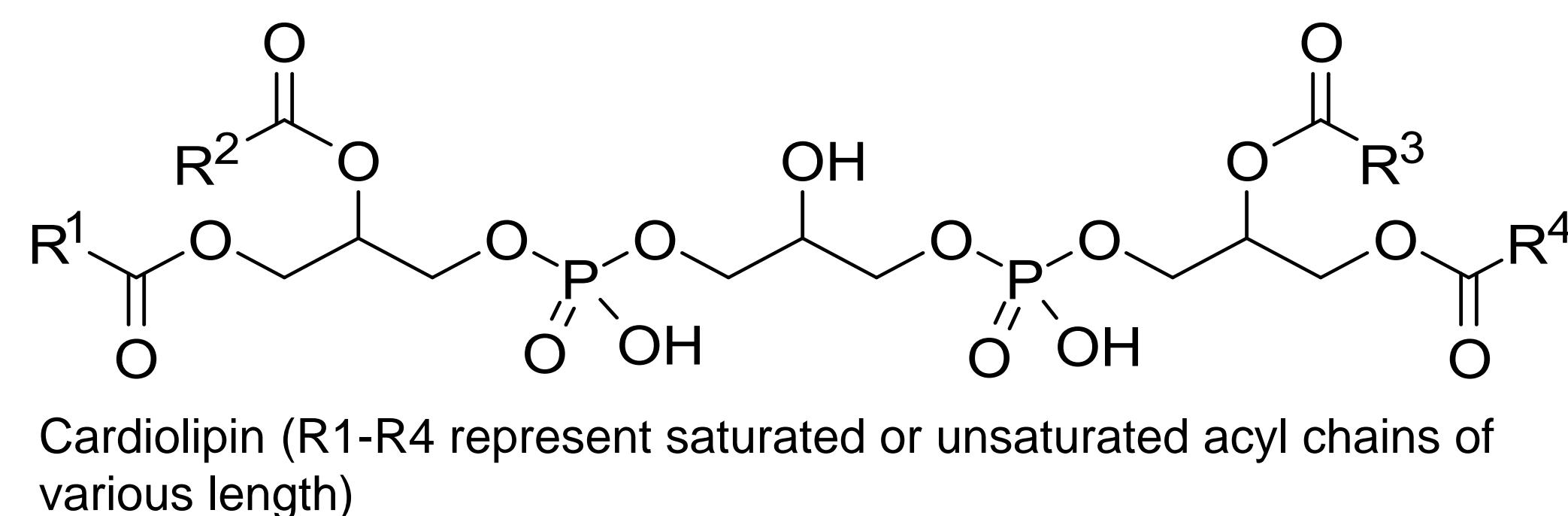


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## Introduction

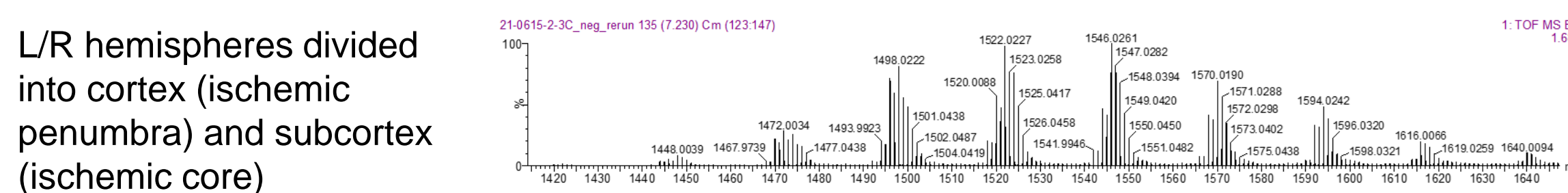
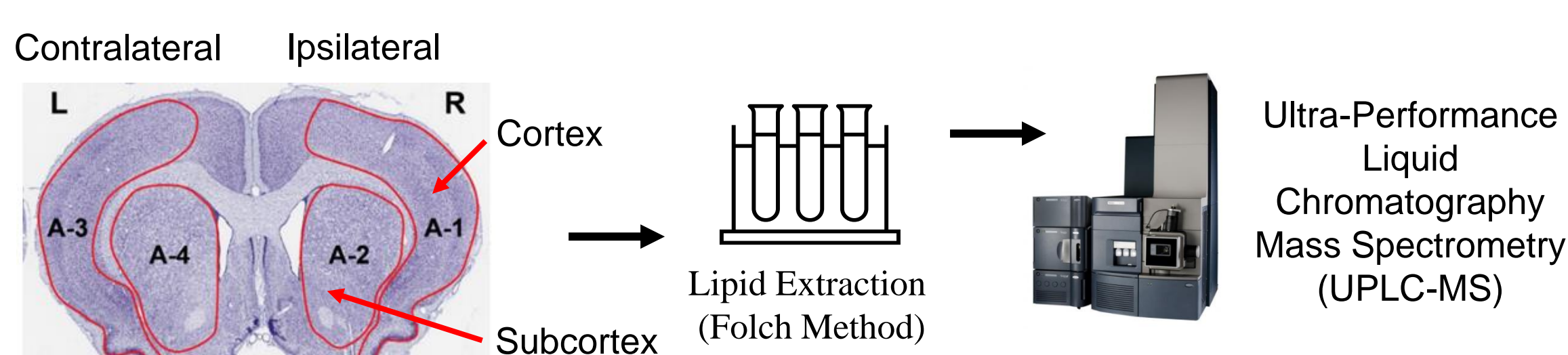
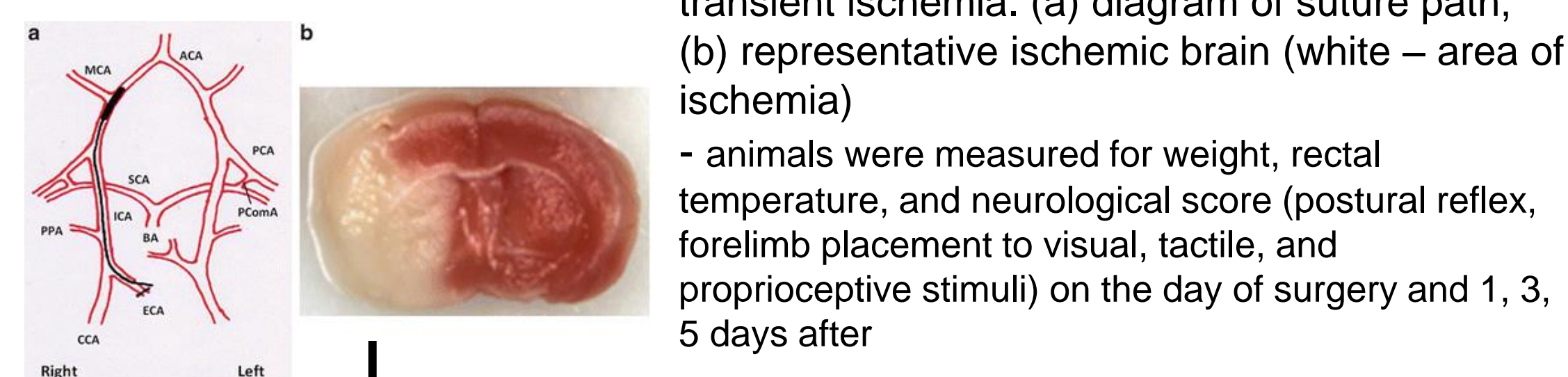
- Stroke accounts for 1 in 6 cardiovascular deaths in the United States
- Ischemia-reperfusion injury is associated with oxidative damage to the mitochondria
- Cardiolipin (CL) represent a group of tetra-acylated phospholipids, located in the inner mitochondrial membrane, functioning as a stabilizer for the respiratory chain
- Following mitochondrial damage, CL translocates to the outer mitochondrial membrane and is involved in signaling cellular mediators of inflammation, autophagy, and apoptosis
- Aim:** Investigate whether ischemic stroke altered the CL lipidome in the ischemic core and ischemic penumbra regions of the brain using mass spectrometry



