Unilateral optogenetic inhibition and excitation of basal ganglia output show opposing effects on left/right lick choices and movement initiation in mice

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Abstract

The functional role of basal ganglia receiving territory of the motor thalamus (BGMT) remains poorly understood. Therefore, to examine how basal ganglia output via the substantia nigra pars reticulata (SNr) and projections to the BGMT control motor behavior, we unilaterally inhibited or excited basal ganglia output neurons and their projections to the BGMT during periods of movement preparation and initiation in mice performing a 2-alternative choice licking task. Using a viral vector carrying the inhibitory opsin DIO-ARCH3 into the SNr of VGAT-cre mice, we inhibited SNr activity starting 0.5 s before the onset of a response window where the mouse must lick right or left depending on the location of a previously delivered air-puff stimulus to the right or left whiskers, respectively. Light activation of ARCH3 in the SNr inhibited SNr activity and biased lick responses towards the lick spout contralateral to the hemisphere of stimulation. In another set of mice, we excited SNr activity using a fast variant of the ChR2 opsin (DIO-hChR2-E123A/T159C), which suppressed licking activity irrespective of the direction of the trial. However, exciting terminals of neural projections from the SNr to the BGMT biased licking responses to the lick spout ipsilateral to the stimulation. These results demonstrate the role of the SNr in directing movement preparation and initiation in behaving mice, broadly supporting classic models of the basal ganglia where decreases in BG output activity act to facilitate voluntary movement through thalamo-cortical pathways.

Significance Statement

This study provides the first evidence of fast reversible manipulations of basal ganglia output directly influencing motor responses. This is also the first study to begin to isolate the effects of basal ganglia output specifically to the motor thalamus on the control of motor behavior. We found that inhibiting the SNr directly led to a contralateral bias in the licking motor response and that direct activation of the SNr suppressed licking activity altogether. Interestingly, selectively inhibiting the SNr projection to the BGMT biased licking responses to the ipsilateral direction. Our observations broadly support classic BG models and further suggests that ascending basal ganglia output to the thalamus and cortex helps to shape the preparation and initiation of sensory guided movement.

Introduction

The basal ganglia (BG) are thought to influence the control of movement by inhibiting and facilitating selected motor programs to accomplish and reinforce goal-directed behavior (Albin et al., 1989, DeLong, 1990). Our long-standing understanding of the BG's role in the control of movement suggests that the output nuclei of the BG provide tonic inhibition over motor areas of the thalamus and brainstem to suppress unwanted movements; pauses in the inhibitory output would therefore facilitate movement initiation (Deniau and Chevalier, 1985, Mink, 1996, Hikosaka, 2007). In support of this general basal ganglia functional model, optogenetic modulation of the basal ganglia direct pathway has been found to be pro-kinetic, leading to a reduction in the inhibition of the SNr (Freeze et al., 2013), the primary basal ganglia output nucleus in the rodent, and disinhibition of downstream targets (Oldenburg and Sabatini, 2015). Additionally, pauses in SNr activity preceded initiation of orienting movements in rats performing an auditory-cued Go-Stop task (Schmidt et al., 2013).

Recent studies have shown that descending motor cortical projections control lateralized (left vs. right) licking in adult mice (Li et al., 2015, Chen et al., 2017). Loops from the cortex to thalamus (including both cerebellar and basal ganglia receiving regions) have also been implicated in controlling the preparation and initiation of lateralized licking, such that neural activity recorded from the motor thalamus in a delayed licking task shows ramping prior to the initiation of licking and inhibiting the thalamus reduces performance to chance (Guo et al., 2017). The basal ganglia are positioned to influence corticothalamic loops and directed licking behavior via inhibitory projections from the output nuclei of the basal ganglia, namely the substantia nigra reticulata (SNr) and the globus pallidus internus (GPi). The objective of this study was to examine how output activity from the basal ganglia influences initiation and suppression of goal-directed behavior and which role is played by output projections to the motor thalamus. To achieve this objective, we trained mice to perform a 2-alternative choice licking task where they must lick a left or right positioned lick spout following a left or right air puff, respectively. In order to further parse the contributions of BG output on cognitive control of movement preparation and suppression, we trained the same mice to perform two variations of this task: first allowing mice to lick before the response window and anticipate the reward delivery ('anticipatory' task variant) and later a variant that required mice to actively withhold licking until the onset of the response window ('withholding' task variant). By training the mice to perform these task variants one after another, we were able to parse the basal ganglia contributions to initiating or suppressing anticipatory movements and the control of rewarded choice preparation and initiation. Using optogenetic methods to inhibit SNr neurons unilaterally, we found that silencing the inhibitory output of the basal ganglia triggered licking contralateral to the side of inhibition, irrespective of the rewarded licking direction. In contrast, exciting SNr projections to the motor thalamus and inhibiting

thalamic activity suppressed contralateral licking and biased licking towards ipsilateral direction. Exciting the SNr directly suppressed licking entirely, irrespective of the lick direction. These results suggest that the basal ganglia output via SNr to the motor thalamus exerts powerful unilateral control over movement preparation and initiation in the context of sensory guided motor behavior.

Methods

Animals:

For optogenetic stimulation and electrophysiological experiments, male and female Vgat-IRES-Cre mice (*Slc32a1*) aged 6-12 months at the start of experiments were used. Mice were maintained on a 12h:12h reverse light cycle and all experiments and behavioral training was performed during the dark portion of the cycle. Mice undergoing behavioral training were provided *ad libitum* food access and were kept on 1-1.5 ml/day water restriction 6 days a week starting at least 3 days prior and for the duration of handling, training, and experimental testing. On day 7 of each week, mice were given free access to water. During behavioral training and testing, mice were given 10% sucrose solution with 0.1% grape Kool-Aid powder. Liquid consumption was measured during testing and mice were supplemented with water to reach the 1-1.5 ml/day volume. All experimental procedures were approved by the Emory University Institutional Animal Care and Use Committee.

Surgery:

Viral Vector Injection and Fiber Placement. For optogenetic AAV vector injection, mice were anesthetized with isoflurane (induction at 3-4%, maintained at 1-2%) and head-fixed on a stereotaxic frame (Kopf). Craniotomies were made unilaterally above the right, SNr. For somatic SNr inhibition, 200nL of AAV5-EF1a-DIO-eARCH3.0-eYFP (ARCH; n = 5, 3 male) or for excitation, 200nL of AAV5-EF1a-DIO-hChR2(E123T/T159C-EYFP (ChR2(ET/TC) or ChR2; n = 4, 2 male) was injected with a nano-injector (Nanoject II) at the rate of 0.46nl/s into the SNr targeting the coordinates (in mm from Bregma): AP -3.2, ML 1.6, DV -4.2). For somatic SNr excitation and SNr-BGMT terminal excitation 200µm, 0.22NA optic fibers (Thor Labs) each with a 1.25mm steel ferrule were then implanted targeting the SNr and the ventromedial thalamus (AP -1.5, ML 0.9, DV -4.0). Following surgical implantation, mice were kept in single housed cages.

Acute Electrode/Optrode Recordings. To record and/or optogenetically manipulate neural populations in the SNr or downstream projection targets, a craniotomy was made above the site targeting these areas and the dura was kept intact. A 4mm diameter and 1mm tall plastic tube was glued in place around the craniotomy and the area was filled with a removable elastomer (Kwik-Cast, World Precision Instruments) to allow for access to the tissue for future experiments.

Behavior:

Prior to behavioral training, a custom stainless-steel head-post was attached posterior to lambda by placing a thin layer of cyanoacrylate adhesive on the skull, followed by a thin layer of dental acrylic (Metabond; C&B Associates). Following recovery, mice were head-

fixed and placed within the behavioral training setup consisting of two 2mm diameter lick spouts placed 5mm apart and two 2mm diameter air-puff tubes that were directed at the C row of whiskers. The lick spouts were connected to a custom lick sensor circuit that recorded time and duration of licks at 200 Hz. Air-puff intensities were calibrated such that whisker deflection was apparent under high-speed video monitoring without signs of freezing or startle behavior from the mouse.

Behavioral Task

Animal training protocols and behavioral paradigm are adapted from previously reported procedures (Guo et al., 2014). The behavioral paradigm is illustrated in Figure 1A. Left and right air-puff/lick trials were selected pseudo-randomly such that the probability of a right/left trial was adjusted based on the ratio of left/right rewarded trials (e.g. for the trial history of the current session consisting of 8 correct right trials and 4 correct left trials, the probability that the next trial would be left was set to right rewards/total rewards = 66%). Each trial was made up of 3 discrete intervals: a “pre-stimulus” period, a “sensory” period where mice received either a left or right air-puff for 1-1.5 s, followed by a 5 s “response” period where the mice lick the left or right spout to indicate which spout they believe will be rewarded and consume the reward. Trials were separated by a variable inter-trial period. During the sensory period, mild non-aversive air-puff stimuli were directed through 2mm-diameter steel tubes towards the whiskers. The tubes were angled at 15 degrees away from the mouse center to isolate air-puff stimuli to the whiskers, avoiding the face of the mouse. The stimuli lasted for 1-1.5 s during training, though for optogenetic experiments during the withholding task, the sample period was held constant at 1 s.

