Glutamate Inputs to the Nucleus Accumbens: Does Source Matter?

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http://dx.doi.org/10.1016/j.neuron.2012.11.008

How the nucleus accumbens integrates information from multiple upstream regions has been a central question for decades. In this issue of *Neuron*, Britt et al. (2012) photostimulate glutamatergic axons from the amygdala, prefrontal cortex, and hippocampus in the nucleus accumbens, characterizing the functional role of each pathway both in vivo and ex vivo.

The nucleus accumbens (NAc) has been described as a crucial convergence point for information about environmental contexts and cues before the selection and execution of a final motor output and has long been known to be important in the processing of reward-related behaviors (Cardinal et al., 2002; Carelli, 2002), specifically in the context of cocaine-induced plasticity (Thomas et al., 2001; Boudreau and Wolf, 2005). What happens at this last stop? Three of the most robust glutamatergic inputs to the NAc are the basolateral amygdala (Amyg), medial prefrontal cortex (PFC), and the ventral hippocampus (vHipp), each probed by Britt et al. (2012) using optogenetic methods (Figure 1). This characterization revealed many novel insights: while Britt et al. (2012) confirmed some assumptions about these limbic systems, they challenged the dogma surrounding NAc information integration.

The most provocative implication of this paper is that Britt et al. (2012) raise "the possibility that the specific pathway releasing glutamate is not as important as the amount of glutamate that is released." This is indeed a new concept that would change the way much of the field thinks about the way that the NAc integrates information: what if the complex computations are actually much simpler than we thought? What if projection origin matters less than projection target?

More than half a century ago, intracranial self-stimulation (ICSS) was first used to identify several fiber tracts, including putative hippocampal outputs, as neural substrates for reward or reinforcement (Olds and Milner, 1954). However, these seminal studies used electrical stimulation—nonspecifically activating multiple cell types and axons of passage—making it difficult to determine the critical neural circuit element with confidence. In another seminal study from the 1990s, elegant in vivo intracellular recordings in anesthetized animals first characterized the role of hippocampal, prefrontal cortical, and amygdalar inputs to the NAc, demonstrating distinct properties of electrical stimulation in each upstream region (O'Donnell and Grace, 1995). O'Donnell and Grace established the unique ability of hippocampal inputs to the NAc to induce changes in membrane potential, commonly referred to as "up and down states"—medium spiny neurons were pushed into step-function-like states in which the cells were slightly depolarized and more excitable in response to prefrontal cortical inputs (O'Donnell and Grace, 1995). Distinct from the bistable responses elicited by fornix stimulation, electrical stimulation of the amygdala produced longer-lasting depolarization with greater onset latency, and electrical stimulation of the prefrontal cortex elicited a fast, but transient, depolarization (O'Donnell and Grace, 1995). Until the development of optogenetic projection-specific targeting approaches, we did not have the ability to manipulate axons originating in specific regions during freely moving behaviors nor to stimulate axons arriving from a known source in acute slice preparations (Tye et al., 2011; Stuber et al., 2011).

Optogenetic-mediated projection-specific targeting leverages the genetically encodable capability of these light-sensitive proteins and allows for the selective activation of specific populations of cells and axons. However, caveats still include the possibility of depolarizing axons of passage that do not form synapses in the illumination field or the induction of backpropagating action potentials (Petreanu et al., 2007), also known as antidromic stimulation, which may scale with stronger illumination parameters, opsin expression levels, and the specific characteristics of the preparation. These early studies in optogenetic projection-specific targeting used local pharmacological manipulations, blocking glutamate receptors in the postsynaptic target region to demonstrate that the behavioral changes observed were indeed due to local effects—ruling out the possible contribution of axons of passage or antidromic activation to the light-induced behavioral change (Tye et al., 2011; Stuber et al., 2011). Stuber and colleagues investigated two of the same projections, specifically testing the ability of amygdalar and prefrontal cortical inputs of the NAc to support ICSS, by expressing channelrhodopsin-2 (ChR2), a light-activated cation channel, in glutamatergic pyramidal neurons of the amygdala or prefrontal cortex and implanting an optical fiber into the medial shell of the NAc. They observed that amygdalar, but not prefrontal cortical, inputs to the NAc supported ICSS (Stuber et al., 2011).

