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Dopamine-Glutamate Neuron Projections to the Nucleus Accumbens Medial Shell and Behavioral Switching

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Abstract

Dopamine (DA) neuron projections to the striatum are functionally heterogeneous with diverse behavioral roles. We focus here on DA neuron projections to the nucleus accumbens (NAc) medial Shell, their distinct anatomical and functional connections, and discuss their role in motivated behavior. We first review rodent studies showing that a subpopulation of DA neurons in the medial ventral tegmental area (VTA) project to the NAc medial Shell. Using a combinatoric strategy, we show that the majority of DA neurons projecting to the NAc Shell express vesicular glutamate transporter 2 (VGLUT2) making them capable of glutamate co-transmission (DA-GLU neurons). In the NAc dorsal medial Shell, all of the DA neuron terminals arise from DA-GLU neurons, while in the lateral NAc Shell, DA neuron terminals arise from both DA-GLU neurons and DA-only neurons, without VGLUT2. DA-GLU neurons make excitatory connections to the three major cells types, spiny projection neurons, fast-spiking interneuron and cholinergic interneurons (ChIs). The strongest DA-GLU neuron excitatory connections are to ChIs. Photostimulation of DA-GLU neuron terminals in the slice drive ChIs to fire in a burst. Finally, we review studies that address specially the behavioral function of this subpopulation of DA neurons in extinction learning and latent inhibition. Taking into account findings from anatomical and functional connectome studies, we propose that DA-GLU neuron connections to ChIs in the medial Shell play a crucial role in switching behavioral responses under circumstances of altered cue-reinforcer contingencies.

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Introduction

Dopamine (DA) neurons in the ventral midbrain are distributed within the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA). Since the first description of these neurons (Ungerstedt, 1971), studies on SNc DA neurons have focused on motor behavior, as loss of SNc DA neurons underpins Parkinson's disease, while studies on the function of VTA DA neurons have been associated with translation of motivation to action (Mogenson et al., 1980). VTA DA neurons projecting to limbic and cortical areas are known to regulate adaptive responses to both positive and negative reinforcers (Salamone and Correa, 2012; Zahm, 2000). In the past two decades, it has become clear that VTA DA neurons are anatomically and functionally heterogeneous and regulate different aspects of motivated behavior (Bromberg-Martin et al., 2010; Chuhma et al., 2017; Lammel et al., 2014; Morales and Margolis, 2017; Sanchez-Catalan et al., 2014; Salamone and Correa, 2012; Volman et al., 2013). This review focuses on the subpopulation of DA neurons projecting to the nucleus accumbens (NAc) medial Shell and their putative behavioral roles.

The medial Shell of the nucleus accumbens

The majority of VTA DA neurons project to the NAc (Breton et al., 2018; Ikemoto, 2007; Swanson, 1982), which is further divided into three subregions, the medial Shell, lateral Shell and the Core (Groenewegen et al., 1991; Voorn et al., 2004). The NAc medial Shell is distinguished from other NAc subregions by dense afferents from the infralimbic prefrontal cortex, the anterior paraventricular thalamus, the ventral hippocampus, parvocellular basal lateral amygdala, dorsolateral septum, lateral hypothalamus, brainstem nuclei (nucleus of the solitary tract of the hypothalamus, pedunculopontine tegmental nucleus and parabrachial nucleus) and medial VTA (Beier et al., 2015; Berendse et al., 1992; Brog et al., 1993; Delfs et al., 1998; Do-Monte et al., 2017; Groenewegen et al., 1991; H. Groenewegen et al., 1999; Heimer et al., 1991; Hunnicutt et al. 2016; Voorn et al., 2004; Zahm, 2000).

The NAc medial Shell is further differentiated from the NAc lateral Shell and Core by its efferents to the medial ventral pallidum, anterior lateral hypothalamus, lateral preoptic area and VTA (Beier et al., 2015; Groenewegen et al., 1999; Usuda et al.,1998; Yang et al., 2018; Zahm, 2000). The neuronal populations targeted in the NAc projecting regions also differ. For example, NAc medial Shell neurons make strong GABAergic connections to VTA DA neurons, while the NAc lateral Shell neurons make strong GABAergic connections to SN GABA neurons that in turn disinhibit neighboring DA neurons (Yang et al., 2018). Thus, NAc medial Shell neurons directly inhibit VTA DA neuron firing and NAc lateral Shell neurons indirectly increase SN DA neuron firing. Overall, these studies identify the medial Shell as a separate functional unit of the NAc.

DA/GLU neurons preferentially project to the NAc medial Shell

In the rodent, DA neurons comprise between 50–60% of VTA neurons (Breton et al., 2018; Nair-Roberts et al., 2008; Yetnikoff et al., 2014) and are dispersed within several subregions, including the parabrachial pigmented nucleus (PBP), paranigral nucleus (PN), caudal linear nucleus (CLi), interfascicular nucleus (IF) and rostral linear nucleus of the raphe (RLi)

(Figure 1A). The dense VTA projections to the NAc subregions are largely ipsilateral and follow a medial-lateral topography (Figure 1B) (Beier et al., 2015; Breton et al., 2018; Ikemoto, 2007; Lammel et al., 2008; Rodríguez-López et al, 2017; Saunders et al., 2018; Swanson, 1982). The NAc medial Shell receives dopaminergic innervation from the posteromedial VTA subdivisions, which include the IF, CLi and PN. The NAc lateral Shell and Core receive dopaminergic innervation from the lateral half of the VTA, which includes the PBP. Simultaneous injections of the retrograde tracer cholera toxin subunit B (CTB) tagged with either Alexa Fluor 594 or 647 in the medial Shell or Core compartments revealed no overlap in the labeled cell population in the rat VTA (Luo et al., 2018). Furthermore, a mouse study using an intersectional strategy to label VTA DA neurons projecting to either the NAc lateral or medial Shell, clearly showed that the labeled axon arbors did not overlap (Beier et al., 2015). Thus, several lines of evidence point to the existence of a distinct group VTA DA neurons projecting selectively to the NAc medial Shell.

Medial VTA DA neurons are distinguished by their ability to co-release glutamate (for review see Trudeau et al., 2014). Studies examining the colocalization of vesicular glutamate transporter 2 (VGLUT2) mRNA and TH immunoreactivity revealed the restricted and high prevalence of dopamine-glutamate (DA-GLU) neurons in the medial VTA (Kawano et al., 2006; Yamaguchi et al., 2011). DA neurons projecting to the NAc medial Shell preferentially express VGLUT2 mRNA in both rat and mouse (Yamaguchi et al., 2011; Yang et al., 2018). A recent mapping study of molecularly defined DA neuron subtypes, confirmed that medial VTA contains DA-GLU neurons and that they project preferentially to the NAc medial Shell (Poulin et al., 2018). DA-GLU neurons were identified using the INTRSECT strategy (Fenno et al., 2014), in which EYFP is expressed only in cells that express *Cre* and *Flp* recombinases. When *Cre*-on (Con) *Flp*-on (Fon) INTRSECT virus is injected in the VTA of double mutant mice,expressing *Cre* under the VGLUT2 promotor and *Flp* under the TH promotor (TH *Flp*;VGLUT2 *Cre* mice), YFP expression is restricted to TH positive (+)/VGLUT2+ VTA neurons.

