Human Brain Tissue Bank and Laboratory,
Semmelweis University, Budapest

2015

The Human Brain Tissue Bank is unique for two reasons:

1) Only microdissected brain samples are stored. The microdissections of 260 different brain regions, areas and nuclei are performed on slides of frozen brains and the samples are kept on -70°C. These samples have been improved to be excellent for neurochemistry, neuroendocrinology, as well as for molecular biological, proteomical and genomical studies.
2) Very short *post mortem* delay. Most of the brains were removed from the skull and frozen within 2-6 hours after death.

Special collections:

- Alzheimer's disease
- „general arteriosclerosis" (non-Alzheimer's dementia)
- stroke
- depressive suicides
- extreme elderly brain samples (over age 85)
-„control" brain samples (without neurological disorders)

Within a country-wide program (Lenhossék Program), the HBTB has been contracted with Hungarian pathological, neurological and neurosurgical departments or laboratories to collect human brains.

Post mortem delay: 1^h - 6^h

permissions:

- family consent or medicolegal cases

obligatory information:

- family report
- medical report (died in public place) or
- clinical report (died in hospital)
- pathological report
- tests for: HIV, hepatitis, syphylis, Tb, drugs
- neuropathological report

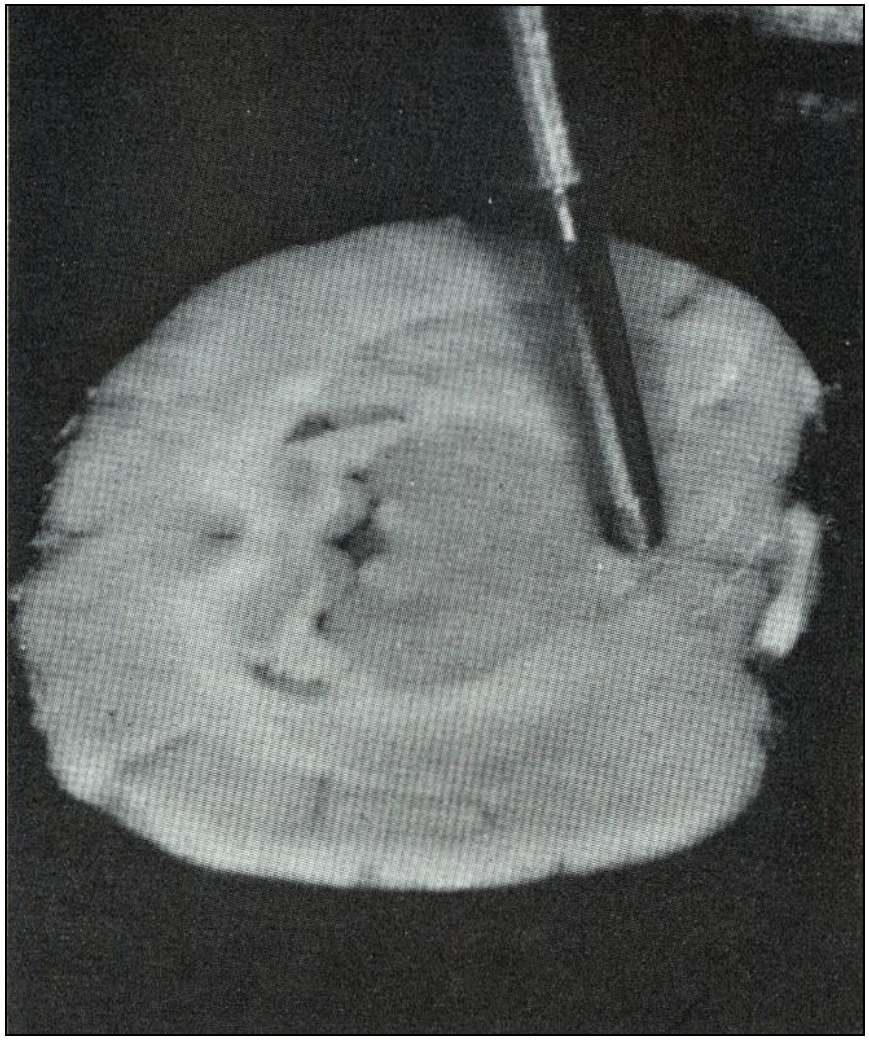
The microdissection of human brain nuclei is based on a simple procedure, called the "*micropunch technique*". This technique was introduced in 1973 to remove small, precisely localized areas from a rat's brain. Later, the technique was adapted for the microdissection of the human brain, and since 1992 the **Human Brain Tissue Bank, Semmelweis University** (HBTB) has been collecting and storing thousands of microdissected human brain nuclei and areas. The bank is located in the Department of Anatomy of the Semmelweis University, Budapest, and it operates under the management of the Laboratory of Neuromorphology of the Hungarian Academy of Sciences.
The Human Brain Tissue Bank, Semmelweis University has been a member of the European Brain Bank consortium, *BrainNet II. Europe*, since 2004.

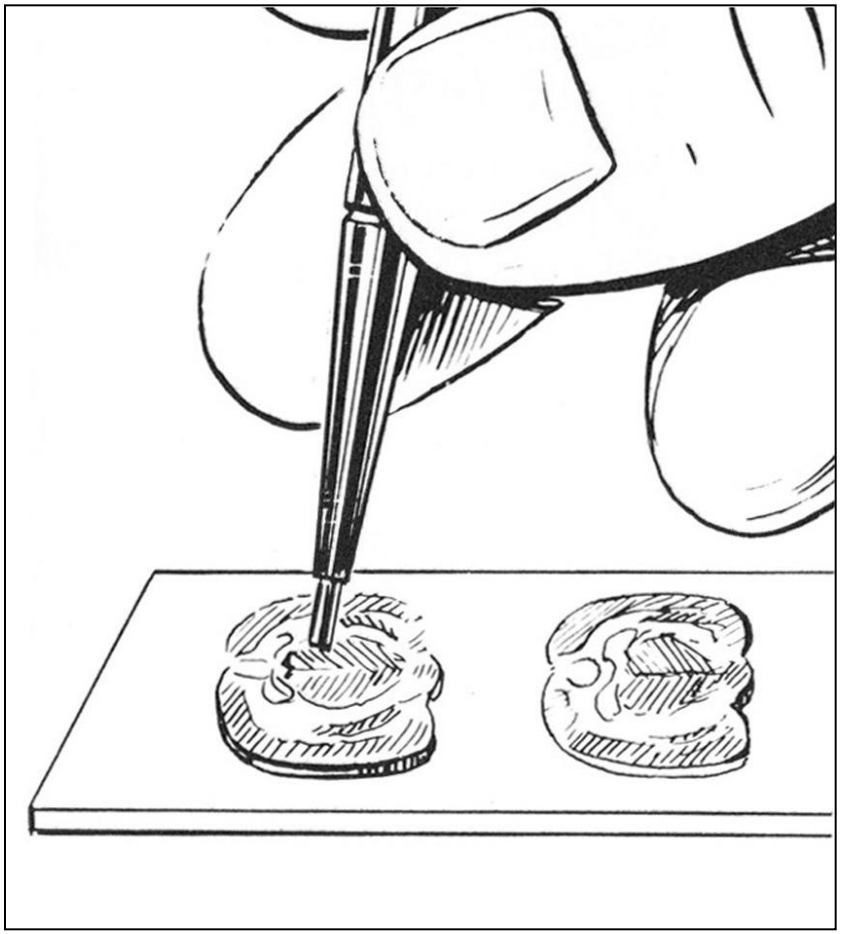
The micropunch technique was published in the *Brain Research* in 1973. Punching out well recognized and precisely localized areas from brain sections is a simple, quick, and reproducible technique, as you will see here later. However, the correct use of technique requires a perfect knowledge of the fine anatomy, or rather, the fine three-dimensional topography of the brain, whether human or from experimental animals. By today, the slogan of the microdissection technique has been realized: "anything that one can recognize and localize anatomically in the brain, can be removed separately."

References:

Palkovits M (1973) Isolated removal of hypothalamic or other brain nuclei of the rat, *Brain Res* 59:449-450

Palkovits M & Brownstein MJ, *Maps and Guide to Microdissection of the Rat Brain*, Elsevier, New York-Amsterdam-London 1988





„Punch needles”

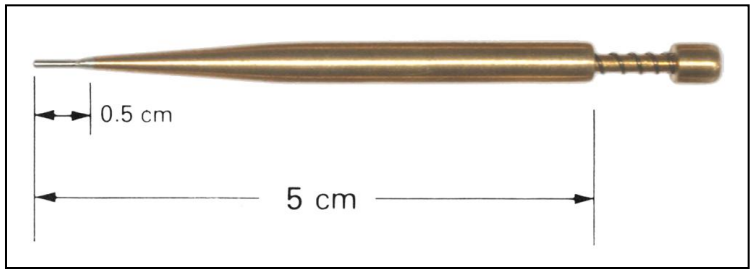
a) The hollow needles are constructed of hard stainless steel tubing mounted in a thicker handle. Their inner diameters vary from 2.0 to 10.0 mm.

b) The needles are equipped with a spring-stylet that fits well into the lumen of the needle and reach 1-2.5 mm beyond the tip of the needle.

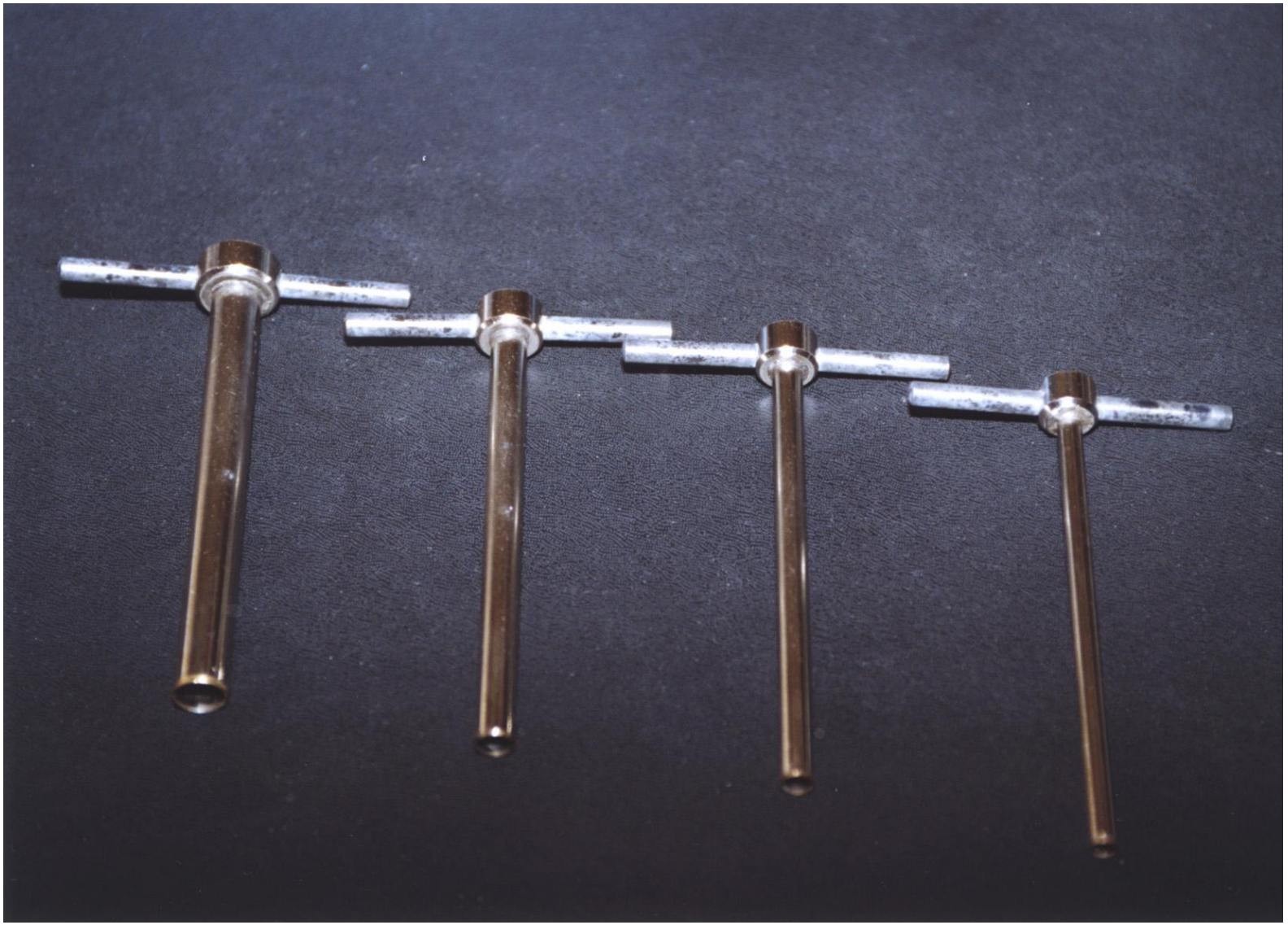
a 

b 

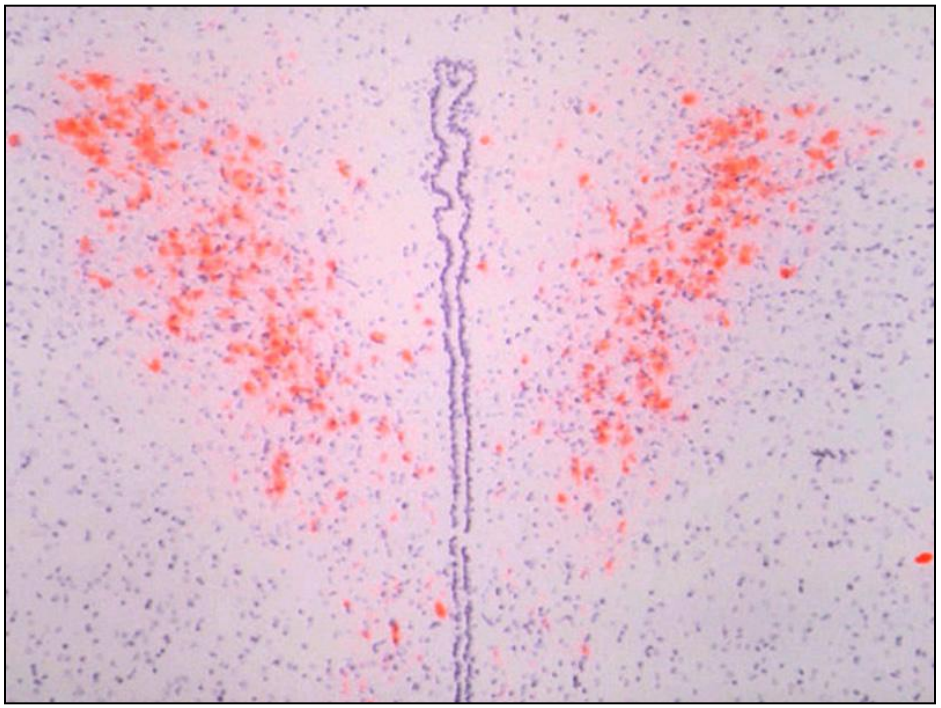
For comfortable handling, needles should be 5.0-7.0 cm long with a thinner end, at least 5-7 mm long, so that the tip of the needle is visible under the microscope.

