$, $F_{food\ port\ (1,9)} = 10.23$, $p < 0.05$, effect of food availability, $t_{lever\ (9)} = 3.62$, $p < 0.05$; $t_{food\ port\ (9)} = 6.59$, $p < 0.05$). BLA axon photo-inhibition increased active lever responding and food port entries specifically when reward was unavailable (effect of photo-inhibition on lever presses, $t_{(9)} = 10.23$*, and food port entries, $t_{(9)} = 6.21$*). **(E)** Comparisons across groups showing PVT and BLA axon photo-inhibition increased lever press ($F_{(2,28)} = 6.93$, $p < 0.01$) and food port entry ($F_{(2,28)} = 3.86$, $p < 0.05$) error scores. For significant post hoc comparisons (*), $t_{(28)} > 5.431$. Error bars represent SEM. * signifies $p < 0.05$.

FIGURE S5. PHOTO-INHIBITION OF BLA NEURONS THAT INNERVATE THE NAC INCREASES INACTIVE LEVER RESPONDING.

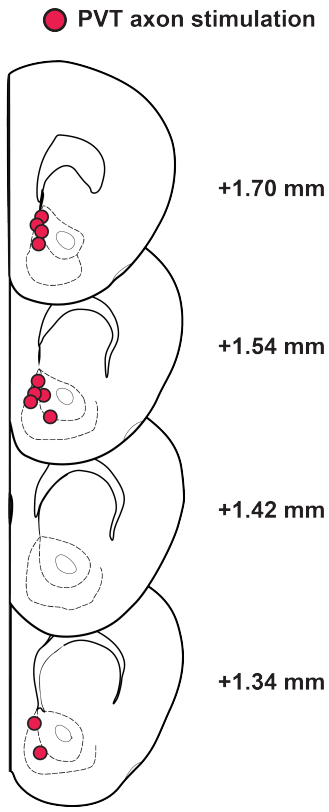
Related to Figure 4.



(A) Photo-inhibition of PVT cell bodies that project to the NAc did not affect inactive lever responding in the VR3 operant task (n = 7; $F_{(1,6)} = 1.17$, $p = 0.32$). **(B)** Photo-inhibition of BLA cell bodies that project to the NAc increased inactive lever responding irrespective of reward availability (n = 8; main effect of photo-inhibition, $F_{(1,7)} = 6.70$, $^{*}p < 0.05$).

FIGURE S6. OPTIC FIBER PLACEMENTS IN THE NAC SHELL FOR THE PVT AXON PHOTO-STIMULATION EXPERIMENTS.

Related to Figure 6.



Each circle represents the bilateral locations of the two fiber optic probes in an individual mouse.

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3.10. CONNECTING THE TEXT: CHAPTERS 3 – 4

In this chapter, we demonstrated that behavioural suppression is a distributed function of multiple NAc circuit elements. Although stimulation of PVT and BLA projections to the NAc can produce divergent behavioural effects (Britt et al., 2012; Zhu et al., 2016), disruption of either pathway increased unproductive reward seeking behaviour, suggesting that the NAc is a critical site for circuit susceptibility to impaired behavioural inhibition.

Chronic deficits in behavioural control, which may arise from dysregulated NAc circuits, are a hallmark of numerous disorders characterized by compulsivity, including OCD, Tourette syndrome, and autism. Since patterned stimulation of mPFC afferents has been shown to alter NAc physiology and drive an OCD-like phenotype in mice (Ahmari et al., 2013), we sought to test whether other NAc afferents play a similar role in regulating compulsivity. In the next chapter we compare the effects of repeatedly activating PVT and vHPC afferents on self-grooming and reversal learning. Although these inputs target NAc subregions distinct from mPFC (Wright and Groenewegen, 1995) and one another (Yang et al., 2019), they are both involved in behavioural suppression, allowing us to test whether these segregated circuit elements can commonly contribute to enduring dysregulations of behavioural inhibition, as suggested by the effects of acutely silencing PVT and BLA inputs in chapter 3.

CHAPTER 4

HYPERACTIVITY OF PARAVENTRICULAR THALAMIC INPUTS TO THE NUCLEUS ACCUMBENS ELICITS COMPULSIVITY.

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4.1. ABSTRACT

Dysregulations of the nucleus accumbens (NAc) and its cortical afferents are implicated in the disinhibition of compulsive behaviors. Recent evidence suggests that hippocampal (vHPC) and thalamic (PVT) inputs to the NAc also contribute to behavioral suppression and may thus play a role in compulsivity. To directly test this hypothesis, we measured the effects of NAc afferent stimulation on repetitive motor behaviors and cognitive flexibility. Brief or repeated activation of PVT inputs enhanced self-grooming acutely and cumulatively over several days, a phenotype which was reversibly blocked by mGluR5 antagonism. vHPC input was not directly involved in self-grooming, but repeated activation of either afferent impaired subsequent performance on a reversal learning task. These data suggest a pathway-specific role for PVT-NAc circuitry in the etiology of compulsions, and highlight the NAc as a convergent site of multiple circuit vulnerabilities to psychiatric disease.

4.2. INTRODUCTION

Compulsions are maladaptive, repetitive behaviors that persist despite adverse consequences (Luigjes et al., 2019). These behaviors feature prominently in numerous psychiatric disorders including autism spectrum disorder (ASD), obsessive-compulsive disorder (OCD), and related disorders such as trichotillomania and dermatillomania. Many of these disorders share symptomology, treatment responses, and exhibit comorbidity, suggesting a common pathophysiological mechanism (Fineberg et al., 2014; Wood and Ahmari, 2015). Despite converging lines of evidence that implicate striatal circuits in compulsive disorders, precise neural mechanisms are unclear and current treatments are inadequate.

The striatum is a basal ganglia (BG) structure that integrates inputs from cortical and subcortical regions to guide habitual and goal-directed behaviors (Mannella et al., 2013). Imaging studies in compulsive patient populations have demonstrated dysregulated cortico-striatal circuits (Breiter et al., 1996; Gu et al., 2008; Leckman et al., 2010; Liu et al., 2022; Odlaug et al., 2016; Page et al., 2009; van den Heuvel et al., 2005), including abnormal ventral striatal morphology in trichotillomania (Isobe et al., 2018) and enhanced resting state connectivity between frontal cortex and ventral striatal regions in OCD (Harrison et al., 2013; Harrison et al., 2009). This striatal hyperactivity is recapitulated in SAP90/PSD95-associated protein 3 (SAPAP3) knock-out (KO) mice that exhibit excessive self-grooming behavior (Ade et al., 2016; Welch et al., 2007). Many compulsive disease models are characterized primarily by excessive self-grooming as it represents a highly conserved repetitive behavior with construct and face validity (Kalueff et al., 2016).

Although genetic mouse models of OCD such as SAPAP3 KOs have penetrant compulsive phenotypes (Berridge et al., 2005; Kazdoba et al., 2014; Peça et al., 2011; Xu et al., 2017), the causal mechanisms of these mutations are hard to decipher because they disrupt brain-wide synaptic transmission and often have small cumulative effects on psychiatric disease susceptibility (Bienvenu et al., 2009; Boccuto et al., 2013; Salatino-Oliveira et al., 2018). Recent circuit models suggest that the genetic and environmental factors that contribute to compulsivity may be reducible to straightforward neural dysregulations that map quite proximally onto specific disease dimensions. For example, repeated stimulation of orbitofrontal cortex inputs to the ventral striatum (OFC-VMS) increases self-grooming and enhances striatal neuron

responsivity to afferent stimulation (Ahmari et al., 2013), simulating altered resting state fronto-striatal connectivity observed in OCD patient populations (Harrison et al., 2013; Harrison et al., 2009). In other studies, inhibition of these fronto-striatal circuit elements have normalized compulsivity (Burguiere et al., 2013; Piantadosi et al., 2022; Ramírez-Armenta et al., 2022), complementing a growing body of deep brain stimulation literature in which DBS targeted to the ventral striatum has successfully alleviated symptoms in treatment-resistant OCD (Mantione et al., 2015; Senova et al., 2019; Welter et al., 2021).

A growing interest in the role of ventral striatal networks in compulsivity has highlighted the nucleus accumbens (NAc) and its afferents as possible regulators of pathological repetitive behaviors (Wood and Ahmari, 2015), a notion that is consistent with the role of the NAc in suppressing inappropriate actions. Much evidence has shown that impaired behavioral suppression is the most common consequence of disrupting the activity of the NAc (Ambroggi et al., 2011; Bracs et al., 1984; Christakou et al., 2004; Reading et al., 1991; Schoenbaum and Setlow, 2003; Stopper and Floresco, 2011) or its afferents (Bercovici et al., 2018; Do-Monte et al., 2017; Lafferty and Britt, 2020; Lafferty et al., 2020b; Piantadosi et al., 2020; Reed et al., 2018), a behavioral feature that is commonly observed in the response inhibition patterns of compulsive patient populations (Chamberlain et al., 2006; Kertzman et al., 2018; Munakata et al., 2011). Although human imaging data has highlighted fronto-striatal pathophysiology in compulsive disorders (Ahmari et al., 2013; Pascoli et al., 2018; Pascoli et al., 2014), patterned stimulation and dysregulated synaptic plasticity of other NAc inputs is thought to underlie disinhibited behavioral states. In particular, low frequency stimulation of ventral hippocampal inputs to the NAc (vHPC-NAc) reduces social avoidance (Bagot et al., 2015), while high frequency stimulation of vHPC input enhances inactive lever pressing (Pascoli et al., 2014). Similarly, opioids have been shown to potentiate paraventricular thalamic inputs to the NAc (PVT-NAc), driving aversive behaviors (Zhu et al., 2016) and disrupting behavioral suppression (Vollmer et al., 2022). Additionally, recordings from the PVT-NAc pathway reveal event-related activity when animals withhold behavioral responses (Reed et al., 2018), and reduced activity of select PVT neuronal ensembles during sucrose seeking (Vollmer et al., 2022). These data suggest that dysregulations of multiple NAc afferents may independently contribute to deficits in behavioral suppression, but their underlying role in eliciting enduring compulsivity remains unclear.

To test the causal role of dysregulated NAc afferents in eliciting compulsive behaviors, repeated photostimulation was targeted to vHPC and PVT axons in the NAc of mice. We found that burst stimulation of PVT-NAc afferents elicited enhanced self-grooming behavior which was blocked by MTEP, a metabotropic glutamate receptor (mGluR5) antagonist. Following repeated PVT-NAc stimulation over multiple days, animals exhibited a persistent compulsive phenotype, comprising excessive self-grooming resulting in facial fur loss, increased marble burying and impaired performance on a reversal learning task. Repeated stimulation of vHPC axons incompletely recapitulated this phenotype, suggesting a pathway-specific role for the PVT-NAc pathway in compulsivity. Taken together, these findings complement previous work that identified a causal role for fronto-striatal pathophysiology in driving OCD-like behaviors (Ahmari et al., 2013), suggesting that the NAc may represent a convergent site of multiple circuit vulnerabilities to psychiatric disease.

