The Role of Oxidative Stress in Inhibitory Interneurons for Cortical Seizure Generation

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Introduction

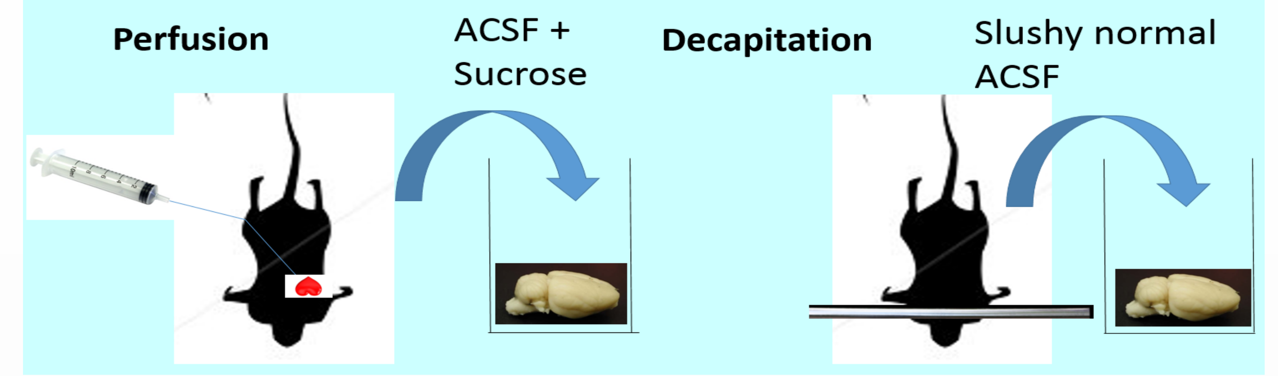
In vitro (in glass) brain slice preparation techniques are widely used for investigations in brain function. Once the slices have been prepared small electrodes can be inserted to record electrical activity. After this immunohistochemistry can be performed to observe protein expression and localisation.

Aims:

1. To distinguish which *in vitro* rat brain slice preparation technique, either perfusion or decapitation, is a suitable model for epilepsy.
2. To distinguish whether the incubation period of slices affects the cell function and viability.

Methods:

Immunohistochemistry on 40μm hippocampal slices that had been prepared by one of the following techniques:

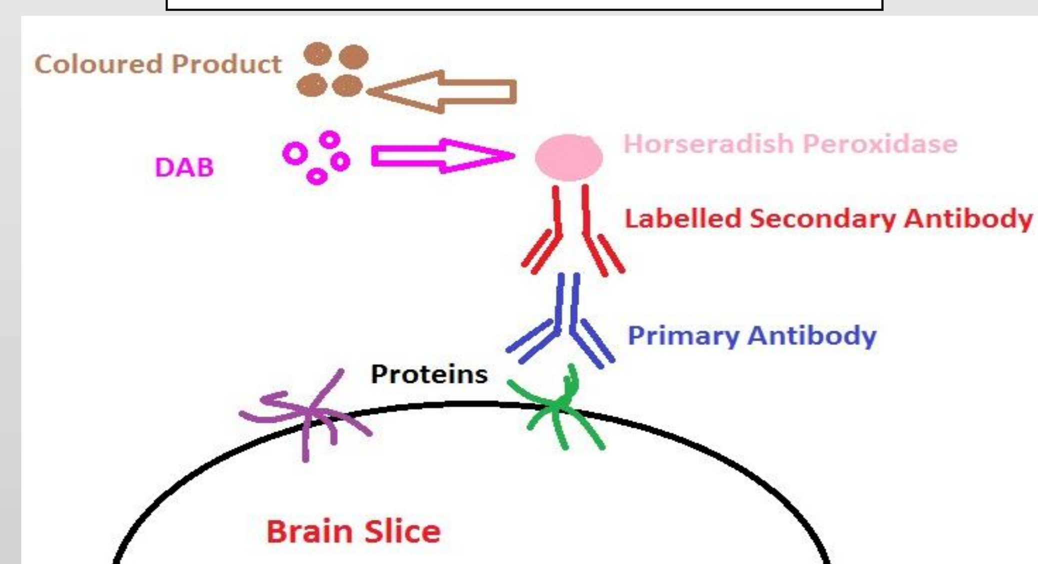


(ACSF = Artificial cerebrospinal fluid, the fluid surrounding the spinal cord)

Primary Antibodies used to Visualise Cellular Markers	Primary Antibodies used to Visualise Inhibitory Interneurons
IBA-1 – Immune cell of the brain	**Pavalbumin (PV)**
GFAP- Neurons metabolic support	**Somatostatin (SOM)**
HIF-α- Oxygen deprivation	**Vasoactive intestinal peptide (VIP)**
Lectin – Scaffold of a neuron	**Calbindin (CB)**
Caspase-3- Cell death	**Calretinin (CR)**
GABA – Inhibitory neurotransmitter	**Cholecystokinin(CCK)**
8- oxo dg – Oxidative stress (DNA damage)	
Cam Kinase II – Excitable neurons in the brain	

Cell density was calculated by counting stained cells in layers 1-3 and 4-6 of the entorhinal cortex (where seizures typically originate from).