## Methods

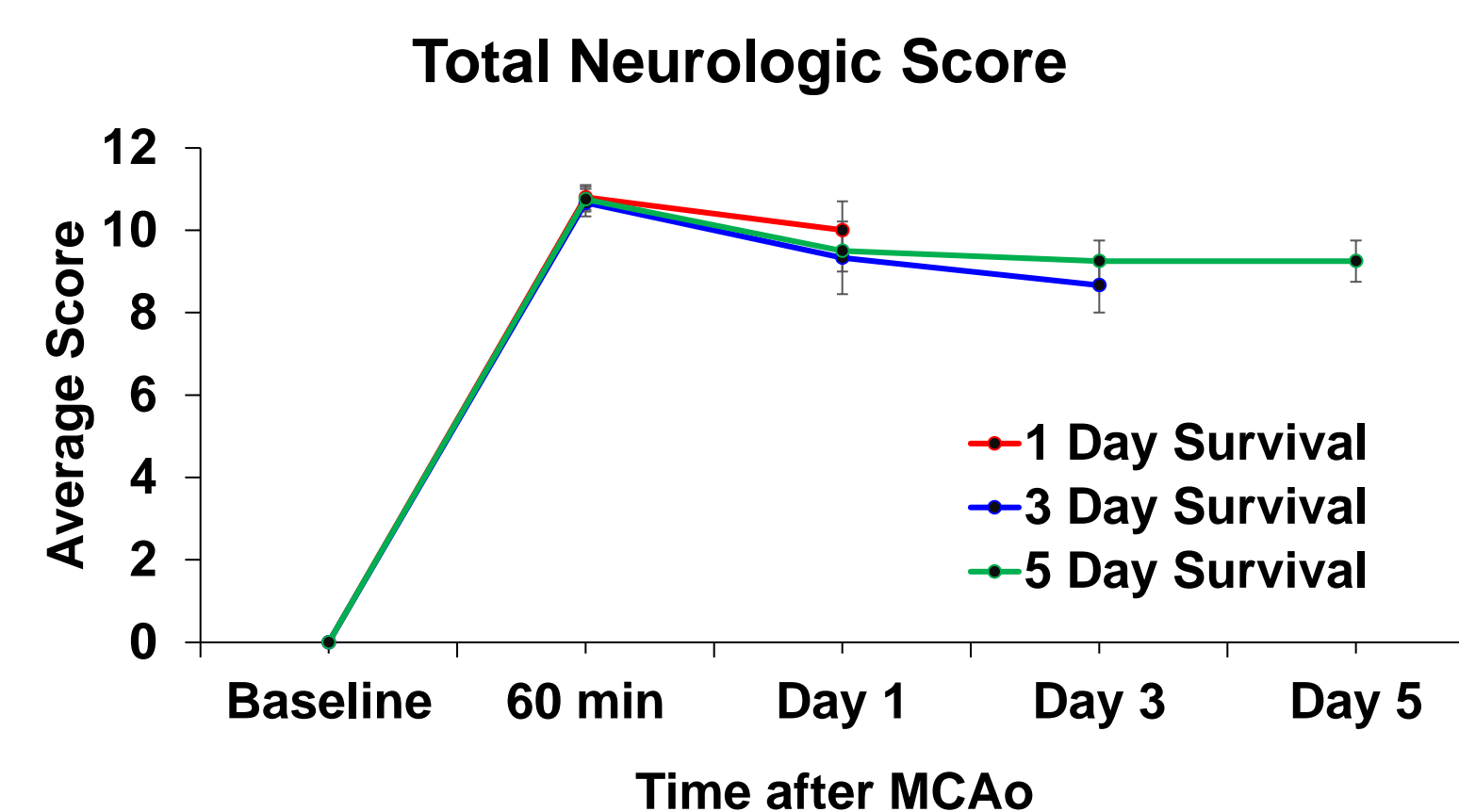
**Animal model:** adult male Sprague-Dawley Rats (280 – 300 g)  
**Group A:** 1 day survival (n=4)  
**Group B:** 3 day survival (n=3)  
**Group C:** 5 day survival (n=4)

**Middle Cerebral Artery occlusion (MCAo)** using the intraluminal suture method – 2 hours transient ischemia. (a) diagram of suture path, (b) representative ischemic brain (white – area of ischemia)  
- animals were measured for weight, rectal temperature, and neurological score (postural reflex, forelimb placement to visual, tactile, and proprioceptive stimuli) on the day of surgery and 1, 3, 5 days after