In this issue of *Neuron*, Britt et al. (2012) put forth an article of impressive breadth, characterizing three pathways from anatomical, electrophysiological,

and behavioral perspectives (Figure 1). Anatomically, Britt et al. (2012) examined the patterns of axons expressing a fluorescent protein in the NAc from the Amyg, PFC, and vHipp, revealing the unique distribution of axons throughout the NAc in exquisite detail across multiple animals (Britt et al., 2012), largely consistent with earlier studies (Voorn et al., 2004). They also investigated the properties of synaptic transmission from each of these pathways using ex vivo whole-cell patch-clamp recording techniques in acute slice preparations of different animals expressing ChR2 in one of the upstream regions (Amyg, PFC, or vHipp). These experiments revealed new insights about the relative strength of light-evoked excitatory postsynaptic currents (EPSCs), showing that vHipp inputs evoked the greatest EPSC amplitudes in the NAc shell, with the PFC inputs evoking the smallest EPSC amplitudes of the three (Britt et al., 2012). This was not a result of varying sensitivity or composition of postsynaptic AMPARs for each input, as demonstrated by the nearly identical amplitudes of quantal release and indistinguishable current-voltage relationships across synapses, respectively (Britt et al., 2012). However, Britt et al. (2012) did observe that the vHipp-NAc synapses showed greater NMDAR-mediated inward currents, which could explain the unique ability of this input to induce the stable depolarization seen in "up and down states" of NAc MSNs (O'Donnell and Grace, 1995).

Electrophysiologically, there is a unique feature that Britt et al. (2012) identified of vHipp-NAc synapses: they were exclusively potentiated after cocaine treatment. In contrast to Pascoli et al. (2012), they did not observe a cocaine-induced potentiation of PFC inputs to the NAc (Pascoli et al., 2012). This might be explained by the fact that Pascoli and colleagues investigated only infralimbic inputs to D1 receptor-expressing medium spiny neurons (MSNs) in the NAc, while Britt et al. (2012) expressed ChR2 throughout the mPFC including both prelimbic and infralimbic regions and recorded from all MSNs. Given the opposing functions observed in both prelimbic and infralimbic cortices as well as D1 and D2 receptor-expressing neurons, this may have resulted in a "zero-sum" effect when pooled together. Perhaps vHipp inputs to the medial shell of the NAc preferentially formed synapses on D1-type MSNs, though testing this hypothesis would require additional experiments.

Behaviorally, the inhibition of vHipp axons in the NAc reduced, while activation increased, cocaine-induced locomotion (Britt et al., 2012). Britt et al. (2012) also demonstrated that illumination of vHipp axons in the NAc supported ICSS and real-time place preference (RTPP).

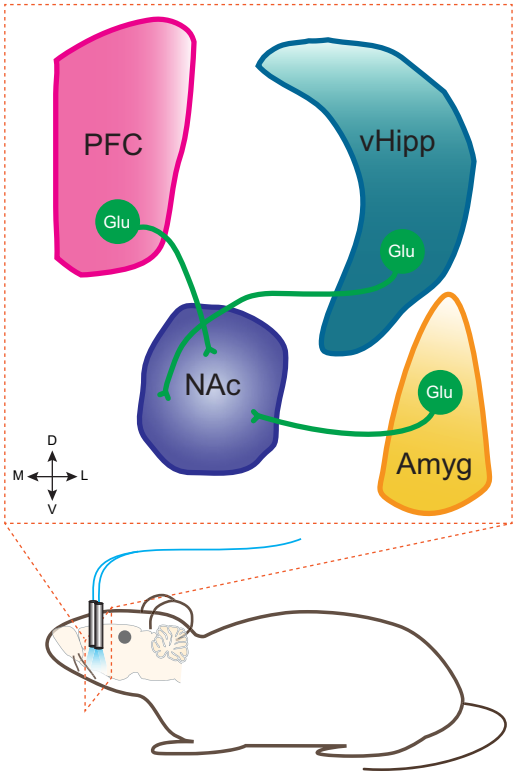

Figure 1. Schematic Representation of Glutamatergic Inputs to the Nucleus Accumbens from the Medial Prefrontal Cortex, Ventral Hippocampus, and Basolateral Amygdala and Their Manipulation in the Freely Moving Mouse

As described by Britt et al. (2012) in this issue of *Neuron*, bilateral activation of axons in the NAc originating from each of these upstream regions were characterized during reward-related behaviors. Although this figure is not drawn to scale and multiple anteroposterior coordinates were collapsed, from a coronal slice perspective, dorsal (D), ventral (V), medial (M), and lateral (L) are indicated. NAc, nucleus accumbens; PFC, medial prefrontal cortex; vHipp, ventral hippocampus; Amyg, basolateral amygdala.

