Changes in spinal connections by descending pathways with ageing

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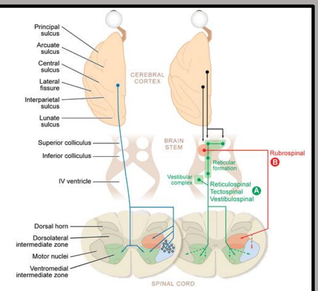
1) Introduction & Aim

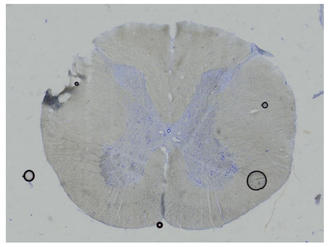
- Both corticospinal and reticulospinal tracts are involved in movement control. (1)
- Corticospinal tract axons are lost during ageing.
- The aim of this project is to investigate how reticulospinal and corticospinal termination patterns change during healthy ageing and sarcopenia.



Different descending pathways of the nervous system.

2) Methodology

- Two old macaque monkeys were injected with adeno-associated virus (AAV) at different sites of their brains. The viruses contain different coloured fluorescent proteins.
- The spinal cord tissues of the monkeys were processed using various histological protocols to enhance terminal visualisation.
- Reticulospinal and corticospinal terminals were counted using Stereoinvestigator software.



Spinal cord tissue stained with cresyl violet for visualisation of its anatomy.

Monkey 1: Karaba

Injection sites on the brain	Type of fluorescent protein
Reticular formation	mCerulean [blue colour]
Old primary motor cortex	GFP [green colour]
New primary motor cortex	mCherry [red colour]

Monkey 2: Lantarese

Injection sites on the brain	Type of fluorescent protein
New primary motor cortex	mCerulean [blue colour]
Old primary motor cortex	GFP [green colour]
Reticular formation	mCherry [red colour]

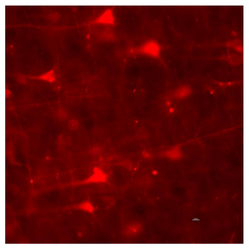
Tables 1 & 2: Types of fluorescent proteins used at different injection sites.

3) Results & Discussion

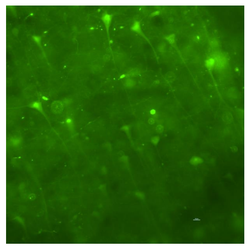
Extra Notes: The grey matter of the spinal cord has been demarcated by yellow lines in some of the images in this section.

a) Intrinsic Fluorescence

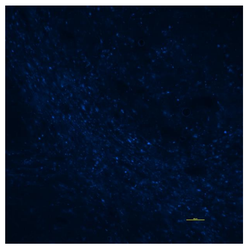
- The intrinsic fluorescence of the viruses was relatively clear at the sites of injection. *(Images below are from Monkey 1: Karaba)*



New Primary Motor Cortex



Old Primary Motor Cortex



Reticular Formation

- However, the intrinsic fluorescence of the viruses at different levels of the spinal cord was unclear and disorganized, with a lot of background fluorescence.

b) Immunohistochemistry (IHC) Staining

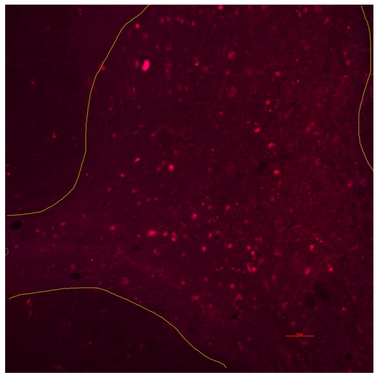
- IHC staining was then carried out to amplify the intrinsic fluorescence of the viruses at different levels of the spinal cord. ***(IHC staining is the use of antibody-based method to detect a specific protein in a sample)***
- Unfortunately, some problems were encountered during IHC staining:
  - There was no primary antibody that was specific to mCerulean.
  - GFP and mCerulean only differ from each other by a few amino acids, so the primary antibody for GFP would also be able to bind to mCerulean as they have almost similar structures, thus causing the results of IHC staining for GFP to be non-specific.

Fluorescent protein	Primary antibody	Secondary antibody
mCherry	1:500 mouse anti-mCherry	1:500 donkey anti-mouse
GFP	1:1000 rabbit anti-GFP	1:500 donkey anti-rabbit
mCerulean	1:1000 rabbit anti-GFP	1:500 donkey anti-rabbit

Table 3: IHC staining for the different types of fluorescent proteins.

