Published in final edited form as:

Trends Neurosci. 2017 June ; 40(6): 325–327. doi:10.1016/j.tins.2017.04.006.

Seq-ing the Circuit Logic of the Basal Ganglia

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Abstract

Recently, Wallace et al. (2017) provide an unprecedented view of the layers of molecular, cellular and circuit complexity involving a basal ganglia output structure, the entopeduncular nucleus. Their findings lend order to chaos by revealing how molecularly and functionally defined cellular subsets are organized into distinct circuitry.

It has been well established that the basal ganglia are heavily interconnected with nearly all cortical regions as well as thalamic, limbic and brainstem regions (Gerfen and Wilson, 1996). As might be inferred from this connectivity, the basal ganglia are implicated in aspects of nearly every action, and are also increasingly recognized to contribute to cognitive and affective states. Yet there are still many gaps in our knowledge of the particular cellular and anatomical substrates for these diverse functional domains and how they interact.

At more macroscopic levels, regional differences in inputs and outputs stream through the basal ganglia structures on a ventromedial/dorsolateral gradient (Haber, 2003), suggestive of parallel circuits. However, the interconnected nuclei of the basal ganglia face a massive compression in total cell numbers from the large striatal regions to the increasingly diminutive distal nuclei, presenting a challenge as to whether and how such parallel circuits are focused onto these nuclei.

In the rodent, a major output nucleus of the basal ganglia is known as the entopeduncular nucleus (EP) which roughly corresponds to the globus pallidus interna, (GPi) in humans, a common target for deep brain stimulation to relieve symptoms of movement disorders like Parkinson's disease and dystonia (Breit et al., 2004). Over the decades, considerable cellular heterogeneity has been described in this tiny but potent nucleus. Subsets of EP cells have been found to release different neurotransmitters, including a population that co-releases GABA and glutamate (Shabel et al., 2014), express distinct calcium-binding proteins like parvalbumin and calretinin (Hontanilla et al., 1997) or neuropeptides like substance P- and somatostatin (Murakami et al., 1989), and show distinctive firing patterns (Nakahishi et al., 1990). For the most part though, because these various EP features have been described in

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isolation, how these different properties map onto each other was unknown leaving much about the overall organizing principles of the EP relatively opaque.

In this issue of *Neuron*, Wallace and colleagues cleverly and comprehensively layer multiple complementary approaches to accomplish a tour-de-force illumination of the organizing principles of this circuitry. Wallace et al. (2017) first employed Drop-Seq technology (Macosko et al., 2015) to obtain genome-wide RNA sequence from more than a thousand single neurons. These data identified two cell clusters that could be assigned to the EP. The two clusters also showed segregated expression of *Sst* and *Pvalb*, genes whose products were previously known to identify distinct EP subpopulations (Hontanilla et al., 1997; Murakami et al., 1989).

The team next employed fluorescent in situ hybridization (FISH) to validate some of the newly identified markers and understand how previously observed heterogeneity in neurotransmitter types mapped to these genetically defined cell populations.

Neurotransmitter types largely co-segregated with cell subsets defined by *Sst* and *Pvalb* expression. *Sst*-expressing cells expressed biosynthetic genes for both glutamate and GABA and were most abundant in the anterior portion of the EP, while the majority of *Pvalb*-expressing cells expressed GABA markers and were concentrated in posterior EP. However, with FISH, a third minor population of neurons was now discernible in the border regions between these two populations. This third population of cells co-expressed *Pvalb* and glutamatergic markers, but not GABAergic markers.

To determine how these molecularly defined subgroups of cells corresponded to the varied anatomical projections of the EP, Wallace and colleagues made clever use of a number of intersectional viral tracing strategies to map the inputs and outputs of the molecularly defined EP neuron subsets. They found that *Sst*-expressing cells project exclusively to a limbic target, the lateral habenula (LHb) while the vast majority of *Pvalb*-expressing cells comprise the commonly described motor output that is GABAergic and projects predominantly to thalamus. Interestingly, the rare population of purely glutamatergic *Pvalb*+ EP neurons was found to project to LHb. These findings establish a critical segregation of limbic and motor streams to subsets of neurons with distinct molecular and neurotransmitter-type signatures. Wallace et al., (2017) directly test the functional consequences suggested by these molecular signatures. Their findings show activity of the expected different neurotransmitter types in their predicted targets (Figure 1).