4.3. RESULTS

To assess the short-term effects of NAc afferent hyperactivity on compulsivity, we targeted expression of ChR2 to vHPC or PVT cell bodies and delivered light intracranially to stimulate axons of these cells in the NAc of mice exploring an open field chamber (**Fig. 1A**). Following a habituation period, mice received axonal photostimulation for 5 mins (40 Hz for 2 s every 10 s). Self-grooming and locomotion were measured before, during, and after this epoch, permitting within-session comparisons (**Fig. 1B**, S1). While intracranial light delivery did not overtly affect the behavior of YFP-only control mice, PVT axon stimulation enhanced the cumulative duration of self-grooming along with grooming bouts in a manner that persisted beyond the end of the photoexcitation epoch (**Fig. 1C-E**). vHPC-NAc activation temporarily enhanced locomotor activity (**Fig. 1F**), but did not otherwise affect grooming measures, suggesting pathway-specific contributions to distinct forms of behavioral disinhibition. Although activation of the PVT-NAc pathway can elicit aversive states (Lafferty et al., 2020b; Zhu et al., 2016), which could drive anxiety- or stress-evoked grooming (van Erp et al., 1994), we did not find PVT axon stimulation altered center occupancy in an open field (**Fig. S2**), which is in line with other studies that report no anxiogenic effects of pathway hyperactivity (Cheng et al., 2018). These data support the idea that PVT-NAc circuits can proximally influence self-grooming behavior and may be poised to trigger compulsive self-grooming when dysregulated.

To determine whether self-grooming triggered by PVT-NAc stimulation is a simple motoric effect or arises in a manner comparable to other rodent models of compulsivity, we tested the behavioral effects of mGluR5 antagonism, which has been shown to attenuate excessive self-grooming in several rodent models of compulsivity (Ade et al., 2016; Mehta et al., 2011; Silverman et al., 2010). An intraperitoneal (i.p.) injection of the mGluR5 negative allosteric modulator MTEP (10 mg/kg) prior to PVT axonal photostimulation in the open field blocked the effect of enhanced self-grooming without affecting basal grooming levels (**Fig. 2A-C, S3**). vHPC-NAc locomotor enhancement was not influenced by MTEP (**Fig. 2D**), further demonstrating that MTEP's effects were specific to stimulation-induced grooming. This finding suggests a convergent mechanism of aberrant glutamatergic signaling in ongoing compulsive behavior which supports recent clinical research into the efficacy of mGluR-targeting drugs in compulsive patient populations (Grados et al., 2015; Stoppel et al., 2021). That said, the effect of PVT-NAc activation is short-lived, and enhanced grooming may be an incidental, non-physiological outcome of synchronous stimulation. We next sought to test whether repeated PVT axonal stimulation can elicit lasting changes in self-grooming.

We hypothesized that enduring dysregulations of PVT-NAc circuitry would contribute to compulsivity over longer timescales, so we targeted optogenetic stimulation to vHPC or PVT axons with 40 Hz bursts spanning 5 mins, repeated on five consecutive days (**Fig. 3A**). Self-grooming was measured in an open field during the 15 min habituation period prior to intracranial light delivery. PVT-NAc stimulation elicited a progressive increase in self-grooming behavior that persisted beyond the final photostimulation epoch (**Fig. 3B,C**) and resulted in facial fur loss three weeks later (**Fig. 3D,E**). No effect of photostimulation was observed in vHPC-NAc mice, indicating a unique role for PVT afferents in driving an enduring and maladaptive alteration in self-grooming. As before, systemic MTEP administration reduced self-grooming to basal levels (**Fig. 3F**).

Although aberrant self-grooming was our primary measure, marble burying was used as an additional convergent measure of compulsivity to validate the generalizability of our circuit model. While burying, burrowing, and digging comprise the typical rodent behavioral repertoire (Deacon, 2006a), rodents that persist in burying harmless stimuli may be exhibiting non-functional repetitive behavior analogous to disease-related compulsivity (Umathe et al., 2012). We found that repeated PVT axonal stimulation increased marble burying several days after

photostimulation (**Fig. 3G**), without affecting anxiety-related open field measures (**Fig. 3H**). Altogether, these data suggest a broader role for PVT-NAc circuits in dysregulating behavioral suppression to produce a compulsive phenotype. Accordingly, we next assessed the consequences of repeated afferent stimulation on a reward seeking task requiring cognitive flexibility and suppression of inappropriate behavioral responses.

Many compulsive patient populations exhibit global deficits in response inhibition, particularly during go/no-go tasks and games requiring task-switching (Chamberlain et al., 2006; Ganos et al., 2014; Gu et al., 2008; Kertzman et al., 2018; Munakata et al., 2011), so we sought to test the performance of over-grooming PVT-NAc mice on a reversal learning task. We trained mice to alternate pressing one of two levers for food reward such that the identity of the rewarding and non-rewarding levers reversed after five consecutive presses on the rewarding lever, measuring these reversals, and perseverative errors on the unrewarding lever (**Fig. 4A**). Across daily sessions, task performance improved as animals carried out more reversals relative to rewards obtained (**Fig. 4B,C**), and reduced the proportion of perseverative errors made (**Fig. 4D**). Once performance stabilized, mice received 5 days of daily axonal photostimulation as above (**Fig. 3A,4A**). We found that activation of PVT afferents impaired task performance two weeks after photostimulation, reducing the number of reversals made per reward without affecting perseverative errors (**Fig. 4E,F**). This result indicates that while animals made a similar proportion of correct lever presses across a session, strings of consecutive presses were frequently interrupted by perseverative errors, suggesting a degree of behavioral inflexibility. Notably, repeated activation of the vHPC-NAc pathway also impaired task performance in the days following photostimulation and up to two weeks later, persistently reducing reversals but only enhancing perseverative errors for a few days (**Fig. 4E,F**). Taken together, these findings suggest that overactivation of multiple NAc afferents can promote ineffective reward seeking that arises from enduring deficits in response inhibition. While we observed only modest effects on perseveration (**Fig. 4F**), frequent returns to the previously rewarded lever are reminiscent of "checking" behaviors observed in compulsive patient populations and rodent models (Benzina et al., 2021), which may better account for deficits in behavioral flexibility.

4.4. DISCUSSION

Disorders such as OCD, ASD and trichotillomania are heterogeneous in terms of clinical presentation and etiology, but patients vary commonly along a few symptom dimensions (Allen et al., 2003). While personalized psychiatry will be necessary to address this heterogeneity (Alda, 2013), better primary therapies must target the particular neural system dysfunctions that drive distinct behavioral deficits. Compulsivity, with its links to aberrant striatal signaling (Breiter et al., 1996; Burguiere et al., 2015; Gu et al., 2008; Leckman et al., 2010; Liu et al., 2022; Odlaug et al., 2016; Page et al., 2009; van den Heuvel et al., 2005), is one such transdiagnostic construct that aligns with symptom axes put forth by frameworks like the Research Domain Criteria (RDoC) (Pittenger et al., 2019). While animal models will never capture the heterogeneity and complexity of mental disorders, constituent elements of compulsivity such as repetitive self-directed motor behaviors and cognitive inflexibility may help us to identify neural circuits for targeted therapeutics. In the present study, we assessed the causal role of NAc afferent hyperactivity in eliciting compulsivity. Although previous work has highlighted the contribution of fronto-striatal circuits to excessive self-grooming, we have identified a complementary role for PVT-NAc inputs. Activation of this pathway elicited both a short- and long-term enhancement in self-grooming behavior, resulting in facial fur loss. Increased marble burying and impaired reversal learning emerged alongside enhanced self-grooming, suggesting a broader impact of axonal stimulation on behavioral inhibition. By comparison, vHPC-NAc stimulation did not affect self-grooming, but hindered performance on a reversal learning task. These results identify a novel NAc circuit vulnerability to compulsivity and are consistent with a distributed role for multiple NAc circuit elements in behavioral inhibition (Lafferty et al., 2020a). Since we have probed neural pathways that parallel corticostriatal inputs, our circuit model of compulsivity will permit investigation of how diverse neural dysregulations give rise to convergent behavioral phenotypes.

Self-grooming is a highly conserved, innate, self-directed sequence of motor behaviors (Spruijt et al., 1992). Aberrant self-grooming is a core feature of numerous rodent models of compulsivity (Ahmari et al., 2013; Berridge et al., 2005; Mangieri et al., 2018; Shmelkov et al., 2010; Ullrich et al., 2018; Welch et al., 2007), and has obvious translational value for disorders involving abnormal self-grooming in humans, including trichotillomania, dermatillomania, and contamination-related OCD (Fineberg et al., 2010; Maraz et al., 2017; Rasmussen and Eisen,

1992). Although we have relied on a binary measure of grooming behavior here, self-grooming comprises a rich repertoire of behavioral syllables that are flexibly sequenced to produce stereotyped and non-stereotyped chains (Berridge et al., 1987; Kalueff et al., 2007). Future characterization of our circuit model of compulsivity should consider self-grooming as a multidimensional variable and identify effects of NAc afferent stimulation that are specific to properties of the grooming sequence. Nevertheless, we have revealed a role for PVT-NAc circuits in repetitive self-grooming, situating the ventral striatum as a regulator of the cognitive and affective processes that disinhibit and reinforce repetitive self-grooming (Wood and Ahmari, 2015).

Drug addiction has long been associated with dysregulated glutamate signaling in the NAc (Scofield et al., 2016), and recent studies in compulsive patient populations and rodent models of compulsivity also implicate glutamate (Mahone et al., 2018; McGrath et al., 2000; Pittenger et al., 2011; Presti et al., 2004). In fact, therapies that target mGluR5 signaling are the subject of ongoing clinical research to treat compulsivity (Grados et al., 2015; Stoppel et al., 2021). mGluR5 is located perisynaptically (Mitrano and Smith, 2007), and is likely activated primarily by glutamate spillover caused by elevated local glutamate release (Baker et al., 2002). mGluR5 signaling regulates the physiology (Niswender and Conn, 2010) and plasticity (Lüscher and Huber, 2010) of the NAc, suggesting a role in shaping ongoing and future behavior. In line with our work, mGluR5 drugs have been shown to bidirectionally influence self-grooming in SAPAP3 KO mice (Ade et al., 2016). Furthermore, PVT axons in the NAc preferentially target mGluR5-containing spines (Mitrano et al., 2010), and high frequency stimulation of NAc afferents has been suggested to trigger mGluR-mediated LTD, even at neighbouring inputs (Pascoli et al., 2014). Aberrant striatal plasticity is a hallmark of repetitive self-grooming models, often involving complex profiles of physiological adaptation that include concurrently weakened synaptic inputs and enhanced striatal excitability (Ade et al., 2016; Hadjas et al., 2020; Shmelkov et al., 2010; Welch et al., 2007). Recent studies have shown that hyperactivity in indirect pathway striatal neurons may be a cause of excessive self-grooming in SAPAP3 KO mice (Piantadosi et al., 2022; Ramírez-Armenta et al., 2022). Although these studies focus on the dorsolateral striatum, others have found that strengthened PVT input to indirect pathway NAc neurons underlies disinhibited behavioral states in models of opiate relapse (Keyes et al., 2020; Zhu et al., 2016). It remains to be seen whether PVT-NAc-mediated enhancements in self-

grooming are driven by aberrant glutamate neurotransmission and ensuing imbalances in direct and indirect pathway neuron activity. Of course, synaptic dysregulations and afferent hyperactivity have complex effects on *in vivo* network activity, so a significant challenge of this future work will be to identify distributed loci of synaptic and physiological alterations that collectively elicit the repetitive self-grooming phenotype (Lafferty et al., 2021).