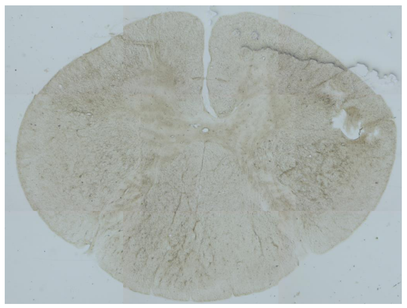
c) Synaptophysin Immunostaining

- Synaptophysin antibody is used to visualize presynaptic vesicles to aid with terminal localization but this needs further optimization.



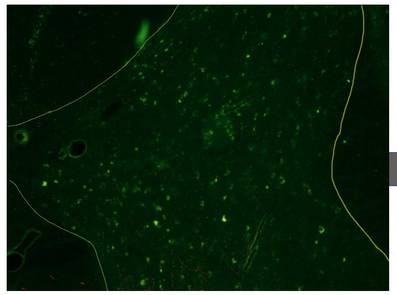
d) DAB Staining

- Chromogenic IHC using DAB is performed on mCherry to remove background fluorescence but this did not work out.

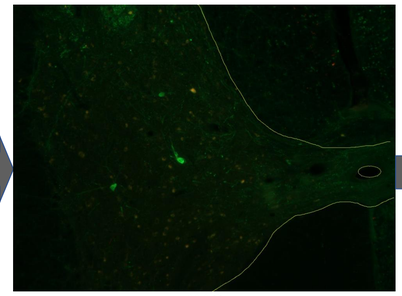


e) IHC Staining + Sudan Black Staining

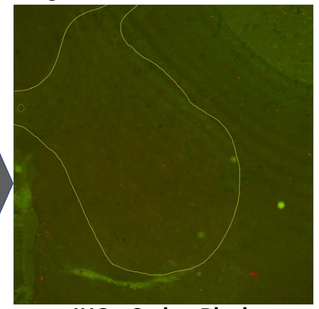
- For this new approach to tissue processing, IHC staining (shown in Table 3) was first carried out and then followed by Sudan Black staining. The purpose of Sudan Black staining was to reduce the background fluorescence.



Intrinsic Fluorescence

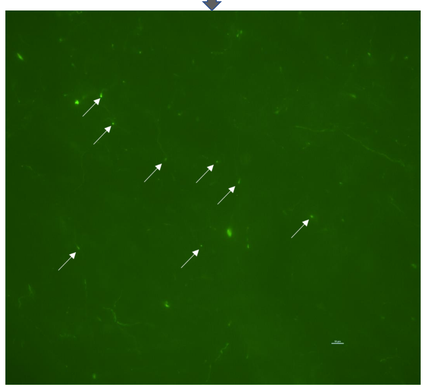


IHC

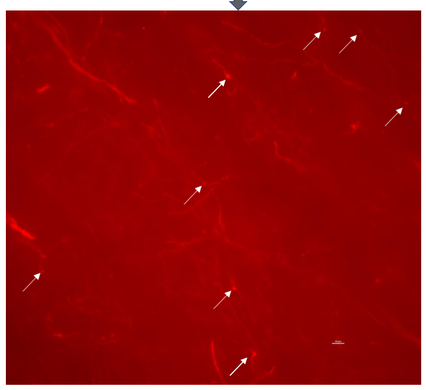


IHC + Sudan Black

- It can be observed that the background fluorescence has been greatly reduced after Sudan Black staining, while leaving behind the intrinsic fluorescence of the viruses, thus enabling the discovery of terminal-like structures.



Corticospinal Terminals



Reticulospinal Terminals

- Structures resembling corticospinal and reticulospinal terminals have been pointed out with white arrows in the two images above this bullet point. Attempts to count and map out the terminals have been carried out but the tissue counting protocol has not been optimized yet.

4) Conclusion

Thus far, the optimal protocol for the processing of the monkeys' spinal cord tissues is IHC staining followed by Sudan Black staining.

5) Future Work

- Further optimize the processing of the monkeys' spinal cord tissues to achieve better visualisation of the viruses.
- Start counting and mapping out the nerve terminals for both reticulospinal and corticospinal tracts when the tissue counting protocol has been fully optimized.

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References:

1. Baker SN (2011) The primate reticulospinal tract, hand function and functional recovery. J Physiol 589:5603-5612.