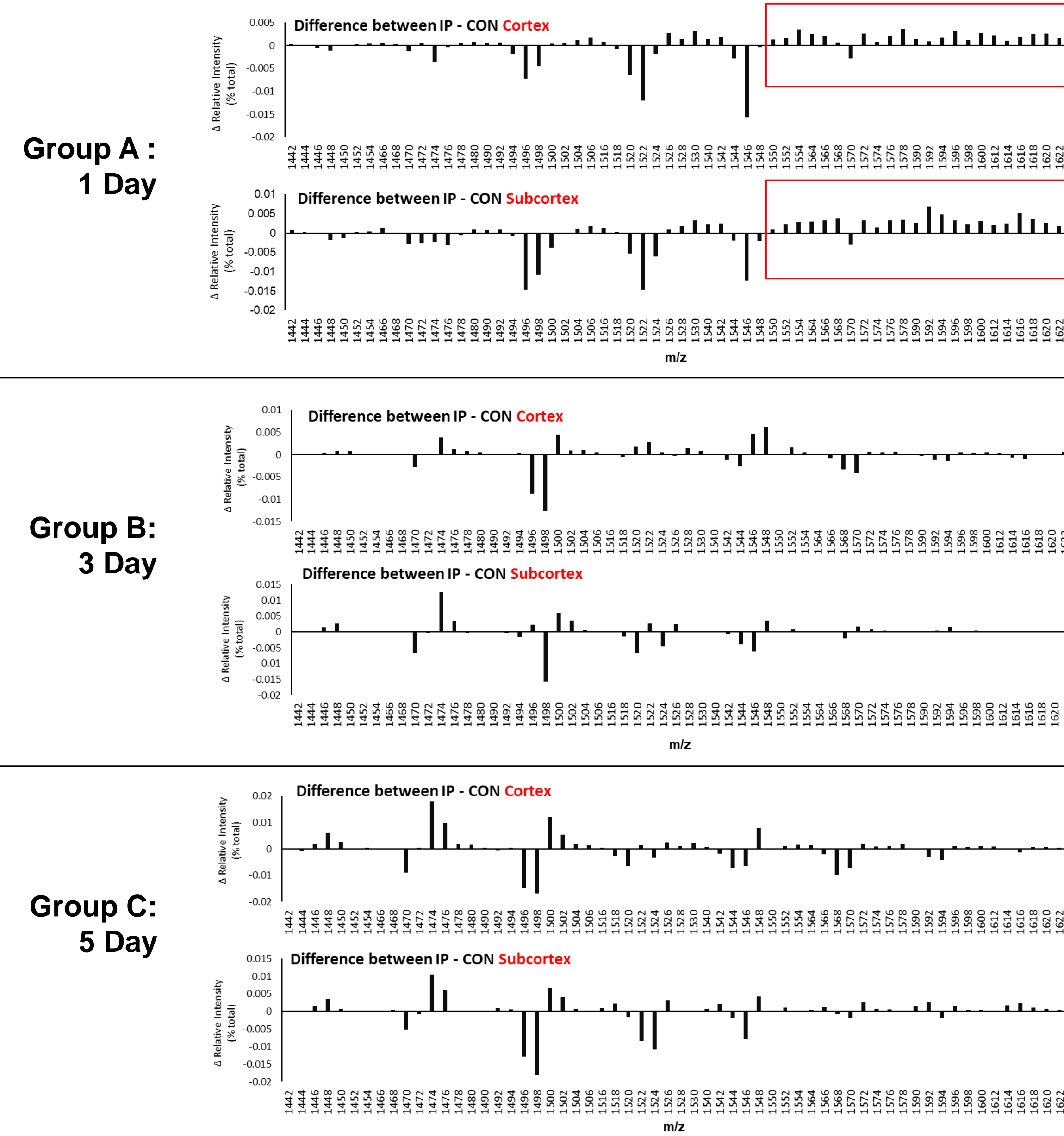
L/R hemispheres divided into cortex (ischemic penumbra) and subcortex (ischemic core)