Dopaminergic signals that converge on the NAc may promote and reinforce aberrant patterns of self-grooming that are themselves gratifying or that relieve anxiety (Fineberg et al., 2014). This idea is supported by evidence that hyperdopaminergic mice exhibit more rigid patterns of self-grooming behavior (Berridge et al., 2005), and that NAc dopamine levels are transiently elevated during self-grooming (Jørgensen et al., 2023). Furthermore, a neural population in the medial paralemniscal nucleus was found to elicit self-grooming and alleviate post-stress anxiety in a manner that raised ventral tegmental area (VTA) dopamine signaling in the NAc (Sun et al., 2022). PVT-NAc stimulation has also been shown to increase NAc dopamine levels (Parsons et al., 2007), suggesting one possible mechanism by which afferent hyperactivity may recruit dopamine's neuromodulatory effects to drive repetitive self-grooming. PVT-NAc proximity to dopamine signaling may also account for differences in the immediacy of enhanced self-grooming elicited in our circuit model and in fronto-striatal models of compulsivity (Ahmari et al., 2013), a notion which is further supported by the relatively sparse and distinct innervation pattern of prefrontal cortex inputs to the NAc (Britt et al., 2012).

Despite the translational value of self-grooming in psychiatric disease models, compulsivity is a multifaceted construct that comprises more than just excessive grooming behavior (Fineberg et al., 2014). Many theoretical models of compulsivity propose that repetitive behaviors emerge as a consequence of impaired behavioral inhibition (Chamberlain et al., 2006; Ganos et al., 2014; Gu et al., 2008; Kertzman et al., 2018; Munakata et al., 2011), suggesting a domain general behavioral deficit that is detectable on a myriad of assays. Disturbances in NAc physiology frequently produce similar deficits, resulting in inefficient reward seeking behavior under many conditions (Ambroggi et al., 2011; Blaiss and Janak, 2009; Bowman and Brown, 1998; Dalton et al., 2014; Floresco, 2015; Floresco et al., 2008; Lafferty and Britt, 2020; Lafferty et al., 2020b; Millan et al., 2015; Peters et al., 2008; Reading and Dunnett, 1995; Reading et al., 1991; Stopper and Floresco, 2011; Yun et al., 2004). To test whether disruptions of NAc physiology that elicit repetitive self-grooming also impair behavioral suppression, we assayed

marble burying and reversal learning tasks and identified dissociable effects of PVT-NAc and vHPC-NAc stimulation.

Previous studies in patient populations have highlighted impulsivity and compulsivity as distinct constructs that reflect inclinations toward unplanned, reactive behavior and repetitive, stereotyped behaviors, respectively (Chamberlain and Sahakian, 2007). Although these behaviors are thought to arise for opposing reasons related to pleasure-seeking and harm-avoidance, respectively (Fineberg et al., 2014), impulsivity and compulsivity are difficult to dissociate in rodents since behavioral enhancement and behavioral disinhibition both cause an increase in measured behavior. While it is possible to make inferences, the underlying motivation for elevated behavior is typically inaccessible and the relevant neurobiological substrates are highly overlapping (Grant and Kim, 2014; Robbins et al., 2012). A growing body of evidence has recognized that impulsivity and compulsivity are likely linked by a shared dysfunctional inhibition of thoughts and behaviors (Fineberg et al., 2014), hence our broad focus on mechanisms of behavioral suppression that implicate striatal systems and their inputs.

Enhanced marble burying reflects a disinhibition of natural rodent behaviors that involves burying harmless stimuli (Deacon, 2006b). This assay remains controversial for its apparent relevance to many psychiatric disease models and its sensitivity to such a wide variety of pharmacological interventions, which undermines its predictive validity (Alonso et al., 2015). Nevertheless, the perseverative features of marble burying and its ease of implementation have made it a benchmark for measuring compulsivity in rodent models. Despite the sensitivity of this assay, we have identified a limited effect of PVT-NAc circuit disruption, rather than brain-wide involvement of a single neurotransmitter system, which provides greater mechanistic insight than typical behavioral screening approaches. Moreover, this effect provides convergent evidence for a general compulsive phenotype, demonstrating the utility of marble burying as a complement to other measures of compulsivity with more face, construct, and predictive validity.

Like enhanced marble burying, impaired reversal learning has long been thought to reflect a deficit in behavioral inhibition which, in these and similar tasks, is often framed as cognitive inflexibility (Jones and Mishkin, 1972). Although this unitary view of response inhibition has been revised to account for dissociable measures of inflexibility, a persistence of inefficient behavioral strategies remains foundational (Izquierdo et al., 2017). For example,

performance on reversal learning tasks can be described in terms of trial-by-trial responses to positive and negative reward outcomes (Izquierdo et al., 2013; Klanker et al., 2015) or in terms of whether the animal has developed a model of the task to predict reversals (Costa et al., 2015), but deficits on either measure implicate overlapping basal ganglia circuits that shape decision making at these different timescales and across a variety of modalities (Izquierdo et al., 2017). Here we show that persistent hyperactivity of vHPC-NAc or PVT-NAc afferents causes a lasting reduction of reversal behavior without impacting perseverative errors or overall reward attainment in a reversal learning task. This profile of behavior indicates that animals will frequently revisit the unrewarded lever, preventing sufficient consecutive rewarded lever presses from triggering reversals. This result is consistent with recent cross-species evidence that reversal learning deficits in OCD patient populations and SAPAP3 KO mice are driven by excessive response lability rather than perseveration (Benzina et al., 2021). In this study, a fraction of individuals alternated task responses despite consistent positive feedback, and measures of this spontaneous change in patients correlated with the severity of checking symptoms. This finding suggests a role for NAc afferent dysregulation in a particular subtype of compulsive behaviors. Future investigations should ply the rich behavioral datasets generated by reversal learning tasks to identify distinct aspects of cognitive flexibility that are altered in rodent models of compulsivity that align with particular clinical subtypes.

Despite the wealth of new studies identifying the role of the PVT-NAc circuit in aversion and behavioral inhibition (Do-Monte et al., 2017; Kessler et al., 2021; Keyes et al., 2020; Ma et al., 2021; Vollmer et al., 2022; Zhu et al., 2018; Zhu et al., 2016), comparatively less emphasis is given to other NAc afferents and their dual role in promoting and suppressing behavior. vHPC-NAc afferents are known to promote spatially-mediated reward seeking (Floresco et al., 1997; Ito et al., 2008), but lesion studies have also implicated vHPC activity in behavioral inhibition under conditions of threat (Bryant and Barker, 2020; McNaughton and Gray, 2000). This contribution to approach-avoidance conflict is comparable to recent theories about the role of PVT-NAc circuits in motivated behavior (Choi et al., 2019; Choi and McNally, 2017) and is consistent with the larger idea that bidirectional control of behavioral gain is a critical distributed feature of NAc circuits (Lafferty and Britt, 2020; Lafferty et al., 2020a; Reed et al., 2018). While we have shown that vHPC-NAc afferents do not influence self-grooming behavior, we have highlighted an important consequence of vHPC-NAc dysfunction in behavioral inhibition. Future

work remains to demonstrate whether this dysregulation is mediated primarily by altered vHPC-NAc connectivity or by effects elsewhere in the network, including disrupted local or distal interactions with prefrontal cortex (Meyer et al., 2019; Pascoli et al., 2014) and amygdala circuits (Jimenez et al., 2018).

Overall, we conclude that hyperactivity of NAc afferents induces enduring deficits in behavioral inhibition, and we highlight a distinct role for PVT-NAc circuits in compulsivity. Our findings complement previous fronto-striatal models of disease by identifying a parallel circuit for induction of repetitive self-grooming. While underlying mechanisms remain to be elucidated, this work suggests that dysregulated glutamate signaling through mGluRs may be a convergent aspect of compulsive disease etiology. The NAc is a critical site for psychiatric disease susceptibility and normalizing its dysfunction represents a promising means of addressing the pathophysiology underlying compulsive disorders.

4.5. MATERIALS & METHODS

4.5.1. EXPERIMENTAL ANIMALS

Male and female C57BL/6 wildtype mice were used. All animals were bred in-house. Mice were housed on a reverse light cycle 12 h photoperiod. At approximately three months of age (25-30 g), animals underwent surgery. Six weeks following surgery, animals undergoing operant training were placed on a restricted feeding schedule to maintain 85-90% of pre-surgery body weight. A daily weight and feeding log were maintained for the duration of all experiments. All experiments were conducted in accordance with the Canadian Council of Animal Care and the McGill Animal Care Committee.

4.5.2. VIRAL CONSTRUCTS AND SURGERY

Prior to surgery, animals were anesthetized using a ketamine (Ventoquinol, 100mg/kg) and xylazine (Bayer, 10mg/kg) cocktail. The skull of the animal was then secured to a stereotaxic frame (Kopf Instruments) and prepared for intracranial virus injections according to standard stereotaxic procedure. 700 nL of virus was injected over a ten-minute period using a Nanoject II Injector with an oil-filled glass micropipette (Drummond Scientific, 3-000-203-G/X) pulled to a tip diameter of 10 µm. All injections were targeted bilaterally. Viruses were obtained from the Canadian Neurophotonics Platform (CNP) and used at 5.0 x 10^{12} GC/mL.

For all axonal photostimulation experiments, AAV5-CamKIIα-hChR2-eYFP or AAV1-hSyn-ChR2-GFP was delivered to the vHPC (distance from bregma: AP -3.6 mm, ML ±3.25 mm, DV -4.25 mm) or the PVT (AP -1.0 mm, ML ±0.35 mm, DV -3.12 mm) of mice. AAV9-CamKIIα-eYFP was targeted to vHPC or PVT of control mice. Ten minutes later, two 200 µm optical fibers (0.37 NA) were implanted above the NAc (10° angle, AP 1.5 mm, ML ±1.4 mm, DV -4.57 mm).

4.5.3. OPTOGENETICS AND BEHAVIORAL TESTING

Optogenetics. Behavioral experiments were carried out 8 weeks after surgery. To test the short-term effects of NAc afferent photostimulation, mice were optically tethered, then placed in an open field chamber (8.5 x 7 inches) and permitted to freely explore for 30 minutes. After a 15 min habituation period in the open field, light was delivered intracranially for five minutes (**Fig. 1B**). This photostimulation consisted of 2 s 40 Hz trains occurring every 10 s for 5 min (473 nm,

10 mW, 5 ms pulse duration). For chronic afferent stimulation, mice received 5 min of intracranial light delivery as described above, repeated on 5 consecutive days, and grooming behavior was measured over the 15 min habituation period prior to photostimulation.

Open field measures. Mouse behavior including self-grooming, locomotion and center/surround occupancy of the open field was recorded in 5 min bins, before, during, and after photostimulation using video tracking software (Ethovision, Noldus Information Technology; 30 Hz recordings). Self-grooming was scored using Noldus' Behavior Recognition Software and the accuracy of automated scoring was quantified by comparison to manually scored grooming on multiple metrics (**Fig. S1**). We found that automated scoring correctly labeled grooming in 82.7 ± 1.7% of 1s bins across five random samples of 4-minute mouse behavior spanning periods during and after axonal photostimulation (**Fig. S1B**). Moreover, the statistics of self-grooming behavior across experimental and control groups did not differ significantly between automated and manually scored conditions (**Fig. S1C,D**). Center and surround occupancy in the open field was quantified for the entire 30 min session. Qualitative fur loss was scored by blind raters 3 weeks after the last day of NAc axonal photostimulation.

