# Quantification of Cox4 in the prefrontal cortex in post stroke, vascular, and other ageing related dementias

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Introduction

- Stroke is a major risk factor for dementia, with a reported 24% incidence of **post-stroke dementia** within 3.8 years following stroke.
- Little is known about PSD pathology compared to cognitively healthy stroke survivors.
- Lesions in the prefrontal circuitry are linked to executive dysfunction observed in vascular related dementias.
- It was previously found that pyramidal cell volumes in Layers III and V (*indicated by arrows*) of the **dorsolateral prefrontal cortex (dlPFC)** of post stroke subjects to be reduced by 30-40% compared to controls and post stroke non-dementia cases[1].
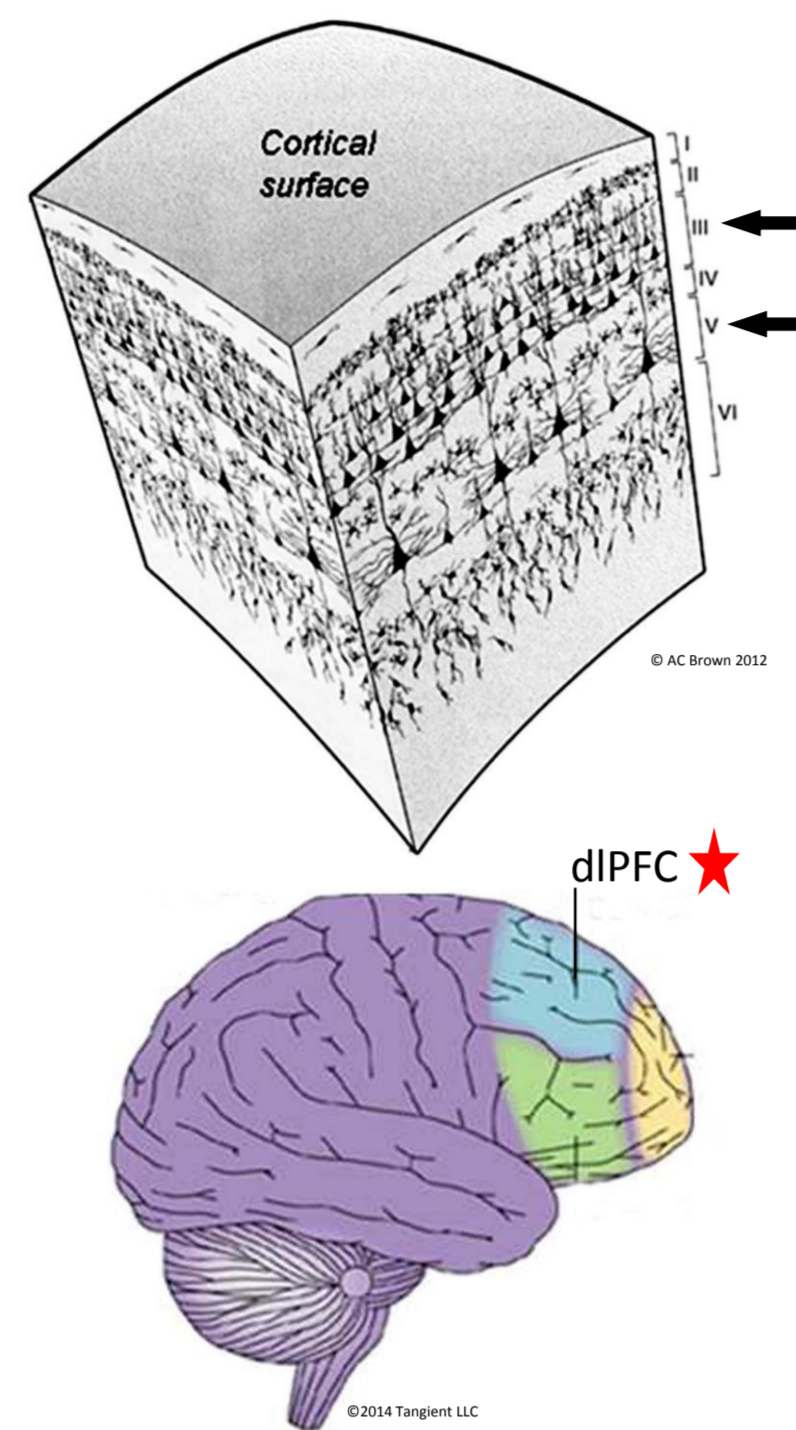
Aims/Objectives

- To quantify **Cytochrome c oxidase subunit IV (Cox4)** in layers III and V pyramidal neurons of the dorsolateral prefrontal cortex (dlPFC) in post mortem demented brains.
- To investigate Cox4's relationship to pyramidal neuron volumes and executive function in post stroke dementias.