Using the same strategy we have confirmed these observations and shown further the exclusiveness of these projections. TH *Flp*;VGLUT2 *Cre* double mutant mice were either injected with the INTRSECT Con/Fon virus (AAV-nEF-Con/Fon hChR2(H134R)-EYFP-WPRE), restricting ChR2-EYFP expression to DA-GLU neurons, or INTRSECT *Cre*-off (Coff) Fon virus (AAV-nEF-Coff/Fon hChR2(H134R)-EYFP-WPRE), restricting ChR2-EYFP expression to DA-only neurons (TH neurons that do not express VGLUT2). VTA injections used previously described methods (Chuhma et al., 2014; Mingote et al., 2017), and the results are presented in Figure 2 and 3. We show first ChR2-EYFP expression induced by a Con/Fon virus requires expression of both *Cre* and *Flp* in DA cells (Figure 2A). We estimated the specificity of each Con/Fon and Coff/Fon virus by counting how many ChR2-EYFP+ VTA cells also coexpressed TH immunoreactivity. Our results show a specificity rate of 93 ± 1.0 % for the Coff/Fon virus and of 87 ± 2.6 % for the Con/Fon virus (Figure 2B). Among all TH immunoreactive cells in the VTA, we found that 71 ± 4.6 % expressed Coff/Fon virus and are thus DA-only neurons, while 31 ± 2.6 % expressed Con/Fon virus and are thus DA-GLU neurons (Figure 2C). This number of VTA DA-GLU neuron is only slightly higher than previously reported (Kawano et al., 2006; Steinkellner et

al., 2018; Yamaguchi et al., 2011). DA-GLU neurons were mostly seen in the medial VTA, IF and PN subregions, and DA-only neurons in the lateral PBP (Figure 2D), consistent with previous *in situ* hybridization studies (Kawano et al., 2006; Steinkellner et al., 2018; Yamaguchi et al., 2011). Although the specificity within the VTA was high for both viruses, TH *Flp* may label some TH negative cells in the interpeduncular nucleus, as reported previously by Poulin and colleagues (2018). This non-specific expression was seen in mice injected with the Coff/Fon virus (Figure 2D, yellow arrows). Thus, the TH promotor appears to be active in GABAergic neurons in this nucleus. These neurons project to the lateral habenula but do not produce TH protein nor release DA (Lammel et al., 2015). Interestingly, Con/Fon virus did not show this ectopic expression, since interpeduncular nucleus GABAergic neurons do not co-express TH and VGLUT2, further validating the combinatoric strategy.

Figure 3A shows the distribution of the ChR2-EYFP+ axons labeled by Con/Fon and Coff/Con viruses within the striatum. In agreement with Poulin et al. (2018), the projections from DA-GLU neurons are restricted to the NAc medial Shell and medial olfactory tubercle. Strikingly axons of DA-only neurons almost completely avoid the NAc dorsal medial Shell (Figure 3A, yellow arrows), while still innervating the NAc lateral Shell and Core and most of the dorsal striatum. Thus, not only do DA-GLU neurons target the NAc medial Shell specifically, but they do so exclusively in the dorsal medial Shell. Whole-cell voltage clamp recordings support the specificity of this combinatoric strategy by showing that glutamate-mediated excitatory postsynaptic currents (EPSCs) are observed only when photostimulating DA-GLU neuron axons in the medial Shell, and not when photostimulating DA-only neuron axons in the Core (Figure 3B), which receives the densest innervation from DA-only neurons.

Effects of DA neuron glutamate cotransmission in the NAc Shell

Selective photostimulation of DA neuron terminals in different brain regions recipient to DA neuron projections in DAT-IREScre; Ai32 mice has enabled comprehensive mapping of DA neuron connections, revealing the remarkable complexity of the ventral midbrain DA neuron signals and their regional heterogeneity (Chuhma et al., 2014; Kabanova et al., 2015; Mingote et al., 2015; Pérez-López et al., 2018; Straub et al., 2014; Stuber et al., 2010; Tritsch et al., 2012; Tecuapetla et al., 2010; Wieland et al., 2014). Several new modes of DA neuron signaling have been revealed. First, DA neuron DA transmission can induce fast synaptic responses. DA transmission in the medial dorsal striatum pauses spontaneous firing of cholinergic interneurons (ChIs) by inducing a subsecond hyperpolarization mediated by D2R coupled to a G-protein coupled inward rectifier potassium channels (GIRK channels) (Cai and Ford, 2018; Chuhma et al., 2014, 2018; Straub et al., 2014). Second, DA neurons synthesize and co-release GABA in the NAc and dorsal striatum (Kim et al., 2015; Tritsch et al, 2012; Tritsch et al., 2014). Only a few DA neurons express mRNA for the GABA synthetic enzyme GAD 65 (Kim et al., 2015; González-Hernández et al., 2001; Tritsch et al., 2014). Instead, DA neurons sustain GABA release via plasma membrane uptake of GABA (Tritsch et al., 2014) and synthesis mediated by aldehyde dehydrogenase 1a1 (Kim et al., 2015). DA neurons do not express vesicular GABA transporter; apparently GABA is loaded into vesicles via vesicular monoamine transporter 2 (Tritsch et al, 2012). Finally, DA

neurons make glutamate-mediated excitatory connections in the NAc and lateral dorsal striatum, but not to the medial dorsal striatum (Cai and Ford, 2018; Chuhma et al., 2014; Chuhma et al.,2018; Mingote et al., 2015; Stuber et al., 2010; Tecuapetla et al., 2010). It appears that in relation to connections to ChIs, DA-GLU neurons signal via ionotropic glutamate in the medial Shell, while they signal via metabotropic glutamate receptors in the lateral dorsal striatum.

The optogenetic mapping of the DA neuron synaptic connections in the striatum clearly defined the NAc medial Shell as a hotspot for glutamate cotransmission (Chuhma et al., 2014; Mingote et al., 2015). Glutamatergic responses were measured in spiny projection neurons (SPN), fast spiking neurons (FSI), and ChI and the amplitude of these responses gives an estimate of cell-specific connection strength (Figure 4A). SPNs and FSIs showed similarly low connection strength. These weak connections are unlikely to drive SPNs and FSIs to fire given their deep resting membrane potentials, suggesting that DA neurons would only drive firing coincident with other glutamatergic inputs. Indeed, it has been shown that when SPNs are slightly depolarized and at membrane potentials approximating the typical *in vivo* up state of these neurons, single pulse stimulation of DA neurons is sufficient to drive firing (Tecuapetla et al., 2010). In contrast, the strength of the DA neuron glutamatergic connections to ChIs was several times greater (Figure 4A). Indeed, burst stimulation of DA neurons drives ChIs to burst fire and then pause (Figure 4B). The burst is driven by the activation of AMPA receptors and the pause by activity-dependent activation of SK3 channels and partially by D2 receptors coupled with GIRK channels (Chuhma et al., 2014). DA neurons in the medial Shell make no apparent GABAergic connections to ChIs (Chuhma et al., 2014).

Why DA neurons make the strongest glutamatergic connections to ChIs is not clear. A recent paper suggested that DA neuron glutamate-only synapses have high release probability (Silm et al., 2019), but the number of glutamate vesicles per synapse or number of synapses per ChI may also contribute to cell-specific connectivity. DA neurons make widespread axonal arborizations that can broadcast signals to many striatal neurons (Matsuda et al., 2009). In the NAc medial dorsal Shell, which only receives projections from DA-GLU neurons, a single DA neuron could excite multiple ChIs and synchronize their activity. The effects of synchronized ChIs activity on local striatal circuits has been studied extensively in the last decade with optogenetic stimulation (Cachope et al., 2012; English et al., 2012; Faust et al., 2015; Nelson et al., 2011; Threlfell and Cragg, 2011; Threlfell et al., 2012; Witten et al., 2010). These studies have revealed that ChIs modulate DA release and SPN activity.

