Brain dissection protocol for amyotrophic lateral sclerosis/motor neurone disease

	Name	Signature	Date
Prepared by			
Approved by			
Approved by			
Revised by			

The SOPHIA (Sampling and biomarker OPtimization and Harmonization In ALS and other motor neuron diseases) project aims to provide a common European strategy for the prioritization and selection of candidate biomarker domains for optimization and harmonization.

(See http://www.neurodegenerationresearch.eu/initiatives/biomarker-transnational-call/results-of-funding-call/sophia/)

Part of this package is an initiative to harmonise autopsy practice for cases of ALS. Below is a recommended autopsy protocol, based on the BrainNet Europe Motor Neurone Disease protocol from 2008 (www.brainnet-europe.org/images/content/en/bilder/MND_Protocols.pdf). Draft versions were circulated to neuropathologists working in SOPHIA-allied centres and this guidance is the result of that consultation.

In appreciation of the fact that many neuropathologists are constrained by limitations in time, funding and storage space, the recommendations focus on 'minimum' and more detailed, 'optional' recommendations. It is hoped that the minimum recommendations will allow useful samples to be collected without placing an excessive burden on the individuals and departments involved. Adherence to more of the optional recommendations will allow a greater diversity of scientific studies to be performed. Furthermore, the aim is to generate materials that can be used by the broader research community and not to burden neuropathology centres with the requirement to generate that data themselves.

The protocol pertains only to nervous system tissues. If a full autopsy is performed, it may be useful to take samples of somatic organs.

It is requested that records of the autopsy and samples taken are made on the 'Progeny' database. This can be found at https://euromotor.umcutrecht.nl. A manual for this database will be separately circulated. For questions regarding the use of the Progeny database, please contact Hermieneke Vergunst by sending an email (h.vergunst@umcutrecht.nl) or calling +31 (0)88 7555887. It is likely that demographic and clinical details of the deceased will already have been recorded on Progeny.

Thanks to Olaf Ansorge, Michael Farrell, Dietmar Thal, Bernd Romeike and Paul Ince for their input.

J Robin Highley

Senior Clinical Lecturer

University of Sheffield

December 2013

Fresh brain dissection

Tissue should be removed and processed as quickly as possible after death. However, even with long post mortem intervals (up to several days) the tissue can still be used to gain useful scientific date, even for some mRNA studies.

If facilities for snap-freezing tissue are available, one cerebral hemisphere, one cerebellar hemisphere, one side of the midbrain and brainstem and select levels of spinal cord should be frozen (see below) or formalin-fixed. Otherwise, the whole brain and spinal cord should be fixed intact.

4% phosphate buffered formalin is the preferred fixation medium.

Cerebrospinal fluid may be extracted from the ventricles, centrifuged to remove red blood cells and frozen.

Snap-freezing protocol

Dissection

The brainstem should be detached from the brain by a high horizontal cut through the midbrain at a level superior to the third nerve, avoiding damage to mesial temporal and thalamic structures.

The cerebellum should be dissected away from the brainstem with incisions through the cerebellar peduncles. The cerebellum should then be divided with a sagittal cut a few millimetres lateral to the midline in order to preserve the vermis. The portion of the cerebellum with the vermis attached should be formalin-fixed. The portion of cerebellum without vermis should be sliced at right angles (approximately 1 cm thickness) to the cortical folia on the superior surface of the cerebellar hemisphere and snap-frozen.

The midbrain and brainstem should be hemisected. Half should be formalin-fixed by placing it cut-surface down in a container with formalin. The other half should be snap frozen.

The forebrain should be hemisected in the mid sagittal plane. The hemisphere to be fixed should either be suspended from the middle cerebral artery in formalin or placed medial surface-down in a container of formalin.

Ideally the hemisected cerebrum should be dissected in a manner that allows easy identification of the motor cortex, bearing in mind that future studies may be performed by scientists whose principal expertise may not be anatomical. This may be facilitated by marking the motor cortex of the precentral gyrus with India ink or tissue marking dye. An alternative is to take blocks of motor cortex directly from the intact brain.