Training for the behavioral task began after a minimum of 10 days following surgery and 5 days following the start of water restriction. Mice were handled during initial days of water restriction to acclimate with handling. On the first day of head-fixation and training, mice were secured in the holder and placed in the behavioral setup with the two lick spouts positioned and centered in front of the mouth of the mouse. During the first day of training, mice could lick both the left or right spout and would receive a sucrose reward following licks at a minimum interval of 10 s. This was primarily used to acclimate the mouse with head-fixation and the positions of the lick spouts. The positions of the spouts were manually adjusted further and closer to the center of the mouse to promote equal licking of both lick spouts. During subsequent days of training, mice were transitioned to a task where the air-puffs signifying the rewarded spout were active, though the reward was automatically triggered (“Auto-Reward”). This typically lasted 1 day of training (~4 50 trial blocks) until the mice became acclimated to the puffs and would display anticipatory licking towards the correct lick spout before the reward was triggered, demonstrating the mice had built the association between puff location and rewarded lick spout. In the next step of training, typically occurring on days 2-3, mice received the air-puffs and were then allowed to lick freely towards both spouts in the Response period and were only rewarded

on licks to the correct spout ("Free-Lick"). Mice during this time learned to make the correct perceptive decision (signified by licking the rewarded spout exclusively during trials). Mice were then introduced to the 'anticipatory' variant of the task (Fig 1C). This variant of the task now penalized incorrect decision licks during the reward period by transitioning straight into a lengthened inter-trial delay period (6-10 s vs. 2-4 s for passed trials). To minimize licking outside of the response interval of the trials, each trial began with a pre-stimulus period where the mouse must withhold licking for at least 2-3 s before air-puff onset. Licking during the last second of this period led to a trial fail and mice were placed back in the inter-trial period awaiting the start of the next trial. Training mice to perform the anticipatory task occurred over 2-5 days. Finally, mice were trained on the second variant of the task that required the mice to withhold licking ('withholding' variant) during the stimulus period until the offset of the air-puff (Fig 1D). To train mice to begin withholding licks during the stimulus period, any lick activity during the air-puff triggered an "early lick" fail and was penalized with an extended inter-trial period. At the beginning, the air-puff was shortened to 0.5 s and gradually increased in steps of 0.25 s until the mice were able to withhold licking and achieve 60% performance with a variable sample period duration between 1-1.5 s. This training typically took an additional 1-2 weeks. Of the 12 mice that were trained in the anticipatory variant of the task, 8 mice were able to subsequently learn the withholding variant.

Optogenetic stimulation:

Before and after each session the output intensity of the light source (either LED or laser) was determined using an optical power meter and sensor (PM100D and S121C, ThorLabs). For SNr inhibition experiments, we used a 593nm yellow laser (Shanghai Dream Lasers) collimated and coupled to a 200micron, 0.22 NA patch cable (Doric Lenses) leading to 8-12mW output from the fiber tip. For somatic SNr excitation we used a 470nm LED (Doric Lenses) coupled to the same patch cable with output power between 1-3mW from fiber tip. Finally, for SNr-BGMT terminal excitation, we used a 470nm blue laser (Shanghai Dream Lasers) with the same patch cables with output power between 8-12mW from the fiber tip. Trials with optogenetic stimulation were randomly intermixed with control trials for a total proportion between 25-50% of trials with light exposure. The optical stimulation trials were also all executed with a fixed 1 s air-puff duration in order to avoid inconsistent relations between stimulation and the timing of anticipatory licking.

Electrophysiology:

During surgical preparation, a craniotomy was made over the future right SNr/BGMT recording sites (-4 to 1mm AP, 0.5 to 2.5mm ML) and covered with Kwik-cast (WPI Inc.). The dura was left intact. A stainless-steel reference skull screw (#19010-10, Fine Science Tools) was placed over the contralateral sensory cortex. A 0.01" diameter steel wire was soldered between the screw and a gold pin to connect to the acquisition system during

recording. The mice were allowed at least 3 days to recover. Following recovery, mice were acclimated to being head-fixed and recording sessions began (one session per day, 2 hours per session). Within the first session of head-fixation, mice showed no overt signs of stress and appeared relaxed. During recording, mice were maintained on a randomized interval reward paradigm where mice were provided with a sucrose reward via right or left lick spout every 30-60 s to encourage quiet wakefulness during the session. At the start of the session, the Kwik-cast cover over the craniotomy was removed. Custom optrodes consisting of a 50-100micron optic fiber (ThorLabs) attached 200-300microns above a micro-electrode (FHC) were lowered into the SNr or BGMT. Raw signals (0.1–10 kHz band-pass filtered) were acquired at 20 kHz, amplified and digitized (RHD2132 headstage, Intan Technologies) and saved (RHD2000 Evaluation System/Interface Software, Intan Technologies). Once unit activity was detected in the SNr or BGMT, optical stimulation with either a yellow (593 nm) or blue (473 nm) laser was delivered for 1 or 2 s continuous pulses every 10 s to stimulate ARCH3 or ChR2 expressing neurons, respectively. For some recordings, the optic fiber was placed in the SNr with a separate electrode lowered in to the BGMT for recording activity downstream of the site of stimulation. After each session, the craniotomy was covered with Kwik-cast and following the final recording session, the mouse was perfused with PBS followed by perfusion with 4% paraformaldehyde and 15% sucrose. The brain was then removed and transferred to a 4% paraformaldehyde/30% sucrose solution for later histological processing.

Data Analysis:

Analysis of behavioral and electrophysiological data was performed in MATLAB (MathWorks). Only behavioral sessions where baseline performance was above 60% were included in analysis. Behavioral trials in which the mouse licked <1 s before the start of the trial (onset of the air-puff) were caught and sent to inter-trial delay. These trials were rare (<2% in trained mice and excluded from analysis). Lick data was pre-processed to remove “artifact licks” (spout contacts shorter than 10 ms and contacts lasting longer than 200 ms typically caused by electrical noise and paw touches, respectively). Trials where the decision lick (first lick during the response period) was classified as an artifact lick were removed from subsequent analyses. To calculate average lick frequencies for the various behavioral and experimental conditions, the onsets of lick contacts were marked and lick contacts across each trial were summed in 50 ms bins and divided by the length of the bin duration. Significance of the performance change in each optogenetic stimulation condition was determined using bootstrapping to account for variability across mice, sessions, and trials. We tested against the null hypothesis that the performance change caused by optogenetic stimulation was due to normal behavioral variability. In each round of bootstrapping, we replaced the original data set with a re-sampled set in which we resampled with replacement from: 1) animals; 2) sessions performed by each animal; and 3) the trials within each session. We then computed the change in

performance on the re-sampled data set. Repeating this procedure 10,000 times produced a distribution of performance changes that reflected the behavioral variability. The P value observed performance change was calculated as the fraction of times bootstrapping produced an inconsistent performance change (for example, if a performance decrease was observed during optogenetic stimulation, the P value is the fraction of times a performance increase was observed during bootstrapping, one-tailed test). Error bars represent the +/- SEM generated from bootstrapping unless noted otherwise.

Results

Mice learn to perform 'anticipatory' and 'withholding' variants of a 2-alternative choice licking task.

In order to understand how the basal ganglia output influences directed licking behavior, we employed a two-alternative choice behavioral task. In order to further parse the contributions of BG output on cognitive control of movement preparation and suppression, we trained the same groups of mice to perform two variations of this task: first, allowing mice to lick before the response window and anticipate the reward delivery, and second, a new variant that required mice to actively withhold licking until the onset of the response period (Fig 1B). By training the same mice to perform these task variations in sequence, we are able to parse the basal ganglia contributions to anticipation and initiation of licking movements (anticipatory task) as well as the control of movement preparation and suppression (withholding task).

Unilateral inactivation of the SNr biases towards contralateral licking behavior.

We began assessing the role of basal ganglia output in the anticipatory variant of the alternative choice licking task using fast, reversible optogenetic inactivation of the SNr through nigral ARCH3 activation (Fig 2A). In awake mice at rest, yellow (593nm) light stimulation of SNr neurons expressing ARCH3 abolished nearly all firing activity for the duration of the stimulation (mean firing rates 17.2Hz baseline vs. 2.61Hz with ARCH inhibition, n = 10 single units, Fig 2BC). Following the offset of the stimulation, the firing rate quickly returned to baseline levels. In behaving mice, we optogenetically manipulated the SNr in the right hemisphere (Fig 2D). To examine the role of the SNr in anticipatory licking activity, the SNr was silenced 0.5 s before the offset of the air-puff and continued 0.5 s into the response period of the 'anticipatory' variant of the task. Inhibiting the right SNr during this period biased licking activity towards the left (contralateral to the inhibited SNr) direction (Fig 2EF). A striking increase in right air-puff trials with left decision licks was observed and the overall success rate to right trials was decreased (Fig 2H). Additionally, the decrease in successful trial performance with right air-puffs was due to an increase no-response trials (Fig 2H, $P<0.001$). In contrast, left air-puff trials showed an increase in successes associated with a decrease in mistaken decision licks to the right (5 mice, 36 sessions, $P<0.001$ (left), $P<0.001$ (right), bootstrap, Fig 2H). Importantly, successful trials showed the same amount and timing of anticipatory licking and the same consummatory lick rate in trials with air-puffs on either side with or without SNr inhibition (Fig 2F). This suggests that the lateralization of decision making to the left with right SNr inhibition was not due to a slowing of movement to the contralateral side, but rather a categorical change in the decision process. In control mice (n = 2) expressing injected

with an EYFP virus without ARCH3, light stimulation had no effect on licking behavior or task performance (2 mice, 11 sessions, Fig 2IJ).

Unilateral inhibition of the SNr decreases reaction time towards the contralateral direction.

