Austin Journal of Anatomy

Open Access

Austin Publishing Group

Perspective

Perspective Research of Specific Neural Projection with Microspheres Retro-Grade Tracing

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Received: July 21, 2016; **Accepted:** July 28, 2016; **Published:** August 16, 2016

Perspective

Brain is the most complex organ of human body and the cerebral cortex is the most component of the brain. The cerebral cortex itself is divided into different regions, each containing specific neuron types. During development, these neurons project to different target region and establish the specific neural projections [1].

Decades of research have confirmed that many functions including cognitive and motivated behavior require the intergration of sensory inputs and motor outputs. In neuroanatomy, it is the specific neural projection which is responsible for transmission of neural signal. Recently, Zingg et al. launched the Mouse Connectome Project, and they generated a cortical connectivity atlas [2]. Although numerous studies have examined neural connections of many region of mammalian brain, the specification and communication of different cerebral region are largely unclear. Especially, the molecular mechanisms that operate the neural projection is elusive until now

In our laboratory, we focus on the projections of dopaminergic neurons in midbrain, these neurons can be divided into mesostriatal, mesocortical and mesolimbic subtype cells with projection to striatum, prefrontal cortex and neucleus accubens respectively. Microsphere retrobeads were bought from Lumafluor Company. This tracer was reported before for neural labeling [3] and demonstrated an Ideal effect. We injected the green retrobeads into prefrontal cortex and striatum and labeled mesocortical and mesostriatal subtype dopaminergic neurons. These neurons can be easily detected under fluorescence microscope without staining (Figure 1).

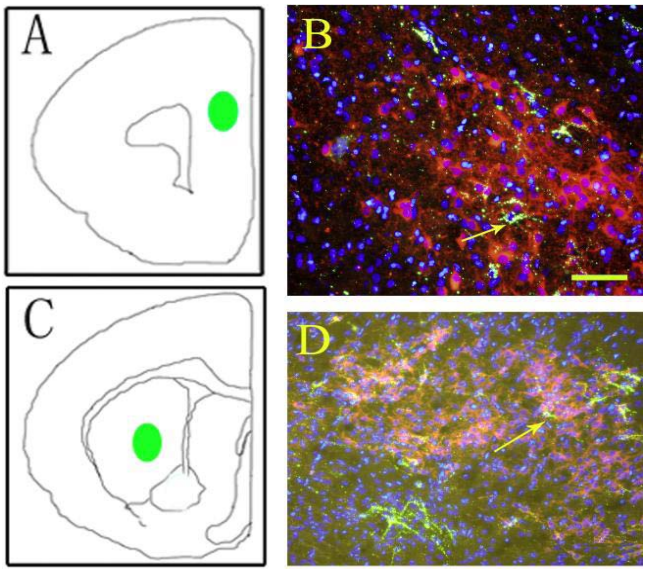

Figure 1: Retro-grade tracing of meosocortical projection (A, B) and mesostrial projection (C, D). A and C show the injection position in cerebral cortex; Arrows in B and D show labeled subtype neurons of mesocortical and mesostriatal projection, respectively.

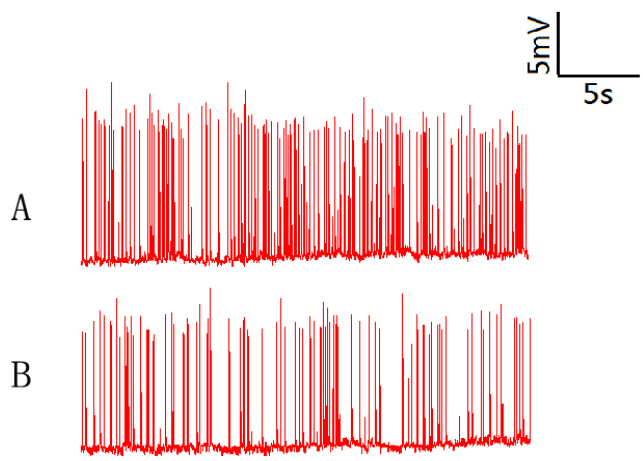

Figure 2: Aripiprazole reduced the frequency and amplitude of spontaneous action potentials. A, aripiprazole 10μM; B, control group.

One advantage of this tracing is that we can analize the physiological function with the brain slice patch. Dong et al in our research group labeled mesocortical subtype neurons and detected effect of aripiprazole, a new type of antipsychotic drug, on electrophysiological properties of labeled neurons with brain slice patch technique. Aripiprazole (10μM) reduced the frequency and amplitude of spontaneous action potentials (Figure 2).

Another advantage of microsphere retrobeads tracing is that the labeled cells can be collected through Fluorescence Activated Cell Sorting (FACS). And the RNA can be extracted from these pure subtype neurons, the further analysis, such as real time PCR and

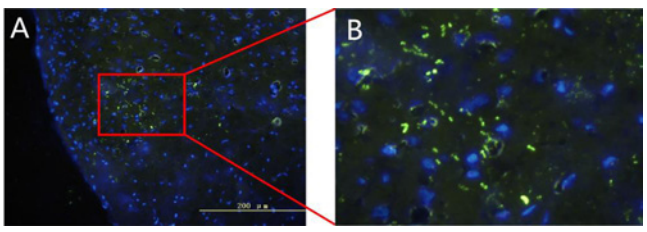

Figure 3: Microsphere retrobeads show nerve regeneration.A, labeled neurons in spinal cord; B, amplification of A.

Citation: Sun J. Perspective Research of Specific Neural Projection with Microspheres Retro-Grade Tracing. Austin J Anat. 2016; 3(2): 1052.

RNA-seq can be performed to explore the molecular mechanisms of specific neural projection.

Additionally, for the first time, we use microsphere retrobeads in peripheral nervous system. In our experiment, we cut sciatic nerve of rat, and the transected sciatic nerve was repared with muscle bridge technique. Eight weeks after transaction, retrobeads were hypodermicly injected into the foot of rat. Labeled neurons were detected in ipsilateral of spinal cord of rat (Figure 3).

In conclusion, microsphere retro-grade tracing provides a good technique for neural projection studies. We can not only labeled subtype neurons, but also can explore the underlying molecular mechanisms in cell specification. Moreover, we have already used this technique in peripheral nervous system for nerve regeneration detection.

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Austin J Anat - Volume 3 Issue 2 - 2016
ISSN : 2381-8921 | www.austinpublishinggroup.com

Citation: Sun J. Perspective Research of Specific Neural Projection with Microspheres Retro-Grade Tracing. Austin J Anat. 2016; 3(2): 1052.