Synchronized burst firing of ChI directly increases DA release by stimulating presynaptic nicotinic acetylcholine receptors (nAChRs) on DA neuron terminals (Cachope et al., 2012; Threlfell et al., 2012). In the NAc Core and dorsal striatum, the ChI-driven DA release does not show frequency-dependent summation, as single or train stimulation of ChIs produces DA transients of similar amplitude (Shin et al., 2017; Threlfell et al., 2012). The lack of summation is due to desensitization of nAChR during train stimulation. In the NAc shell, nAChRs on DA neuron terminals show less desensitization due to elevated acetylcholinesterase activity (Shin et al., 2017). This reduces the inhibitory effect at higher

frequencies, allowing frequency-dependent increases in DA release. Thus, the NAc Shell local circuit and molecular environment appears to be suitable for establishing a positive feedback loop in which DA neuron burst activity induces further DA release through frequency-dependent activation of presynaptic nAChRs. The activation of nAChR will also increase release of cotransmitters, glutamate and GABA. DA neurons make glutamatergic connections to both ChIs and SPNs, and GABA connections to SPNs (Chuhma et al., 2014; Tritsch et al., 2014). However, both GABA and glutamate synapses show short-term depression when DA neurons are stimulated at burst firing frequencies (Mingote et al., 2017; Straub et al., 2014; Tecuapetla et al., 2010; Tritsch et al., 2014), limiting the facilitating effect of nAChR activation. So, the main effect of stimulating DA neuron presynaptic nAChRs will be to increase DA release.

Synchronized activation of ChI also drives inhibitory responses in SPNs both in the NAc and striatum (English et al., 2012; Faust et al., 2015; Nelson et al., 2014; Luo et al., 2013; Witten et al., 2010). This inhibition is disynaptic and recruits local GABAergic circuits. In the dorsal striatum, ChI-driven inhibition is mediated by the activation of presynaptic nAChR in some classes of GABA interneurons and GABA-releasing DA neurons (English et al., 2012; Nelson et al., 2014; Tepper et al., 2018). GABAergic responses induced by nAChR activation are similar in SPNs expressing either D1 or D2 receptors (Luo et al., 2013), suggesting that synchronized ChI activity induces a general inhibition of striatal outputs. In NAc medial Shell, the optogenetic activation of ChIs inhibits 81% of SPNs recorded *in vivo* (Witten et al., 2010). This effect is blocked by mecamylamine, and thus mediated by nAChR and most likely disynaptic (Witten et al., 2010). The GABA neurons mediating ChI-driven SPN inhibition in the Shell remains unknown. Although nAChR induced GABA release from dorsal striatum DA neuron terminals, the contribution of GABA corelease in NAc mShell is likely to be minimal, because of short-term depression and limited GABA cotransmission in the region (Straub et al., 2014). Further research is necessary to determine the involvement of GABA interneurons, since these cells may play a critical role in mediating the inhibitory effects of ChIs on SPN activity.

As schematized in Figure 5, DA neurons in the NAc Shell make strong glutamatergic connections to ChIs and may synchronize their activity. Synchronized bursting of ChIs triggers a cascade of events affecting the excitability of SPNs. This feed-forward system has two phases; the first phase is a rapid and transient inhibition of SPN activity, followed by a second phase with multiple modulatory components. The initial inhibition of SPNs involves the activation of presynaptic nicotinic receptors on GABA interneurons and GABA-releasing DA neurons. The second phase is mediated by the activation of G-protein coupled muscarinic and DAergic receptors in SPNs. These modulatory effects are less characterized in the NAc Shell (Goldberg et al., 2012; Surmeier et al., 2011). In general, M2-class muscarinic and DA D2 receptor signaling increases the excitability of SPNs, while M1-class muscarinic and DA D1 receptor signaling decreases the excitability SPNs. The effects depend on the divergent expression of these receptors in different subpopulations of SPNs, adding further complexity beyond what is illustrated in the schematic.

Salient events activate DA neurons projecting to the NAc Shell

DA neurons modulate motivation through their actions in the NAc (Floresco, 2015; Salamone and Correa, 2012). Burst firing of DA neurons is often observed during aversive, appetitive or novel events, and during the presentation of cues in the environment predicting positive or negative reinforcement (Bromberg-Martin et al., 2010; Hamid et al., 2016; Saddoris et al., 2015). In the NAc Shell, activity of DA neurons reflects salience of events and instigates responses directed towards salient stimuli (Saddoris et al., 2015; Wyvell and Berridge, 2000). For example, microdialysis and voltammetry studies have shown increases in DA release in NAc medial Shell when animals consume food or enter a novel environment (Bassareo and Di Chiara, 1997; Gambarana et al., 2003; Rebec et al., 1996; Roitman et al., 2008). Salient aversive events are associated with a slight decrease in DA release, which is immediately followed by a large increase in DA release at the end of the aversive stimulus(Budygin et al., 2012; de Jong et al., 2019). Increases in DA release associated with food and novelty rapidly dissipate with repeated exposure (Bassareo and Di Chiara, 1997; Bassareo and Di Chiara, 1999; Gambarana et al., 2003; Rebec et al., 1996; Segovia et al., 2011), while those associated with inescapable shock do not (Budygin et al., 2012), in agreement with increases in DA release tracking general salience. Cues predicting the delivery of food also increase DA release in the NAc Shell and the amount of DA released is positively correlated with the amount of food delivered (Sackett et al., 2017). Thus, an important factor controlling the activity of DA neurons projecting to the NAc medial Shell is the relevance of stimuli in the environment, which incorporate different dimensions of salience related to novelty and previous experience with an appetitive or aversive reinforcer. As such, DA neurons projecting to the NAc medial Shell convey alerting signals that track alterations in the environment to promote changes in behavioral output.

DA neurons projecting to the NAc medial Shell signal changes in contingencies and promote behavioral switching

DA neuron control of motivated behavior involves the capacity to facilitate switching between behaviors (Eveden and Robbins, 1983; Oades, 1985; Weiner and Feldon, 1997; Redgrave et al., 1999). Changes in DA transmission in the NAc Shell modulate the degree to which competing behavioral repertoires interfere with ongoing behavior. For example, DA receptor antagonism in the medial Shell does not block food consumption (Baldo et al., 2002; Berridge and Robinson, 1998; Nowend et al., 2001; Salamone and Correa, 2012) but alters the microstructure of feeding; animals eat the same amount of food in fewer and longer bouts, without engaging in other common behaviors, such as grooming or locomotion (Baldo et al., 2002). Reduced DA transmission decreases switching, while increased DA is associated with switching to a new behavioral strategy. In a decision-making paradigm, increases in DA release in the NAc medial Shell are greater when rats made choices under ambiguous conditions (St. Onge et al., 2012). Similarly, increases in DA release observed during performance of a set-shifting task suggest a role in shifting but not in acquisition (Stefani and Moghaddam, 2006).

Evidence that DA-GLU neurons in the medial Shell modulate behavioral switching is supported by studies using paradigms in which stimulus-reinforcer contingencies are altered, such as extinction. During extinction, a stimulus that was previously associated with a primary reinforcer is presented several times without consequence. This situation requires a shift in behavior and new learning so that the animal stops responding to the stimulus and deems it irrelevant. Animals form two separate memories, one about stimulus-reinforcer association and another about the stimulus-nothing association. Behavioral responses to the stimulus after conditioning depend on how strongly the stimulus elicits one or the other memory and produces a behavioral switch (Westbrook and Bouton, 2010).

There is evidence that in extinction increasing activity of DA neurons projecting to the NAc mShell facilitate switching, while inhibiting them disrupts switching. During fear extinction, a subpopulation of VTA DA neurons increases their activity with omission of the expected aversive event, i.e. at offset of the presentation of the conditioned stimulus (Bromberg-Martin et al., 2010; Salinas-Hernández et al., 2018). In mice trained to associate a tone with a shock and during extinction, photostimulation of VTA DA neurons at tone offset promotes a switch from fear responding to extinction and facilitates extinction learning (Salinas-Hernández et al., 2018). The photoinhibition of VTA DA neurons during the same period disrupts switching from fear responding to extinction and impairs extinction learning. DA-GLU neurons projecting to the NAc Shell are activated by the omission of an expected shock during extinction (Badrinarayan et al., 2012; de Jong et al., 2019). Inhibiting DA-GLU neurons projecting to the NAc Shell, but not the NAc Core, impairs extinction learning (Luo et al., 2018). These observations suggest that DA-GLU neurons projecting to the NAc Shell modulate extinction. Figure 6 illustrates hypothesized changes in the NAc medial Shell during fear extinction. DA-GLU neuron burst firing during shock omission may synchronize ChI activity, triggering a cascade of events which produce prolonged increases in DA release and the inhibition of SPNs activity (Figure 5).

