Long-range Connectivity Between Prefrontal Cortex and Cerebellum in the Mouse

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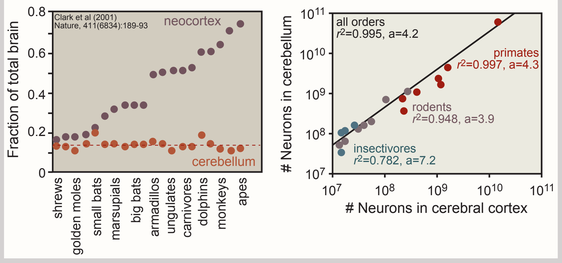
ABSTRACT

The cerebral and cerebellar cortices are functionally parceled, and although both cortices are indirectly interconnected it is unclear how functional segregation in one maps onto the other. This uncertainty arises from the multisynaptic nature of their connections and the limits of tracing methods that label single synaptic steps. Recent work in primates, including humans, indicates the presence of distinct cortico-cerebellar anatomical loops that govern cognition and action. Here, using retrograde transsynaptic transport of pseudo rabies varies, we extend work in primates to anatomically define the long-range circuits between the frontal cortex and cerebellum of the mouse. We find that a spatially specific set of cerebellar deep nuclear cells project to various region of the mouse prefrontal cortex. These studies begin to define a model system to address non-motor functons of the cerebellum and how such functions may falter in psychiatric disorders.

INTRODUCTION

Across species the cerebral cortex (CX) and cerebellum (CB) have evolved in tandem increasing in size relative to the rest of the brain. These two structures are linked by a set of topographic, multisynaptic connections and, together with their co-evolution, point to complementary roles in information processing. Of the approximately 86 billion neurons in the human brain, 69 billion (80%) are found in the CB and, irrespective of species, for every 1 neuron in the CX there are 4 in the CB (Fig 1). The CB, thus, likely serves an integral and general role in overall brain function, yet the exact nature of that role remains unknown.

Figure 1. Evolutionary scaling between the cerebral cortex and cerebellum



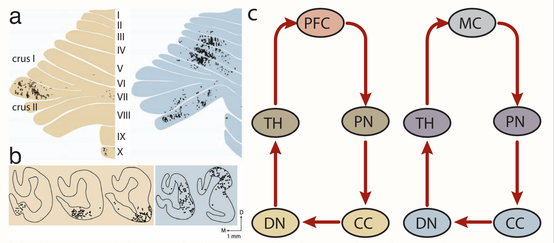
Although traditionally viewed as a structure for fine motor control, there is now substantial evidence from functional imaging and neuropsychological studies that indicate a role for the CB in non-motor functions. Moreover, the uniformity of CB architecture suggests it performs a general computation offering predictions and corrections on its inputs regardless of modality. Interestingly, recent anatomical work in primates shows multisynaptic afferents and efferents between the CB and dorsolateral prefrontal cortex (DLPFC), a region intimately involved in the flexible, goal-oriented control of behavior (Fig 2). This provides a potential structural correlate of why CB lesions can produce discoordinated mental activity analogous to uncoordinated bodily movement.

Here, we investigate the extent and nature of anatomical connections between PFC and CB of the mouse, a major model system for human behavioral disorders. We use a strain of pseudorabies virus (PRV) that expresses green fluorescent protein (GFP) to map specific transsynaptic connections across the brain (Fig 4). We have infected various regions of PFC (the anterior cingulate, infralimbic/prelimbic, and orbitofrontal cortex) of adult male mice, and allowed the virus to spread for 3-6 days before examining neuronal expression patterns ex vivo. We observed a pattern of spatially restricted GFP-labeled cells across the posterior poles of all three nuclei of the CB. Ongoing work will trace the converse direction from CB to PFC and will determine whether these connections form closed loops as they do in primates. Our findings may help establish a neuroanatomical framework for interrogating non-motor functions of the CB.

METHODS

Using retrograde trans-synaptic transport of rabies varies, Kelly and Strick (2003) have shown that the CB areas that send projections to DLPFC, area 46, (particularly crusI/II) are separate from those projecting to MC, area M1, (mainly lobules IV-VI) and that these projections go through distinct areas of the deep cerebellar nuclei (DN)(Fig 2).

Figure 2. Cognitive and motor loops of the cortico-cerebellar system



a) Left, areas of the cerebellar cortex in the monkey that project to the prefrontal cortex (PFC, area 46) and those, Right, that project to the motor cortex (MC). b) The output through dentate nucleus eventually arriving in the neocortex take segregated paths, with those going to PFC, gold, located ventrally and those to MC, blue, located more dorsally. c) A schematic of the segregrated cortico-cerebllar loops. Note that these loops are also segregated at the level of thalamic nuclei (TH) and pontine nucleus (PN). Adapted from Strick et al. (2010).

We used a similar approach in mice (Fig 3) using the pseudorabies virus Bartha strain that expresses GFP (PRV-142) (Fig 4).