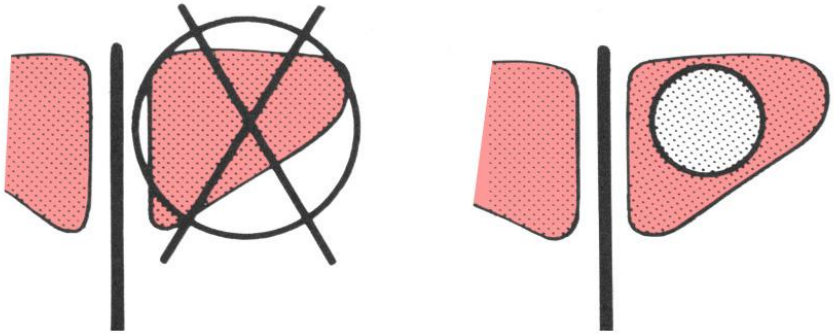


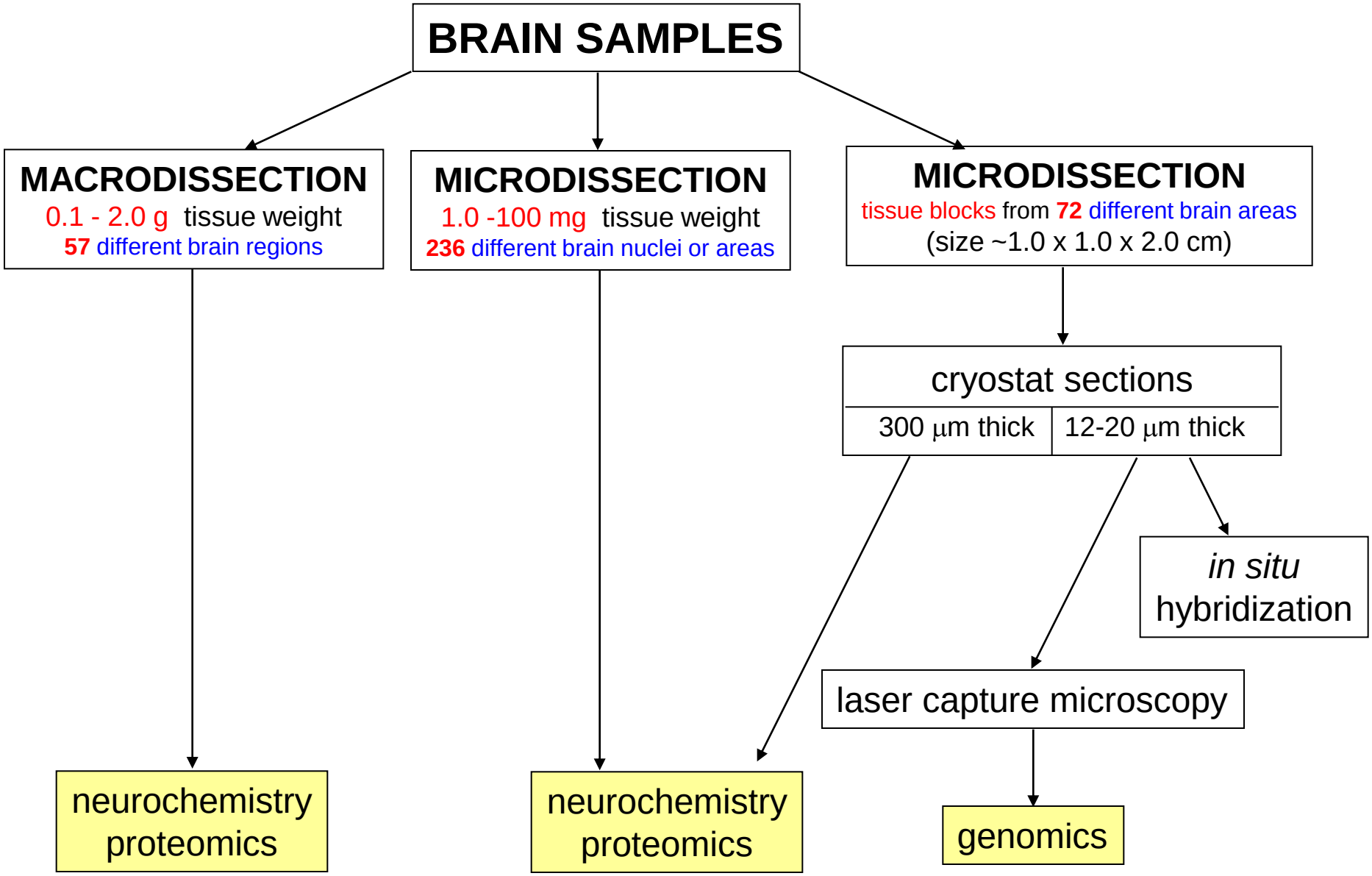
„Needles” for macrodissection



General rule that the inner diameter of the needle should be as large as possible, but smaller than the smallest diameter of the brain nuclei or areas

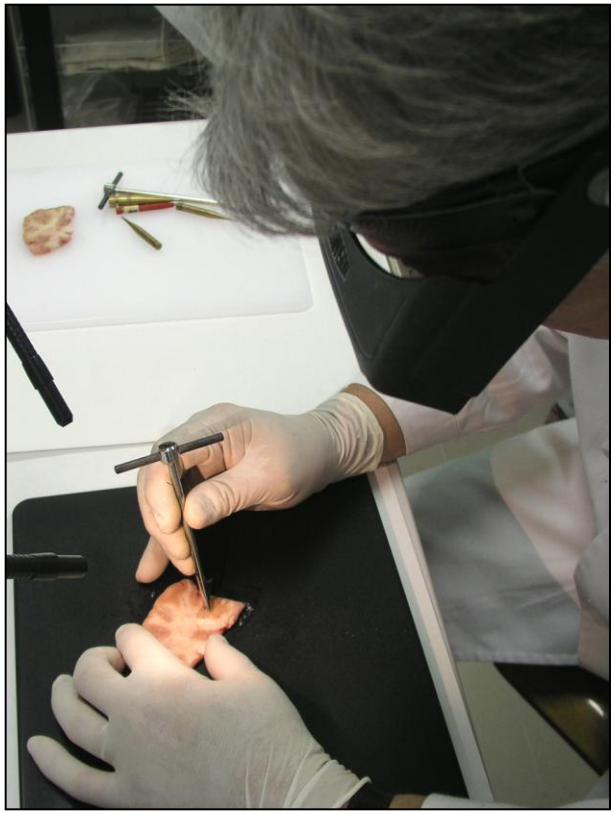


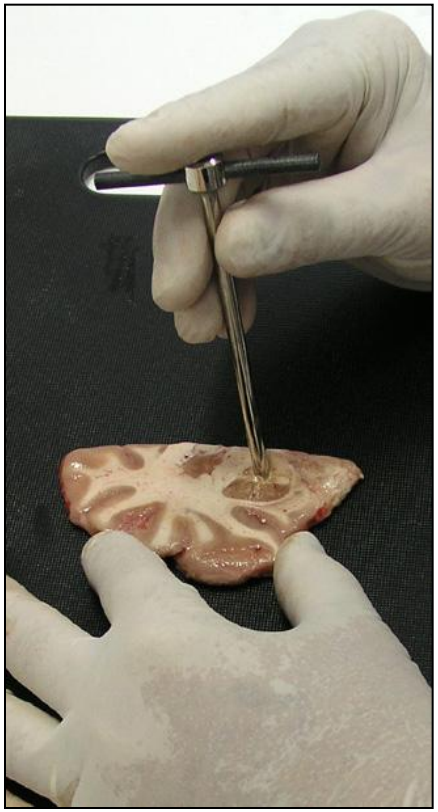


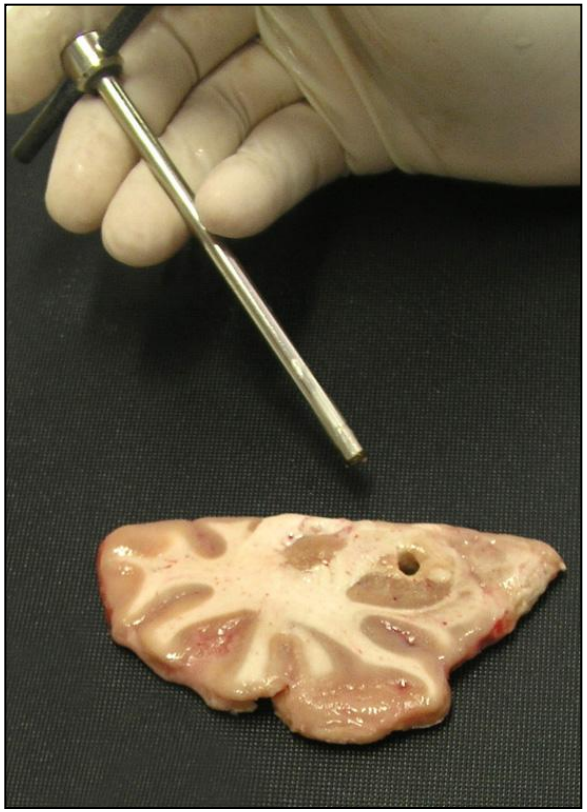

BRAIN SAMPLES
MACRODISSECTION
0.1 - 2.0 g tissue weight
57 different brain regions
MICRODISSECTION
1.0 -100 mg tissue weight
236 different brain nuclei or areas
MICRODISSECTION
tissue blocks from 72 different brain areas
(size ~1.0 x 1.0 x 2.0 cm)
cryostat sections
300 μm thick
12-20 μm thick
in situ hybridization
laser capture microscopy
genomics
neurochemistry proteomics
neurochemistry proteomics

Macrodissection from serial sections

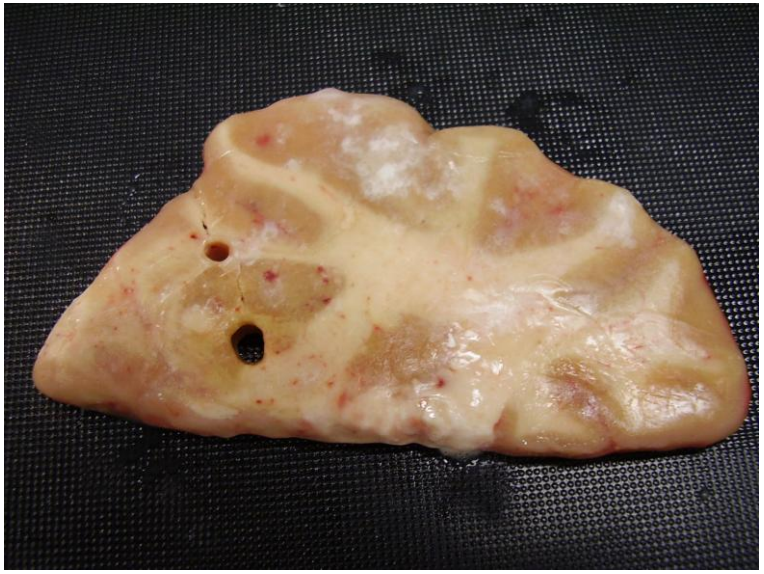
Brain nuclei can be removed from sections that have been placed on a black rubber plate. By using upper illumination, the black plate provides a contrasting background.