Extinction of reward-associated conditioned responses may undergo similar modulation by Shell-projecting DA neurons. During extinction of conditioned responses to food, DA release measured by microdialysis is increased in the NAc Shell but not in the Core (Bassareo et al., 2017). A voltammetry study showed large and prolonged increases in DA release in the NAc Shell during the early phases of extinction (Saddoris et al., 2015). These prolonged increases in DA release were also observed in fear extinction (Badrinarayan et al., 2012; de Jong et al., 2019) and fit the positive feedback loop described in Figure 5 in which DA-GLU neurons drive the synchronization of ChI activity and induce further increase in DA release through activation of nAChRs. Nevertheless, the involvement of DA neurons in extinction of appetitive responses remains controversial. A subpopulation of DA neurons shows a pause in firing during reward omission (Cohen et al., 2012; Schultz, 2007) and opposing that pause by optogenetic stimulation of VTA DA neurons during reward omission slows extinction (Steinberg et al., 2013), suggesting that the neurons code for a negative reward prediction error (Cohen et al., 2012; Schultz, 2007; Steinberg et al., 2013).

Subpopulations of DA neurons projecting to different subregions of the NAc serve different motivational functions (Bromberg-Martin et al., 2010). Measuring DA release voltammetrically during a learned instrumental chain schedule showed that Shell-projecting

DA neurons respond to salient and alerting events, while Core-projecting DA neurons track changes in prediction errors (Saddoris et al., 2015). DA neurons that show an increase in firing may target the NAc Shell selectively, and facilitate extinction by alerting to the presence of unexpected events and promoting switching behavior; while DA neurons that show a pause in firing may target the NAc core selectively and facilitate extinction by signaling a negative error prediction. Thus, photostimulation NAc-Shell would facilitate extinction learning (as described in aversive conditions by Luo et al. 2018), while photostimulation of NAc-projecting DA neurons would slow extinction (as described in appetitive conditions by Steinberg et al., 2013). Future research should test this hypothesis by directly comparing the stimulation of these two subpopulations of neurons during extinction in both aversive and appetitive conditions.

Studies on latent inhibition support a role for DA-GLU neurons in behavioral switching. As in extinction, animals in a latent inhibition experiment are exposed to conflicting contingencies; a stimulus is first presented several times until it becomes irrelevant and it is then paired with a primary reinforcer. In this circumstance, animals need to shift from a stimulus-nothing association to a stimulus-reinforcer association; however, the pre-exposure interferes with this process and reduces the associative strength between the stimulus and reinforcer, revealing latent inhibition (Lubow, 2010). Latent inhibition has been mostly studied using aversive stimuli. In the pre-exposure phase, a tone is presented several times without consequences; in a following conditioning phase, the tone is paired with a mild shock; and in final test phase, the tone is present alone and the amount of freezing is measured. Animals that are pre-exposed to the tone freeze less to the tone in comparison with animals that were not pre-expose to the tone and just received tone-shock pairings. The decrease in freezing in pre-exposed animals reflects latent inhibition (Moser et al., 2000; Weiner and Feldon, 1997).

Latent inhibition is modulated by DA release in the NAc Shell during conditioning. An *in vivo* microdialysis showed that presentation of a tone paired with a shock increases DA release in the Shell. However, this increase in DA release during conditioning was eliminated when animals were pre-expose to the tone and showed latent inhibition (Murphy et al., 2000). Intra-NAc injections of amphetamine during the conditioning phase, which increase DA levels, disrupt latent inhibition and facilitate switching (Young et al., 2005; Moser et al., 2000). Intra-NAc haloperidol injections during conditioning, which block DAergic signals through D2 receptors, enhance latent inhibition and block switching (Joseph et al., 2000). Thus, the extent of latent inhibition expression depends on the activity of Shell-projecting DA-GLU neurons during the conditioning phase, when the animal first encounters conflicting contingencies. Only a few studies examined latent inhibition using appetitive stimuli and it is not clear how DA neurons modulate this type of latent inhibition (Killcross et al., 1994; Moser et al., 2000). Impairing DA neuron glutamate cotransmission enhances latent inhibition and blocks switching (Mingote et al., 2017), suggesting that changing DA-GLU neurons into DA-only neurons interferes with learning a new role in situations of conflict.

As reviewed above, there is evidence that Shell-projecting DA-GLU neurons track salient events, such as changes in stimulus-reinforcer contingencies. Studies on fear conditioning

and latent inhibition point to a critical role of Shell-projecting DA-GLU neurons in situations of conflict, helping determine how quickly or efficiently learning of new contingencies develops.

How DA neuron GLU cotransmission in the NAc medial Shell might facilitate behavioral switching

Studies examining behavioral effects of lesions or inactivation of the NAc Shell suggest that a major role of the NAc Shell is to suppress competing behaviors that interfere with ongoing goal-directed responses (Floresco, 2015). Thus, in fully predicted circumstances, activation of SPNs in the NAc Shell promotes a *Stay on task* mode by inhibiting projection areas and blocking competing behavior patterns (Figure 7A). However, in ambiguous circumstances, inhibition of SPNs promotes a *Switch task* mode by disinhibiting projecting areas (Figure 7B). This hypothesis is supported by several studies showing that NAc medial Shell, but not Core, lesions disrupt latent inhibition and facilitate switching (Gal et al., 2005; Jongen-Rêlo et al., 2002; Pothuizen et al., 2005). With disinhibition of NAc Shell projection areas, previously blocked competing behaviors can be expressed, allowing animals to test new behavioral responses, setting an opportunity for new learning. Selecting an optimal strategy from *unlocked* competing behaviors requires other parallel striatal circuits, such as the NAc core and the Striatum (Floresco, 2015; Sharpe et al., 2019). Once a new goal-directed behavior is established, the NAc Shell goes back to *Stay on task* mode.

We hypothesize that transition between the two modes is driven by the activation of Shell-projection DA-GLU neurons (Figure 7). These neurons are activated by conflicting contingencies and promote a *Switch task* mode by synchronizing ChI activity and inducing a rapid inhibition of SPNs. The role of NAc Shell ChIs in behavioral switching is supported by a recent study of ChIs in extinction in a cocaine-context association (Lee et al., 2016). Optogenetic activation of ChIs enhanced extinction and facilitated switching, while inhibition suppressed extinction and blocked switching. In our model, we propose that ChI activation leads to inhibition SPN activity and disinhibition of NAc Shell projection areas. As new learning progresses and a new behavioral response develops, DA-GLU neuron activity would decrease and promote *Stay on task*. This hypothesis is supported by fear conditioning studies revealing an increase in DA neuron activity in the early stages of extinction and a decrease in activity during late stages (Badrinarayan et al., 2012; Salinas-Hernández et al., 2018).

Overall, the studies reviewed here support a role for DA-GLU neurons in behavioral switching, however several links between cell activity and behaviors are still missing. For example, DA-GLU neuron induced synchronization of ChI activity in the NAc Shell should be assessed *in vivo.* A recent study recorded the activity of NAc cells while photostimulating DA neurons and found that 25% of the recorded cells increased their firing within 50 ms of stimulation, most likely mediated by monosynaptic connections (Wang et al., 2017). The effect was eliminated in mice lacking VGLUT2 in DA neurons, showing that the postsynaptic effects depend on glutamate cotransmission. However, the postsynaptic cells were not identified in this study. Future studies should take advantage of *in vivo* calcium

imaging techniques to measured ChI synchronized activity and its links to DA neuron activity (Rehani et al., 2019). Stimulation of DA-GLU neurons induces a subsequent ChI-driven inhibition of SPNs, which is supported by work done in the dorsal striatum and *in vivo* recordings from the NAc Shell by Witten and colleagues (2010). Nevertheless, further research is required to identify which GABAergic neurons are activated by acetylcholine and mediate the overall inhibition of SPNs activity.