While they replicated the finding that photostimulation of Amyg axons in the NAc could support reward-related behaviors (Stuber et al., 2011), in contrast to earlier work from this group, they found that illuminating ChR2-expressing PFC neurons could also support ICSS. This discrepancy can be reconciled by several experimental details; Britt et al. (2012) performed a more robust activation of PFC axons in the NAc by using bilateral stimulation and illumination parameters at a 50% higher frequency and train duration. This difference highlights the importance of titrating optogenetic experimental parameters in much the same way as pharmacological experiments, using light and/or viral "dose-dependent curves."

Finally, yet another surprising result emerged from this study with their ability to support ICSS with nonspecific MSN activation (Britt et al., 2012). In the NAc (Lobo et al., 2010), D1 and D2 receptor-expressing cells showed opposing effects on reward-related behaviors. However, when examining the data from these studies, the degree to which activation of D1 receptor-expressing neurons was positively reinforcing may have overpowered the aversive properties of D2 receptor-expressing neuronal activation in the NAc, leading to a net effect of positive reinforcement. This finding led Britt et al. (2012) to suggest that perhaps the source of glutamatergic innervation was less important than the bulk amount of glutamate released into the medial shell of the NAc.

While this might not be true in physiological settings, where glutamate release is governed by the natural spiking of neurons rather than robust trains at frequencies only seen in bursting pyramidal neurons, Britt et al. (2012) certainly put forth a host of new questions. The subtleties of this study need to be explored, particularly given the caveats that the Amyg, vHipp, and PFC are all robustly and reciprocally connected to each other. While they may provide direct input to the NAc, further experiments are needed to confirm that monosynaptic

input from each of these inputs is sufficient to support reward-related behaviors. An important caveat to note for nearly all optogenetic studies published to date is that the use of cylindrical optical fibers with blunt-cut tips creates a relatively narrow and small cone of light that may not capture all of the axon terminals expressing ChR2—particularly in large structures such as the NAc, which is organized spherically rather than cylindrically. Here, Britt et al. (2012) looked only at the medial shell of the NAc, but other recent studies in the NAc core or lateral shell could have different effects, as recently suggested (Lammel et al., 2012). Another possibility raised by Lammel and colleagues is that multiple distinct experiential qualities could support ICSS, including salience, alertness, motivation, and hedonic pleasure in addition to general reward and reinforcement (Lammel et al., 2011). It would also be interesting to characterize the ultrastructural organization across the NAc of axonal terminals arriving from the vHipp, PFC, and Amyg—how often do these axon terminals synapse onto the same cell, and how are these interactions assembled (axoaxonal synapses, on the same dendritic arbor, etc.)?

To conclude, even with the recent flood of insights toward causal relationships between the brain and behavior facilitated by optogenetic approaches (Tye and Deisseroth, 2012), there is still much to do. The paper from Britt et al. (2012) in this issue of *Neuron* makes an important contribution to the field by providing multiple new insights, raising provocative new questions, and opening the floodgates even wider than before to invite more research in this exciting new arena of systems neuroscience.

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Rules Got Rhythm

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http://dx.doi.org/10.1016/j.neuron.2012.11.003

Intelligent agents must select and apply rules to accomplish their goals. In this issue of *Neuron*, Buschman et al. (2012) demonstrate that oscillatory neuronal coupling is key to rule processing in monkey prefrontal cortex, notably when rules change during tasks.

Our lives are governed by rules. Whether we are engaged in sports, school, traffic, shopping, or work, it is necessary to know "the rules of the game." Knowledge of rules is indispensable in projecting the consequences of our actions and predicting which action may help us achieve a particular goal (Miller and Cohen, 2001; Bunge, 2004).

The concept of a "rule" refers to a learned association between a stimulus (e.g., a red traffic light) and a response (stopping the car) that can guide appropriate behaviors. A typical feature of rules is that the mapping between stimulus and action is context dependent—a yellow traffic light may suggest pressing the brakes or the gas, depending on other contextual signals (Miller and Cohen, 2001). Of critical importance in real-life environments is the ability to flexibly switch between rules. A change of rules can dictate that the same stimulus warrants a different course of action than it did a few minutes before (e.g., either filling or cleaning your favorite coffee mug).