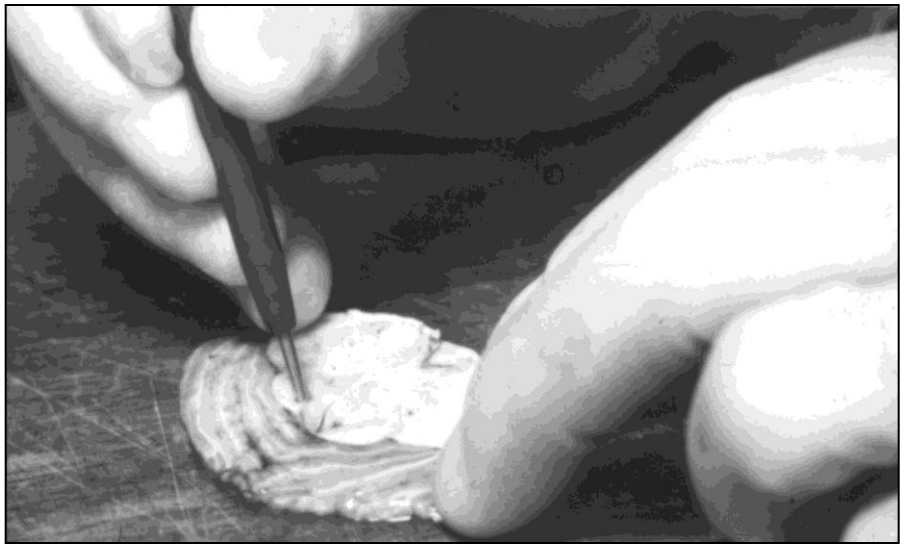


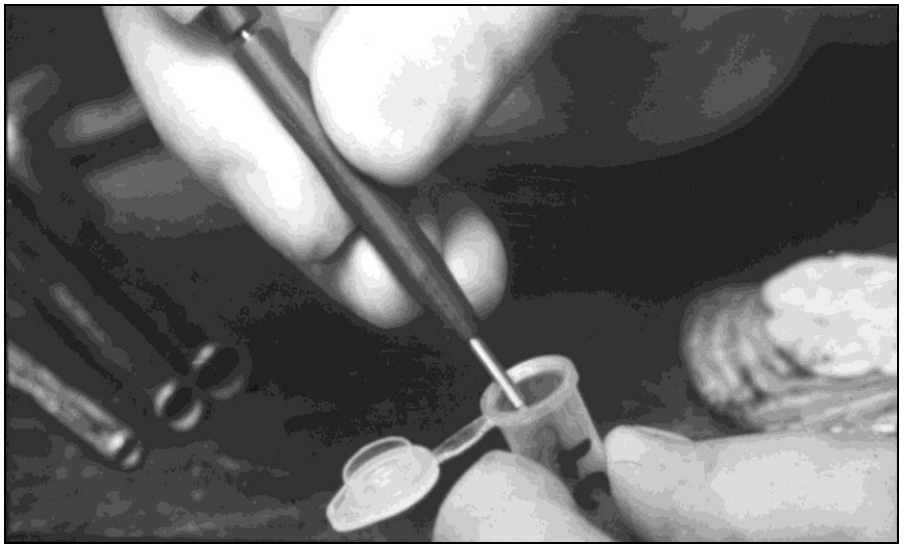




Microdissection from serial section by using small „punch needles”

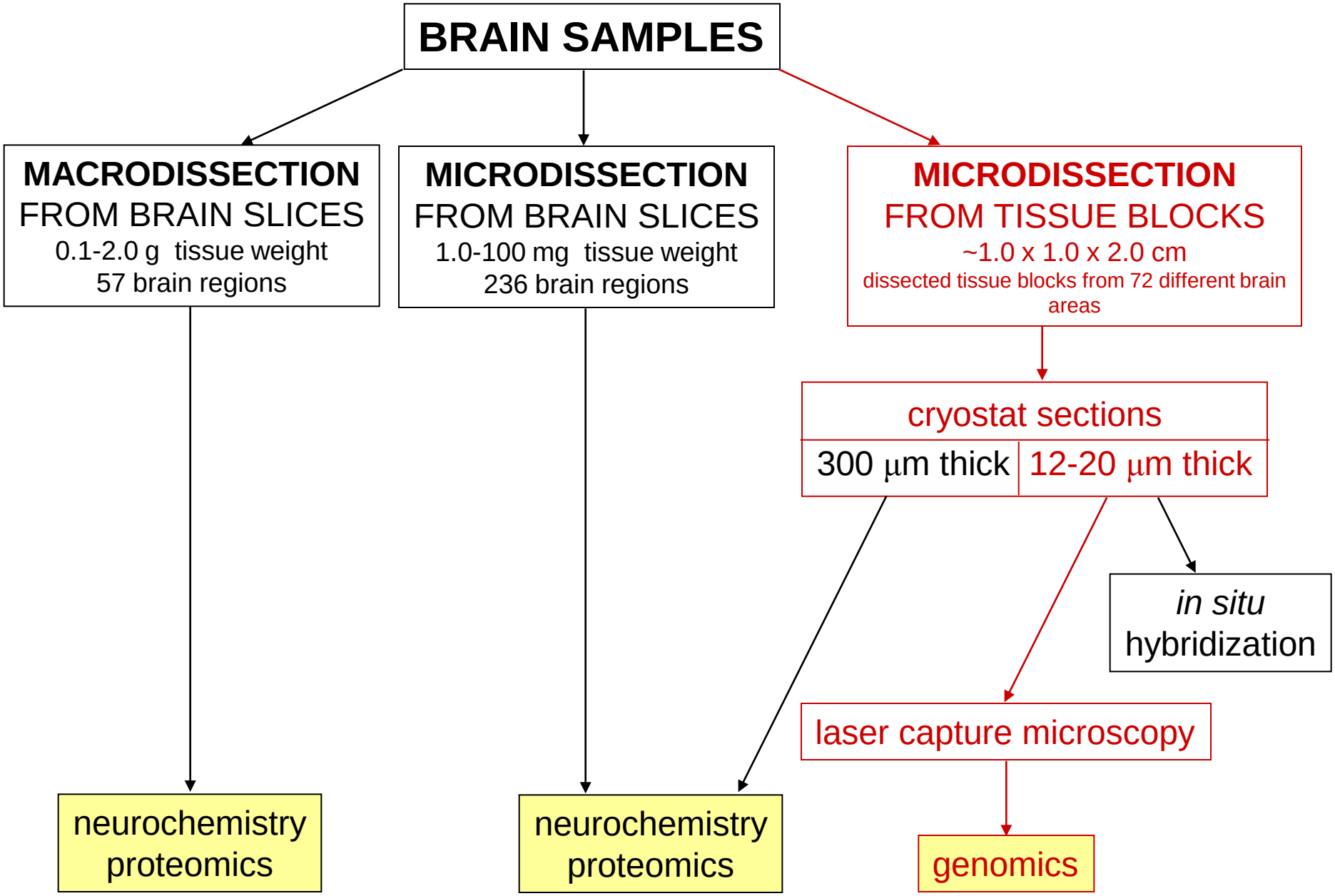






The dissected tissue pellet can be pushed out with the stylet into a tube or dish, or directly into the microhomogenizer.

If several samples are punched from the same brain nucleus, the pellets can be collected in the lumen of the needle and pushed them all at once.


BRAIN SAMPLES
MACRODISSECTION
FROM BRAIN SLICES
0.1-2.0 g tissue weight
57 brain regions
MICRODISSECTION
FROM BRAIN SLICES
1.0-100 mg tissue weight
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MICRODISSECTION
FROM TISSUE BLOCKS
~1.0 x 1.0 x 2.0 cm
dissected tissue blocks from 72 different brain areas
cryostat sections
300 μm thick
12-20 μm thick
in situ hybridization
laser capture microscopy
genomics
neurochemistry proteomics
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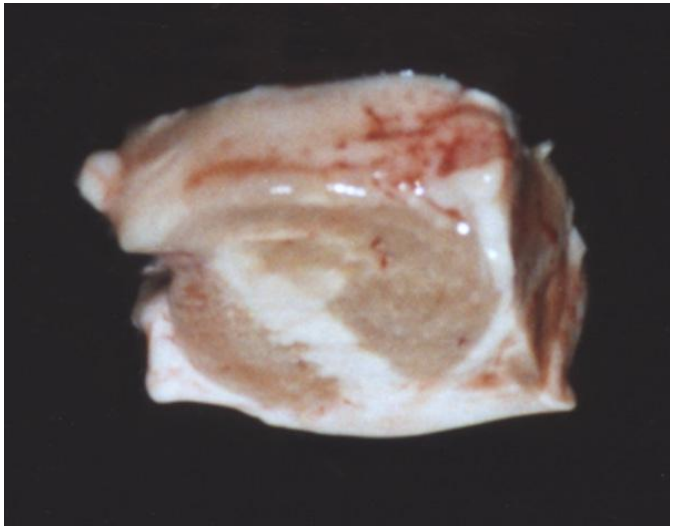
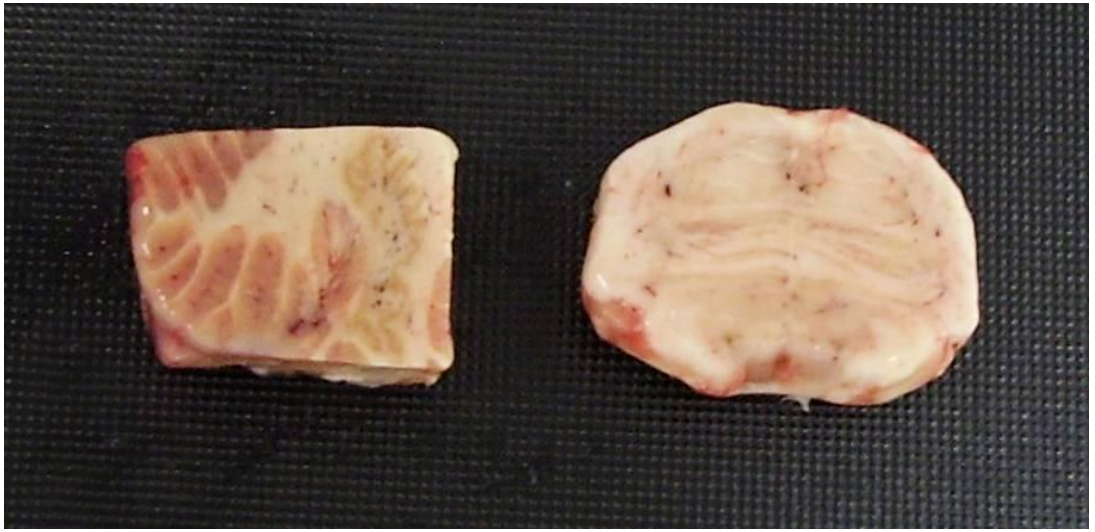
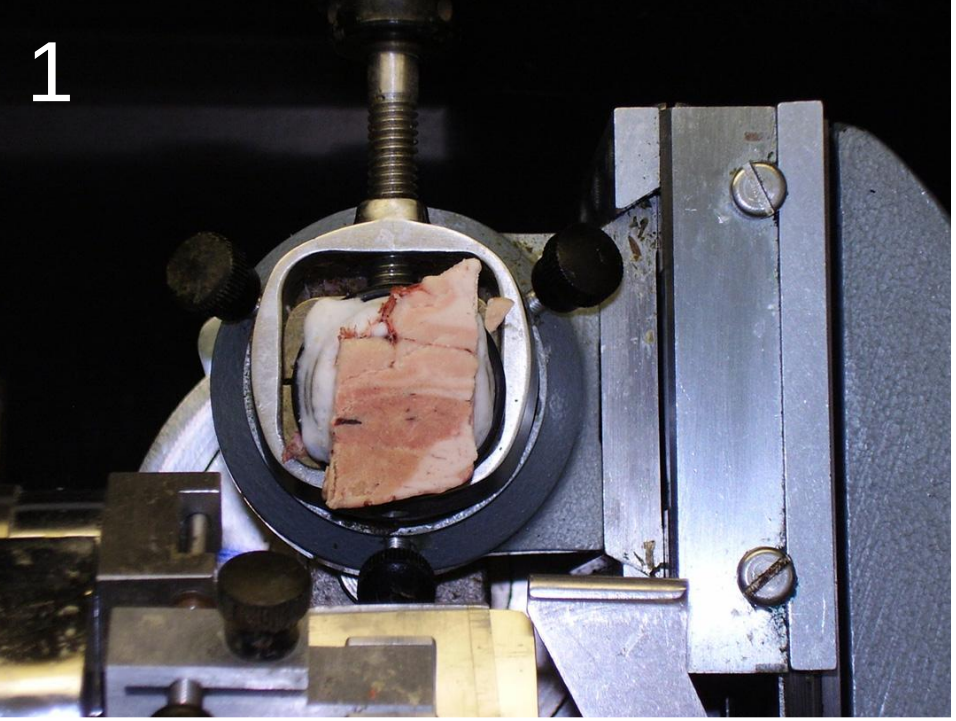
About 10 x 10 x 20 mm large, anatomically recognized and topographically oriented blocks are cut out from the brain immediately after removal from the skull (generally 20-40 brain areas), frozen on dry ice, ready for cryostat sectioning without thawing of the tissue. Tissue blocks packed in aluminium foil or in airtight bags are stored at -70°C.