Finally, perhaps the biggest challenge will be to determine the neural circuits in NAc Shell that are disinhibited by DA-GLU neuron burst activity in situations of conflicting contingencies, such as in extinction or in latent inhibition. Exploiting the ability of optogenetic techniques to suppress the activity of specific NAc outputs selectively may be very useful. Indeed, this technology has already been use to dissect NAc Shell outputs controlling feeding (Baldo and Kelley, 2007; Maldonado-Irizarry et al., 1995). Optogenetic inhibition of D1-expressing SPNs rapidly stimulates feeding by disinhibiting the lateral hypothalamus, while stimulation blocks feeding (O'Connor et al., 2015). The work of Berridge and colleagues, further revealed that rostral NAc Shell and DA D1 receptors control feeding, while the caudal NAc Shell and DA D1 and D2 receptors control fearful behaviors (Faure et al., 2008; Richard and Berridge, 2011). Other studies have shown that different subregions of the NAc Shell control either aversive or appetitive responses (Al-Hasani et al., 2015) and this may reflect divergent input-output relationships within the NAc Shell (Groenewegen et al., 1999; Reed et al., 2018; Yang et al., 2018). Dissecting these neural circuits will require a systematic analysis of the effects of silencing different outputs along the anterior-posterior axis of the NAc Shell during behaviors associated with either appetitive or aversive outcomes.

Since the first report of glutamate cotransmission in DA neurons in the late 1990's (Sulzer et al., 1998), the function has been gradually elucidated. The recent development of new technologies to manipulate subpopulation of DA neurons selectively revealed that DA-GLU neurons are located in the medial VTA, preferentially project to the NAc medial Shell and control the activity of ChIs. A better understanding of how DAGLU neurons modulate NAc Shell associated-circuits will be crucial in establishing the function of DA-GLU neurons in motivated behavior. Here we have described a series of testable hypotheses about the functions of DA-GLU neurons, both at the synaptic and behavioral levels, which should promote more research in this area and advance our understanding of the DA system.

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Highlights

- Dopamine-glutamate neurons project selectively to the Nucleus medial Shell
- Dopamine-glutamate neurons in the NAc medial Shell make ChI burst fire
- Dopamine-glutamate neurons are involved in behavioral switching
- Proposed mechanism through which dopamine-glutamate neurons gate a “switching task mode”

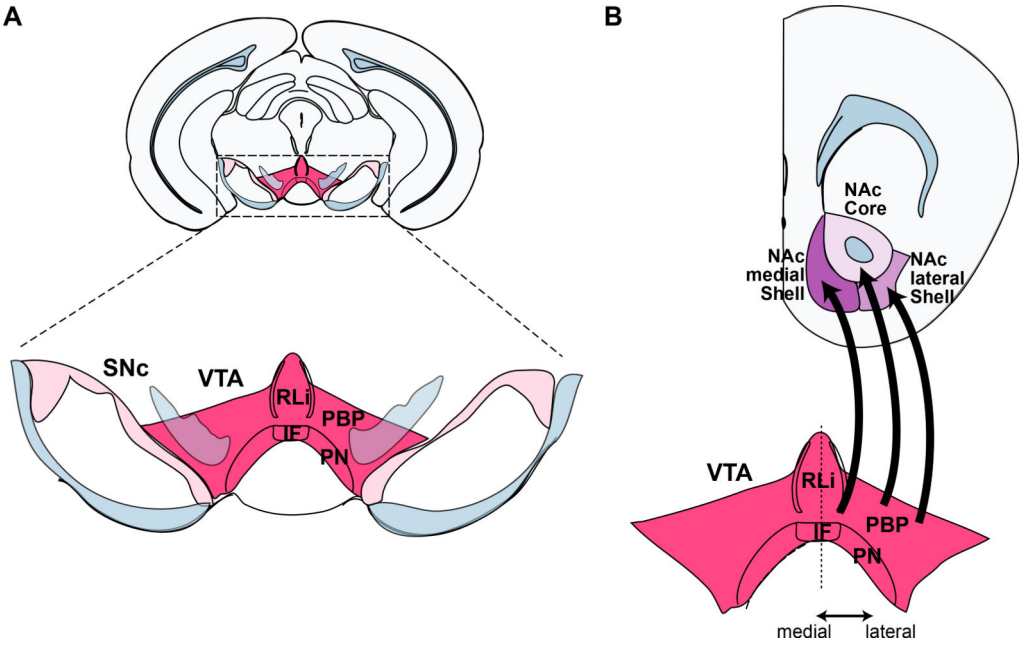

Figure 1 –. The ventral tegmental area and its projections to the NAc.

A. Coronal section illustrating the location of the VTA in the ventral midbrain (upper), and its subregions (lower). **B.** VTA projections to the NAc medial Shell, lateral Shell and Corefollow a medial-lateral topography. Due to its more caudal location, the caudal linear nucleus (CLi) is not shown. Color coding: light blue, fibers; light pink, substantia nigra pars compacta (SNc); dark pink, ventral tegmental area (VTA); dark violet, nucleus accumbens (NAc) medial Shell; light violet, NAc lateral Shell; pale violet, NAc core. Abbreviations: IF, interfascicular nucleus; PBP, parabrachial pigmented nucleus; PN, paranigral nucleus; RLi, rostral linear nucleus of the raphe; VTA, ventral tegmental area.