## Results – Neurological Score



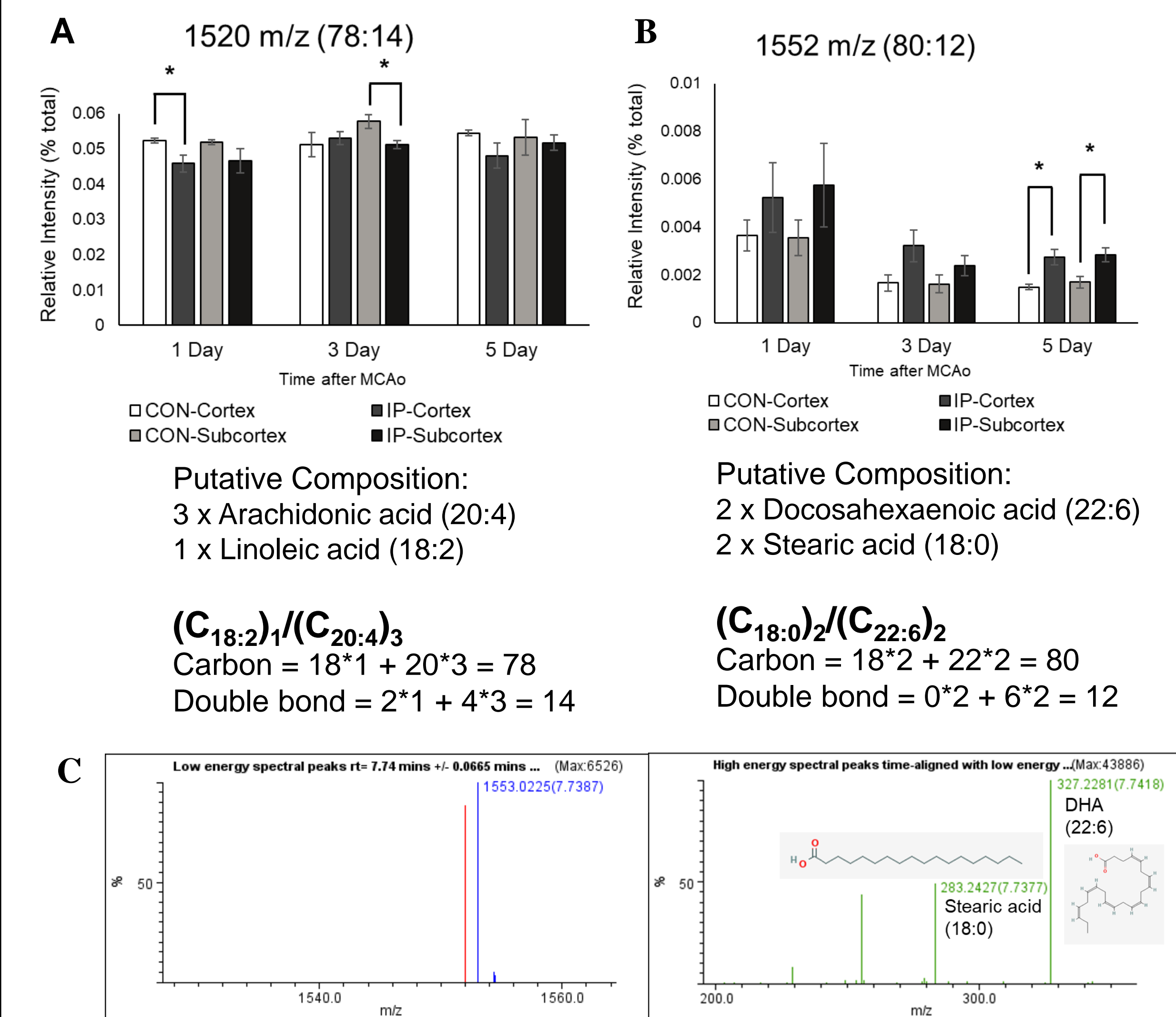
**Figure 1. MCAo produced consistent neurological deficits.** Total neurologic score represents the summation of postural reflex, visual forward stimuli, visual sideways stimuli, tactile dorsal stimuli, tactile lateral stimuli, and proprioceptive score on the affected side. Total score range from 0 (no deficit) – 12 (maximal deficit). Physiological parameters showed no significant differences between study groups.

## Results - MS Analysis



**Figure 2. CL species shift towards higher carbon clusters 1 day after MCAo.** CL species with mass/charge ratio (M/Z) between 1442 – 1622 were selected for further analysis. CL in the cortex and subcortex ipsilateral (IP) to MCAo was compared to the cortex and subcortex from the contralateral (CON) hemisphere from groups A, B, and C. The relative difference between ipsilateral to contralateral was plotted to visualize shifts in CL distribution.

## Results – MS Analysis



**Figure 3. Specific changes in CL species.** (A) 1520 m/z (78:14, # acyl chain carbons/# double bonds) was significantly decreased in the ipsilateral cortex (1 day post MCAo) and subcortex (3 days post MCAo). The putative composition of arachidonic acid and linoleic acid was proposed based on literature reports of similar mass. (B) 1552 m/z (80:12) was significantly increased in the ipsilateral cortex and subcortex (5 days post MCAo). The putative composition proposed of docosahexaenoic acid (DHA) and stearic acid was supported by MS/MS daughter ion findings. (C) Left – low energy parent ion (1552 m/z with its 1553 m/z carbon-13 isotope). Right – high energy daughter ion (327.2 m/z, DHA; 283.2 m/z, stearic acid).

## Conclusions

- Ischemic injury produces subtle shifts in CL profile after 1, 3, or 5 days of MCAo with a noticeable shift towards higher carbon clusters 1 day post injury.
- CL species 1520 m/z (78:14), 1552 (80:12) are significantly decreased and increased respectively after MCAo. Species 78:14 contains arachidonic acid while 80:12 contains DHA in the side chains. Arachidonic acid and DHA are associated with respective pro-inflammatory and anti-inflammatory properties.
- Changes in acyl group composition suggest structural changes in the mitochondria and different acyl groups may participate in pro/anti-inflammatory signaling thus the CL lipidome may be malleable to exogenous lipid mediators aimed to rescue mitochondrial dysfunction.
- Future work will continue with MS/MS identification of CL species and CL modifications such as oxidation and cleavage