Method

- 60 cases (10 X Control, PSD, PSND, VaD, AD and Mix).
- Neuropsychometric measures were available for post-stoke cases through the CogFAST study.
- Serially cut 10µm wax-embedded paraffin tissue blocks were stained with anti-Cox4 antibody and visualised using diaminobenzidine (DAB). 10 images were taken per case at 10X magnification using a Zeiss Axioplan 2 microscope.
- **Image analysis:** Using Image Pro software, Cox4 was quantified by measuring **Per Area (P/A)** and **Integrated Optical Density (IOD)** from 10 images of layer III and 10 images of layer V for each case, *see Image 1*. Pyramidal neuronal counts were performed on 10 images and the mean was calculated for each case.
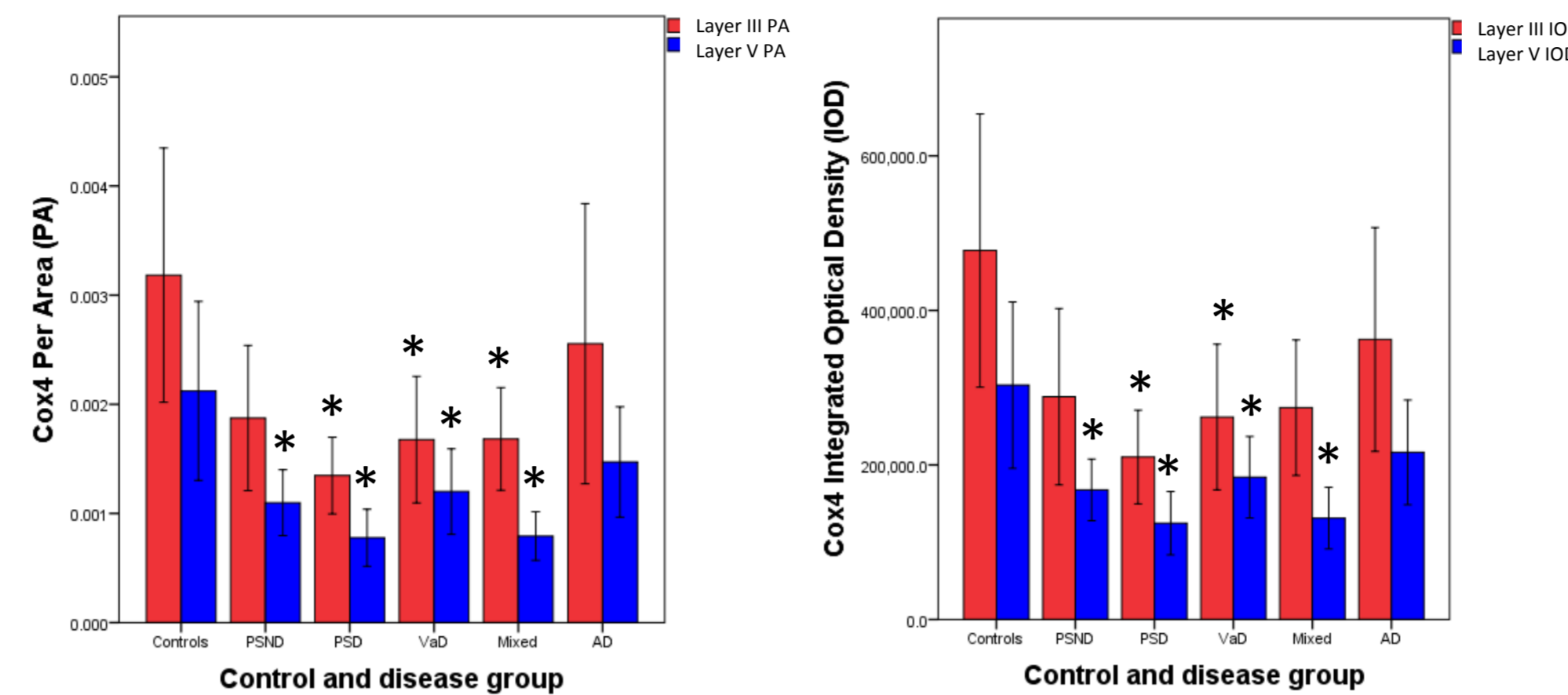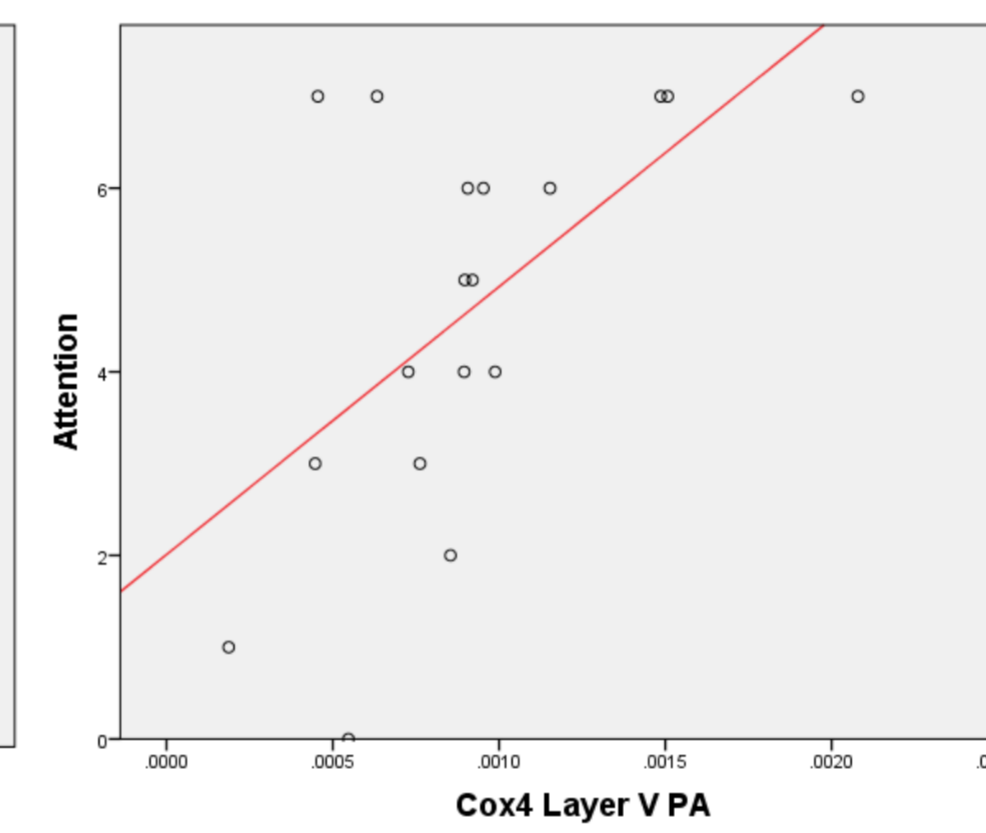