To test the hypothesis that SNr optogenetic inhibition interferes with the ability to withhold unwanted anticipatory movements, we activated ARCH during the 2nd half of the air-puff stimulus in the same mice after further training them to perform the 'withholding' variant of the task. A subset (3 of 5) of the mice were able to learn this variant of the task. After achieving 60% performance, we again tested the effect of optogenetic manipulation starting 0.5 s before the offset of the 1 s air-puff. This manipulation again lateralized the execution of trials, but with additional features compared to the 'anticipatory' task (Fig 3). Right air-puff trials again resulted in a significantly increased proportion of wrong direction decision licks (Fig 3E, $P<0.01$, bootstrap). Nevertheless, overall correct performance in the withholding version of the task was slightly increased in right air-puff trials with optogenetic SNr inhibition (3 mice, 17 sessions, $P=0.041$, bootstrap), opposite of the observed change in the anticipatory task. By further examining the breakdown of failure types (Fig 3E), we found a reduction of failures due to early licking ($P<0.001$, bootstrap), indicating a higher success rate of suppressing anticipatory licking in the 'withholding' task when SNr was inhibited. In contrast, left air-puff trials were associated with a significant increase in such early licks ($P<0.01$, bootstrap). This supports the hypothesis that inhibiting SNr output did not only have direct consequences for guiding direction of licking, but also impaired the ability to withhold licks towards the contralateral direction to stimulation and facilitated withholding to the ipsilateral direction. The average lick frequency traces from correct trials (Fig 3C) with optogenetic stimulation were again similar to control trials, suggesting that the licking process when initiated was not itself disrupted. However, success trials did show a shorter reaction time to lick left and a longer reaction time to lick right compared to baseline left and right trials (Fig 3 HI, $P<0.01$ (left), $P<0.01$ (right), bootstrap). This suggests that when the mice were able to withhold licking during the optogenetic stimulation, suppressing basal ganglia output biases preparation of movement or motor readiness, such that the mouse is able to respond more quickly to the contralateral spout and more slowly towards the ipsilateral direction. Control mice expressing EYFP in the SNr but no ARCH (n = 2 mice), showed no significant changes in trial outcomes between baseline and optogenetic stimulation trials (Fig 3G).

Unilateral excitation of the SNr impairs both contra- and ipsilateral licking activity.

Since inhibiting basal ganglia output resulted in a contralateral bias in movement initiation and preparation, the classic model of basal ganglia rate coding leads to the prediction that increasing basal ganglia output would exert an opposite effect on licking activity, namely a reduction of correct decisions to lick in the direction contralateral to stimulation.

To test this prediction, we used the neural activator, ChR2(ET/TC), to increase output activity from the SNr (Fig 4A). In awake mice, blue light (473 nm) stimulation of the SNr using a 1 s continuous pulse increased the already highly active SNr to approximately double the baseline firing rate for the duration of the stimulation (mean firing rate at baseline = 13.9Hz vs. opto = 49.9Hz, n = 8 single units, Fig 4BC). In mice performing the anticipatory variant of the behavioral task (n = 4 mice, 16 sessions, Fig 4D), exciting the right SNr suppressed both left and right licking activity for the duration of the stimulation after anticipatory licking had already started in the first 0.5 s of the air-puff (Fig 4EF). Following the offset of the optogenetic perturbation, mice often resumed licking towards the correct spout (Fig 4EF), indicating that they remembered the air-puff direction through the period of movement inhibition. However, successful task performance significantly decreased in both left and right lick trials with ChR2 stimulation (Fig 4H, $P<0.01$ (left), $P<0.01$ (right)). This decrease in performance was both a result of licking the wrong spout direction (Fig 4H, $P<0.01$ (left), $P<0.001$ (right), bootstrap) and not licking during the response period (Fig 4H, $P<0.05$ (right), bootstrap), suggesting a partial loss of the neural representation of lick direction during the motor inhibition. In control mice (n = 2) injected with an EYFP virus without ChR2, blue light stimulation had no effect on licking behavior or task performance (Fig 4IJ). These results give a more complex picture of bidirectional motor control with SNr inhibition or excitation than predicted by the classic rate model. Particular striking was a complete bilateral lick cessation during ChR2 induced SNr rate increases, which was distinctly different from the lateralized effects of ARCH inhibition.

Unilateral excitation of the SNr in 'withholding' task variant improves contralateral withholding of anticipatory licks and suppresses licking bilaterally.

A subset of the ChR2 expressing mice (3 of 4) were further trained to perform the withholding version of the task. In agreement with the results for the anticipatory task, licking was generally suppressed until after the offset of the stimulation (3 mice, 11 sessions, Fig 5BC. Unlike for the anticipatory variant of the task, however, SNr excitation led to an increase in percent correct for left trials (Fig 5E, $P<0.05$). While this effect seems opposite to our hypothesis, examining the failure outcomes shows that this change was driven primarily by a decrease in the percent of left early lick fails (Fig 5E, $P<0.001$), indicating an improvement in the ability to withhold premature licking predominantly to the contralateral side. Optogenetic excitation of the SNr also led to an increase in no response trials towards the left direction (Fig 5E, $P<0.05$) and a trend towards decision licks in the wrong direction (Fig 5E, $p=0.071$). However, wrong decision licks were much reduced compared to the anticipatory variant of the task, suggesting that the additional training during the withholding stage had strengthened the neural representation of air-puff direction and made it more resilient to the ChR2 induced period of lick suppression. On trials where the mice did lick following the offset of the optogenetic stimulation, reaction time was significantly increased compared to control trials for both the right and left lick

directions (Fig 5E) due to the suppression of licking in the initial 0.5 s after air-puff offset when light stimulation was still on. Mice expressing EYFP, but no ChR2 in SNr did not demonstrate any changes in licking performance (Fig 5FG). Overall, these results give further support to the strong bilateral motor suppression of unilateral SNr excitation.

Unilateral excitation of nigrothalamic terminals in BGMT biases licking towards the ipsilateral direction in the anticipatory task variant.

The effects described for somatic SNr inhibition and excitation may be due to due descending projections from the SNr towards the brainstem las well as ascending projections to BGMT. To selectively target the ascending pathway from the SNr to the BGMT, we stimulated ChR2 expressing SNr terminals in BGMT in the same mice used for somatic stimulation through a second fiber implanted over the BGMT. In contrast to somatic stimulation we found that SNr terminal stimulation in BGMT in the anticipatory variant of the task suppressed only contralateral licking behavior and did not affect ipsilateral licking (4 mice, 25 sessions, Fig 6BC). Successful trial execution was significantly impaired for left lick trials but remained unchanged for right licks (Fig 6E, $P<0.001$ (left), bootstrap). This change in performance was due to an increase of left air-puff failed trials both by not responding (Fig 6E, $P<0.001$, bootstrap) as well as licking the wrong direction (Fig 6E, $P<0.001$, bootstrap), which in this case was caused by licking the right spout instead of the left during SNr-BGMT terminal stimulation. This last effect in particular is in stark contrast to the results with somatic SNr excitation, suggesting that ascending thalamic route leads to a lateralized effect on motor preparation, whereas descending outputs may globally suppress the brainstem lick pattern generator.

Unilateral excitation of nigrothalamic terminals in BGMT in the withholding task variant facilitates suppression and increases reaction time for contralateral licking.

Lastly, we again compared changes in the withholding task to those in the anticipatory variant. Licking activity was similarly impaired towards the left direction while it remained intact towards the right direction (3 mice, 13 sessions, Fig 7BC). The proportion of successful task performance in the ‘withholding’ variant was almost unchanged with SNr-BGMT terminal stimulation. However, there was a significant decrease in withholding failures (early lick trials) towards the left direction (Fig 7E, $P<0.05$, bootstrap). This suggests SNr output to BGMT also contributes to the ability of the mouse to withhold movement, though only towards the contralateral direction. In this version of the task, lick reaction times in the response period to the left direction were significantly longer than baseline (Fig 7HI, $P<0.001$, bootstrap). Right reaction times were not significantly changed. This supports the conclusion that exciting SNr output specifically in BGMT exerted a lateralizeed influence on motor preparation and initiation.

Discussion

Our results demonstrate that excitation and inhibition of basal ganglia output via the SNr drives lateralized and opposing influences on the cognitive control of directional licking. The SNr sends inhibitory projections towards both the motor thalamus and the superior colliculus, two structures directly implicated in the control of licking in mice (Li et al., 2015, Rossi et al., 2016). Our results shed new light on a distinct role of the ascending thalamic pathway by demonstrating bilateral lick suppression when the descending pathway is involved, but differential lateralized effects for the BGMT pathway.

An important feature of our behavioral task training was the use of two different task variants that entailed different cognitive demands (Fig 1). This 'anticipatory' task did not require the mouse to withhold movement during air-puff delivery and we observed a steady increase in anticipatory licking activity approaching the start of the response period. This licking was in in the direction of the correct target (Fig 1C), therefore revealing the completion of stimulus evaluation and motor preparation as early as 1 s before air-puff offset (Fig 1C). Optogenetic excitation of the SNr unilaterally suppressed anticipatory licking towards both lick spouts, whereas excitation of SNr terminals in the BGMT suppressed licking contralaterally (Fig 6E). In mice performing a similar directional licking task, suppressing activity in either the VM thalamus or the anterior lateral motor cortex (ALM), through either optogenetic or muscimol inactivation selectively disrupted contralateral licking, while leaving ipsilateral licking unaffected (Li et al., 2015, Li et al., 2016, Chen et al., 2017, Guo et al., 2017, Svoboda and Li, 2017). Our results suggest that this thalamo-cortical motor planning process can be gated by basal ganglia output. The BGMT is well situated anatomically to influence ipsilateral cortical behavior, with single-cell tracing studies depicting large projections branching across ipsilateral layer 1 of sensory and motor cortices (Kuramoto et al., 2009, Kuramoto et al., 2015).