Indirect Immunohistochemistry



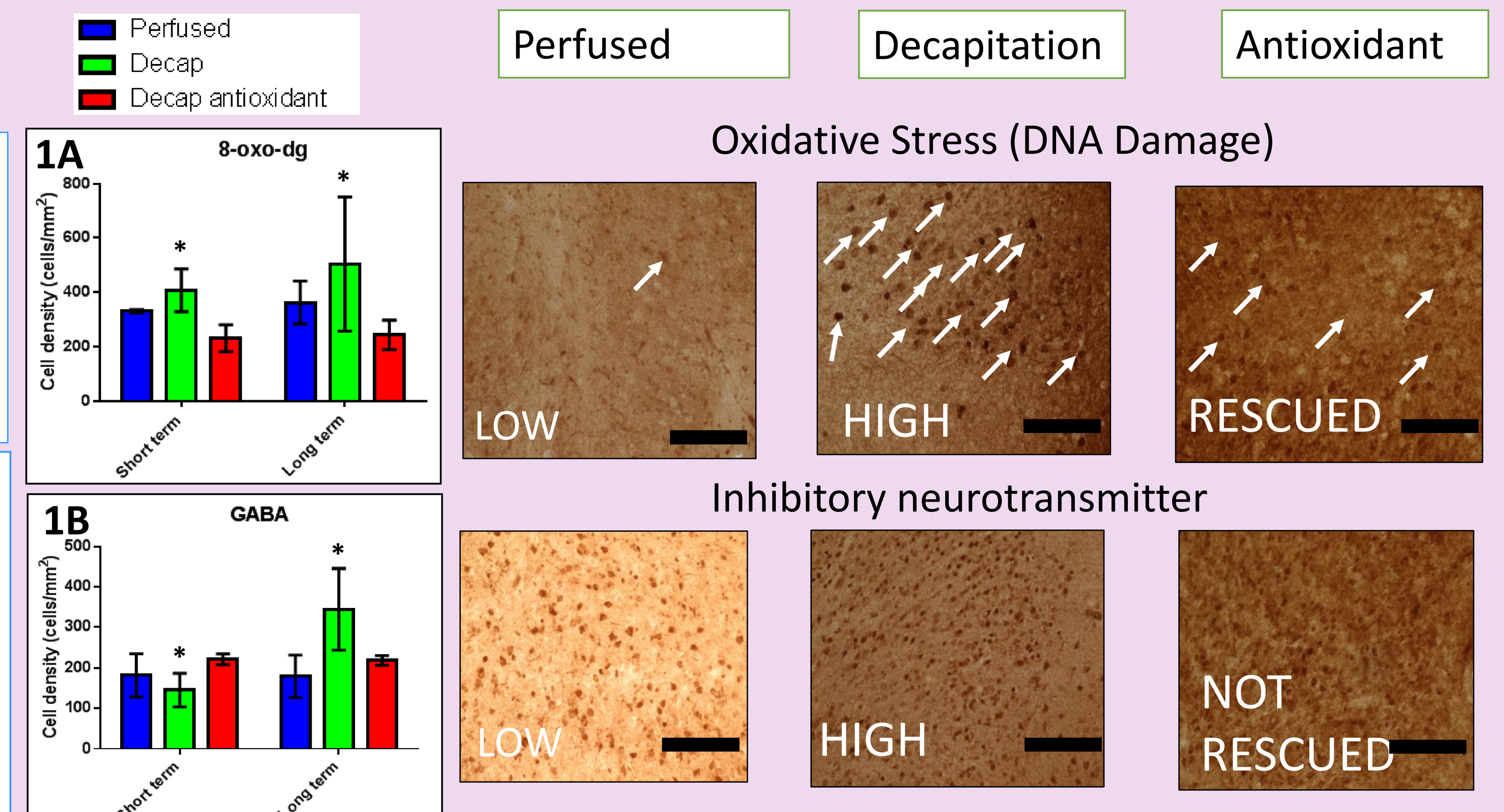


Figure 1. Less damage was observed from slices obtained from perfused than decapitation technique. A: Less oxidative damage with perfused than decapitation and could be reduced (rescued) by the addition of antioxidants to the ACSF. B: increase in inhibitory neurotransmitter in the decapitation compared to perfusion and could not be rescued with the addition of antioxidants. (This was the opposite of expected due to contamination in the antibodies in B) * indicates result was significantly different from perfused.