Figure 3. Allen Brain Atlas reference maps showing areas of frontal cortex infected with PRV

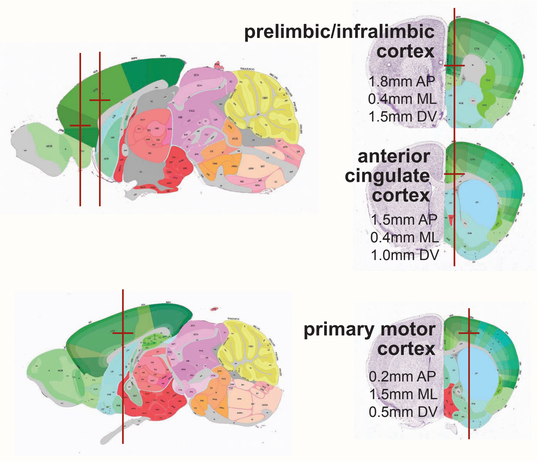
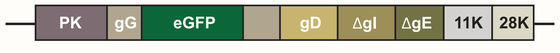


Figure 4. Pseudorabies virus used for retrograde transsynaptic tracing



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RESULTS

Figure 5. Brain-wide expression of PRV-GFP in the mouse brain with PFC infection

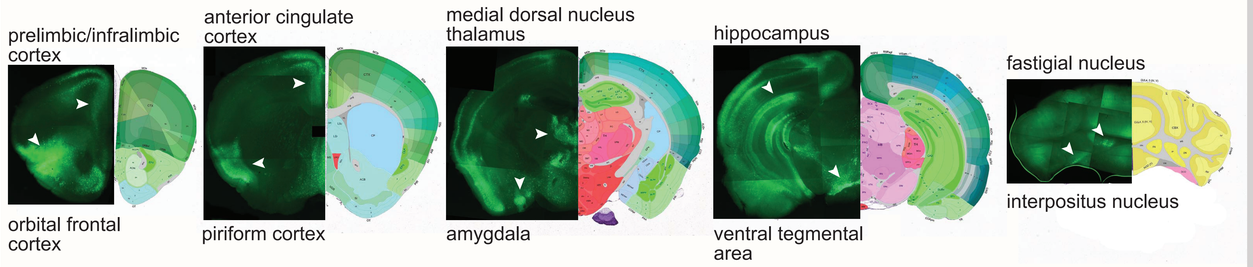


Figure 6. Viral infection into the medial prefrontal cortex labels cells throughout the ventral posterior pole of the cerebellar deep nuclei

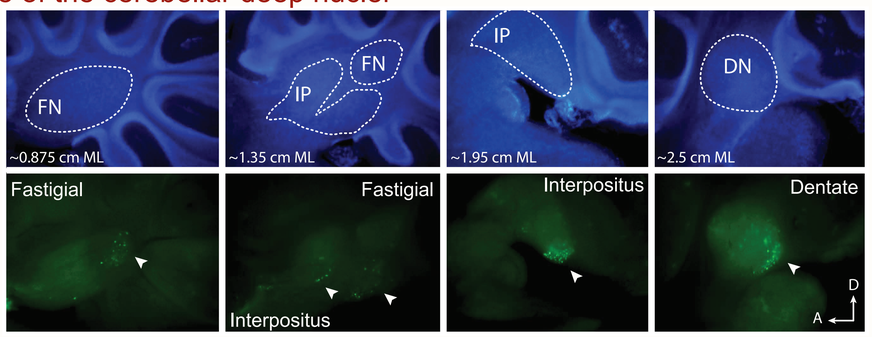


Figure 7. Select population of deep nuclear cells project to anterior cingulate cortex

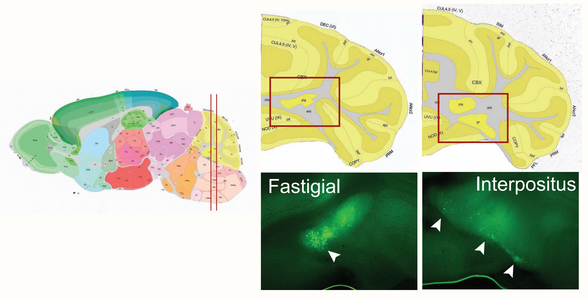


Figure 8. Select population of deep nuclear cells project to prelimbic/infralimbic cortex

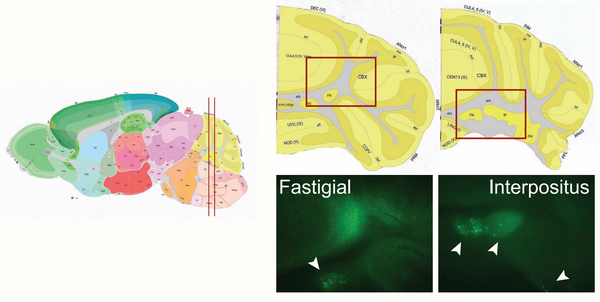
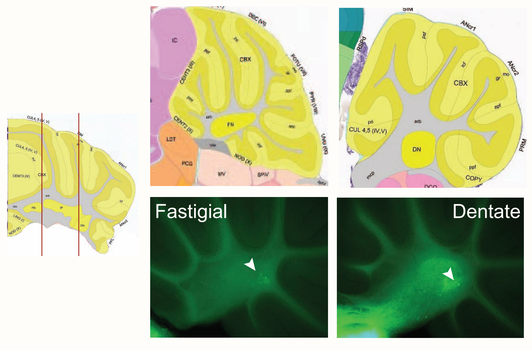


Figure 9. Same areas of cerebellar deep nuclei that project to PFC also project to primary motor cortex



FUTURE DIRECTIONS

All three deep nuclei of the cerebellum project to PFC. This projection orginates from a spatially restricted ventral posterior area of the nuclei.

This same area of the deep nuclei also projects to primary motor cortex.

Co-injection of PRV-GFP/PRV-RFP into PFC and motor cortex can determine if these cells merely comingle or if they send diverging projections to frontal cortex.

Future experiments will determine the areas of cerebellar cortex that project to different areas of PFC and if these are separate from those projecting to motor cortex and whether they receive specific inputs from PFC to form specific cortico-cerebellar loops.