Having revealed how the outputs layered onto the molecularly, regionally, and functionally defined subsets of EP neurons, Wallace et al. turned to how inputs from the two major sources, striatum and GPe intersected with these subgroups. Again, a picture of relative isolation of the motor stream was supported, in that the *Pvalb*+ purely GABA subset received inputs from the striatal matrix, but not the limbic-innervated “patch” (striosome) regions. Similarly, although GPe includes a mixture of cell types, those found to project to the GABAergic *Pvalb* EP neurons were the canonically described inhibitory *Pvalb*-expressing neurons in the motor circuitry.

Limbic-projecting EP cell subsets (Sst/Glu/GABA and Pvalb/Glu) received input from both patch and matrix regions of the striatum, more or less in proportion to their cell numbers, consistent with observations in EP neurons that were defined solely by their projection to LHB (Stephenson-Jones et al., 2016). The limbic projecting EP neurons also received more diverse GPe inputs, approximately 1/3 from *Pvalb* cells, but nearly 2/3 from another subset defined by *Nkx2.1* expression. Given the large convergence of striatal neurons onto EP neurons, it would be interesting to further know whether the striatal matrix inputs that both EP populations receive include common neurons, or are segregated into distinct channels as the EP outflow demonstrates.

From this study, the Drop-Seq results themselves offer a treasure trove of molecular handles to investigate. Although given how new this approach is, some caution is warranted as the field works to establish standards for quality controls in the sequence reads and bioinformatics approaches - lest one find one's self tantalized by "fool's gold." In this study, the authors quickly moved to key validation steps, such as the *in situ* and functional studies, for major observations. The current gene expression datasets suggest some interesting themes. For example, Gene Ontology (GO) analysis indicates that the genes that were differentially expressed between the two cell clusters within the EP are most strongly associated with neurogenesis, development and cell projection organization (pers. obs.). It is intriguing to consider what drives these transcriptional differences, and how they are dynamically contributing to maintaining the distinct connectivity of these neighboring cell types. Certainly, a challenge to stem cell replacement therapies is to not only differentiate into the appropriate functional cell type, but also to establish appropriate connectivity. Datasets such as these in the EP provide opportunities to test the bounds of plasticity in this regard.

Overall, the organizing principles revealed in this extensive and layered analysis confirm the backbone of the basal ganglia circuit model for the motor circuitry (Gerfen and Wilson, 1996), but also substantially clarify our understanding of how limbic circuits propagate through it. These new findings reveal how the limbic circuitry is more complex and that much remains to be explored about how it is regulated to influence mood and motivation and in turn, influences motor circuitry. The refined understanding of the transcriptional, anatomical and functional differentiation of the limbic and motor outputs of the basal ganglia achieved in the present study can now be harnessed to accomplish this.

Neurosurgeons targeting the EP homolog in humans, GPi, for relief from movement disorders have long been aware of the presence of distinct subregions with differing efficacy for the control of movement, particularly targeting the most ventral and medial aspect of GPi. In addition, it is an ongoing clinical challenge to address the possibility and mechanisms of affective disorders occurring as side effects of DBS in this circuitry. Not surprisingly, the basal ganglia is also an emerging target for a number of affective disorders, such as Tourette's and Obsessive Compulsive Disorder. The findings by Wallace et al., (2017) suggest many new opportunities to manipulate the basal ganglia circuitry to disambiguate these behavioral domains. However, just as the picture of functionally distinct but interwoven populations was made possible by the layering of multiple new technologies, optimal therapies may also demand clever layering of modalities. The recent development

by Tadross and colleagues of “DART” technology that uses genetic tags to localize the action of traditional drugs is one promising example of such opportunities (Shields et al., 2017).

Acknowledgments

This work was supported by NIH NS064577 (N.C.).

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Figure 1. Different subpopulations of EP neurons