To test the effects of mGluR5 signaling on stimulation-evoked self-grooming, mice received an intraperitoneal (i.p.) injection of either 3-((2-Methyl-4-thiazolyl)ethynyl)pyridine (MTEP) (5-20 mg/kg) or saline 10 min before placement in the open field (**Fig. 2A, 3A**), counterbalanced across days.

Marble Burying. Polycarbonate rat cages (20 cm X 48 cm X 20 cm) were filled with 5 cm of unscented mouse bedding material. Standard glass toy marbles (of assorted styles and colors, 5.2 g) were gently placed on the surface of the bedding in 5 rows of 4 marbles. Marbles were washed in mild laboratory detergent and rinsed prior to each use. Mice were kept in a well-lit room for 60 min prior to the behavioral assay and then placed in the testing chamber. After 30 min, animals were returned to their home cages and marbles were scored as 'buried' if two-thirds of their surface was covered by bedding (Angoa-Pérez et al., 2013). Marble burying was assessed before and after repeated NAc afferent stimulation (**Fig. 3A**).

Reversal Learning Task. Mice were trained in sound attenuating chambers that had two levers available on either side of a centrally located food receptacle (Med Associates). A houselight and

speaker were located on the opposite side of the chamber. Behavioral data were collected using Med Associates software.

Food deprived mice were placed in an operant chamber for 30 min sessions. Availability of a rewarded and inactive lever varied throughout training. Rewarded lever presses always resulted in delivery of 30 μL of a 15% sucrose solution (m/v), a tone presentation (4.8 kHz, 80dB, 5 s duration) and 10 s retraction of any extended levers. Inactive lever presses only caused 10 s lever retractions. Training consisted of four FR1 phases: standard, reversed, mixed, and reversal learning. During standard FR1 training, mice had access to a single rewarded lever whose spatial position was counterbalanced within groups. This phase continued until mice obtained 30 rewards in a single session. Mice then underwent reversed FR1 training, in which the position of the rewarded lever was now on the opposite side of the food receptacle. Once animals obtained 30 rewards in a single session, they transitioned to mixed FR1 training in which the rewarded lever randomly switched positions following each lever press. After two days of training, mice typically obtained at least 20 rewards between presses on each lever. During the last phase of training, mice had access to both a rewarded and inactive lever and underwent reversal learning. Here, five consecutive presses on the rewarded lever resulted in a reversal, in which the identity of the rewarded and inactive levers was swapped. Presses on the inactive lever were counted as perseverative errors. After 21 days of training, baseline task performance was assessed over 7 days and animals then received daily axonal stimulation for five days. Following stimulation, reward seeking behavior was measured for an additional 7 days, and then 2 weeks later during a single reversal learning session (**Fig. 4A**). Reversals were normalized to the total number of rewards obtained to account for daily fluctuations in overall reward seeking, and perseverative behavior was computed as the number of inactive lever presses made as a fraction of total lever presses. Performance across training was also quantified by normalizing the number of reversals made in each session to a chance number of reversals.

4.5.4. HISTOLOGY

At the end of each behavioral experiment, animals were anesthetized with 270 mg/kg Euthansol (Merck) and transcardially perfused with 4% paraformaldehyde (PFA, Sigma-Aldrich). Brains were removed, post-fixed in PFA for 24 h, and then transferred to PBS for 48 h. Tissue was then

sliced into 60 μm sections on a vibratome (Leica VT1000s) and mounted on microscope slides with a MOWIOL plus DAPI (Sigma-Aldrich) solution.

4.5.5. QUANTIFICATION AND STATISTICAL ANALYSIS

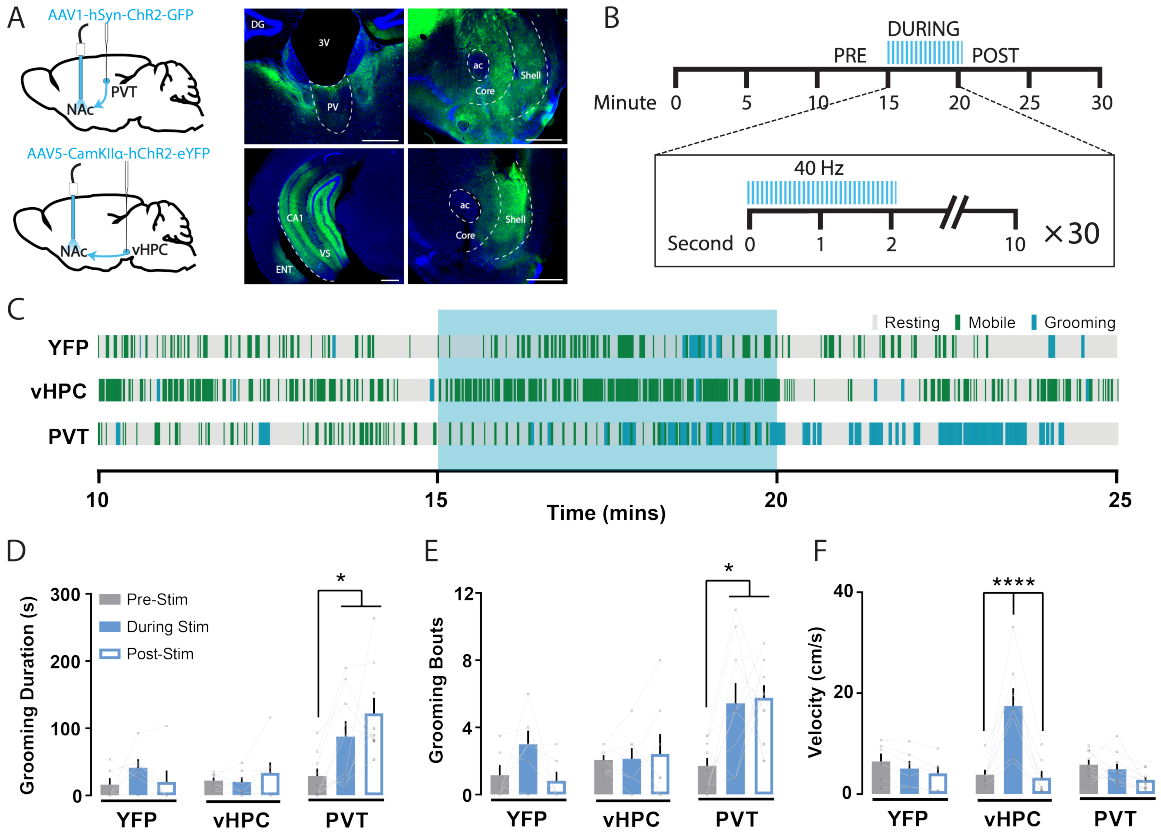
Two-tailed paired t-tests, repeated measures one-way ANOVAs (with Geisser-Greenhouse correction), and mixed two-way ANOVAs were used for statistical comparisons of behavior across photostimulation conditions and across pathways.

Sidak's multiple comparisons tests were conducted for all ANOVA *post-hoc* tests. The significance of all statistical tests was determined using $\alpha = 0.05$. Unless otherwise indicated, all data are reported as the mean ± SEM.

Fur loss scores and marble burying data are better modeled by a Poisson distribution than a normal distribution (Lazic, 2015). A Poisson generalized linear model was fit to these data and significant regression coefficients (β) were used to analyze the relative contributions of pathways and photostimulation conditions to these behaviors. For these analyses, data are reported as mean ± 95% confidence interval (CI).

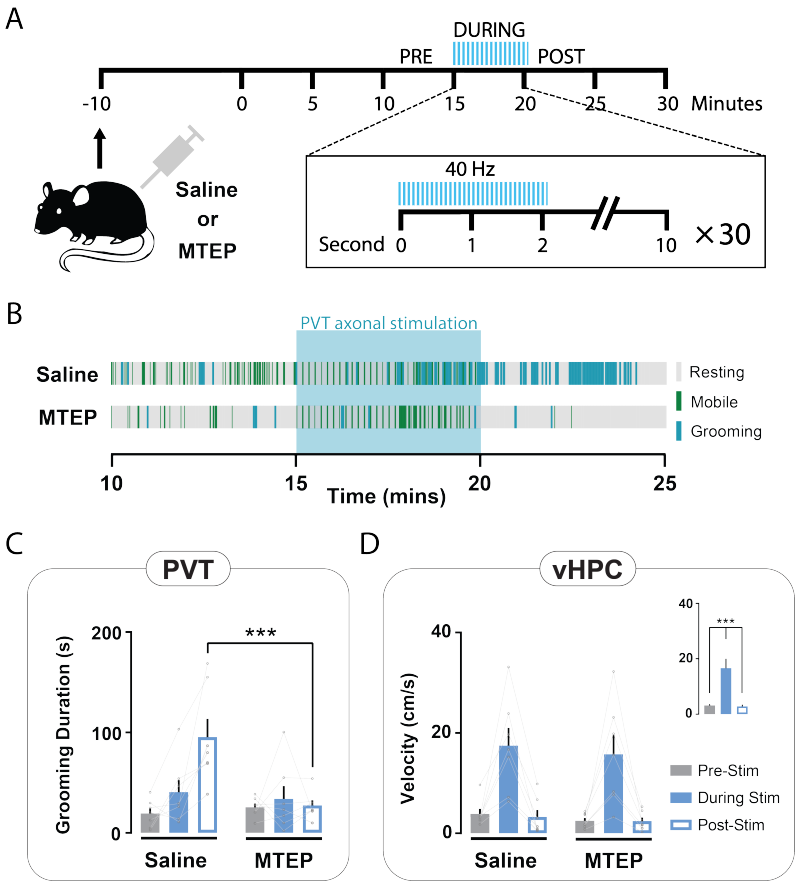
4.6. FIGURES

FIGURE 1. BRIEF STIMULATION OF PVT-NAC AFFERENTS ENHANCES SELF-GROOMING BEHAVIOR.