Several studies have shown that the basal ganglia exert lateralized control of motor circuits (Sakamoto and Hikosaka, 1989, Hikida et al., 2010, Tai et al., 2012, Dominguez-Vargas et al., 2017) with D1 pathway activation biasing towards contralateral movements and D2 pathway activation biasing towards ipsilateral movement. Similar to D1 pathway activation, we found that SNr inhibition increased performance for contralateral trials and decreased performance on ipsilateral trials. We were able to expand the understanding of this directional bias, showing that these changes were due to decreased left wrong direction fail trials and increased right no-response trials, respectively (Fig 2H).

The 'withholding' variant of our lick task required mice to actively withhold licks for the duration of the air-puff (Fig 1D). By adding this withhold demand, we were able to investigate the role of the SNr on movement suppression and movement initiation by

measuring early lick fail trials and reaction time following the offset of the air-puff, respectively. A number of studies have demonstrated a role for the basal ganglia in movement preparation or “motor readiness” (Alexander and Crutcher, 1990, Jaeger et al., 1993, Ding and Gold, 2013). We found that optogenetic inhibition of the SNr during the withholding variant of the task marginally increased performance (Fig 3F), seemingly contradicting performance changes observed in the anticipatory task. However, when we analyze changes in fail trial outcomes, we observed significant increases in early licking towards left trials and decreases in early licking for right trials. This finding supports previous evidence demonstrating a role for the basal ganglia and specifically the SNr in controlling suppression of lateralized movement. Regarding motor preparation, we found lateralized changes in reaction times, such that SNr inhibition led to faster reaction times towards the left and slower reaction times towards the right spout (Fig 3HI). When we instead excited the SNr, we found opposite changes in task behavior, with left trials showing improved performance due to a decrease in the number of early licking fail trials (Fig 5E). As seen in the anticipatory task, average licking activity was suppressed towards both lick spouts for the duration of SNr ChR2 stimulation (Fig 5C). This effect was also apparent when quantifying reaction times, showing that SNr excitation delayed responses towards both left and right spouts (Fig 5HI). As seen in the basal ganglia and motor cortex, the motor thalamus also shows ramping activity underlying movement preparation (Tanaka, 2007, Guo et al., 2017). Our results suggest that excitation of SNr projections to BGMT may be suppressing such ramping activity in thalamo-cortical loops, both aiding in preventing unwanted movements and also impairing contralateral movement preparation.

A recent set of studies have implicated the SNr projection to the superior colliculus (the nigrotectal pathway) in the control of licking behavior (Rossi et al., 2016, Toda et al., 2017). In Rossi et al. 2016, researchers optogenetically bilaterally excited SNr projections at the level of the superior colliculus which led to diminished, though not completely suppressed, licking towards the lick spout positioned in front of the mouse. The authors additionally investigated SNr projections to the midbrain reticular formation (mRF), another brain area associated with control of licking behavior (Li et al., 2015), but found no change in licking activity during SNr-mRF terminal excitation. Our results showing that unilateral excitation of the SNr near completely suppressed licking could therefore be explained via projections to the SC. Consistent with this explanation, anatomical tracing experiments demonstrate that the SNr projects to SC bilaterally, while projections to the thalamus are ipsilateral (Deniau and Chevalier, 1992, Liu and Basso, 2008). Why somatic inhibition of the SNr did not facilitate licking bilaterally (opposite of SNr excitation) requires further examination, but may suggest that the thalamic pathway is more susceptible to disinhibition than the collicular pathway.

Several studies have implicated the SNr in the control of other aspects of behavior, including locomotion (Roseberry et al., 2016) and saccadic eye movements (Hikosaka 1989). To determine whether our manipulations inadvertently influenced any other aspects of movement, we recorded high-speed video of the mouse pupil and face for a subset of trials across mice. Interestingly, we did not observe any movements (including eye, whisker, fore-limb) associated with our optogenetic manipulations (data not shown). This suggests that the behavioral effects seen following SNr manipulations may be dependent on task context or the animal's behavioral state, such that in mice running in an open field, the effects of SNr perturbations would be most apparent with respect to locomotion while in animals performing saccade tasks, the effects are apparent in eye movements.

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Figures and Legends

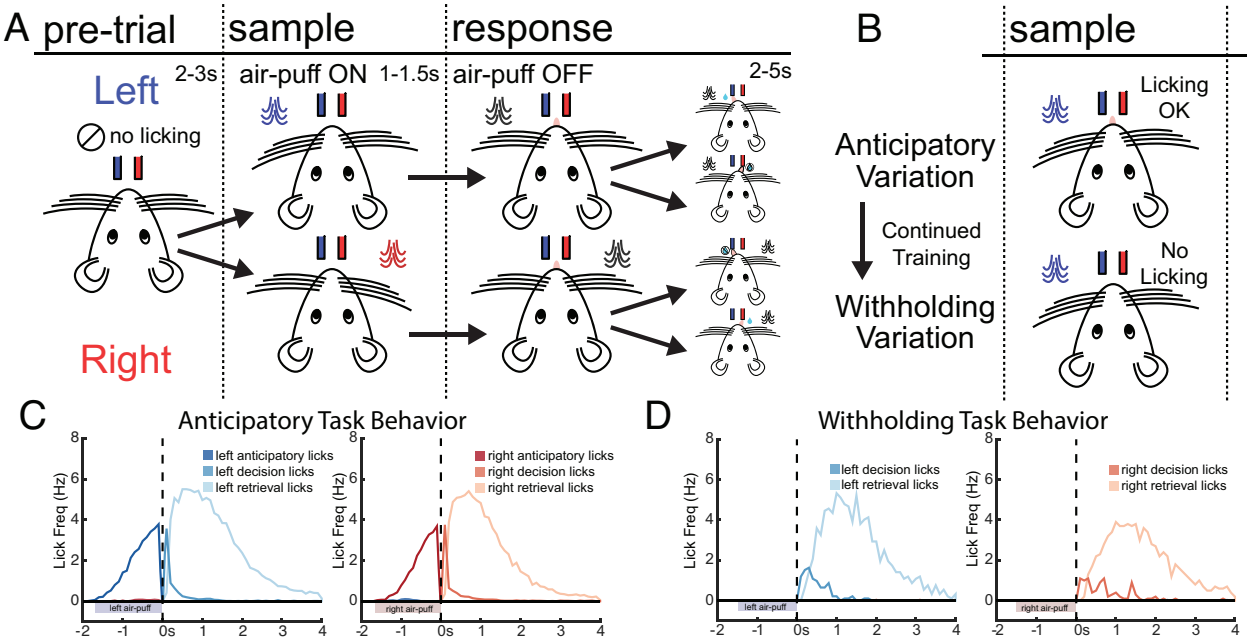

Figure 1. Mice learn to perform anticipatory and withholding variants of a 2-alternative choice licking task.

A. Illustration of the alternative choice licking task. Each trial begins with a pre-trial period lasting 2-3 s where the mouse cannot lick either spout. During the sample period, an air-puff is directed at either the left or right whiskers and remains on for 1-1.5 s. The response period begins after the offset of the air-puff and the first lick during this period is counted as the decision lick. If the decision lick is correct (towards the left spout for left air-puff or right lick spout for right air-puff) the mouse is provided a liquid reward from the correct spout. If the mouse does not provide a lick for the duration of the response period (2-5 s) or licks the incorrect spout no reward is provided. **B.** Mice are trained in two subsequent variations of the licking task. First, they are trained in the anticipatory variation where they are allowed to lick towards either spout during the air-puff in anticipation of the response period and potential reward delivery. After they have learned this task, the same mice are further trained in the withholding variation of the task where the must suppress licking during the duration of the air-puff. **C.** Average lick frequency traces for correct trials from mice performing the anticipatory variant of the task (N = 12 mice). **Left.** For left trials (n = 1151 trials), Mice begin to show anticipatory licking (dark blue) after the onset of the air-puff and the first lick after the offset of the air-puff is counted as the decision lick (middle blue). The retrieval licks (light blue) are the licks corresponding to the mouse retrieving the sucrose reward after a correct decision lick. **Right.** Same format as left trials, but instead showing right trials (n = 1098). Anticipatory, decision, and retrieval licks are depicted as dark red, middle red, and light red, respectively. **D.** Average lick frequency traces for correct trials from mice performing the withholding variant of the task (N = 8 mice). **Left.** For left trials (n = 397 trials), mice successfully withhold licking activity during the air-puff and once again the first lick after the offset of the air-puff is counted as the decision lick (middle blue). The retrieval licks (light blue) are the licks corresponding to the mouse retrieving the sucrose reward after a correct decision lick. **Right.** Same as for left, but now for right lick trials (n = 403 trials).