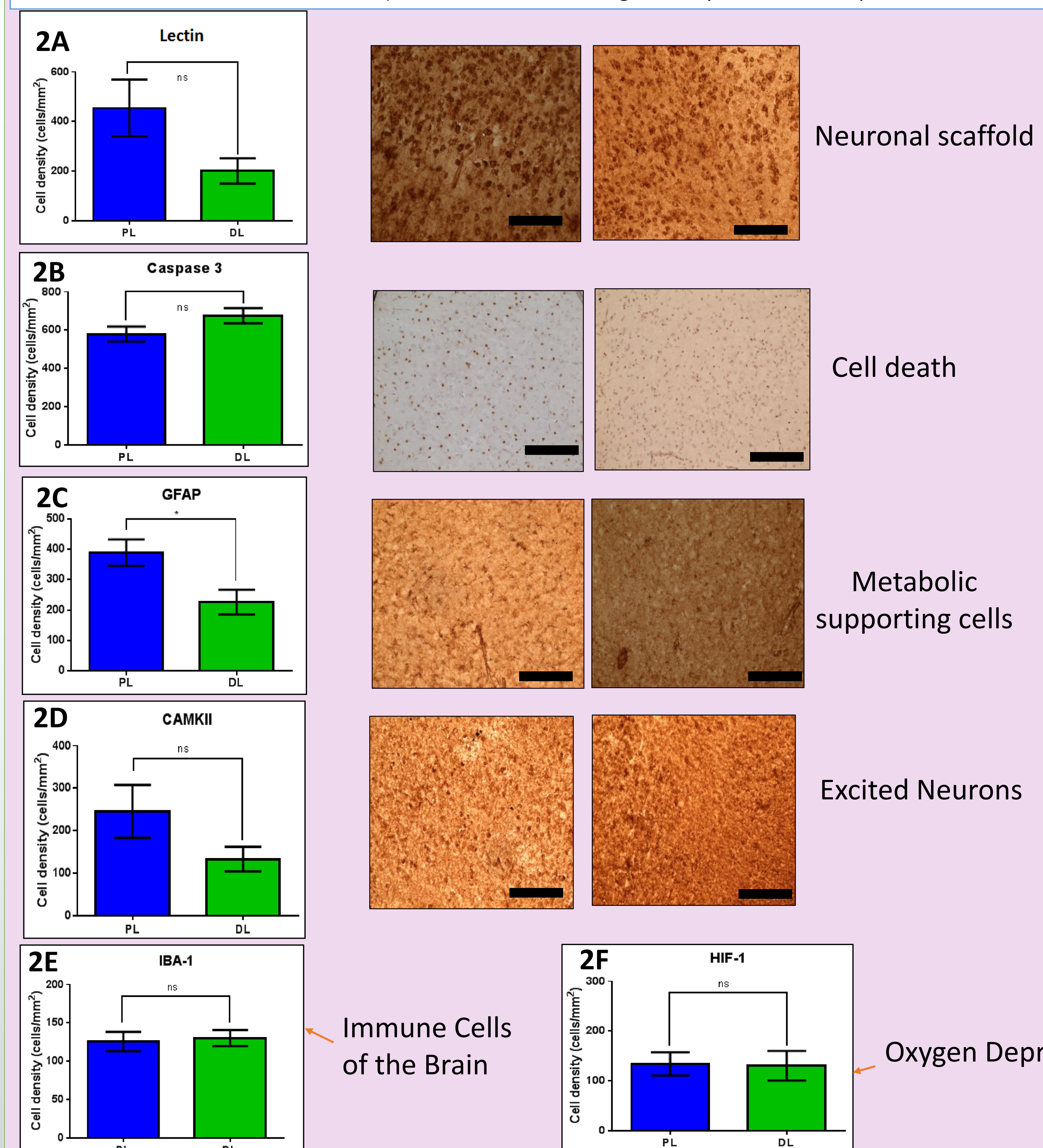


Figure 2: Results A to F were not significant. A and F show no change between the two techniques. B, C, D and E did see some slight changes but would require more repeats to make them significant.