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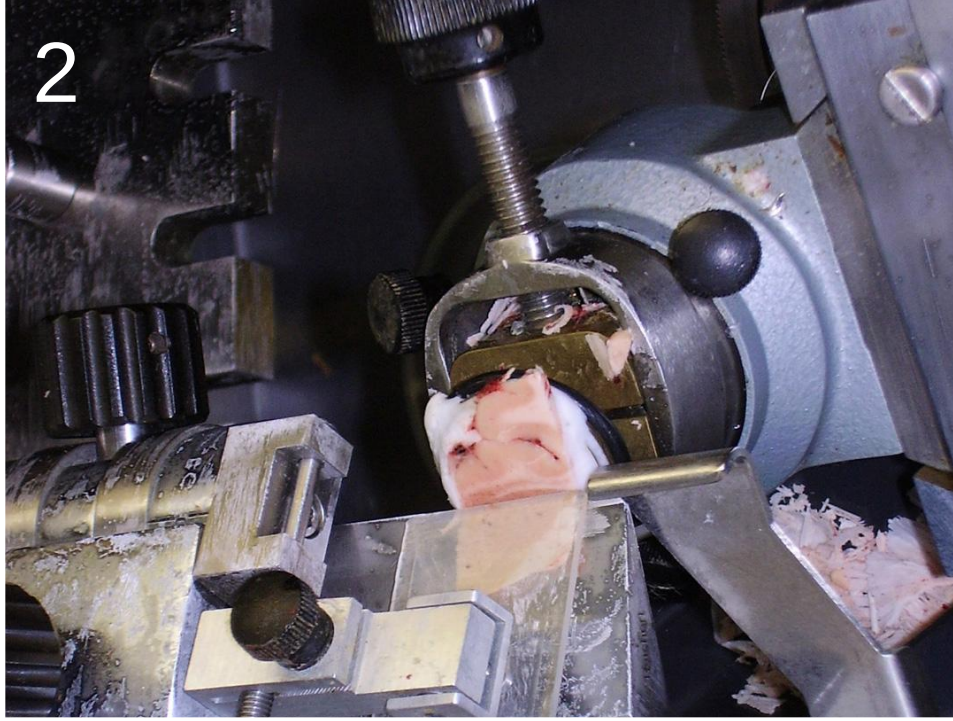
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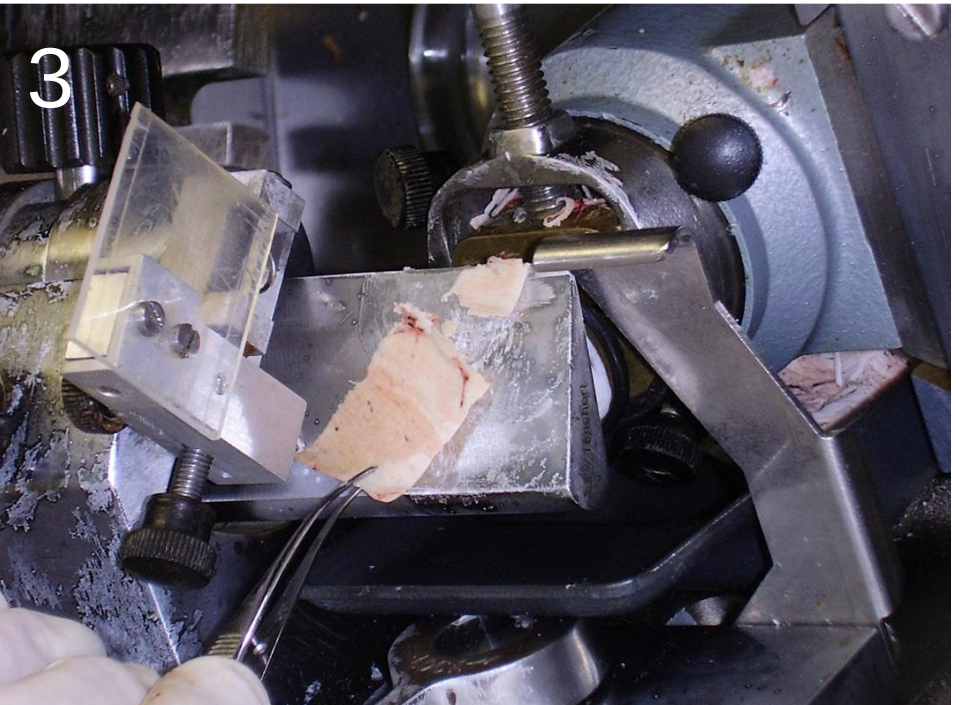
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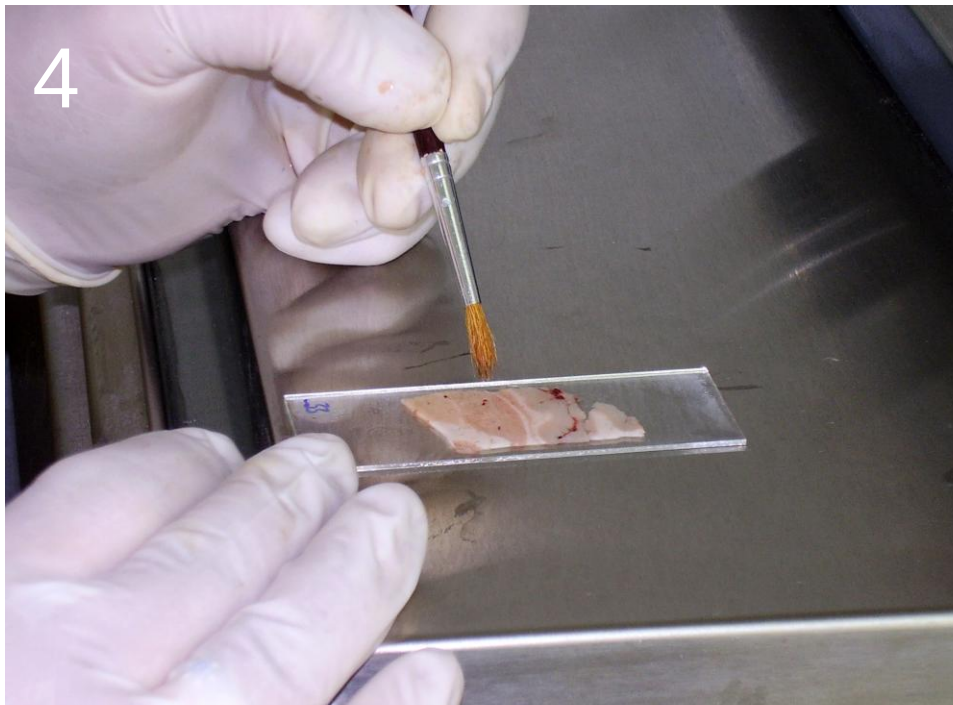
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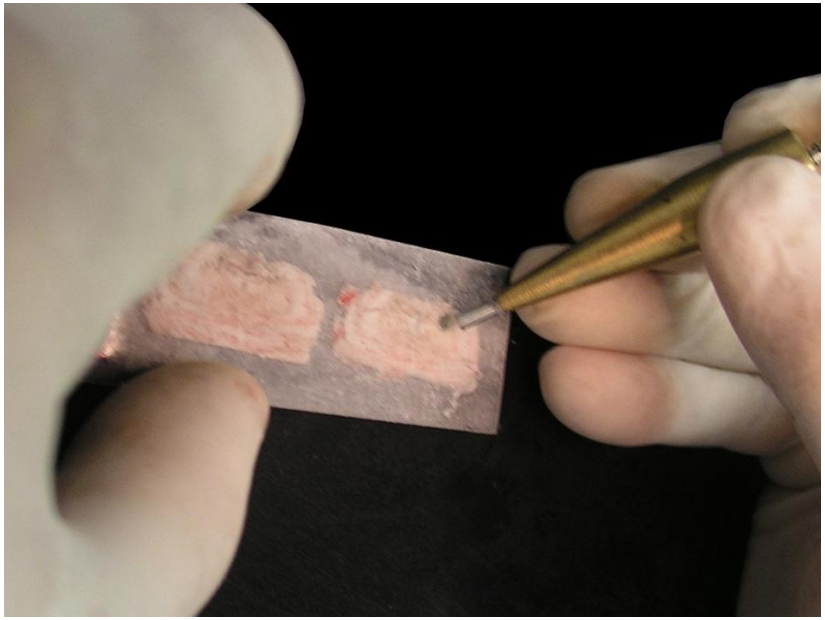
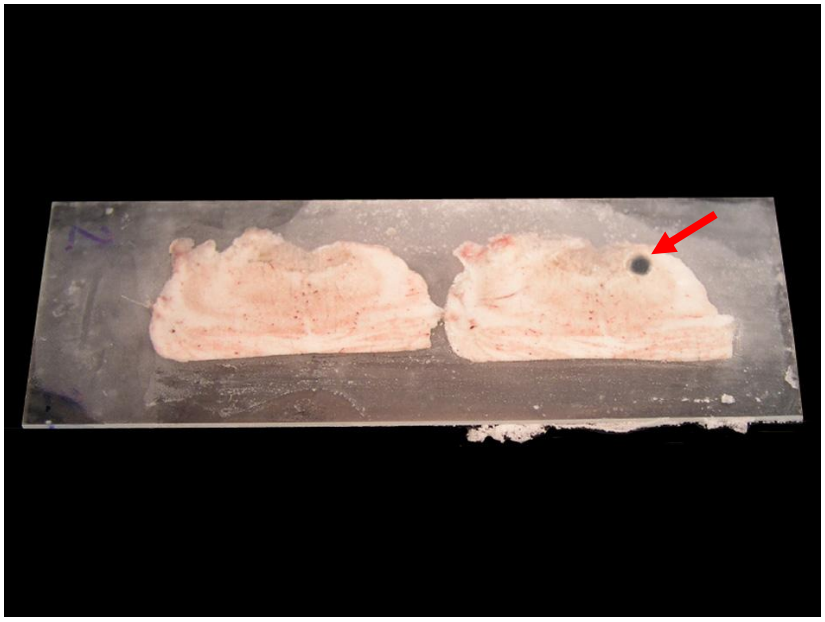
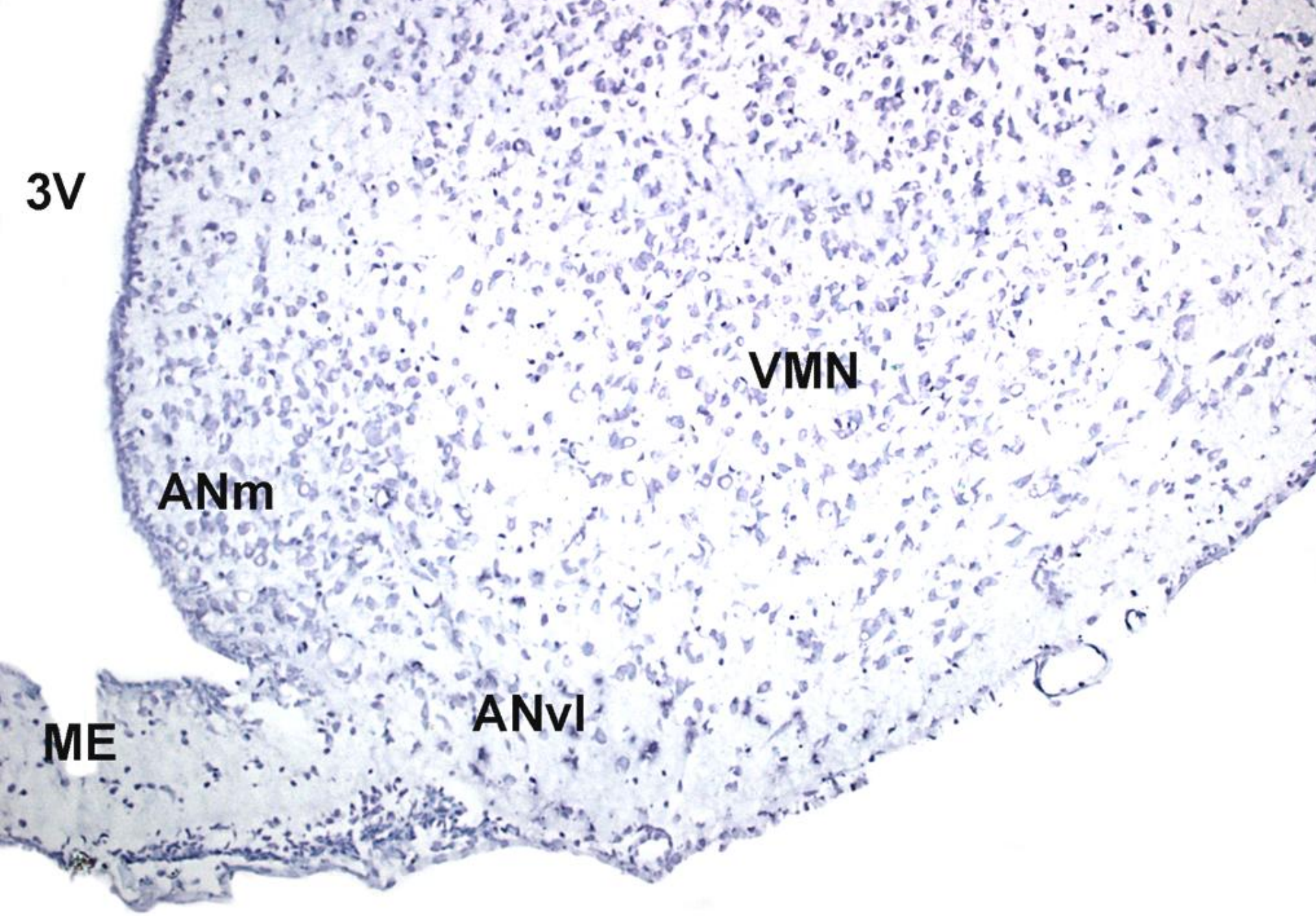
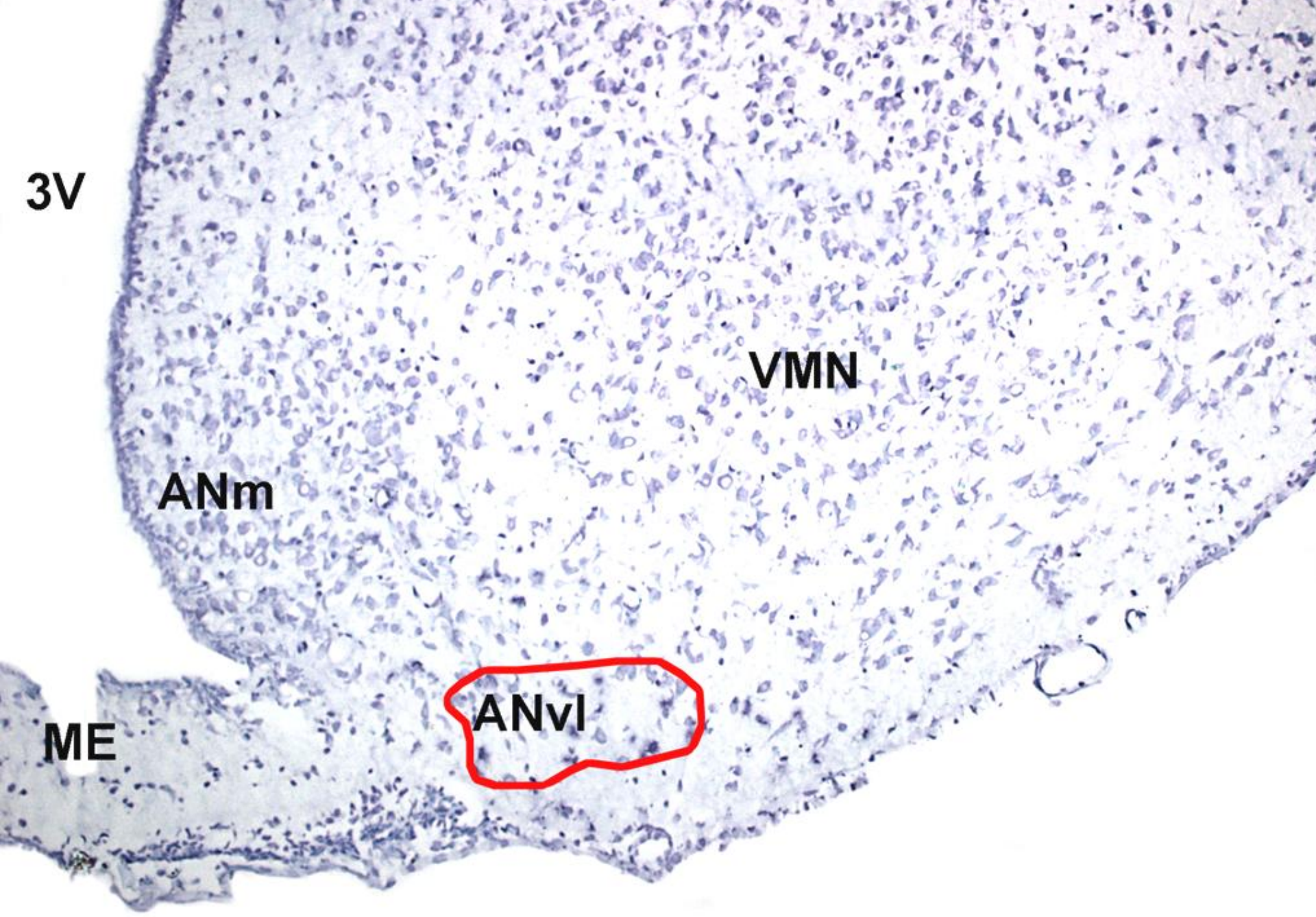




