Mouse Brain Tissue Collection and Analysis

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We use this protocol and it's working

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ABSTRACT

This protocol describes the dissection and collection of coronal sections of the striatum and midbrain from a mouse brain. The tissue can be used in a number of applications and here we describe two: the measurement catecholamine levels using high-performance liquid chromatography (aided by a neurochemistry core) and the distribution of the vesicular monoamine transporter (VMAT2) using a radioligand binding assay.

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Tissue dissection

1 Prepare solutions

1.1 For measurement of brain catecholamine by HPLC, use cold Hank's Balanced Salt Solution (HBSS) containing 10 mM HEPES and 20 mM glucose

1.2 For [^{3}H]-Dihydrotetrabenazine binding, dissection into cold SHT buffer (320 mM sucrose, 10 mM HEPES/Tris, pH 7.4) with 0.4 mM EDTA and Complete Protease Inhibitor Cocktail (Roche)

2 Euthanize mice by inhalation of CO_2

3 Decapitate mouse and remove brain

4 Place brain into a rodent brain matrix (RBM-2000C, Protech International Inc.)

5 By inserting razor blades into the slots of the brain matrix, collect two 1 mm coronal sections containing the striatum

> **Note**
>
> Be sure to flip the slices and look at both sides, selecting the sections that contain most of the striatum

6 Remove the cortical tissue and cut out striatum, collecting tissue in cold solution (HBSS or SHT depending on application)

7 From the same brain, dissect the midbrain using the brain matrix
Transfer this region onto parafilm. Remove top 70% of the dorsal side and collect the ventral side (containing the VTA and substantia nigra) into cold solution (HBSS or SHT depending on application). Do not separate hemispheres; there will be one sample per mouse.

Measurement of catecholamine levels by HPLC

8 Transfer tissue to Eppendorf tubes and flash freeze in liquid nitrogen. Immediately transfer to -80 °C .

9 Tissue is shipped on dry ice to the Vanderbilt Neurochemistry Core (https://lab.vanderbilt.edu/vbi-core-labs/neurochemistry-core/), where tissue catecholamine levels is measured by HPLC with coupled electrochemical detection.

[3H]-Dihydrotetrabenazine binding

10 Disrupt midbrain and striatal tissue with 12 strokes of a Dounce homogenizer at 500 RPM in cold SHT buffer

11 Sonicate tissue for 30 seconds

12 Sediment the debris in a centrifuge at 2000 g for 2 minutes

13 Collect supernatant

14 Measure protein content with bicinchoninic (BCA) assay

15 Dilute 50 µg protein into SHT buffer and add 10 nM (+)-a-dihydrotetrabenazine [9-O-methyl-^{3}H] (ARC; 80 Ci/mmol); incubate at 30 °C for 30 minutes
Binding should be performed in triplicate for each sample; measure non-specific binding by adding 10 µM non-radioactive benazine in the assay

16 Stop reaction by filtration through a Supor 200 0.2 µm filter (PALL); wash 3 times in ice-cold SHT buffer with 20 mM tetrabenezine (Fluka)

17 Measure radioligand signal and normalize specific binding to the amount of membrane protein added to the reaction