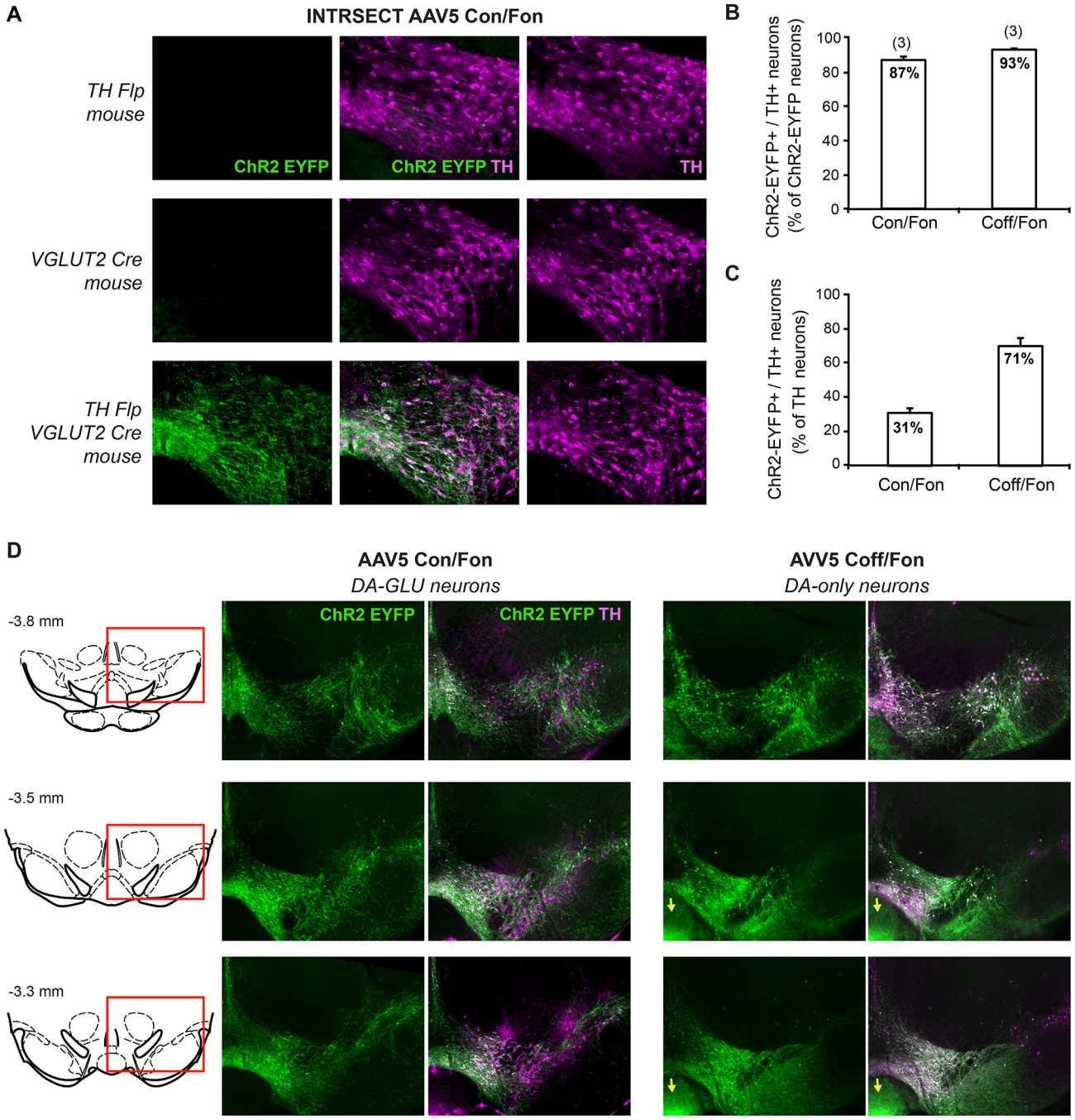

Figure 2 –. Visualization ofDA-GLU and DA-only neurons using the INTRSECT combinatoric strategy.

A. Photomicrographs of the VTA assessing expression of ChR2-EYFP after intra-VTA injections of INTRSECT AAV5 Con/Fon in three different mutant mice: TH *Flp*, VGLUT2 *Cre*, and TH *Flp*;VGLUT2. Cells and processes labeled by the Con/Fon virus and showing EYFP immunoreactivity (left photomicrograph) are only visible in mice that express both *Cre* and *Flp*, validating the combinatoric strategy. **B.** Graphs displaying the specificity of the INTRSECT Con/Fon and Coff/Fon viruses, measured as the percentage of ChR2-EYFP

positive neurons expressing TH in the VTA. Numbers of mice used are indicated above the bars. **C.** Summary of average percentage of TH+neurons in the VTA expressing either INTRSECT AAV5 Con/Fon or Coff/Fon. **D.** Photomicrographs of the VTA showing the distribution of the cells expressing either AAV5 Con/Fon (DA-GLU neurons; left panels) or AAV5 Coff/Fon (DA-only neurons; right panels). Ectopic expression of the AAV5 Con/Fon in the interpeduncular nucleus is indicated by yellow arrows. Numbers above the schematic coronal slices (left) are distance from bregma. These results are original data that have not been been previously published.

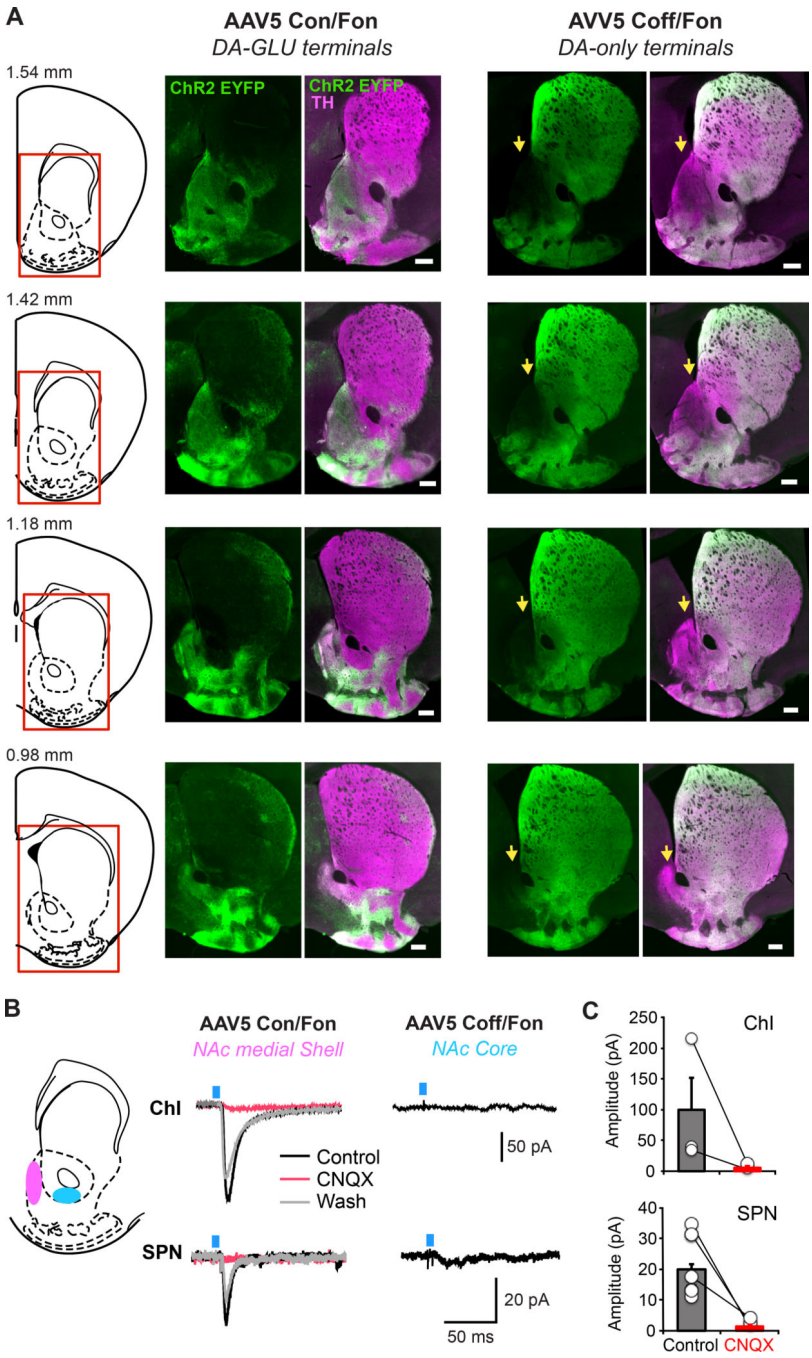

Figure 3 –. Projections of DA-GLU and DA-only neurons to the Striatum.

A. Photomicrographs of the striatum taken at different anterior-posterior positions, showing distribution of DA terminals labeled with ChR2-EYFP driven by either INTRSECT AAV5 Con/Fon (DA-GLU neurons; left panels) or Coff/Fon (DA-only neurons; right panels) in the NAc. Numbers above the schematic coronal slices (left) indicate distance from bregma. Areas in the NAc medial dorsal Shell lacking DA-only labeled terminals are indicated by yellow arrows. **B.** Schematic of a coronal section (1.42 mm from bregma) indicating locations of patch-clamp recordings in the NAc medial Shell (pink) and NAc Core (blue)

(left). Representative traces of EPSCs generated by single-pulse photostimulation (blue bar) at 0.1 Hz recorded from ChIs and SPNs are shown. Traces are averages of 10 consecutive recordings. EPSCs were observed when photostimulating DA terminals labeled by Con/Fon virus and recording in the NAc medial Shell. Responses were blocked by bath application of the AMPA receptor antagonist CNQX (40 0μM, red trace; wash, gray trace). EPSCs were not seen when photostimulating DA terminals labeled by Coff/Fon virus. ince the medial Shell lacks DA-only neuron terminals, DA-only neuron terminal stimulation and recording was done in the NAc Core. **C.** Summary of average EPSC amplitudes in ChIs and SPNs, before and after CNQX in mice injected with the Con/Fon virus is shown. These results are original data that have not been previously published.