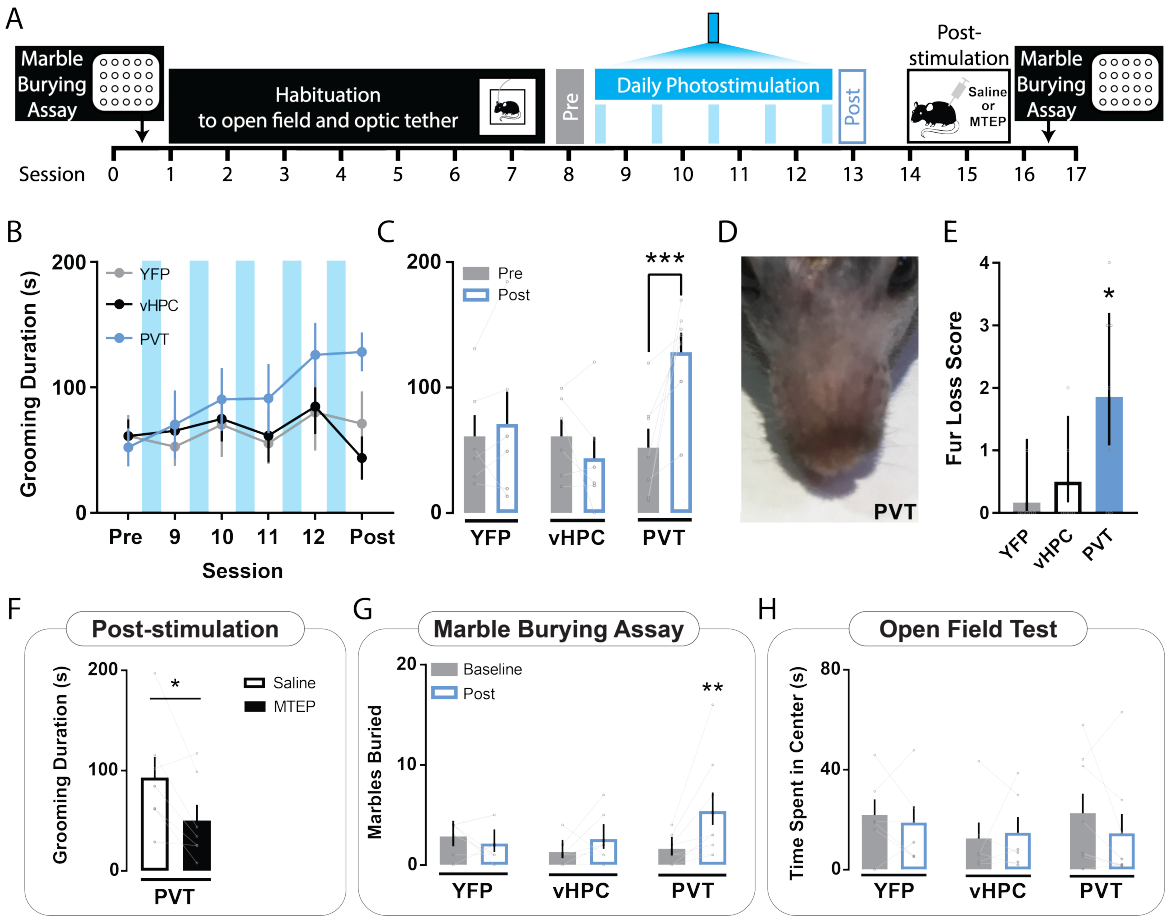
A) Schematic of virus injections (left) alongside representative coronal brain slices showing sites of virus delivery in vHPC and PVT (top right) and axonal fluorescence in the NAc (bottom right). Scale bars, 500 µm. **B)** Schematic of 5 min photostimulation delivered during 30 min session in an open field. **C)** Ethograms from representative mice before, during and after axonal photostimulation. **D)** Summary of cumulative self-grooming (N = 22) and **E)** number of grooming bouts in relation to photostimulation. PVT axon activation (n_{PVT} = 9) enhanced grooming duration ($F_{(4,38)} = 3.20$, $p < 0.05$) and grooming bouts during and after light delivery ($F_{(4,38)} = 2.97$, $p < 0.05$). For the significant post hoc comparisons of self-grooming across PVT stimulation epochs, $t_{(38)} > 3.02$, *$p < 0.05$. **F)** Summary of the effect of axonal photostimulation on locomotion. Running velocity was enhanced only during vHPC afferent activation (n_{vHPC} = 7; $F_{(4,38)} = 14.54$, $p < 0.0001$; post-hoc comparisons of stimulation epochs in vHPC mice, $t_{(38)} > 7.89$, ****$p < 0.0001$). Error bars represent SEM.

FIGURE 2. mGLUR5 SIGNALING IS NECESSARY FOR ENHANCED SELF-GROOMING ELICITED BY PVT-NAC STIMULATION.



A) Schematic of drug injection relative to axonal photostimulation. **B)** Ethogram from a representative mouse and **C)** summary showing the effect of a 10 mg/kg MTEP injection on behavioral changes induced by PVT axonal photostimulation ($n_{PVT} = 7$). Compared to a saline injection, MTEP attenuated post-stimulation enhancement in cumulative self-grooming ($F_{(2,12)} = 10.35$, $p < 0.01$; post-hoc comparison of drug effect on post-stimulation grooming, $t_{(12)} = 5.52$, ***$p < 0.001$). **D)** MTEP injection did not affect locomotor increases induced by vHPC-NAc stimulation ($n_{vHPC} = 7$; $F_{interaction\ (2,12)} = 0.12$, $p = 0.89$; $F_{stimulation\ epoch\ (2,12)} = 17.59$, $p < 0.001$; post-hoc comparisons of velocity across stimulation epochs, $t_{(12)} > 5.07$, ***$p < 0.001$). Error bars represent SEM.

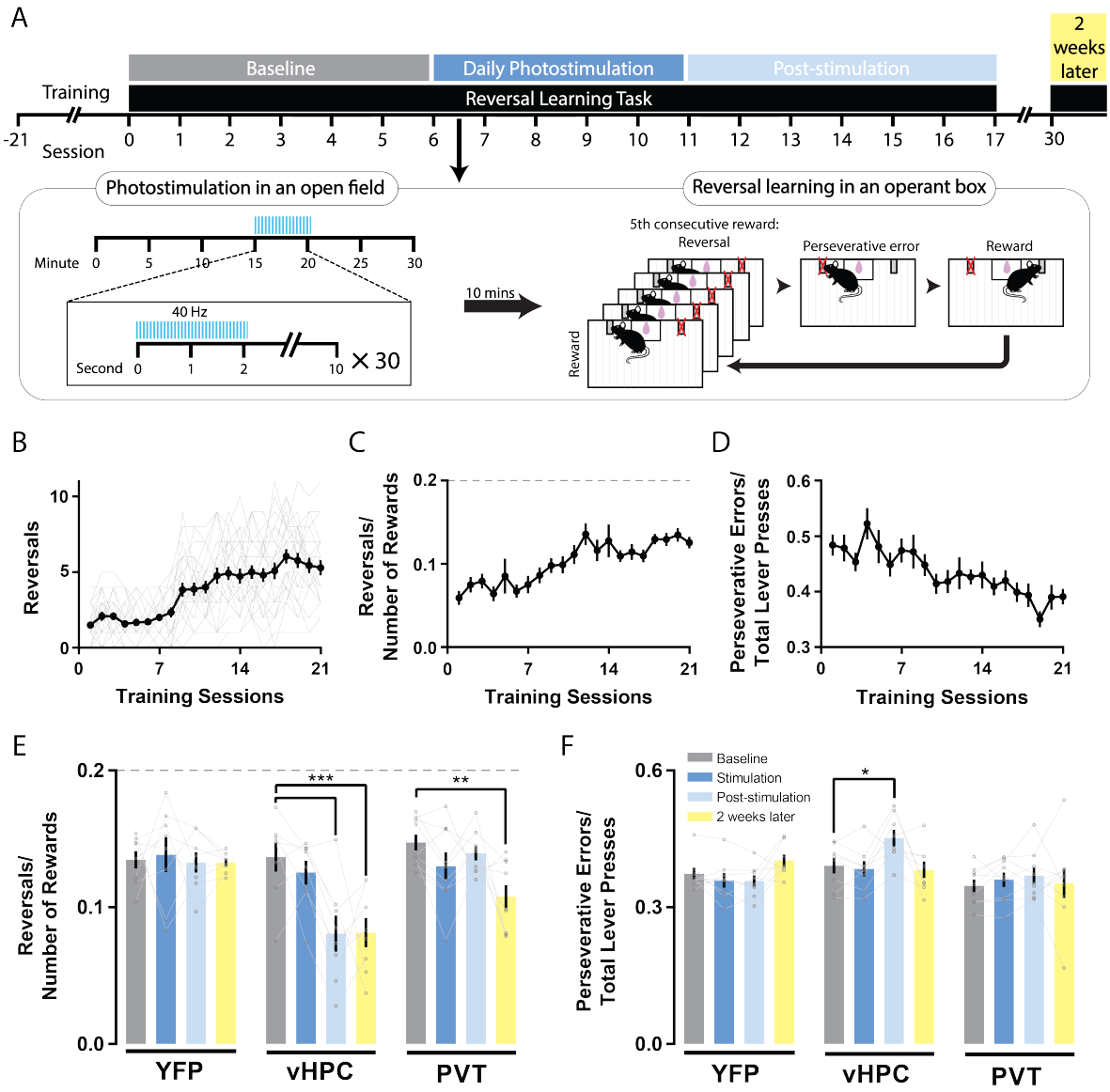
FIGURE 3. REPEATED STIMULATION OF PVT-NAC AFFERENTS ELICITS A PROGRESSIVE AND LASTING INCREASE IN SELF-GROOMING BEHAVIOR.



A) Schematic of experimental time course. Behavior was measured before, during and after 5 days of daily axonal photostimulation. **B)** Summary of cumulative self-grooming (N = 19) across daily photostimulation session, measured during the 15 min habituation period prior to intracranial light delivery. **C)** Comparison of self-grooming during pre- and post-stimulation days as shown in B. 5 days of daily PVT axon photostimulation ($n_{PVT} = 7$) cumulatively enhanced self-grooming ($F_{(2,16)} = 7.94$, $p < 0.01$; post-hoc $t_{(16)} = 4.59$, ***$p < 0.001$). **D)** Representative image and **E)** summary of facial fur loss induced by repeated NAc afferent stimulation. PVT-NAc mice exhibit elevated facial fur loss (Poisson GLM: $R^2 = 0.36$; $\beta_{PVT} = 2.41 \pm 1.04$; $t_{(16)} = 2.32$, *$p < 0.05$). Error bars represent 95% CIs. **F)** MTEP attenuated enhanced self-grooming induced by repeated PVT axon activation ($t_{(6)} = 2.91$, *$p < 0.05$). **G)** Summary of marble burying behavior measured before and after daily photostimulation sessions. Repeated activation of PVT axons increased number of marbles buried (Poisson GLM: $R^2 = 0.21$; $\beta_{PVT\ after}$

$_{photostimulation}$ = 1.48 ± 0.47; $t_{(38)}$ = 3.12, **p < 0.01). Error bars represent 95% CIs. **H)** Summary of centre occupancy in an open field before and after daily photostimulation sessions, showing no effect of axonal stimulation ($F_{(2,17)}$ = 0.67, p = 0.52). Error bars represent SEM unless otherwise stated.