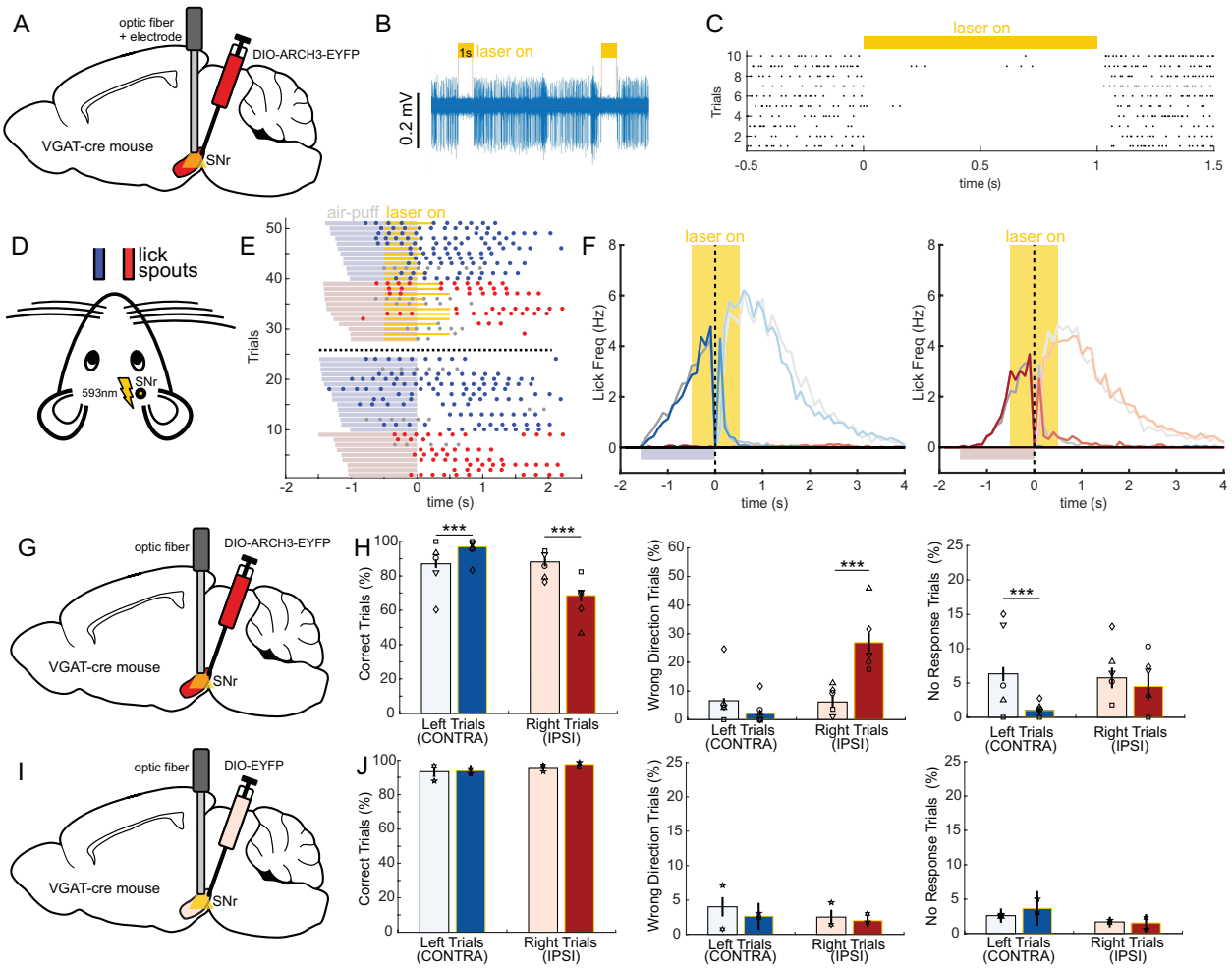

Figure 2. Unilateral inactivation of the SNr biases towards contralateral licking behavior

A. Diagram of optogenetic vector injection and optic fiber targeting. Cre-dependent AAV2-DIO-ARCH3-EYFP was injected in the right SNr of VGAT-cre transgenic mice and optic fiber attached to a microelectrode was lowered into SNr. **B.** Example single-unit recording of an SNr neuron expressing ARCH3. 593nm laser stimulation silences firing activity for the duration of the light pulse and firing quickly resumes following the offset of the light stimulation. **C.** 10 stimulation trials aligned to the onset of stimulation that show the consistency of ARCH3 inhibition of SNr activity. **D.** Illustration showing mouse orientation with respect to right and left lick spouts positioned in front of the mouse and optogenetic illumination through fiber implanted over the SNr. **E.** Example behavioral session showing licking activity for right and left optogenetic and baseline trials. Trials are arranged by optogenetic stimulation condition (stim trials top, baseline below) and length of air-puff presentation. Blue and red horizontal lines depict the presentation of the air-puff during the sample period and yellow lines are the periods of optogenetic SNr inhibition. In this example, optogenetic inhibition of right SNr disrupts right (ipsilateral) licking behavior such that the mouse is biased towards licking the left (contralateral) spout. **F.** Average lick frequency traces for correct trials with (colored lines) and without (gray lines) optogenetic right SNr inhibition. For left trials (n = 300 opto, 247 baseline, **left**) and right trials (n = 185 opto, 301 baseline, **right**) mice show anticipatory licking activity towards the correct spout that increases until the start of the response period. Anticipatory licks are depicted by the darkest colored line, followed by decision licks (middle color) and retrieval licks (lightest colored line). **G.** Behavioral effects of unilateral SNr optogenetic inhibition in the anticipatory task. **H.** Changes in

performance between left (blue) and right (red), off (light) and on (dark) stimulation. Bar height represents mean across all sessions (n = 35 sessions), with shapes representing the mean for each mouse (5 mice). Error bars represent SEM (bootstrap, 10000 iterations) and p values based on bootstrap (see methods, ***p<0.001, **p<0.01, *p<0.05). **Left.** B Compared to interleaved baseline trials (light blue/red columns), licking performance during right SNr inhibition (dark blue/red columns) produced a significant increase in the percent of left trials correct (p<0.001) and significant decrease in the percent of right trials correct (35 sessions, 5 mice). **Middle.** Optogenetic inhibition of the right SNr caused a significant increase (P<0.001) in the percent of right wrong direction fail trials (incorrectly lick left during right trials). **Right.** For left lick trials, SNr inhibition significantly reduced the number of no response trials (P< **I.** Behavioral effects of optogenetic stimulation in control mice (n = 2) injected with cre-dependent EYFP. **J.** Same as H, but for control stimulation. No significant changes in performance were found for percent trials correct (**left**), wrong direction (**middle**), or no response trials (**right**).

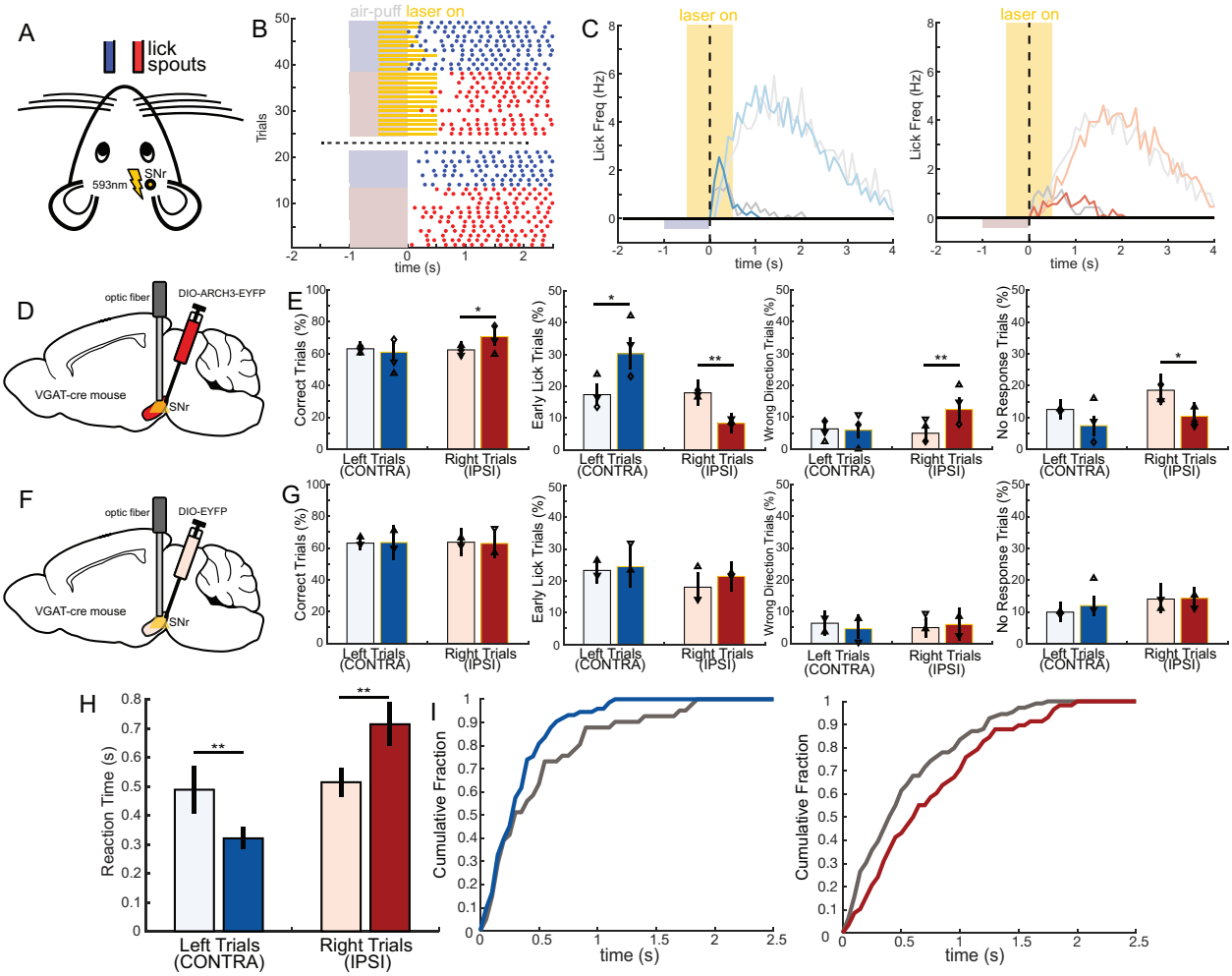

Figure 3. Unilateral inhibition of the SNr decreases reaction time towards the contralateral direction