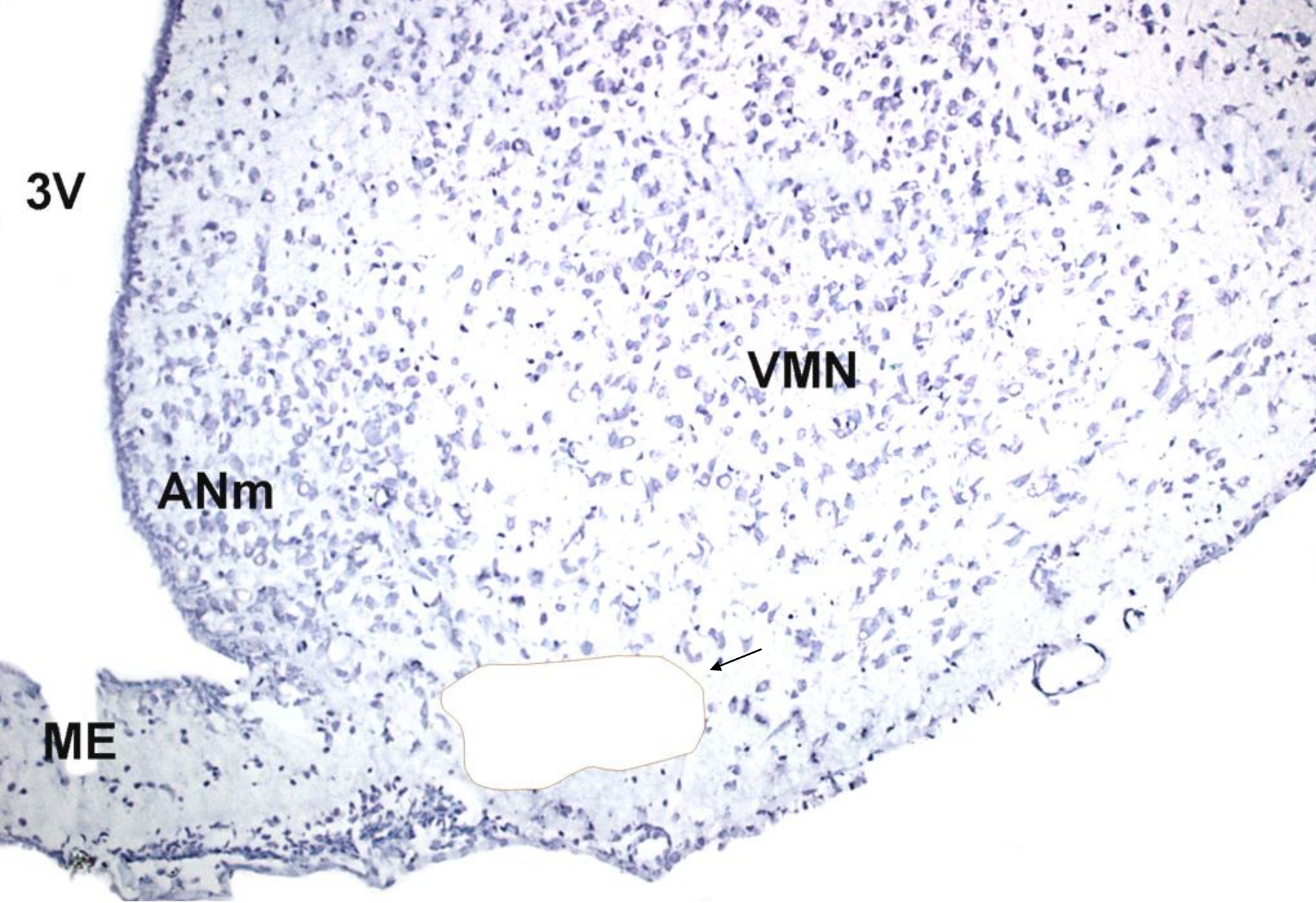
Microdissection with laser capture microscopic technique



Section (20 μm) from the hypothalamus stained with methylenblue



Area to be dissected is outlined on the screen and cut out by a laser beam

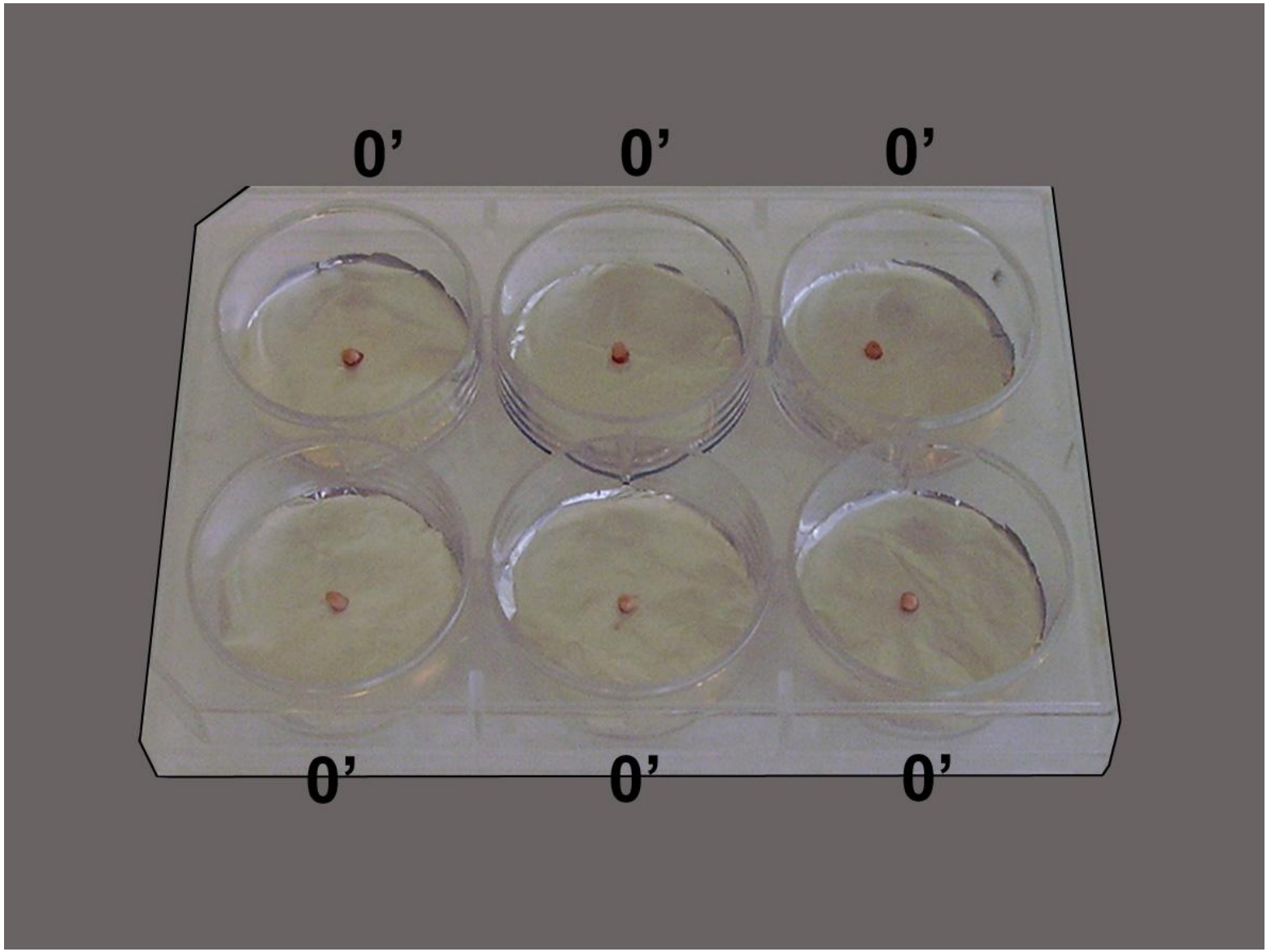


The dissected brain area has been sucked into a miniature Eppendorf-like tube by the vacuum system

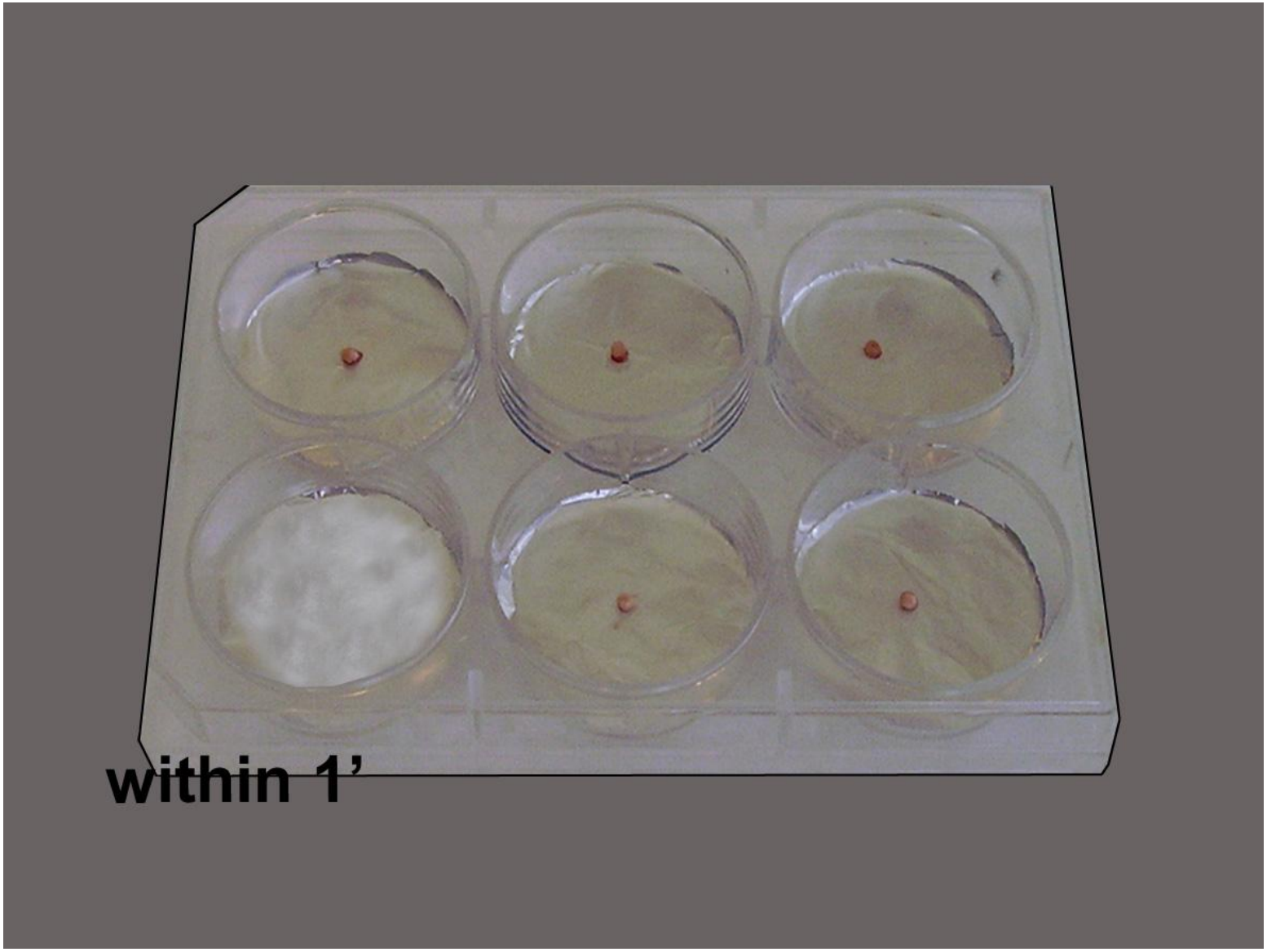
Testing for short ex-vivo *(„post mortem")* alterations