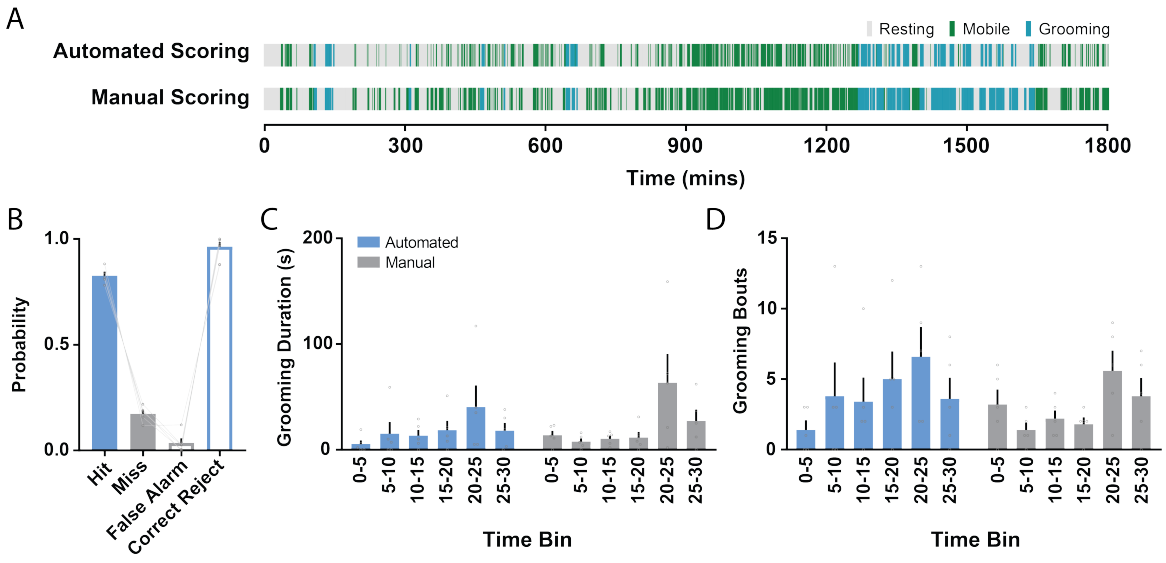
FIGURE 4. REPEATED STIMULATION OF NAC AFFERENTS IMPAIRS PERFORMANCE ON A REVERSAL LEARNING TASK.



A) Schematic of experimental time course. Task performance was measured before, during and after 5 days of daily axonal photostimulation. **B-D)** Summary of task acquisition (N = 24). Number of reversals **(B)** and reversals normalized to rewards obtained **(C)** increased across daily sessions. The dotted gray line represents optimal reversal behavior; 1 reversal for every 5 rewards obtained. Number of perseverative errors measured as a fraction of total lever presses also decreased over training **(D)**. **E)** Summary of normalized reversals in relation to daily photostimulation. Reversals were reduced following vHPC (n_{vHPC} = 8) or PVT (n_{PVT} = 8)

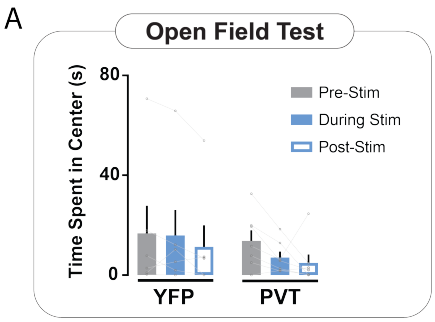
afferent activation ($F_{(6,66)} = 3.345$, $p < 0.01$). In vHPC mice this effect emerged immediately after stimulation and persisted up to two weeks later (post-hoc comparisons to baseline reversals, $t_{(66)} > 4.31$, ***$p < 0.001$), but PVT mice only exhibited deficits at the two-week followup (post-hoc comparison to baseline reversals, $t_{(66)} = 3.28$, **$p < 0.01$). **F)** Summary of the proportion of perseverative errors made in relation to daily photostimulation. vHPC afferent activation enhanced perseverative errors in the 7 days following daily photostimulation ($F_{(6,66)} = 2.874$, $p < 0.05$; post-hoc $t_{(66)} = 2.89$, *$p < 0.05$). Error bars represent SEM.

FIGURE S1. SELF-GROOMING MEASURES ARE SIMILAR BETWEEN AUTOMATED AND MANUALLY SCORED VIDEO.



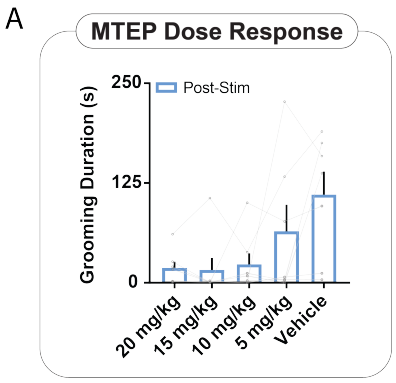
A) Ethogram from a representative mouse showing comparable behavioral classification between automated and manual scoring. **B)** Signal detection statistics showing that automated video scoring correctly classified grooming behavior with 82.7 ± 1.7% accuracy (n = 5). **C-D)** Comparison of self-grooming statistics obtained with automated or manual scoring. The distribution of cumulative grooming durations across a 30 min session **(C)** did not differ significantly between scoring conditions (n = 5; $F_{(1,4)} = 1.93$, $p = 0.24$), nor did the count of grooming bouts **(D)** ($F_{(1,4)} = 6.55$, $p = 0.06$).

FIGURE S2. BRIEF STIMULATION OF PVT-NAC AFFERENTS DID NOT AFFECT ANXIETY-LIKE BEHAVIOR IN AN OPEN FIELD.



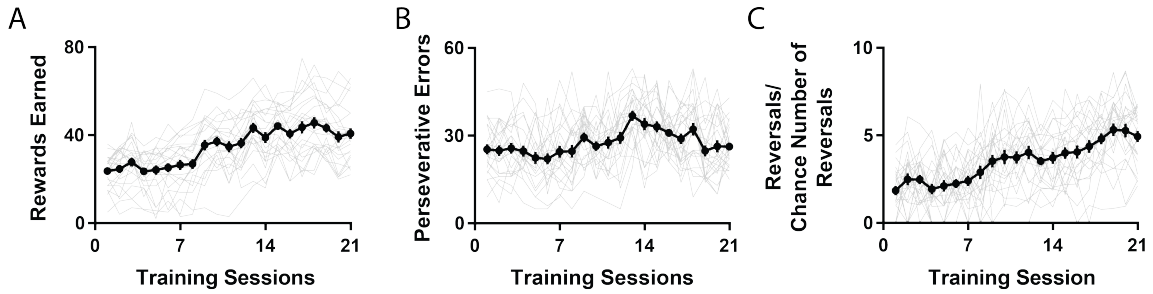
A) Summary of centre occupancy in an open field before, during and after intracranial light delivery, showing no effect of PVT-NAc axonal photostimulation ($n_{YFP} = 6$; $n_{PVT} = 7$; $F_{(2,22)} = 0.70$, $p = 0.51$).

FIGURE S3. ENHANCED SELF-GROOMING ELICITED BY PVT-NAC STIMULATION IS ATTENUATED BY MTEP INJECTION IN A DOSE-DEPENDENT MANNER.



A) Summary of MTEP's effects on self-grooming measured in the 5 min following PVT axon photostimulation (n = 7). Higher doses more strongly attenuated enhanced self-grooming ($F_{(2.43,14.59)} = 4.627$, $p < 0.05$).

FIGURE S4. SUMMARY OF REVERSAL LEARNING TASK PERFORMANCE.



A-C) Summary of rewards earned **(A)**, raw perseverative errors made (B), and reversals normalized within-animal and within-session to the chance number of reversals made given the total number of lever presses made **(C)** (N = 24).

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CHAPTER 5 – DISCUSSION

5.1. SUMMARY

In this thesis we validated a technique for projection-specific silencing and then leveraged it to inhibit NAc afferents and cell types during a reward seeking task that incorporated recurring periods of reward unavailability. We also asked whether transient stimulation of NAc afferents can elicit lasting deficits in behavioural suppression that manifest as compulsivity. We demonstrate that optical stimulation of D1/D2 NAc neurons and PVT/BLA-NAc afferents can produce divergent behavioural effects but that all of these circuit elements contribute collectively to suppression of unproductive reward seeking. We also found that repeated stimulation of PVT-NAc afferents leads to excessive self-grooming and impaired performance on a reversal learning task, while vHPC-NAc hyperactivity only partially recapitulated this phenotype. Altogether, these data complicate the straightforward dichotomy proposed by most models of basal ganglia function and suggest that behavioural inhibition is a core function of NAc circuits that is distributed across its cell types and inputs. These findings indicate that the NAc is a critical node in a behavioural inhibition network, and that the diverse etiologies of compulsive disorders may converge mechanistically in dysregulated NAc afferents. We propose that circuit-specific therapies may be valuable for normalizing aberrant NAc signaling that gives rise to compulsive behaviours.

This chapter will assume a broad perspective by examining conceptual challenges to the circuit-based approaches leveraged throughout this thesis. Implications of experimental results and more proximal technical caveats are considered in detail in the Discussion sections of chapters 2, 3 and 4. Here, we focus instead on key developments in our field that reframe the thesis' core questions.

In the following sections we propose a dominant role for the NAc in behavioural inhibition and revisit the standard go/no-go model of basal ganglia function. An evolutionary perspective colours most of this discussion and posits that behavioural facilitation and inhibition are not functions of single brain regions but have evolved repeatedly to control increasingly complex behavioural repertoires. In this view, effective behavioural control is an emergent consequence of interactions between basal ganglia, cortex and motor effectors, which provides a significant challenge to the reductive approaches applied in modern circuit neuroscience (and throughout this thesis). To address these hurdles, we identify behavioural approaches and

computational tools that will complement a circuit-based approach to the study of the NAc and its afferents and that we believe will prove critical in the era of big data neuroscience.

5.2. THE NUCLEUS ACCUMBENS AS A BEHAVIOURAL INHIBITOR

5.2.1. ARCHITECTURE FOR BEHAVIOURAL INHIBITION

The neural circuit architecture of the basal ganglia suggests that it may function primarily as a behavioural inhibitor. Although direct pathway MSNs project to midbrain output structures like the substantia nigra pars reticulata (SNr) and the entopeduncular nucleus (EP) to disinhibit descending motor commands and recurrent thalamocortical projections, the default state of these output structures is tonically inhibitory. Since MSN activity is kept sparse by a relatively hyperpolarized resting membrane potential (O'Donnell and Grace, 1993) and low excitability (O'Donnell and Grace, 1995), inhibitory SNr and EP neurons exhibit a high resting discharge rate at baseline (Benhamou and Cohen, 2014; Sanderson et al., 1986), resulting in sustained inhibition of descending motor commands. For a neural system in which behavioural output is continuously attenuated, it is perhaps unsurprising that the most common consequence of neural disturbances is a release of unhelpful, context-inappropriate behaviours. In the NAc, it is worth noting that many D1 neurons send axon collaterals to the ventral pallidum, which is the primary target of 'indirect pathway' D2 neurons (Kupchik et al., 2015). This blurred distinction between the traditional go/no-go pathways may contribute to a skewed role for ventral striatal circuits in behavioural inhibition.

Despite the prominent role for NAc circuits in behavioural inhibition, there remain clear examples of NAc circuit perturbations that decrease behavioural output (Ambroggi et al., 2008; Cole et al., 2018; Floresco et al., 1997; Hikida et al., 2010; Ito et al., 2008; Sangha et al., 2014; van Holstein and Floresco, 2020; Yawata et al., 2012). These results suggest that a fraction of NAc network states is important for facilitating behaviour under specific task conditions. How do we reconcile the action selection view with the broader importance of NAc circuits in behavioural inhibition?