A. Illustration showing mouse orientation with respect to right and left lick spouts positioned in front of the mouse and optogenetic illumination through fiber implanted over the SNr. **B.** Example behavioral session showing trial-by-trial licking activity (early lick trials excluded) for right and left optogenetic and baseline trials. Trials are arranged by optogenetic stimulation condition (stim trials top, baseline below). Blue and red horizontal lines depict the presentation of the air-puff during the sample period and yellow lines are the periods of optogenetic SNr inhibition. In this example, optogenetic inhibition of right SNr shifts lick onset for left (contralateral) trials closer to the start of the response window appears to slightly delay right (ipsilateral) licking behavior. **C.** Average lick frequency traces for correct trials with (colored lines) and without (gray lines) optogenetic right SNr inhibition. For left trials (n = 71 opto, 140 baseline, **left**) and right trials (n = 89 opto, 127 baseline, **right**) mice show faster decision licking activity on left trials compared baseline lick decisions. Decision lick activity is depicted by the darker shaded lines and retrieval licks by the light shaded lines. **G.** Behavioral effects of unilateral SNr optogenetic inhibition in the withholding task. **H.** Changes in performance between left (blue) and right (red), off (light) and on (dark) stimulation. Bar height represents mean across all sessions (n = 16 sessions), with shapes representing the mean for each mouse (3 mice). Error bars represent SEM (bootstrap, 10000 iterations) and p values based on bootstrap (see methods, **p<0.01, *p<0.05). **Left.** Compared to baseline trials (light blue/red columns), licking performance during right SNr inhibition (dark blue/red columns) produced a marginally significant increase in the percent of right trials correct (p=0.0418, 16 sessions, 3 mice). **Middle Left.** Optogenetic inhibition of the right SNr caused

a significant increase in the percent of left early lick fail trials (P=0.016) and a significant decrease in the percent of right early lick trials (P<0.01). **Middle Right.** Optogenetic inhibition of the SNr increased the percentage of wrong direction right trials (P<0.01). **Right.** Right SNr inhibition produced a slight trend and a marginally significant decrease percentage of no response trials for left (P=0.23) and right (P=0.048) trials, respectively. **F.** Behavioral effects of optogenetic stimulation in control mice injected with cre-dependent EYFP. **G.** Same as H, but for control stimulation (n = 2 mice, 7 sessions). No significant changes in performance were found for correct (**left**), early lick (**middle left)**, wrong direction (**middle right**), or no response trials (**right**). **H.** Mean reaction times for left and right trials with and without optogenetic SNr inhibition. Reaction times for left trials were significantly decreased (P<0.01) and for right trials were significantly increased (0.01) during SNr inhibition trials. **I.** Cumulative distribution plots showing the changes in the distribution of reaction times for left (**left**) and right (**right**) lick trials. Optogenetic stimulation trials are colored blue/red and baseline distributions are gray. The reaction time distribution shifts towards shorter reaction times for left trials (**left**) and towards longer reaction times for right trials (**right**).

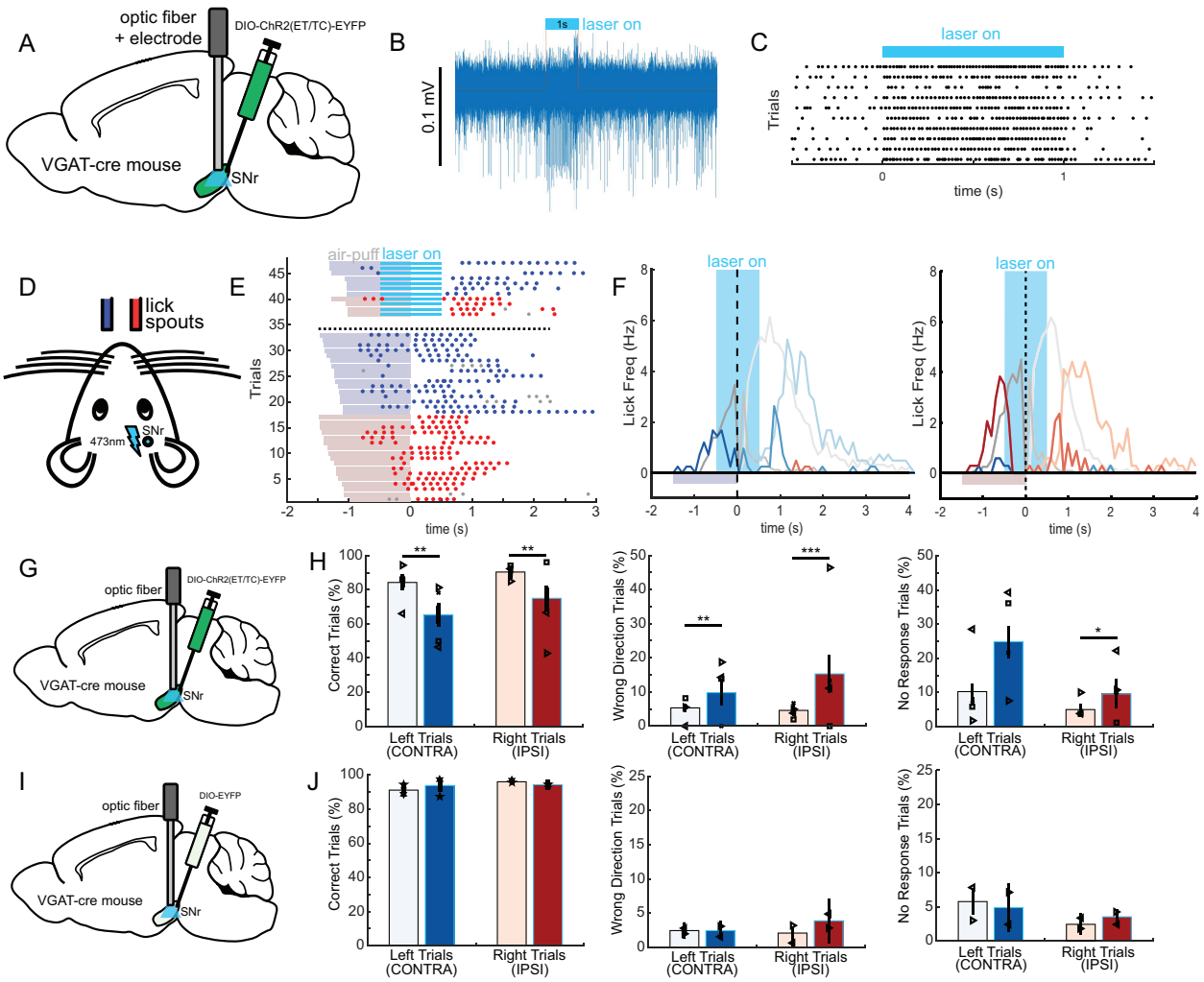

Figure 4. Unilateral excitation of the SNr impairs both contra- and ipsilateral licking activity

A. Diagram of optogenetic vector injection and optic fiber targeting. Cre-dependent AAV2-DIO-hChR2(E123T/T159C)-EYFP was injected in the right SNr of VGAT-cre transgenic mice and optic fiber attached to a microelectrode was lowered in to SNr. **B.** Example multi-unit recording of an SNr neuron expressing ChR2(ET/TC). 473nm laser stimulation increases firing activity for the duration of the light pulse and firing quickly resumes following the offset of the light stimulation. **C.** 10 stimulation trials aligned to the onset of stimulation that show the excitation of SNr activity across trials (single unit isolated from recording shown in B). During ChR2 excitation, mean firing rate increased from 13.9Hz to 49.9Hz (n=10 neurons). Firing rate increase of example neuron is 14.4Hz vs. 46.3Hz during optogenetic stimulation. **D.** Illustration showing mouse orientation with respect to right and left lick spouts positioned in front of the mouse and optogenetic illumination through fiber implanted over the SNr. **E.** Example behavioral session showing licking activity for right and left optogenetic and baseline trials. Trials are arranged by optogenetic stimulation condition (stim trials top, baseline below) and length of air-puff presentation. Blue and red horizontal lines depict the presentation of the air-puff during the sample period and light blue lines are the periods of optogenetic SNr excitation. In this example, optogenetic excitation of right SNr disrupts both right (ipsilateral) and left (contralateral) licking behavior for the direction of the optogenetic stimulation, though licking resumes following the offset of the light. **F.** Average lick frequency traces for correct trials with (colored lines) and without (gray lines) optogenetic right SNr excitation. For left trials (n = 96 opto, 168

baseline, **left**) and right trials (n = 103 opto, 153 baseline, **right**) mice show anticipatory licking activity towards the correct spout that increases until the start of the response period for baseline licking, though is suppressed following the onset of light stimulation for optogenetic trials. Anticipatory licks are depicted by the darkest colored line, followed by decision licks (middle color) and retrieval licks (lightest color). **G.** Behavioral effects of unilateral SNr optogenetic excitation in the anticipatory task. **H.** Changes in performance between left (blue) and right (red), off (light) and on (dark) stimulation. Bar height represents mean across all sessions (n = 16 sessions), with shapes representing the mean for each mouse (4 mice). Error bars represent SEM (bootstrap, 10000 iterations) and p values based on bootstrap (see methods, ***p<0.001, **p<0.01, *p<0.05). **Left.** Compared to baseline trials (light blue/red columns), licking performance during right SNr excitation (dark blue/red columns) produced a significant decrease in the percent of left and right trials correct (p<0.01, 16 sessions, 4 mice). **Middle.** Optogenetic excitation of the right SNr caused a significant increase in the percent of right wrong direction fail trials for both left (P<0.01) and right (P<0.001) trials. **Right.** For left lick trials, SNr excitation showed a trend towards increase percentage of left no response trials (P=0.094) and a marginally significant increase in no response trial percentage for right lick trials (P<0.014). **I.** Behavioral effects of optogenetic stimulation in control mice injected with cre-dependent EYFP. **J.** Same as H, but for control stimulation (n = 2 mice, 8 sessions). No significant changes in performance were found for percent trials correct (**left**), wrong direction (**middle**), or no response trials (**right**).