Samples from same brain areas with
0 to 360 min *ex-vivo* changes

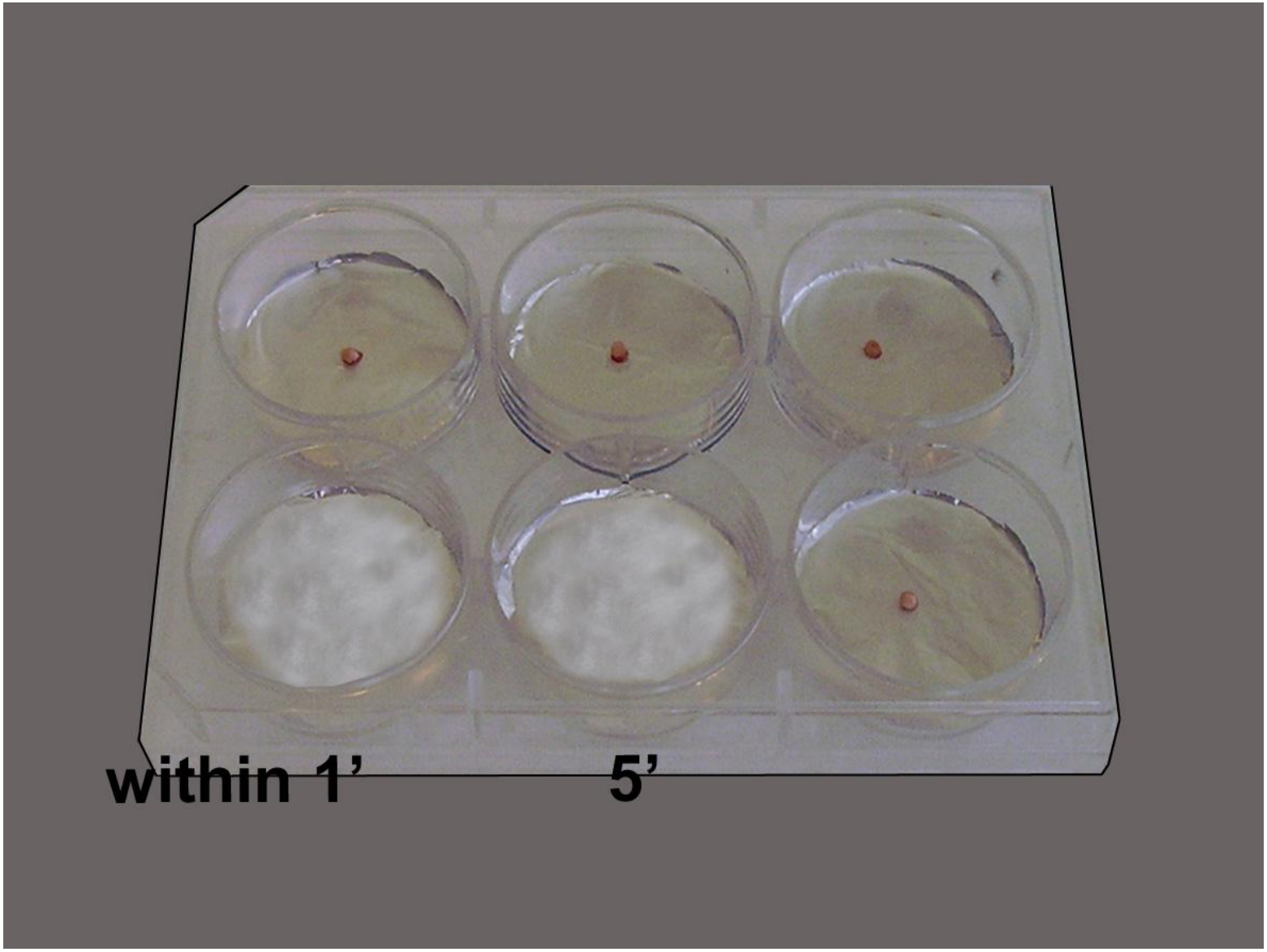
Step 1. Tissue samples from the same brain areas, immediately after removal (0’). One drop saline on each samples.



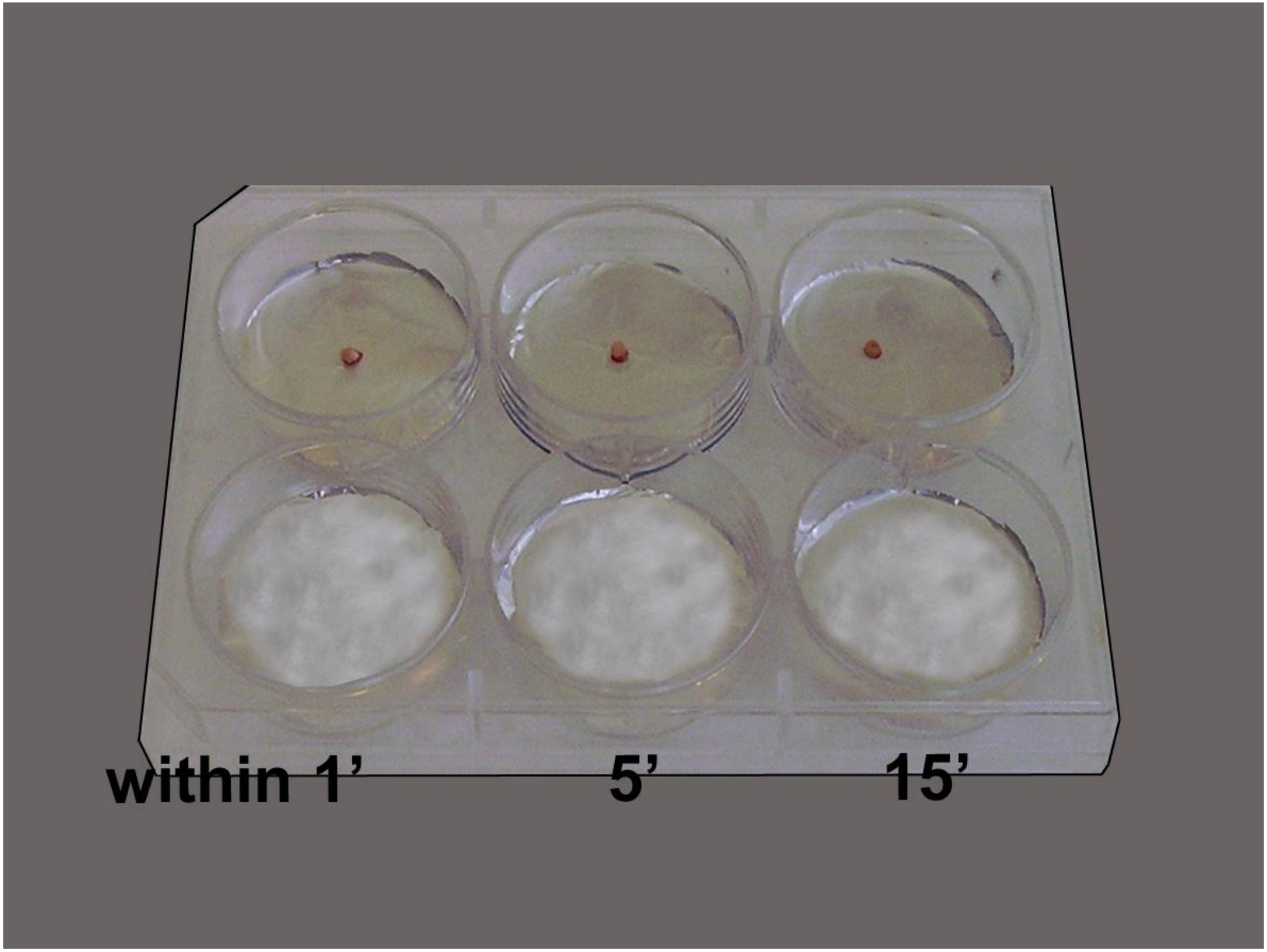
Step 2. The first sample has been covered with dry ice powder within 1’ after removal.



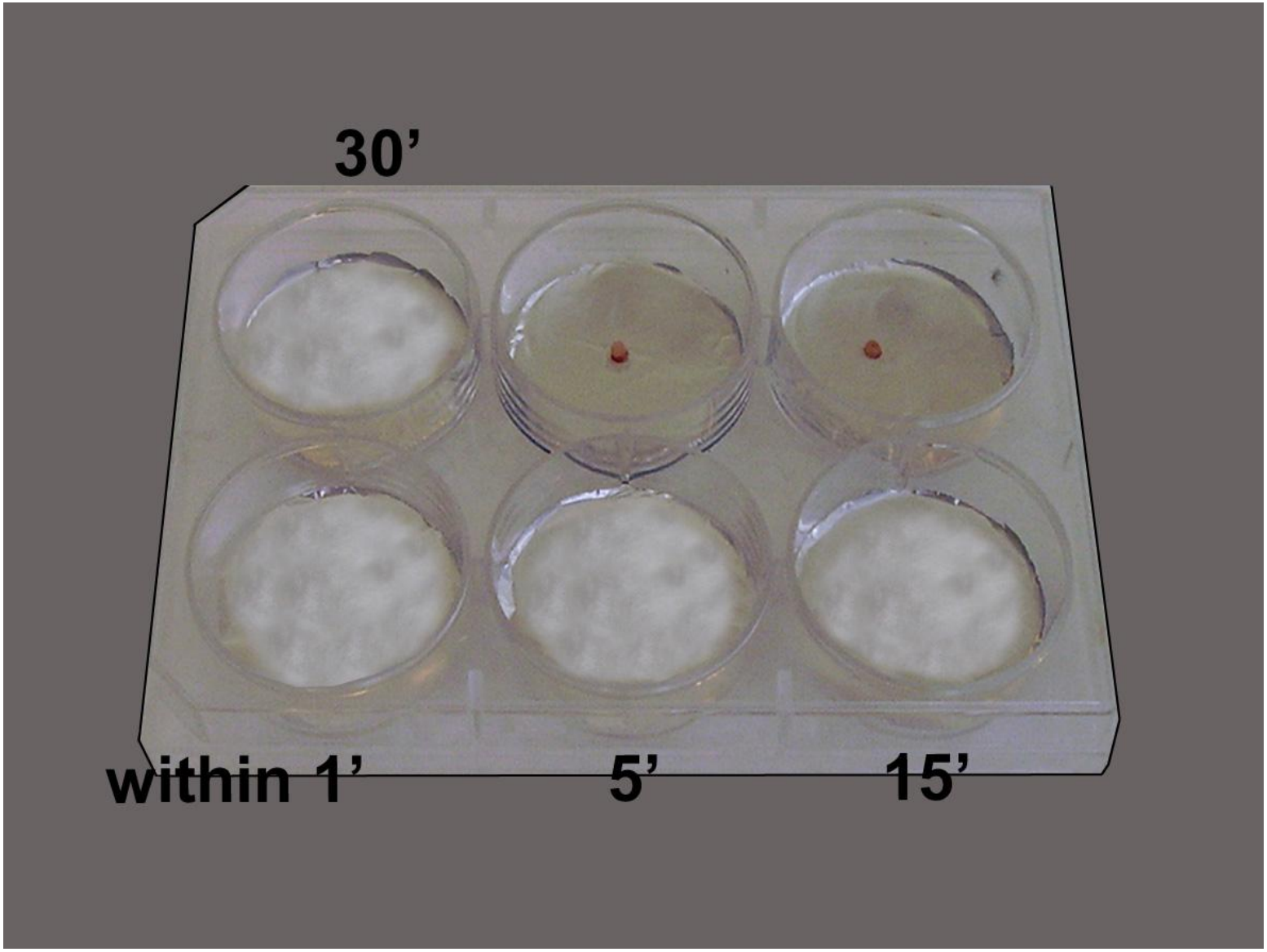
Step 3. The second sample has been covered with dry ice powder after 5’ after removal.