5.2.2 AN EVOLUTIONARY PERSPECTIVE

Here we examine striatal and NAc function through an evolutionary lens, recognizing that the basal ganglia evolved within a broader ancestral system (Cisek and Hayden, 2022). From this

perspective, the evolution of new neural subsystems must arise from a unified nervous system that can already support adaptive behaviour. Recognizing that biological mechanisms of behaviour are phylogenetically linked motivates a biologically grounded taxonomy of behaviour. Lineages of evolving brain structures define a hierarchy of neuroanatomical innovations and their corresponding behavioural functions (Cisek, 2019). This approach, termed "phylogenetic refinement", does away with the tidy dichotomies discussed in section 1.1.1, in part by grounding behavioural categories in something besides intuition.

Under this view, the basal ganglia are one of many brain systems participating in behavioural control. Before the basal ganglia evolved, dopamine signaling emerged in the common ancestor of eumetazoans (jellyfish, worms, insects, vertebrates, etc.) (Barron et al., 2010). Across these phyla, dopamine is thought to signal high local nutrient levels in order to govern the transition between long-range exploration and local exploitation of a nutrient-rich environment (Hills, 2006). Even in this "rudimentary" form, animals were capable of a kind of "decision-making", prioritizing one motor programme over another. The evolution of the hypothalamus and the tectum saw the emergence of additional action selection modules, including foraging and defensive behaviours (Albuixech-Crespo et al., 2017; Holland and Holland, 1999), along with visually-guided escape/approach behaviours (Lacalli, 1996), respectively. Over 120 million years later, the common ancestor of vertebrates evolved, coinciding approximately with the emergence of the basal ganglia and the pallium, a cortex precursor (Redgrave et al., 1999). These animals saw an immense expansion of their behavioural repertoires. Approach and avoidance behaviours could be guided more effectively by leveraging rich stimulus information available in the world. The evolving cortex was thought to specify complex action opportunities, or affordances, on the basis of detailed olfactory and visual representations (Cisek, 2007). It is believed that the basal ganglia evolved in tandem with the pallium to support transitions between gross behavioural modes (e.g., foraging vs. nest-building), while the pallium exerted finer control over skilled behaviours (e.g., reaching and grasping).

The evolutionary perspective provides three key insights:
(1) Action selection is not a unitary process. In other words, behavioural facilitation and inhibition have evolved many times and been placed under the control of distinct neural systems that attend to ever more abstract features of the world in service of increasingly flexible

behaviour. While dopamine might have initially represented nutrients levels to trigger transitions between behavioural modes, the cortex evolved to represent abstract behavioural affordances that rely on detailed predictions about what the world can offer. Antagonistic processes likely play out locally within these systems but also between these systems, and the basal ganglia form but one subsystem that mediates these interactions. While this insight is perhaps obvious, it is worth saying because it reframes the core question of this thesis. Dynamic control of behavioural output arises not from the basal ganglia themselves, but from their *interactions* with other neural subsystems that carry out domain-specific behavioural facilitation and inhibition.

(2) The ventral striatum evolved in the context of a broader neural system. Since interactions between the NAc, its inputs, and downstream motor effectors collectively contribute to behavioural control, it is challenging to identify a causal role for specific circuit elements. Selective pressures shape the functions of multiple neural circuits simultaneously in service of adaptive behaviour. Consequently, specific brain regions, pathways, and cell types cannot be divorced from the networks they are embedded in. In section 5.4, we submit that the search for circuit-specific causes of behaviour may be futile and that cross-regional population dynamics are the key explanatory unit of behaviour.

(3) Phylogenetic refinement suggests that transitions between gross behavioural modes are mediated through interactions across the basal ganglia and cortex. Since the basal ganglia evolved in cooperation with the ancestral dopamine system, it is possible to make inferences about how this neuroanatomical innovation exapted the existing explore/exploit system (Gould and Vrba, 1982). Although phasic dopamine signaling is thought to underlie positive reinforcement, tonic dopamine signaling may still play a role in regulating behavioural transition statistics (Schultz, 1998). Under high reward conditions, tonic dopamine levels are elevated (Beierholm et al., 2013) and behaviour becomes stereotypic (Berridge et al., 2005; Hills, 2006), suggesting an exploitative behavioural strategy. Lesions of striatum have also been shown to impair behavioural sequencing (Berridge and Whishaw, 1992), particularly involving gross movements (Lemke et al., 2019). Conversely, skilled fine movements are unaffected by striatal lesions, and rely on cortical activity (Sauerbrei et al., 2020). Moreover, subpopulations of dorsal and ventral striatal neurons signal transitions between behavioural syllables (Chen et al., 2021; Jin et al., 2014; Klaus et al., 2017; Nelson et al., 2021). A role for the NAc in regulating behavioural transition statistics could also account for behavioural disinhibition arising so readily

from neural circuit perturbations. Manipulations of NAc circuits that enhance transitions between behavioural modes may be interpreted as an enhancement of unproductive, off-target behaviours, while those that decrease transitions may be interpreted as persistence in a particular behavioural mode. A more detailed treatment of this model can be found in section 5.4.2.

These three insights identify core issues that arise from the work presented in this thesis. In the following sections we leverage this evolutionary perspective to highlight behavioural approaches that support phylogenetically-motivated behavioural categories (section 5.3) and novel computational tools that address the challenges of studying integrated neural circuits (section 5.4).

5.3. TOWARDS BETTER BEHAVIOURAL MEASURES

5.3.1. NATURALISTIC BEHAVIOURS

Ethological descriptions of behaviour can be an excellent complement to traditional trial-based behavioural assessments (Datta et al., 2019). Ethology emphasizes the importance of natural behaviours, behaviours that animals typically perform in response to ecologically relevant stimuli (Tinbergen, 1963). Unlike trial-based behavioural assays used in comparative psychology, naturalistic behaviours are typically self-motivated and may reveal the naturally evolved functions of neural circuits. In this framework, behaviour is viewed hierarchically and can be decomposed into sequences of behavioural components. For example, syntactic grooming chains discussed in chapter 4 can be represented as flexible sequences of behavioural syllables, obtained through hand-scoring (Berridge et al., 1987) or unsupervised behavioural clustering (Hadjas et al., 2020). In this case, ethological analyses identify a conserved cephalocaudal pattern of self-grooming, comprising four distinct phases (Kalueff et al., 2016). This approach would have been an excellent complement to our binary description of grooming behaviour and could have revealed more about how PVT-NAc dysregulation affects the structure of self-grooming. Additionally, unsupervised behavioural clustering can be used to identify hidden patterns in the data, permitting the discovery of new behavioural motifs that may more aptly map onto neurobiological variables than traditional psychological terms (Pereira et al., 2022; Wiltschko et al., 2020). By eschewing prescriptive behavioural categories (section 1.1.1), we are able to adopt a behavioural taxonomy that is informed by the inherent structure of the

behavioural data, complementing the phylogenetic refinement approach. Overlap in these taxonomies would be suggestive of true functional categories of behaviour.

Behavioural clustering can reveal relationships between neural activity and behaviour at multiple timescales. While the dorsolateral striatum may regulate transitions between short-lived motor elements, the ventromedial striatum, with its relation to affect and cognition, may underpin transitions between longer-lasting behavioural modes. Testing this hypothesis will require the holistic, multiscale description of behaviour that is provided by pose-estimation and clustering methods such as SLEAP (Pereira et al., 2022) and MoSeq (Wiltschko et al., 2020). Naturalistic foraging tasks which recruit NAc-typical behavioural repertoires, including approach and avoidance, have already been used to examine the neural correlates of transitions between explore/exploit behavioural modes (Pearson et al., 2009) and behavioural strategies (Hayden et al., 2011). More recently, a study showed that dopaminergic signaling in the dorsolateral striatum shapes the sequencing and likelihood of future behavioural syllables during unstructured naturalistic exploration (Markowitz et al., 2023). The notion that dopamine in ventral striatal structures plays a similar role, albeit over longer timescales, is compatible with the role of NAc circuits in regulating behavioural sequences and transitions between them.

5.3.2. INTERPRETABLE BEHAVIOURS

A significant challenge in studying the neural correlates of NAc-mediated behavioural control is that the space of behaviours regulated by the NAc is somewhat opaque. Since NAc afferents come from highly integrative brain structures, their innervation patterns provide little assistance in the way of behavioural grounding. Conversely, in the dorsal striatum, the space of relevant motor behaviours is given by the anatomically-segregated basal ganglia loops that receive topographically organized input from the motor homunculus (Lee et al., 2020). Studying the behavioural contributions of basal ganglia loops is straightforward when a single relevant behaviour can be identified (e.g., striatal control of the anterior forelimb; Foster et al., 2021), given that behavioural control can be grounded in muscle contractions and limb positions – parameters which can be readily measured. We may progress in understanding the role of the NAc in behavioural control by turning to intuitions gleaned from the study of motor striatum.

The value of pivoting to dorsal striatum is further motivated by an evolutionary perspective. In lamprey, a low vertebrate whose ancestors diverged from the vertebrate lineage at

its inception, basal ganglia circuits are highly conserved (Stephenson-Jones et al., 2011). These animals have a very limited behavioural repertoire, and their basal ganglia comprises a motor-regulatory striatum akin to dorsolateral striatum in higher vertebrates. As mammals have a massively expanded set of motor programmes, it is likely that this basal ganglia module for control of motor behaviour has been exapted repeatedly for control of an ever-expanding behavioural repertoire (Gould and Vrba, 1982). This idea is supported by evidence that different striatal subregions tend to have similar cytoarchitecture (Mannella et al., 2013; Voorn et al., 2004). Taken together with the fact that new brain areas must be scaffolded on existing neural circuits (Cisek, 2019), it is likely that the mammalian striatum contains a repeated functional motif. If we can grasp the computations being carried out in motor areas, perhaps we can begin to examine homologous computations occurring in ventral striatal regions.

Herein lies the value of trial-based behavioural assays. To test the role of a dorsal striatal subregion in its input-defined behavioural function, we can restrict our attention to the motor response of interest and record every behavioural measure relevant to its execution. This approach has already been applied to examine the consistency of activated dorsal striatal neural ensembles during a skilled reaching task (Kondapavulur et al., 2022). In a similar assay, bidirectional gain control of reaching vigour can be finely tuned by manipulating D1 and D2 neurons in the dorsomedial striatum (Yttri and Dudman, 2016). Ventrolateral striatum has been shown to play a distinct role in regulating licking behaviour, and unilateral stimulation of the indirect pathway in this region reduces contraversive licking while enhancing ipsiversive licking (Lee and Sabatini, 2021). The power of these studies lies in the clarity of their behavioural readouts and their proximity to the underlying neural activity. With time we may be able to adapt this type of approach for use in NAc circuits.