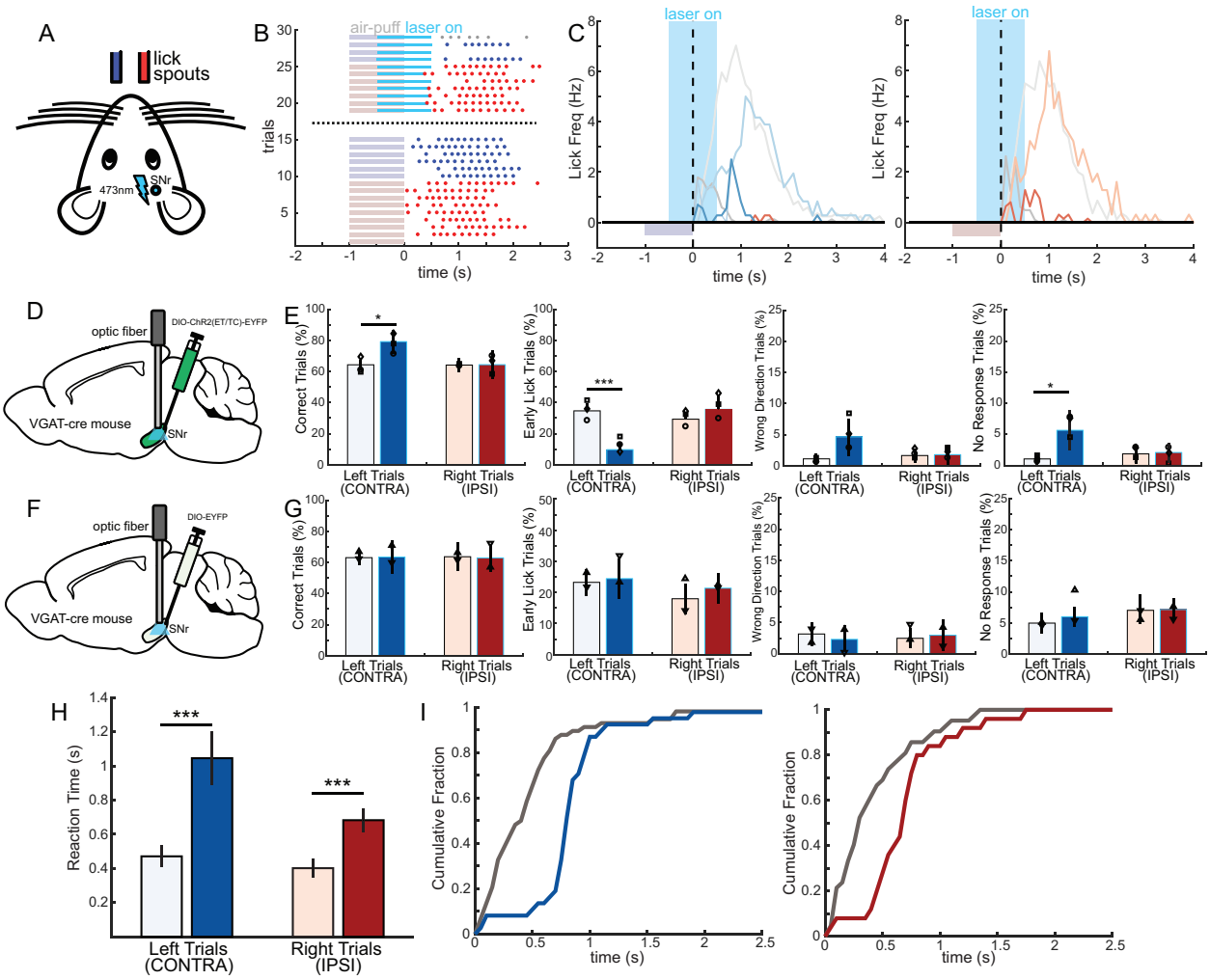

Figure 5. Unilateral excitation of the SNr increases reaction time towards both lick directions

A. Illustration showing mouse orientation with respect to right and left lick spouts positioned in front of the mouse and optogenetic illumination through fiber implanted over the SNr. **B.** Example behavioral session during the withholding task showing trial-by-trial licking activity (early lick trials excluded) for right and left optogenetic and baseline trials. Trials are arranged by optogenetic stimulation condition (stim trials top, baseline below). Blue and red horizontal lines depict the presentation of the air-puff during the sample period and light blue lines are the periods of optogenetic SNr excitation. In this example, optogenetic excitation of right SNr delays lick onset for both right (ipsilateral) and left (contralateral) licking behavior. **F.** Average lick frequency traces for correct trials with (colored lines) and without (gray lines) optogenetic right SNr excitation. For left trials (n = 63 opto, 132 baseline, **left**) and right trials (n = 53 opto, 127 baseline, **right**) mice show delayed decision licking activity towards both spouts compared to baseline lick decisions. Decision lick activity is depicted by the darker shaded lines and retrieval licks by the light shaded lines. **G.** Behavioral effects of unilateral SNr optogenetic excitation in the withholding task. **H.** Changes in performance between left (blue) and right (red), off (light) and on (dark) stimulation. Bar height represents mean across all sessions (n = 12 sessions), with shapes representing the mean for each mouse (3 mice). Error bars represent SEM (bootstrap, 10000 iterations) and p values based on bootstrap (see methods, ***p<0.001, **p<0.01, *p<0.05). **Left.** Compared to baseline trials (light blue/red columns), licking performance during right SNr excitation (dark blue/red columns) produced a significant decrease in the

percent of left and right trials correct (p=0.023, 12 sessions, 3 mice). **Middle Left.** Optogenetic excitation of the right SNr caused a significant decrease in the percent of left early lick fail trials (P<0.001). **Middle Right.** Optogenetic excitation SNr did not produce any changes in direction licking behavior. **Right.** For left lick trials, right SNr excitation produced a significant increase percentage of no response trials (P=0.014) **F.** Behavioral effects of optogenetic stimulation in control mice injected with cre-dependent EYFP. **G.** Same as H, but for control stimulation (n = 2 mice, 8 sessions). No significant changes in performance were found for correct (**left**), early lick (**middle left)**, wrong direction (**middle right**), or no response trials (**right**). **H.** Mean reaction times for left and right trials with and without optogenetic SNr excitation. Reaction times for both left and right trials were significantly during SNr excitation (P<0.001). **I.** Cumulative distribution plots showing the changes in the distribution of reaction times for left (**left**) and right (**right**) lick trials. Optogenetic stimulation trials are colored blue/red and baseline distributions are gray. Both right and left lick responses are primarily delayed until after the offset of the optogenetic stimulation, shifting the distribution towards the right compared to baseline responses.

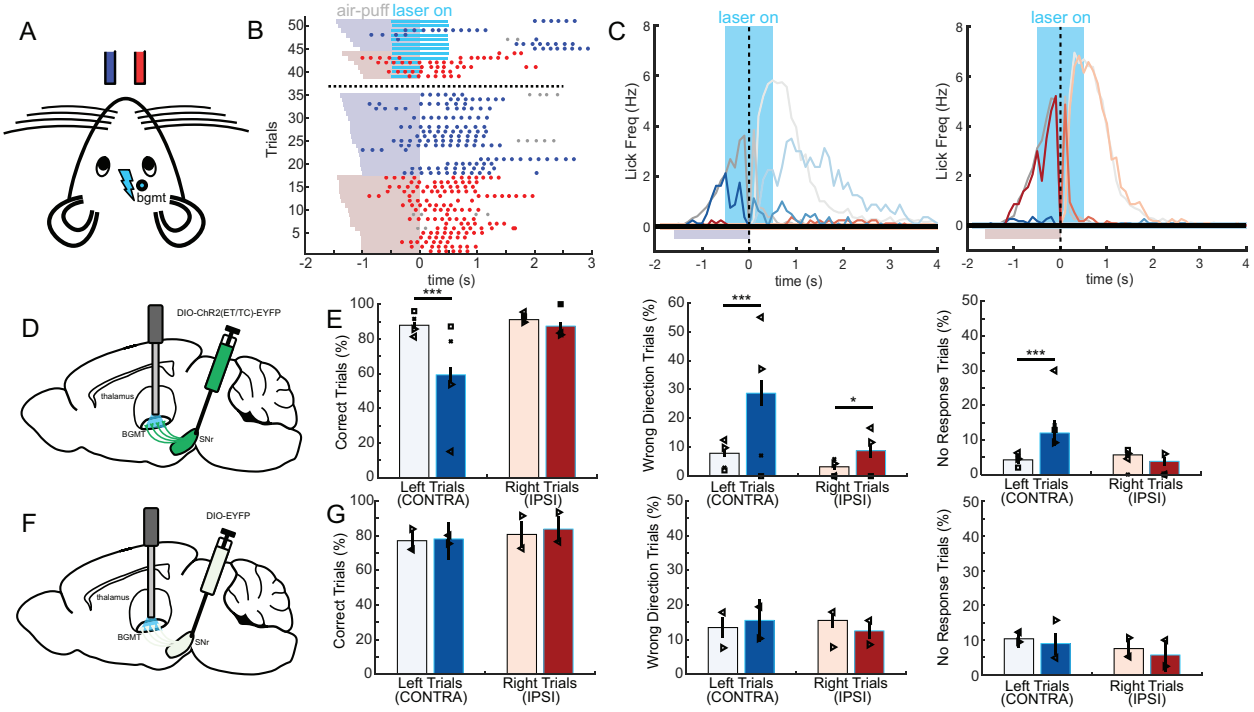

Figure 6. Unilateral excitation of SNr projections to the BGMT biases licking towards the ipsilateral direction