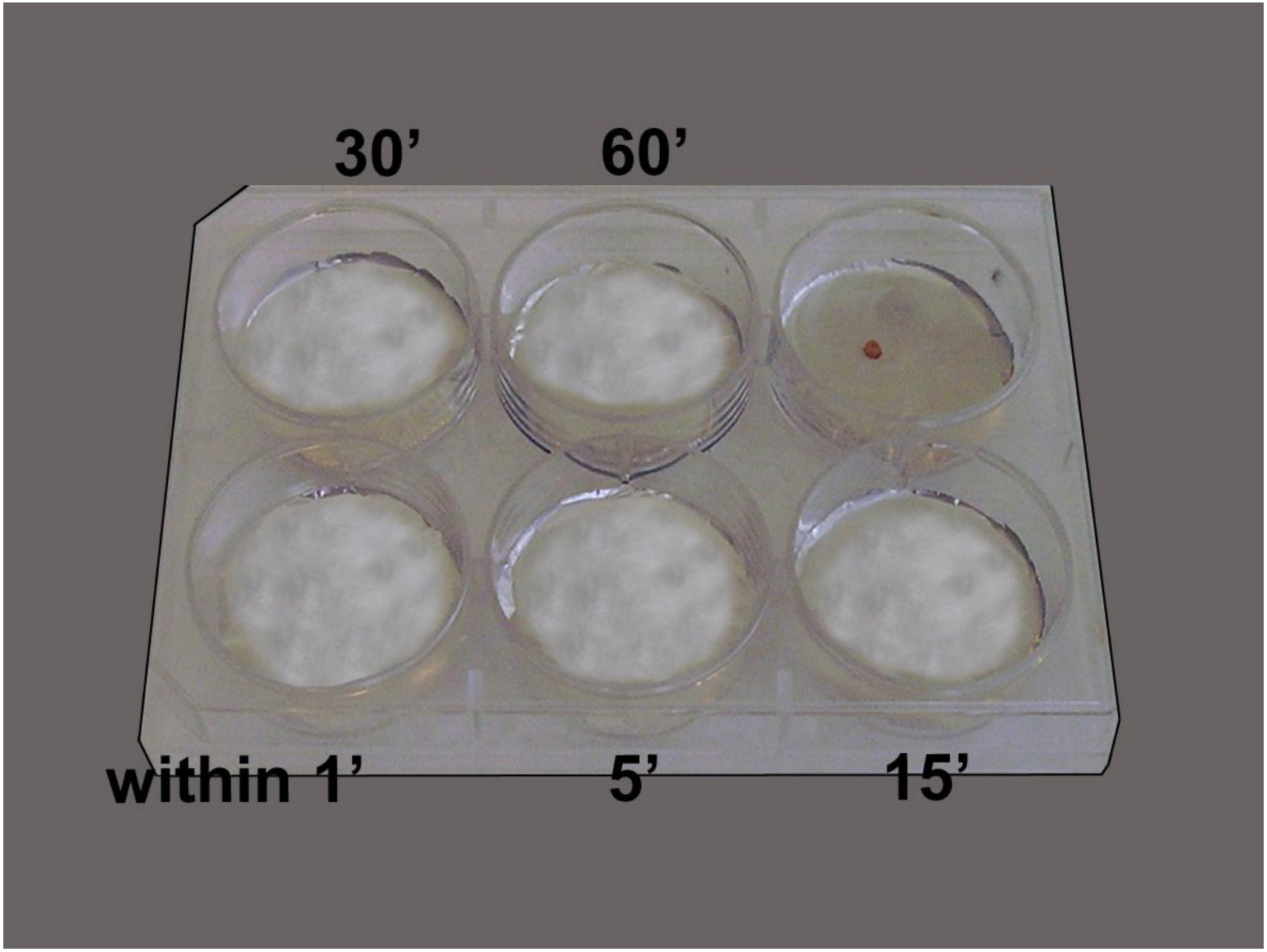
Step 4. The third sample has been covered with dry ice powder after 15’ after removal.



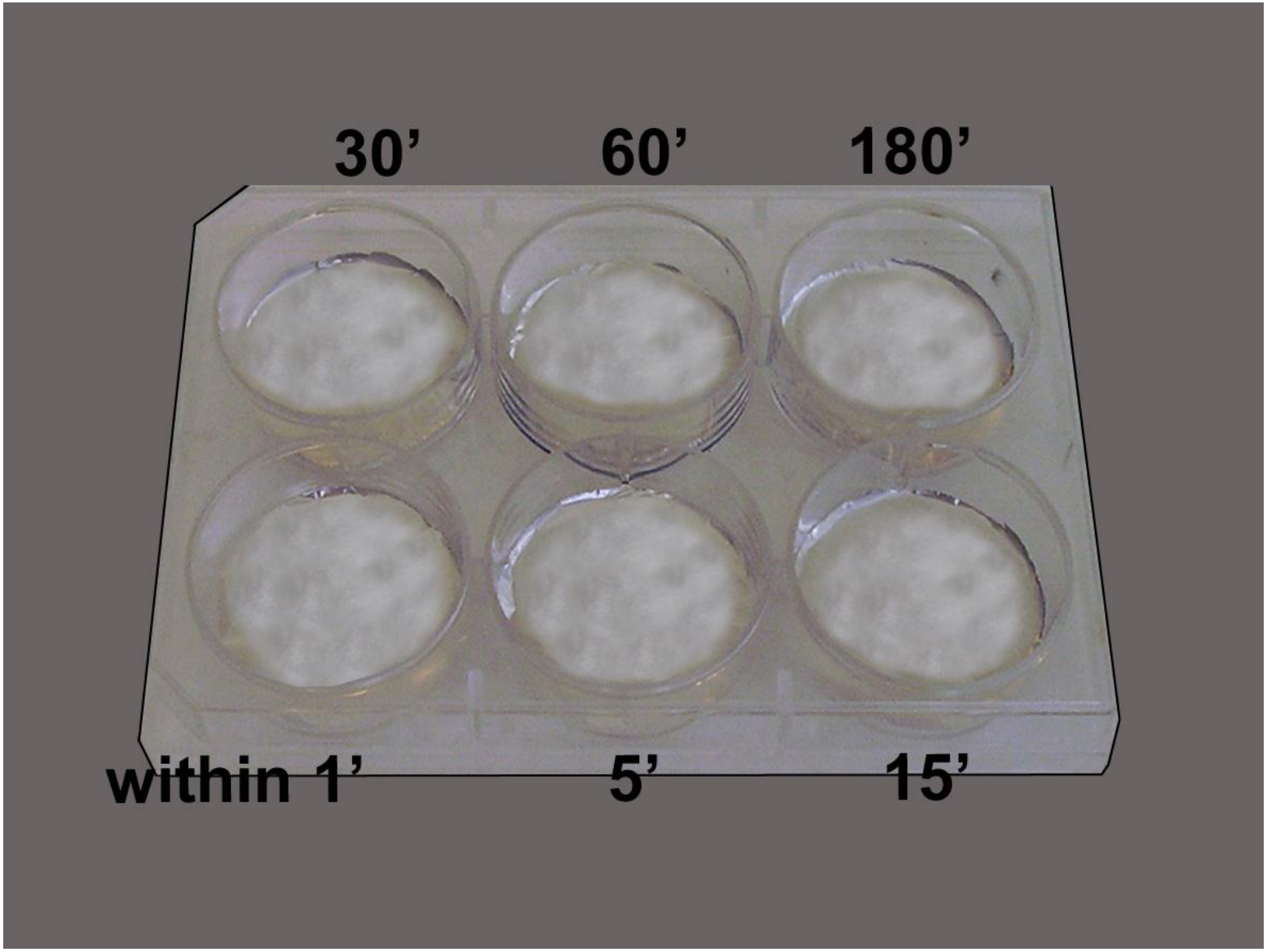
Step 5. The fourth sample has been covered with dry ice powder after 30’ after removal.



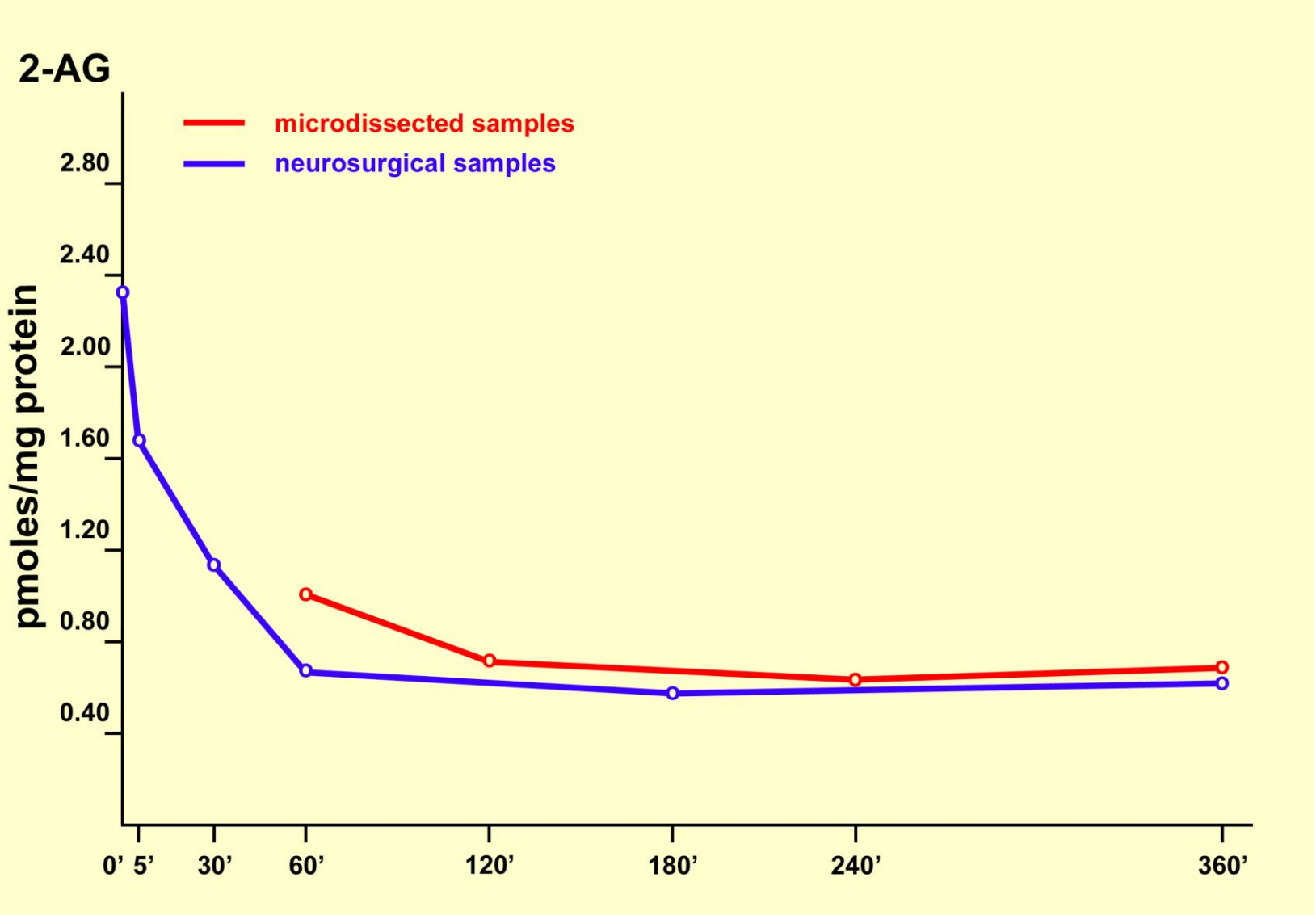
Step 6. The fifth sample has been covered with dry ice powder after 60’ after removal.



Step 7. The sixth sample has been covered with dry ice powder after 180’ after removal.