scale bar = 10μm

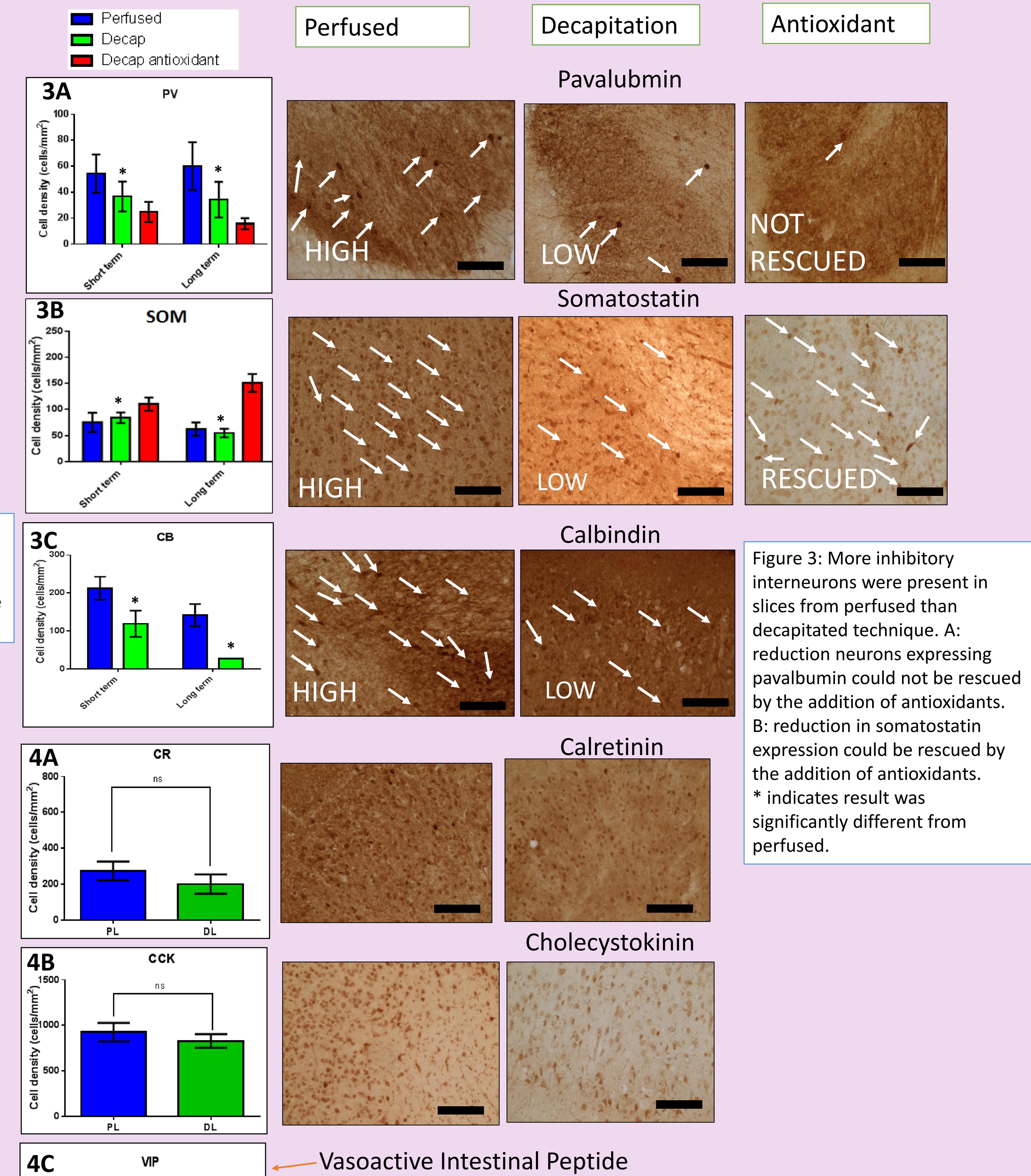


Figure 3: More inhibitory interneurons were present in slices from perfused than decapitated technique. A: reduction neurons expressing pavalbumin could not be rescued by the addition of antioxidants. B: reduction in somatostatin expression could be rescued by the addition of antioxidants.
* indicates result was significantly different from perfused.

Figure 4: there was no significant difference in cell density of inhibitory interneurons expressing CR,CCK or VIP.

Conclusion

The perfusion method should be used in preference to the decapitation method because:

- excitable (seizure or epileptic) activity is easier to induce in decapitation slices
- interneurons are susceptible to oxidative damage
- cell function and viability of inhibitory interneurons is compromised with the decapitation preparation