Genetic labeling strategies for functional analysis of human neocortical cell types and microcircuits in ex vivo brain slices

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Abstract

What is the cellular and functional architecture of the human neocortex, and how does it differ from that of other mammals? It is a widely held believe that the remarkable evolutionary expansion of the neocortex holds the key to our uniquely human abilities. Can the origins of our higher cognitive abilities be traced back to the functional contributions of distinctive neocortical cell types, or perhaps to structural divergence in neocortical wiring? To begin to address these longstanding questions necessitates access to living human brain tissue for functional studies, a daunting task with many logistical challenges. We have successfully established a local network of neurosurgeons providing routine access to vital human neocortical tissue resected during brain surgery. We have begun a program with the goal of cellular level dissection of the human neocortical circuit based on systematic analysis of intrinsic and synaptic properties, cellular morphology, and transcriptomics. In the course of this work we have observed that acute human neocortical brain slices prepared from these neurosurgical specimens exhibit a remarkable viability that greatly exceeds the viability of rodent brain slices. This observation led us to establish a platform for human *ex vivo* brain slice culture and to explore rapid virus-mediated gene transfer into human neurons over the course of one week *in vitro*. With this approach we have achieved high density viral transgene expression in excitatory and inhibitory neuron populations. This has enabled targeted patch clamp recording of transgene expressing neurons for measurement of intrinsic and synaptic properties, as well as precise manipulation of neuron firing with light using Channelrhodopsin2. The feasibility we have demonstrated opens up many new avenues for applying modern molecular genetic tools to study the functional architecture of the human brain. We now describe progress in our efforts to achieve cell type specificity by restricting virus-mediated transgene expression to genetically defined neuronal populations. We hope to harness our emerging transcriptomic and epigenetic data to guide the rational design of novel vectors. Progress in this area is essential to gain access to the many diverse cell types of the human neocortex, an obligate step towards more precise cellular and circuit level investigations, as is currently possible in model organisms like rodent, fish, fly, and worm. Furthermore, the development of novel cell type-specific viral vectors will enable more rapid progress in a wide range of research areas, from human gene therapy for brain disorders to the functional manipulation and dissection of the neural circuitry basis of behavior in non-human primates.