5.4. INTERROGATING INTEGRATED CIRCUITS

5.4.1. A CRISIS OF CAUSALITY

In the traditional box-and-arrow model of brain function, neural circuits carry out serial computations (Barack and Krakauer, 2021). In this model, each box in a circuit diagram carries out a local function and this information is transferred and transformed downstream at the next box, eventually resulting in a behavioural output. Under this view, complex cognition can be explained using the same algorithmic tools that Sherrington used to explain reflex arcs

(Sherrington, 1906). However, deep brain structures like the NAc, are not amenable to this approach. A tremendous hurdle of studying highly integrative structures like the NAc and its inputs is that they reciprocally communicate and are embedded in a series of recurrent neural loops. In systems such as these, where the function of system components is highly interdependent, manipulations of a single neuron or brain region propagate throughout the network and influence activity in distal circuits (Wolff and Ölveczky, 2018). As specific as we may try to be, studying the effects of circuit perturbations does not reveal the precise causal role of a particular cell type or pathway for behaviour, but rather is an exercise in generating non-physiological network states and studying *their* relationship to behaviour. This complication is likely why many loss of function experiments produce similar, short-lived behavioural deficits (Carrera and Tononi, 2014). Compensatory processes in distal neural circuits are constantly at work to promote brain homeostasis in spite of our circuit perturbations. Common intuitions about causal mechanisms may not apply to a system like this. While we can identify causal dependencies – e.g., NAc afferent activity is necessary for suppression of unproductive reward seeking – there may not be a straightforward causal relationship between neural activity in a single pathway or cell type and the production of behaviour (Barack et al., 2022). Emerging views in this field express the importance of population coding schemes as the key explanatory unit of behaviour, rather than the bulk activity of cell types or pathways (Barack and Krakauer, 2021). Dimensionality reduction techniques (Chaudhuri et al., 2019; Low et al., 2018) and dynamical systems theory (Churchland et al., 2012) offer useful frameworks for linking time-varying emergent features of neural activity to behaviour in a manner that describes how the system as a whole evolves in tandem with the environment.

5.4.2. INTERPRETABLE CIRCUIT PERTURBATIONS

In section 1.3.1, we noted that task-relevant events in a discriminative stimulus task elicit heterogeneous neural responses in the NAc and its afferents (Chen et al., 2023; Nicola et al., 2004; Pedersen et al., 2022; Reed et al., 2018). For example, the activity of single neurons often tracks numerous task-relevant variables, and neighboring neurons can exhibit mixed increases and decreases in activity during lever pressing behaviour and food port entries. This heterogeneous encoding scheme does not lend itself to a Sherringtonian view of neural circuits (Barack and Krakauer, 2021) and is notably similar to mixed selectivity encoding schemes

observed throughout the brain (Burgos-Robles et al., 2017; de Vries et al., 2020; Kira et al., 2023; Klaus et al., 2017; Libby and Buschman, 2021; Nair et al., 2023; Otis et al., 2019). In both cortical and deep brain structures like the hippocampus and hypothalamus, neural response heterogeneity is being leveraged to identify neural spaces, or low-dimensional manifolds, which act as basins of attraction for the activity of neural populations. While a raw neural recording will be high dimensional, on account of the number of units recorded, dimensionality reduction techniques such as principal components analysis (PCA) can collapse this high dimensional space to a few orthogonal dimensions that maximally capture the variance in the raw data. In a tower-counting task, where mice obtain rewards by correctly reporting which side of a virtual track had more towers, orthogonal representations of space and accumulated evidence (i.e., number of towers observed) can be abstracted from large-scale neural recordings in the hippocampus (Nieh et al., 2021). “Movement” of the hippocampal population through this neural space is a compact representation of neural dynamics that correlates tightly with a time-evolving description of an integrated position-evidence variable (Shenoy and Kao, 2021). Efficient use of orthogonal neural spaces has been proposed as a way for the same neural population to concurrently represent several task-relevant variables (Semedo et al., 2019). Similarly, recordings from ventromedial hypothalamus (VMH) have revealed a dominant dimension of neural activity that rests along a line. Movement of neural activity along this axis correlates tightly with escalating aggression and transitions to qualitatively distinct behavioural modes (sniffing, followed by dominance mounting, followed by attacking) (Nair et al., 2023). This study used a dynamical systems approach which models neural trajectories through a low-dimensional space by estimating the underlying dynamical forces that constrain the paths traced by the neural population as a whole (Ebitz and Hayden, 2021). If we imagine VMH activity as a ball rolling around a landscape, then the line attractor is a valley that draws the ball down into it. The topology of the landscape represents the local dynamics that shape the ball’s path. This approach is powerful because it provides mathematical grounding to make predictions about the rules that constrain the evolution of neural activity during ongoing behaviour. Moreover, this technique makes it possible to identify patterns of neural activity in the population that correspond to preparatory/cognitive aspects of behavioural control that cannot be obviously linked to outwardly measured behavioural variables. Most importantly, this framework can be

used to address problems of causality in neural circuit manipulations by examining how particular circuit perturbations set new initial states from which neural dynamics can evolve.

Dynamical systems approaches have already proven invaluable in motor control fields and have begun to make their way into the basal ganglia. In motor cortex, time-varying population activity has been modeled as a dynamical system in which rotational dynamics are thought to correspond to preparatory activity, setting the initial conditions for an ensuing neural trajectory that triggers a motor response (Churchland et al., 2012). More recently, motor cortex recordings during a skilled reaching task have revealed that these dynamics are supported by patterned input from motor thalamus. These authors demonstrate that local perturbations in primary motor cortex (M1) and thalamus disrupt these autonomously generated dynamics, resulting in aberrant neural trajectories that elicit aberrant reaching (Sauerbrei et al., 2020). They find that different patterns of stimulation targeted to thalamic inputs set up different initial conditions in M1 from which the neural dynamics evolve. The resulting 'artificial' neural trajectories vary in their similarity to physiological trajectories, permitting key comparisons with the kinetics of ensuing reach behaviour. The capacity to describe how a circuit perturbation affects neural population dynamics is an invaluable tool because it allows us to ground our manipulations in comparison to a physiological reference.

Exciting new work has combined multisite recordings in motor cortex and dorsal striatum during a prehension task to examine how low-dimensional neural dynamics compare between these two regions and to identify how acute or chronic manipulations of either region affect these dynamics and alter behaviour. In this body of work, the authors demonstrate that low-dimensional neural trajectories in dorsal striatum can be predicted by M1 spike trains after animals are trained on skilled reaching (Lemke et al., 2021). Canonical correlation analysis (CCA) is a dimension reduction technique that can be used to identify linear combinations of neurons in two regions that are maximally correlated, identifying shared axes of variations in a low-dimensional neural space (Veuthey et al., 2020). In deep neural networks, this approach has been used to quantify the degree to which representational space is shared across layers of the neural network (Raghu et al., 2017). In simultaneous recordings from M1 and dorsal striatum, this approach has demonstrated that cross-regional coupling, as measured by the canonical correlation between single cell firing rates in each area, is reduced when a new reach target is

introduced in a well-learned reaching task (Kondapavulur et al., 2022). Using a dynamical systems approach, these authors show that variable reaching behaviour elicited by these feedback errors is caused by more variable autonomous dynamics in both M1 and dorsal striatum. Circuit perturbation experiments show that this "exploration" of both neural and behavioural space may reflect transitions between labile and rigid modes of motor control, since lesions of M1 or DLS disrupt cross-regional activity and elicit variability in fine and gross movements, respectively. These results are consistent with the idea that coupling between motor areas and the striatum plays a key role in stabilizing, refining, and transitioning between patterns of neural activity that generate effective motor responses. In this view, cortical representations of actions are preferred regimes in a dynamical system (attractor states), and transitions between these regimes may be facilitated by interactions with the striatum (Athalye et al., 2018; Djurfeldt et al., 2001).

Taken together, this body of work provides a rough model of basal ganglia function grounded in population dynamics (Ebitz and Hayden, 2021), which is consistent with a role for the striatum in behavioural transitions. In this model, disruptions of striatal cell types and afferents are likely to enhance the variability of corticolimbic neural ensembles resulting in release of behavioural control and more exploratory (rather than exploitative) behavioural modes. This prediction is consistent with the notion that most forms of the perseverative behaviour that we observed following NAc circuit manipulations were generally disinhibited behavioural modes: sampling of previously unrewarded or extinguished actions and increased checking behaviour in our PVT-NAc circuit model of compulsivity. Perhaps, as previously suggested, aberrant NAc activity alters transition statistics between different behavioural modes and dynamical cortical regimes. If cortical dynamics become trapped in a deep basin of attraction following NAc disruption, this could manifest as persistence in a particular behavioural mode. Conversely, excessive switching of cortical dynamics would likely elicit context-inappropriate behavioural output. This model recontextualizes imbalances of behavioural facilitation and inhibition as disordered transition statistics between behavioural modes, which would usually manifest as disinhibition in traditional behavioural tasks. There is evidence that striatal activity correlates with behavioural transitions (Chen et al., 2021; Jin et al., 2014; Klaus et al., 2017; Nelson et al., 2021), and is important for organizing the hierarchical structure of behaviour (Geddes et al., 2018; Jin et al., 2014; Markowitz et al., 2023). It is tempting to assume that dorsal

striatal functions have been exapted for use in ventral striatal circuits but direct evidence in support of this model has yet to be collected.

This body of work demonstrates the feasibility of using computational approaches to interpret circuit perturbations and extract population dynamics from mixed neural responses recorded in the NAc during behavioural tasks. These techniques implicitly recognize that the striatum functions as one part of a densely recurrent network by grounding manipulations in complete descriptions of how they steer neural activity through low-dimensional spaces. In chapter 4 we examined how patterned activation of NAc afferents elicits dysregulated behaviour and discussed how patterned stimulation can inspire DBS protocols that normalize neural circuit dysfunction. Understanding the consequences of patterned stimulation on neural dynamics will provide mechanistic insight into the etiology of circuit dysfunctions and motivate novel circuit-based therapies, particularly in the realm of compulsive disorders. Although we may be able to infer ventral striatal function by its homology to dorsal striatal circuits, it will be valuable to focus on the NAc to initiate more mechanistically-grounded preclinical work relevant to psychopathology.

5.5. CONCLUSION

This thesis broadly surveys the role of multiple NAc afferents and cell types in behavioural inhibition using classical circuit neuroscience techniques, including optogenetics and calcium imaging. We identify the NAc as a site of susceptibility where disruptions of any NAc circuit element disinhibits behaviour on a number of measures, highlighting a site of potential mechanistic convergence for compulsive disorders characterized by deficits in behavioural control. We address the dominant idea that the balance between behavioural facilitation and inhibition arises from antagonistic interactions between NAc circuit elements, finding instead that the integrated activity of this densely recurrent network is most important for behavioural suppression. Enduring disruptions of NAc physiology give rise to enduring impairments in behavioural inhibition, suggesting future avenues for development of circuit-based therapies of compulsivity, such as opto-inspired deep brain stimulation. This work highlights a significant challenge of using circuit neuroscience approaches to study densely interconnected neural circuits implicated in cognitive and affective behaviours. Consequently, we spotlight newly adopted computational techniques that parse the underlying structure of rich behavioural and

neural datasets. It is now possible to ground circuit perturbations in descriptions of the population dynamics they elicit and correlate these dynamics to granular, multiscale descriptions of behaviour. By focusing on homologous circuits in dorsal striatum, we expect that we will soon be able to make inferences about the computations performed by the NAc and grasp how it transforms information arising from its afferents. As experimental tools develop at an accelerating pace, the era of large-scale neuroscience is well and truly upon us (Gao and Ganguli, 2015; Stevenson and Kording, 2011). Neuroscientists and psychologists, steeped in tradition, would do well to confront the profound computational challenges that hide in the systems they study, sooner rather than later.

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