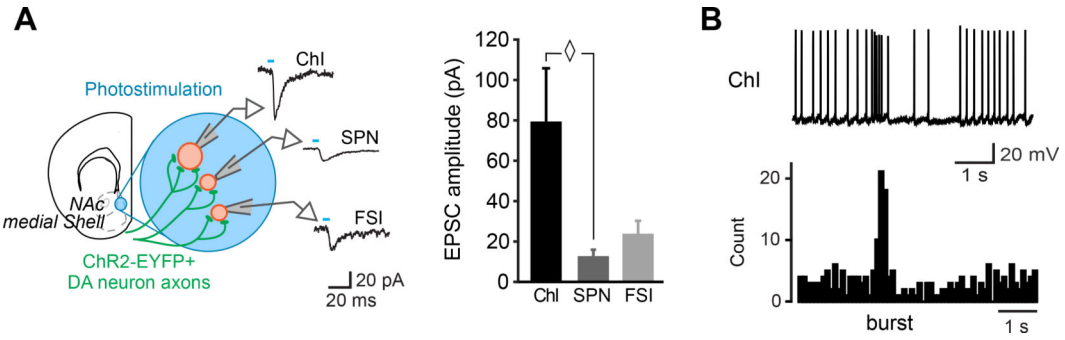

Figure 4 –. Functional connectivity of DA/GLU neurons in the NAc medial Shell.

A. Schematic of a coronal slice (1.34 mm from bregma) indicating the location of the patch-clamp recordings in the NAc medial Shell (left). DA neuron excitatory responses evoked by photostimulation (blue circles) were measured from ChIs, SPNs and FSI. On the left is the summary of average EPSC amplitude after single-pulse photostimulation (modified from Chuhma et al. 2014). **B.** Effect of photostimulation mimicking DA neuron bursting (5 pulses at 20 Hz) on ChI firing. A representative trace is shown on top, with peristimulus histograms summing ten consecutive traces (0.1 s bin) below (modified from Mingote et al., 2017). Abbreviations: ChI, cholinergic interneuron; SPN, spiny projection neuron; FSI, fast spiking interneuron.

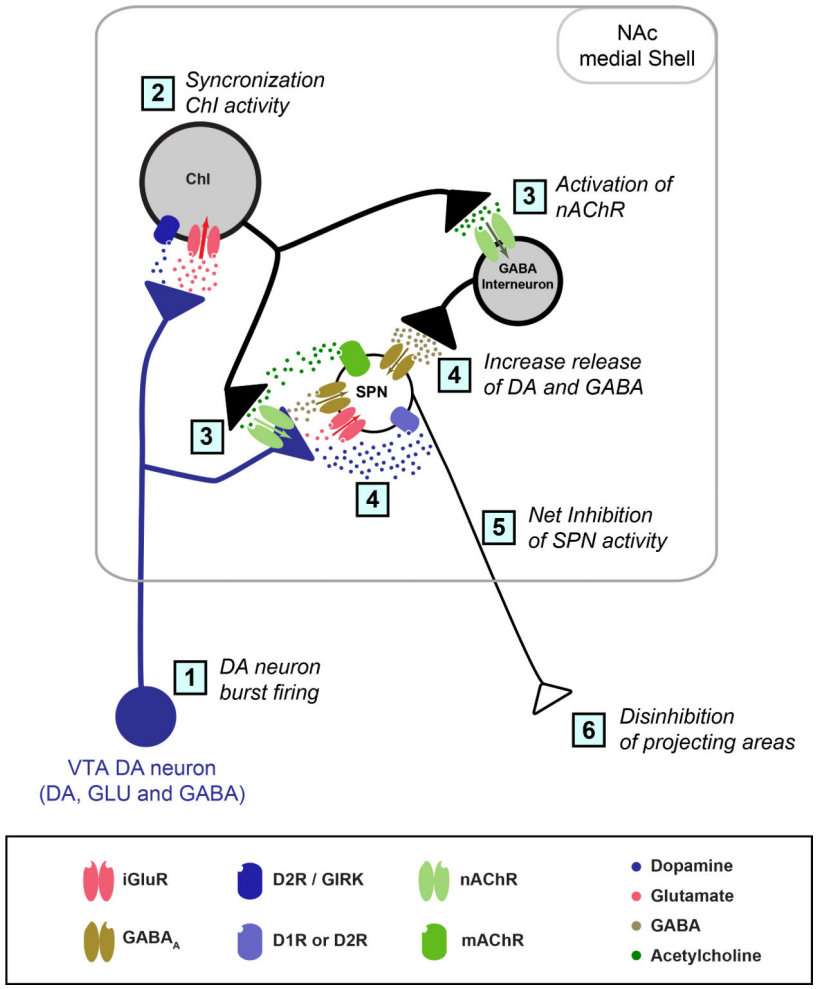

Figure 5 –. DA neuron glutamate cotransmission in the NAc medial Shell.

Simplified schematic of the NAc Shell local circuit showing the cascade of events triggered by DA neuron activity. DA neurons evoke DA and glutamate signals at their synaptic connections to ChIs, while they evoke DA, glutamate and GABA signals at their connections to SPNs. The following sequence of events are hypothesized: **1)** DA-GLU neuron burst firing; **2)** Synchronization of ChIs activity in the NAc medial Shell by DA-GLU excitatory inputs; **3)** Increased acetylcholine release and activation of presynaptic nAChRs in DA neurons and GABA interneurons, **4)** an overall increase in DA and GABA release; **5)** $GABA_A$ receptor activation in SPNs induces rapid and transient inhibition of SPN activity. **6)**. Decrease in GABA release from SPNs leads to disinhibition of NAc Shell projection areas. Transmitter release sites are shown as one presynaptic terminal per postsynaptic NAc cell type. Note that the modulatory effects mediated by muscarinic and DAergic receptors in SPNs, which are hypothesized to alter the excitability of SPN on a longer time scale, are not shown. Abbreviations: ChI, cholinergic interneuron; SPN, spiny projection neuron; ChR2, channelrhodopsin; NpHR, halorhodopsin; iGluR, ionotropic glutamate receptor; D1R, dopamine D1 receptor; D2R, dopamine D2 receptor; D2R / GIRK, dopamine D2 receptor coupled with G protein-activated inward rectifier potassium channels; nAChR, nicotinic receptor; mAChR, muscarinic receptor.