A. Diagram showing mouse orientation with respect to right and left lick spouts positioned in front of the mouse and optogenetic illumination through fiber implanted over the BGMT. **B.** Example behavioral session showing licking activity for right and left optogenetic and baseline trials during the anticipatory task. Trials are arranged by optogenetic stimulation condition (stim trials top, baseline below) and length of air-puff presentation. Blue and red horizontal lines depict the presentation of the air-puff during the sample period and blue lines are the periods of optogenetic SNr-BGMT excitation. In this example, optogenetic excitation of right SNr-BGMT projections disrupts only left (contralateral) licking behavior during optogenetic stimulation trials. **C.** Average lick frequency traces for correct trials with (colored lines) and without (gray lines) optogenetic right SNr-BGMT terminal excitation. For left trials (n = 95 opto, 310 baseline, **left**) and right trials (n = 128 opto, 297 baseline, **right**) mice show anticipatory licking activity towards the correct spout that increases until the start of the response period for baseline licking, though anticipatory licking is suppressed following the onset of light stimulation for left (contralateral) optogenetic trials. Anticipatory licks are depicted by the darkest colored line, followed by decision licks (middle color) and retrieval licks (lightest color). **D.** Behavioral effects of unilateral SNr-BGMT optogenetic excitation in the anticipatory task. **E.** Changes in performance between left (blue) and right (red), off (light) and on (dark) stimulation. Bar height represents mean across all sessions (n = 25 sessions), with shapes representing the mean for each mouse (4 mice). Error bars represent SEM (bootstrap, 10000 iterations) and p values based on bootstrap (see methods, ***p<0.001). **Left.** Compared to baseline trials (light blue/red columns), licking performance during right SNr-BGMT terminal excitation (dark blue/red columns) produced a significant decrease in the percent of left trials correct (p<0.001, 25 sessions, 4 mice). **Middle.** Optogenetic excitation of the right SNr-BGMT terminals caused a significant increase in the percent of left (P<0.001) and right (P=0.02) wrong direction fail trials. **Right.** For left lick trials, SNr-BGMT terminal excitation caused a significant increase in the percentage of left no response trials (P<0.001). **F.** Behavioral effects of optogenetic stimulation in control mice injected with cre-dependent EYFP. **G.** Same as H, but for control stimulation (n = 2 mice, 7 sessions).

No significant changes in performance were found for percent trials correct (**left**), wrong direction (**middle**), or no response trials (**right**).

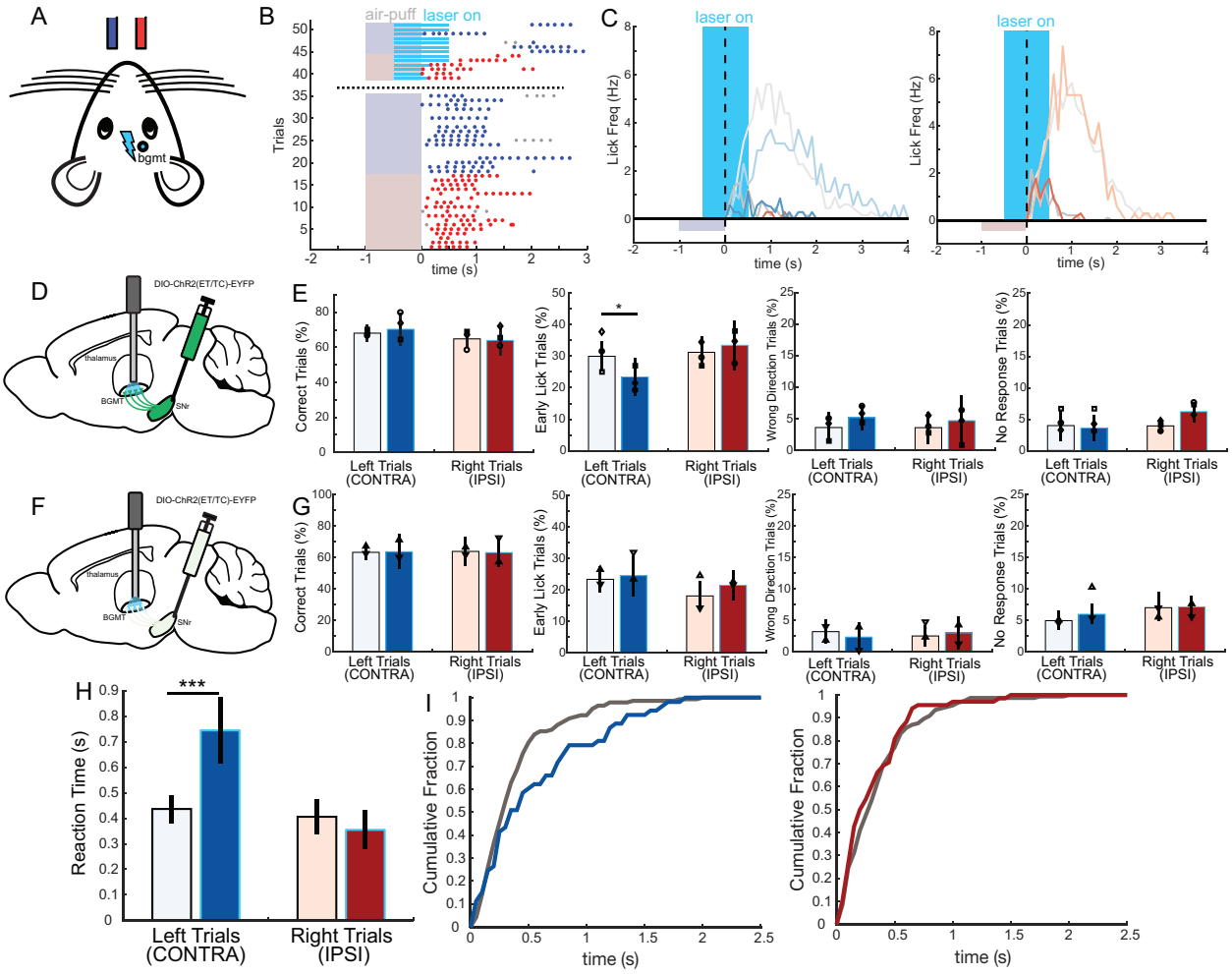

Figure 7. Unilateral excitation of nigrothalamic terminals facilitates suppression and increases reaction time for contralateral licking

A. Illustration showing mouse orientation with respect to right and left lick spouts positioned in front of the mouse and optogenetic illumination through fiber implanted over the SNr. **B.** Example behavioral session during the withholding task showing trial-by-trial licking activity (early lick trials excluded) for right and left optogenetic and baseline trials. Trials are arranged by optogenetic stimulation condition (stim trials top, baseline below). Blue and red horizontal lines depict the presentation of the air-puff during the sample period and light blue lines are the periods of optogenetic SNr-BGMT terminal excitation. In this example, optogenetic excitation of right SNr projections to BGMT delays lick onset for both right (ipsilateral) and left (contralateral) licking behavior. **F.** Average lick frequency traces for correct trials with (colored lines) and without (gray lines) optogenetic right SNr-BGMT terminal excitation. For left trials (n = 87 opto, 245 baseline, **left**) and right trials (n = 91 opto, 197 baseline, **right**) mice show similar licking activity during baseline trials, though during left optogenetic trials, decision and retrieval licking appears delayed. Decision lick activity is depicted by the darker shaded lines and retrieval licks by the light shaded lines. **G.** Behavioral effects of unilateral SNr-BGMT optogenetic terminal excitation in the withholding task. **H.** Changes in performance between left (blue) and right (red), off (light) and on (dark) stimulation. Bar height represents mean across all sessions (n = 13 sessions), with shapes representing the mean for each mouse (3 mice). Error bars represent SEM (bootstrap, 10000 iterations) and p values based on bootstrap (see methods, *p<0.05). **Left.** Compared to baseline trials (light blue/red columns), performance in optogenetic stimulation trials (dark

blue/red columns) remains unchanged. **Middle Left.** Optogenetic excitation of the right SNr projections to BGMT caused a significant decrease in the percent of left early lick fail trials (P=0.023). **Middle Right.** Optogenetic excitation of SNr-BGMT did not produce any changes in percentage of wrong direction fail trials. **Right.** No response fail percentages were not altered by optogenetic stimulation of SNr projections to the BGMT. **F.** Behavioral effects of optogenetic stimulation in control mice injected with cre-dependent EYFP. **G.** Same as H, but for control stimulation (n = 2 mice, 8 sessions). No significant changes in performance were found for correct (**left**), early lick (**middle left)**, wrong direction (**middle right**), or no response trials (**right**). **H.** Mean reaction times for left and right trials with and without optogenetic SNr excitation. Reaction times for left, but not right trials were significantly increased during SNr excitation (P<0.001). **I.** Cumulative distribution plots showing the changes in the distribution of reaction times for left (**left**) and right (**right**) lick trials. Optogenetic stimulation trials are colored blue/red and baseline distributions are gray. Left lick responses are delayed during optogenetic stimulation trials (**left**) while the response distribution for right trials is unchanged compared to baseline (**right**).