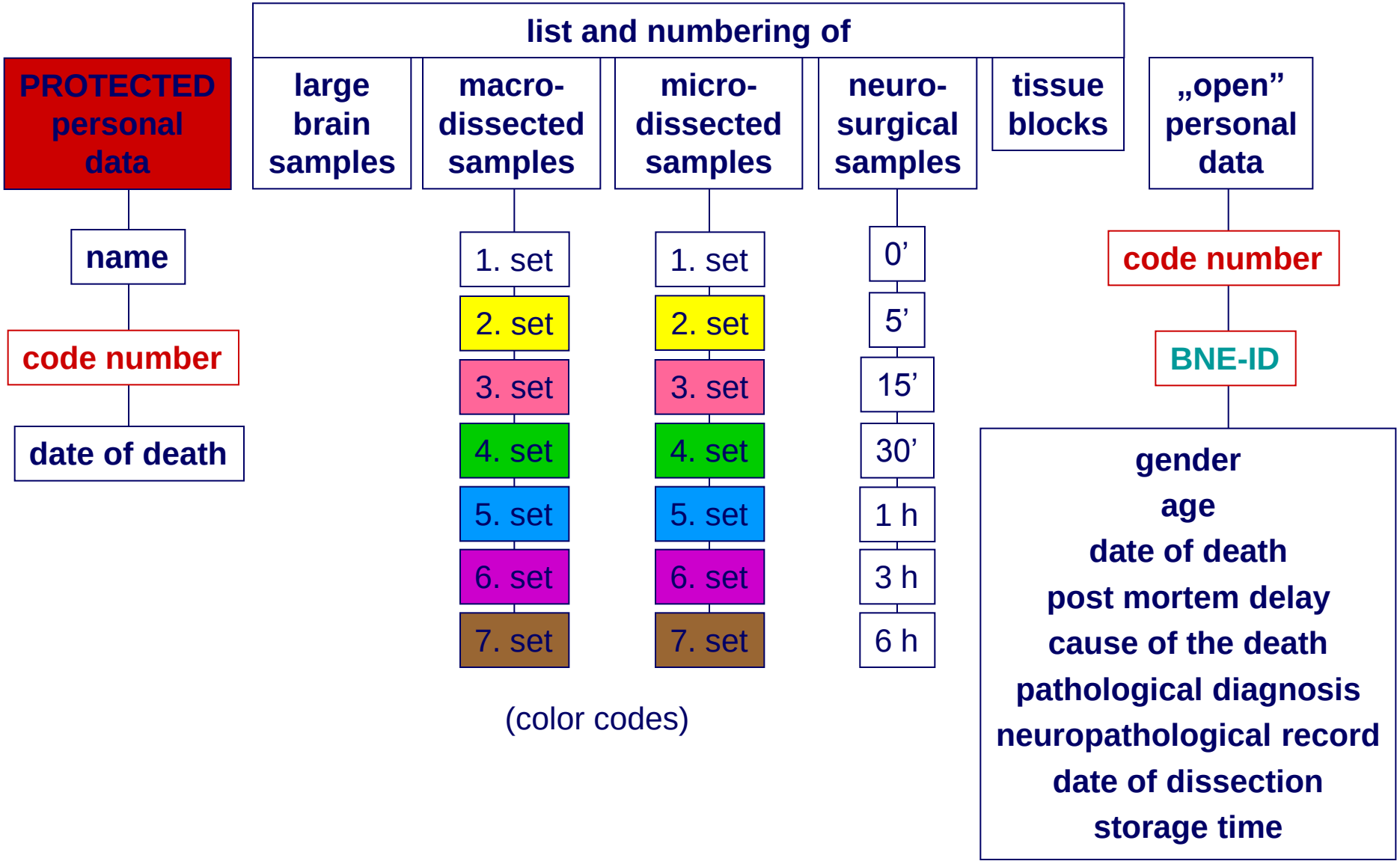


2-ARACHIDONOYL GLYCEROL

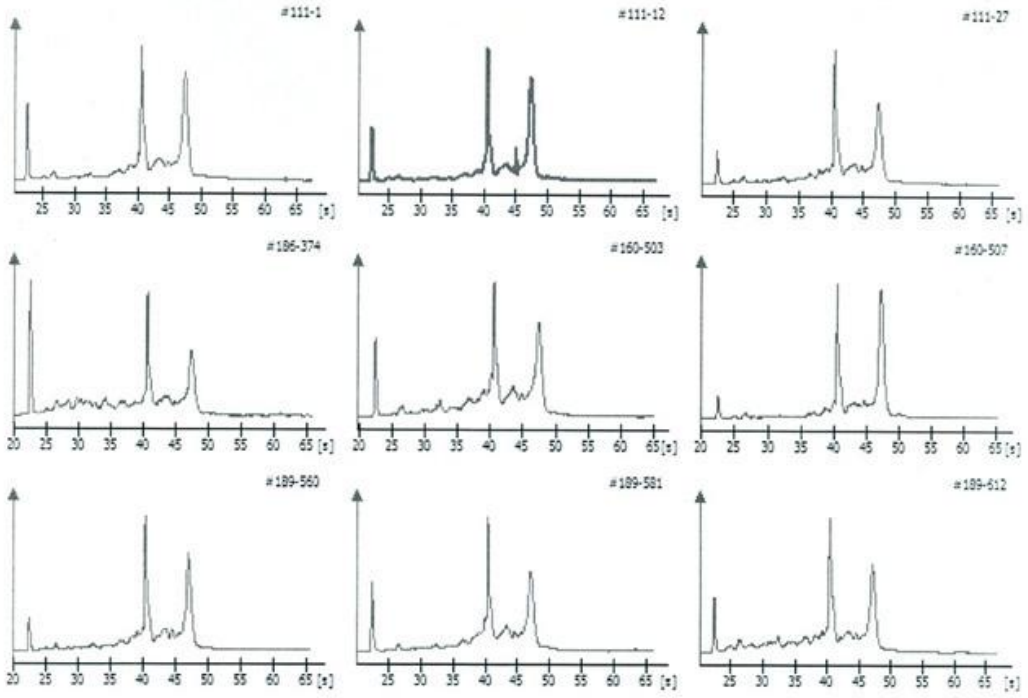


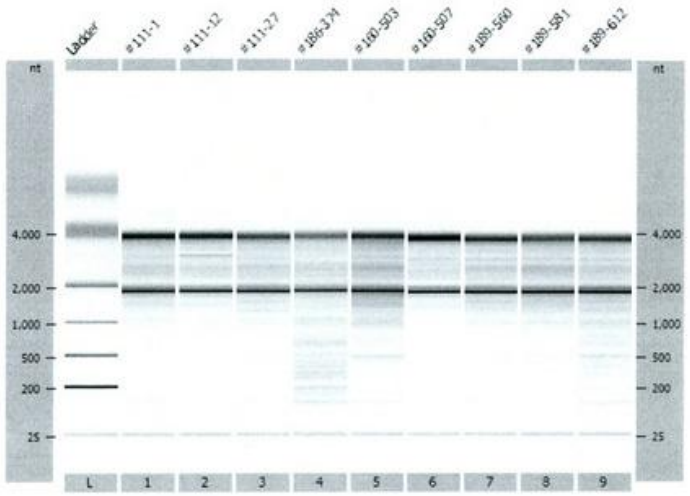
Database

The database contains several “compartments”. In order to fully respect personal rights, information about deceased persons is divided into *restricted* and *“open” compartments*. The restricted compartment contains the personal data of the subjects. The 7 parallel sets of the microdissected and macrodissected samples fill up two compartments.



RNA quality in different brain samples





RNA was extracted using the Qiagen lipid tissue kit and the quality was checked by the Bioanalyser.

Dept. of Cellular and Molecular Neuroscience
Imperial College, Charing Cross Campus
London W6 8RF

„The samples have good quality RNA."

The activity of the Human Brain Tissue Bank, Semmelweis University, Budapest has been authorized by the Committee of Science and Research Ethic of the Ministry of Health of Hungary (ETT TUKEB) and the Semmelweis University Regional Committee of Science and Research Ethic strictly for research studies. The tissue samples are made available to qualified researchers or research laboratories world-wide after signing a statement about scientific collaborations with the HBTB (Form No. 1.) and a declaration of the proper use and the fate of the microdissected human brain samples provided by the HBTB (Form No. 2.).

Form No. 1.

Human Brain Tissue Bank, Semmelweis University

STATEMENT

Date: .....

There is a scientific collaboration between the .... and the Human Brain Tissue Bank, Semmelweis University. We are requesting microdissected human brain samples for this study.

The dissected human brain samples are for medical/biological research examinations. They will be studied only in the course of research project(s) indicated below which have scientific aims. No samples will be provided any of these samples to anybody else, or any other research laboratories. The use and the fate of the brain samples will be reported to the Human Brain Tissue Bank, Semmelweis University after the final step of the research procedures.

Title of the project(s): .....

Approved by: .....

No. of approval: .....

Techniques will be applied: .....

Name of the senior investigator: .....

Postal address of the laboratory: .....

.....

Phone: .....

Fax: .....

e-mail: .....

.....
senior investigator

Form No. 2.

Human Brain Tissue Bank, Semmelweis University

ACKNOWLEDGEMENT

Date: .....

We received today ..... human brain samples from the Human Brain Tissue Bank, Semmelweis University. The dissected human samples are exclusively for medical research examinations. This material will be studied in the course of a research project. We will not provide any of these samples to anybody else.

Title of the project: .....

We will let know the Human Brain Tissue Bank, Semmelweis University about our scientific results obtained by studying of these samples, and about the fate of the samples.

Name of the senior investigator: .....

Name of the Institute: .....

Postal address: .....

Phone: .....

Fax: .....

e-mail: .....

.....
senior investigator

HBTB közlemények

	darab	*impakt faktor*	*idézettség*
1991-2015	**83**	**337.7**	**4955**

2014

Du L, Merali Z, Poulter MO, Palkovits M, Faludi G & Anisman H (2014) Catechol-O-methyltransferase Val158Met polymorphism and altered COMT gene expression in the prefrontal cortex of suicide brains, *Prog Neuropsychopharmacol Biol Psychiatry* 50: 178-183
IF: 4.025 (2013)

Fuxe K, Borroto-Escuela DO, Romero-Fernandez W, Palkovits M, Tarakanov AO, Ciruela F & Agnati LF (2014) Moonlighting proteins and protein-protein interactions as neurotherapeutic targets in the G protein-coupled receptor field, *Neuropsychopharmacology* 39: 131-155
IF: 7.833 (2013)

2015

Durrenberger PF, Fernando FS, Kashefi SN, Bonnert TP, Seilhean D, Nait-Oumesmar B, Schmitt A, Gebicke-Haerter PJ, Falkai P, Grünblatt E, Palkovits M, Arzberger T, Kretzschmar H, Dexter DT & Reynolds R (2015) Common mechanisms in neurodegeneration and neuroinflammation: a BrainNet Europe gene expression microarray study, *J Neural Transm* 2014 Aug 13. [Epub ahead of print]
IF: 2.871 (2013)

Ádori C, Glück L, Barde S, Yoshitake T, Kovacs GG, Mulder J, Maglóczky Z, Havas L, Bölcskei K, Mitsios N, Uhlén M, Szolcsányi J, Kehr J, Rönnbäck A, Schwartz T, Rehfeld JF, Harkany T, Palkovits M, Schulz S & Hökfelt T (2015) Critical role of somatostatin receptor 2 in the vulnerability of the central noradrenergic system: new aspects on Alzheimer's disease, *Acta Neuropathol* 129: 541-563
IF: 9.777 (2013)

Dobolyi A, Bagó AG, Gál A, Molnár MJ, Palkovits M, Adam-Vizi V & Chinopoulos C (2015) Localization of SUCLA2 and SUCLG2 subunits of succinyl CoA ligase within the cerebral cortex suggests the absence of matrix substrate-level phosphorylation in glial cells of the human brain, *J Bioenerg Biomembr* 47: 33-41
IF: 2.708 (2013)

Dobolyi A, Ostergaard E, Bagó AG, Dóczi T, Palkovits M, Gál A, Molnár MJ, Adam-Vizi V & Chinopoulos C (2015) Exclusive neuronal expression of SUCLA2 in the human brain, *Brain Struct Funct* 220: 135-151
IF: 4.567 (2013)

Neuropeptides

Luteinizing hormone-releasing hormone	Palkovits et al., Endocrinology, 1974
Thyrotropin-releasing hormone	Brownstein et al., Science, 1974
Somatostatin	Brownstein et al., Endocrinology, 1976
Growth hormone releasing factor	Arimura et al., Peptides, 1984
Corticotropin-releasing hormone (CRF)	Palkovits, Brownstein, Vale, Fed Proc, 1985
Vasopressin	Zerbe and Palkovits, Neuroendocrinology, 1984
Substance P	Brownstein et al., Brain Res, 1976
Cholecystokinin	Beinfeld and Palkovits, Brain Res, 1982
Enkephalins	Kobayashi et al., Life Sci, 1970 Zamir et al., Brain Res, 1985
Dynorphins	Zamir et al., Brain Res, 1983, 1984
Pro-opiomelanocortin derived peptides, ACTH, α-MSH, β-END	de Kloet, Palkovits, Mezey, Pharm Ther, 1981
Atrial natriuretic peptide (ANF)	Bahner et al., Hypertension, 1988
Tuberoinfundibular peptide of 39 residues	Dobolyi, Palkovits, Usdin, J Comp Neurol, 2003
Apelin / apelin receptors	Reaux et al., J Neurochem, 2001 / Neuroscience, 2002

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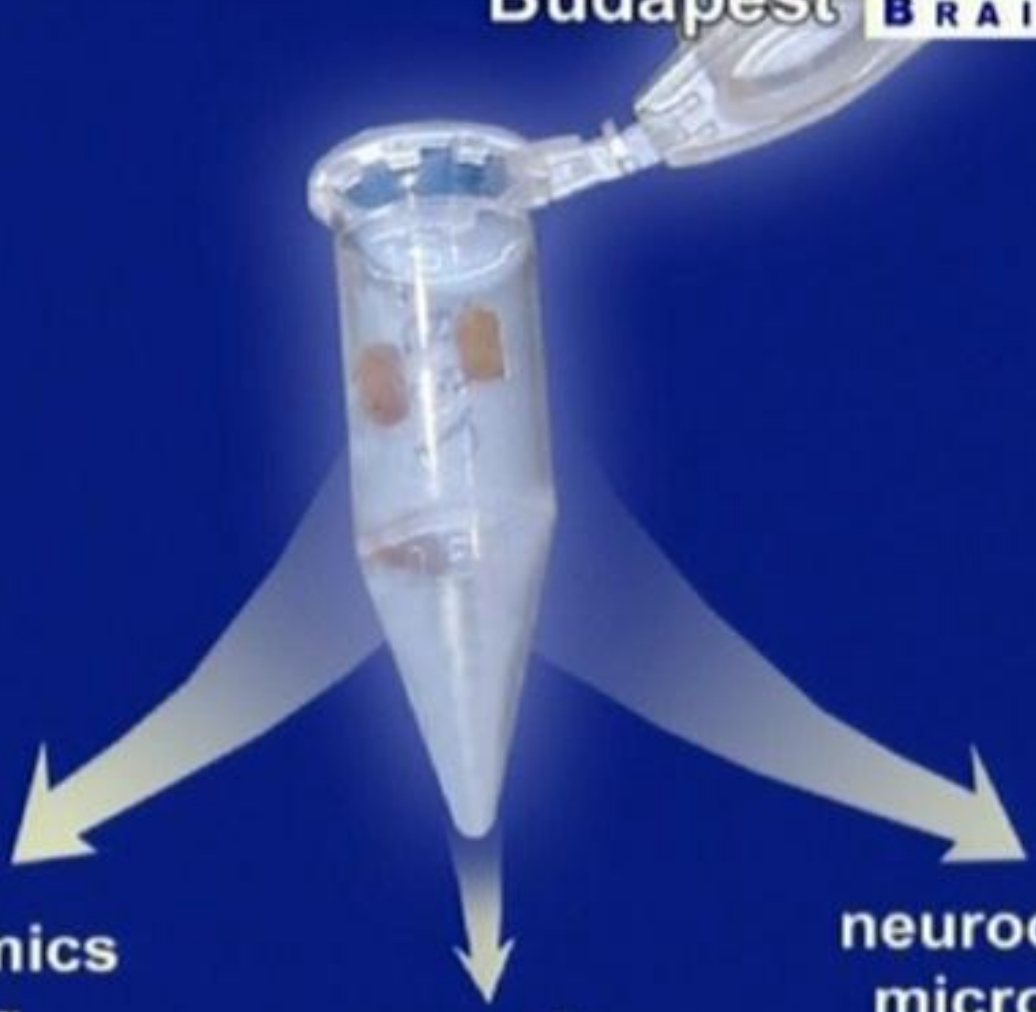

proteomics
2 mg

genomics
10 ug

neurochemical
microassays
50-500ug