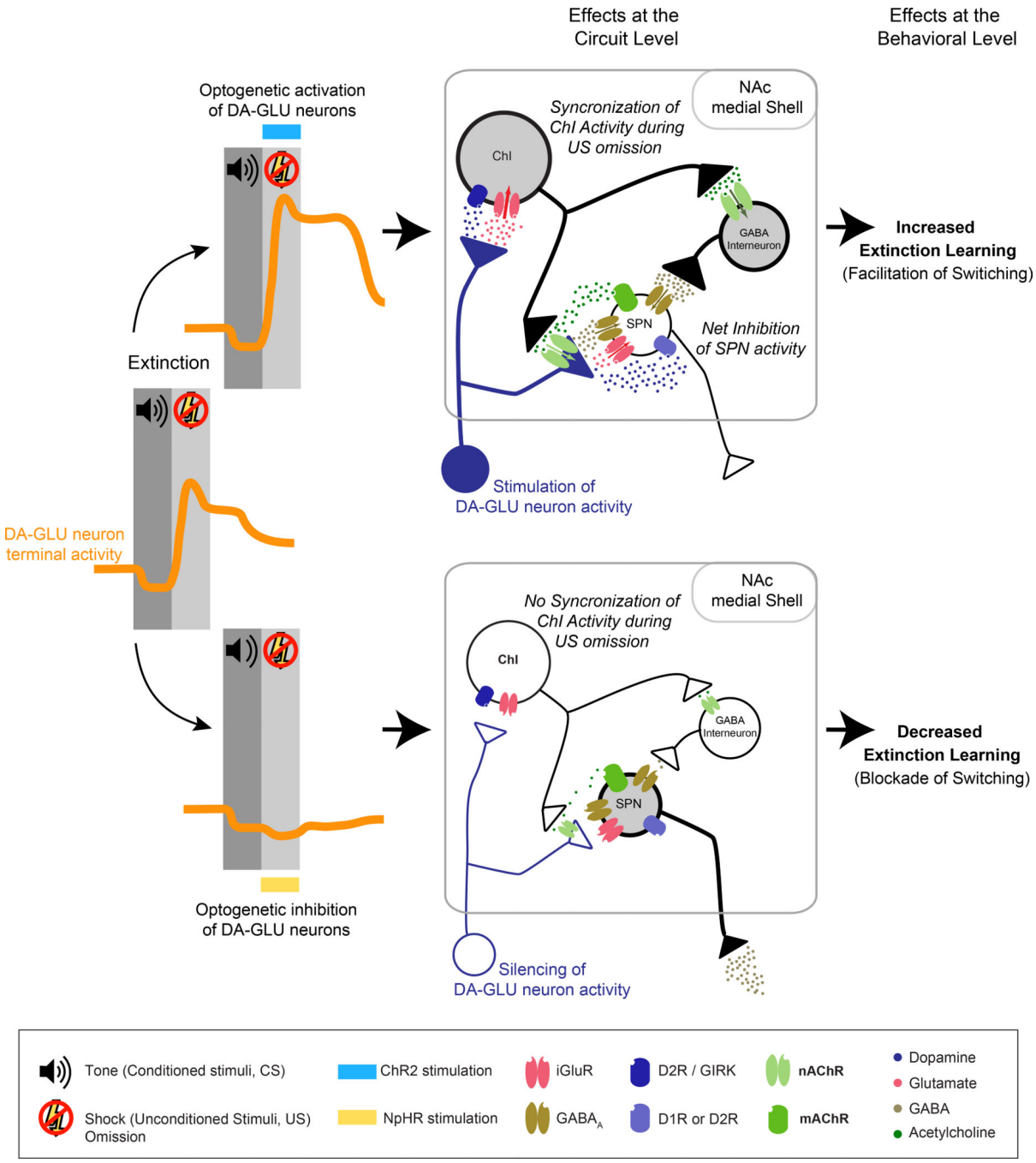

Figure 6 –. Modulating DA-GLU activity in the NAc medial Shell during fear conditioning.

The panel on the left shows the activity of DA-GLU neuron terminals (orange line) in the NAc medial Shell during fear extinction. Tone presentation (dark gray bar) is associated with a slight decrease in DA-GLU neuron activity, while shock omission (light gray bar) is associate with a prolonged increase in their activity (based on findings from Badrinarayan et al., 2012; de Jong et al., 2019; Salinas-Hernández et al., 2018). The upper panel on the right illustrates the activity of DA-GLU neurons after optogenetic stimulation during shock omission and hypothesized circuit and behavioral effects. At the circuit level, the burst firing

of DA-GLU neurons synchronizes ChIs activity. The net effect on SPN excitability is a rapid and transient inhibition of all SPNs (based on findings from Witten et al., 2010). At the behavioral level, the photostimulation of DAGLU neurons facilitates switching and increases extinction learning (based on findings from Luo et al., 2018; Salinas-Hernández et al., 2018). The lower panel, illustrates the activity of DA-GLU neurons after optogenetic inhibition. At the circuit level, the inhibitory manipulation prevents DA-GLU neurons from synchronizing ChIs and the subsequent net inhibition of SPNs. At the behavioral level, the inhibition of DA-GU neurons during shock omission blocks switching and slows extinction (based on findings from Luo et al., 2018; Salinas-Hernández et al., 2018). For detailed information on the local circuit diagrams refer to Figure 5 caption.

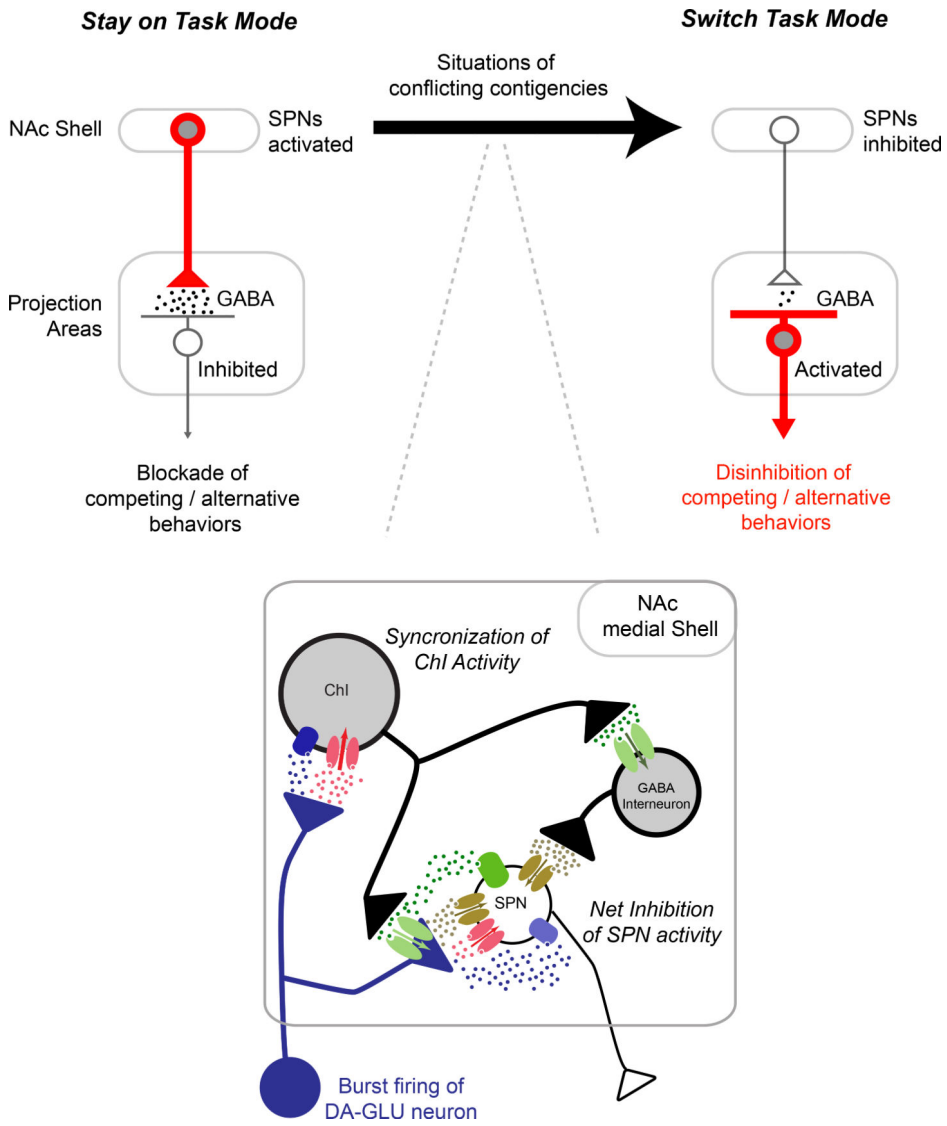

Figure 7 –. How DA/GLU neurons facilitate behavioral switching.

Schematic on the left shows NAc Shell connections to projection areas when animals engage in well-predicted goal directed behaviors, which promote *Stay on Task* (based on suggested function by (Floresco, 2015). In this mode, SPNs in the NAc Shell are active, inhibiting projection areas and blocking expression of competing behaviors that could interfere with the ongoing task. Situations of conflicting contingencies (black arrow) activate DA-GLU neurons and gate the NAc Shell into a *Switch Task* mode. The NAc Shell local circuit diagram (inset) shows how DA-GLU neurons synchronize ChIs activity and produce a rapid and transient inhibition of SPN activity. (For detailed information on the local circuit changes induced by DA-GLU neurons refer to Figure 5 caption). So in the *Switch Task* mode, most of the SPNs in the NAc Shell are silent, leading to less GABA release in projection areas. The disinhibition of these areas releases previously blocked behaviors and allows for the exploration of new behavioral strategies.