Graph 1
Quantity of Cox4 stain expressed as a percentage of the total image area in Layers III (red) and V (blue) of the dlPFC- Per Area (PA). * represents significant difference compared to controls.

Graph 2
Quantity of Cox4 stain expressed as an average pixel intensity per stained cell in Layers III (red) and V (blue) of the dlPFC- Integrated Optical Density (IOD) * represents significant difference compared to controls.
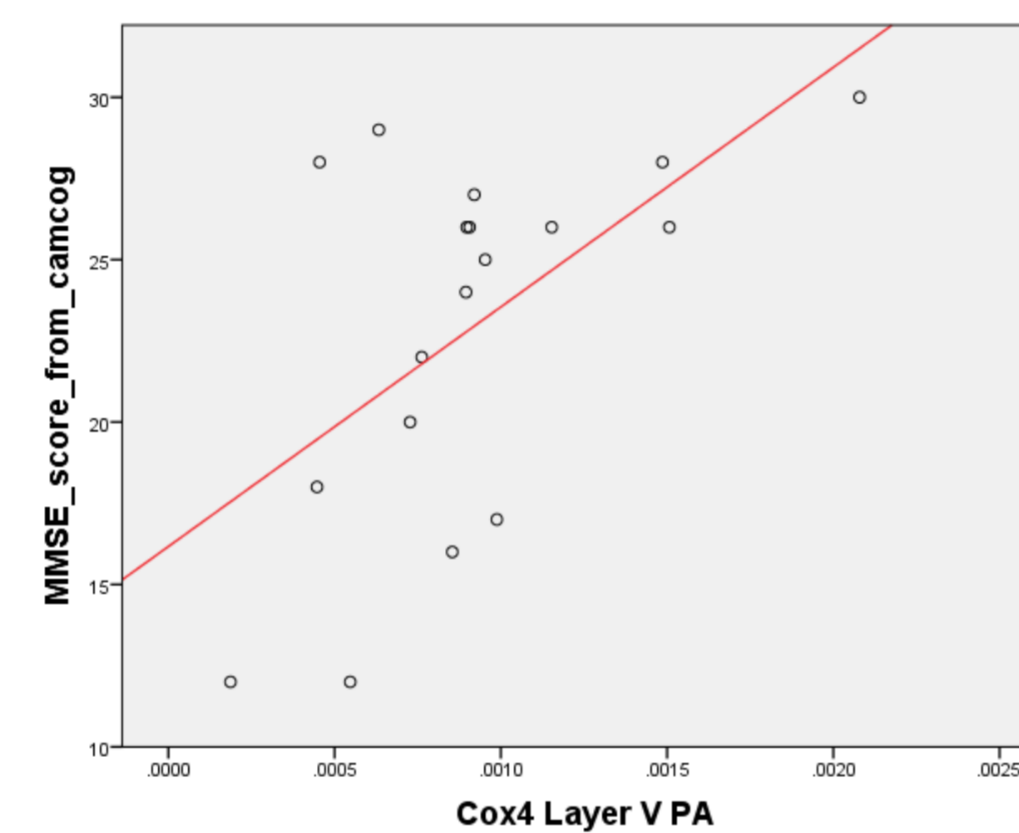
Graph 3
Significant correlation (P=0.047) between Layer V Cox4 Per Area and MMSE scores for Post stroke cases