Following this, the whole hemi cerebrum may be cut into serial coronal slices of approximately 1cm thickness and snap-frozen. Alternatively, blocks of fresh brain (as per the protocol for fixed brain dissection below) may be snap frozen. Sampling the brain in this manner reduces the necessary freezer storage space required and is also believed to avoid freezing artefacts and reduces the amount of post-freezing tissue handling.

For the spinal cord, the dural covering is opened along its length, and the anterior surface (identified by the prominent anterior spinal artery in the midline) placed uppermost. The T1 nerve rootlet is identified as it is the most inferior of the large rootlets that form the brachial plexus. Using this landmark other nerve root levels can be identified. Select levels should be formalin-fixed or snap frozen, ideally following the protocol below (from the BrainNet Europe guidelines):

Formalin fixation	Freezing
Upper cervical to C4	
	C5-C6
C6-C8	
	T1
T2-T4	
	T5
T6-T8	
	T9
T10-T12	
	L1-L4
L5 and remainder	

Other samples that may usefully be taken include:

- Dorsal root ganglion
- Femoral nerve at the level of the inguinal ligament
- Sural nerve
- Skeletal muscle
- Somatic organs

Freezing process

Samples of brain to be snap frozen should be either a) sandwiched between two metal plates that have been cooled to -70^{O}C with liquid nitrogen or b) laid on a metal plate that has been cooled with liquid nitrogen.

Dissection of fixed brain and spinal cord

The brain should be cut using relatively standard procedures: The brainstem (if still attached) should be removed by a high horizontal cut through the midbrain at a level superior to the third nerve, avoiding damage to mesial temporal and thalamic structures. The cerebellum should be dissected away from the brainstem with incisions through the cerebellar peduncles.

As with the fresh forebrain, the fixed hemisphere should be dissected in a manner to maximise the ease of identification of the precentral gyrus. This may be done by either painting the precentral gyrus or taking blocks of precentral gyrus before slicing the forebrain. Following this, the whole hemicerebrum may be cut into serial coronal slices of approximately 1cm thickness and snap-frozen.

Prolonged storage of wet tissue in formalin results in increased masking of protein epitopes and thus their suitability for immunohistochemistry. On this basis, it is advisable to process as much tissue as possible to paraffin, even if blocks thus processed are to be archived and not examined.

Blocks to be taken should include:

1. Cervical spinal cord*
2. Thoracic spinal cord
3. Lumbar spinal cord*
4. Medulla oblongata (at mid olive, 1-2mm superior to the obex of the fourth ventricle to include the nuclei of the XII cranial nerve, dorsal vagal nucleus and nucleus ambiguus)*
5. Superior precentral gyrus*
6. Mid precentral gyrus*

7. Inferior precentral gyrus
8. Frontal neocortex*
9. Temporal neocortex (superior and middle temporal gyri)*
10. Occipital neocortex
11. Hippocampus at the level of the lateral geniculate nucleus*
12. Basal ganglia at the level of the anterior commissure to include the nucleus basalis of Meynert
13. Amygdala
14. Midbrain at the level of the third nerve*
15. Pons including the locus ceruleus
16. Cerebellum including the dentate nucleus*
17. Thalamus at the level of the mammillary bodies

*=essential for full diagnosis and characterisation

As a minimum, the essential blocks should be stained with Haematoxylin and Eosin together with immunohistochemistry for ubiquitin or p62. The latter tends to give more clear and easily interpreted results.

Further immunohistochemistry may include TDP-43 and FUS. CD68 may also be used to elucidate pyramidal tract degeneration. Glial pathology may be assessed with a combination of tinctorial as well as CD68 and GFAP immunohistochemistry.

Ideally, other major neuropathological processes should be assessed. This would include immunohistochemistry for β-amyloid, phosphorylated tau and α-synuclein and comment on significant vascular pathology.