Graph 4
Significant correlation (P=0.014) between Layer V Cox4 Per Area and Attention scores for Post stroke cases
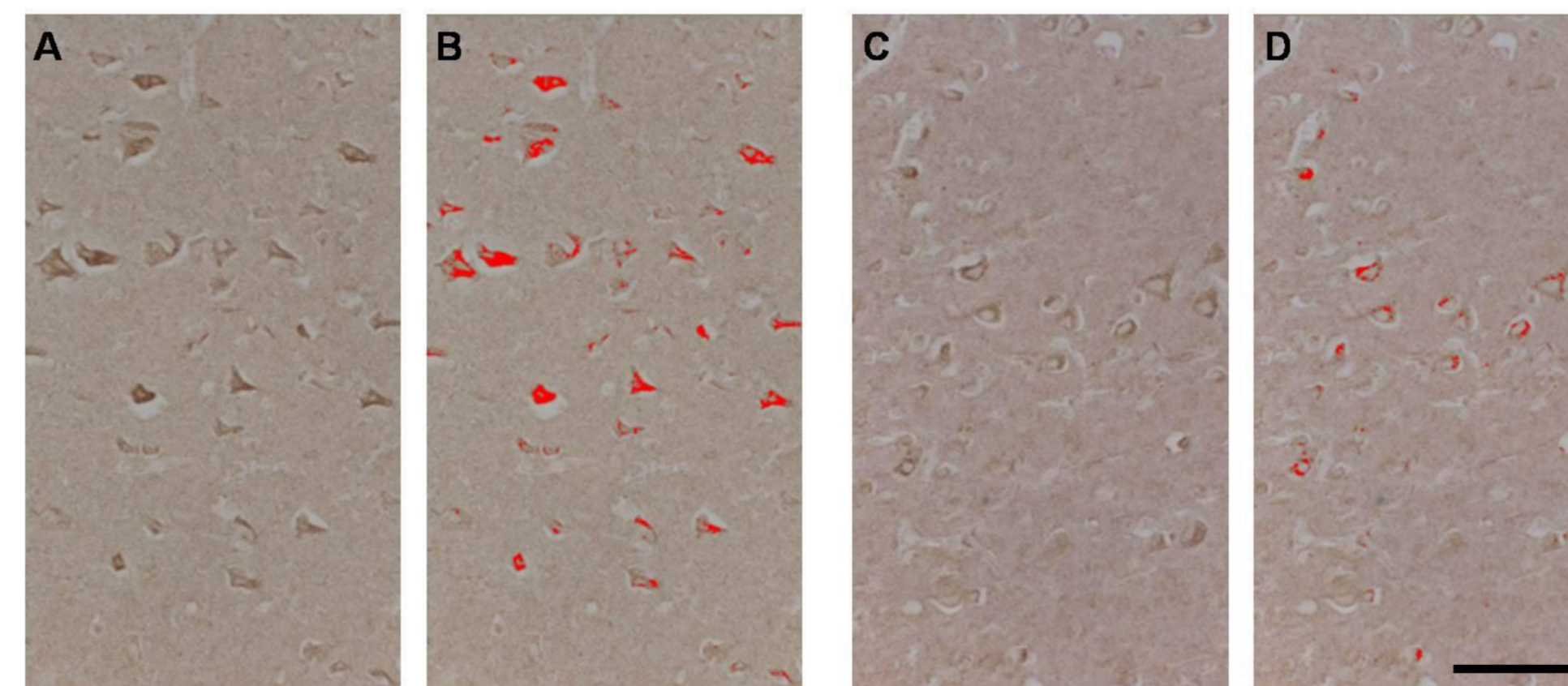

Image 1. 2D densimetric analysis comparison between control subject and those suffering post stroke dementia. A = Control Cox4 +ve neurons, B = Control Cox4 +ve neurons + interface, (red) C = PSD Cox4 +ve neurons, D = PSD Cox4 +ve neurons + interface (red). Bar represents 100 microns for A, B, C and D

Results

Per Area (PA) analysis of Cox4 showed a significant decrease in **Layer III** in PSD (P=0.019), VaD (P=0.028) and Mix (P=0.034) when compared to controls. **Layer V** showed a significant decrease in PSND (P=0.019), PSD (P=0.004), VaD (P=0.049) and Mix (P=0.004) compared to controls. A trend between PSND and PSD patients was found in Layer V, with P/A of PSD comparably less than PSND (P=0.086). *See Graph 1*

IOD analysis showed a significant decrease in **Layer III** in PSD (P=0.034) and VaD (P=0.041), with a trend in Mix (P=0.070) compared to controls. **Layer V** showed a significant decrease in PSND (P=0.019), PSD (P=0.003), VaD (0.049) and Mix (P=0.004) compared to controls. *See Graph 2*

On examination of relationships between Layer V P/A data from PSND and PSD patients with neuropsychometric measures, we found correlations with Memory-Learning (rho= -0.553, P=0.017), attention (rho=-0.566, P=0.014) and MMSE score from CAMCOG examination (rho=-0.474, P=0.047). Layer V IOD data from PSND and PSD patients correlated with attention (rho=-0.502, P=0.034). *See Graphs 3 and 4*

The number of Cox4 +ve pyramidal neurons in layer III significantly decreased in PSND (P=0.001), PSD (P=0.013), VaD (0.001) and Mix (P=0.001) compared to controls. Layer V showed a similar decrease, with PSND (P=0.002), PSD (P=0.002), VaD (P=0.031), Mix (P=0.002) and AD (P=0.019) all showing a significant decrease in pyramidal neurons compared to controls.

Discussion

- Cox4 was stained regardless of neuronal type. Layers III and V contain high numbers of pyramidal neurons and so we infer that the quantitative changes observed were attributed to pyramidal neurons.
- It is difficult to delineate from this study whether the quantitative changes in Cox4 are attributable to a decrease in expression in all pyramidal neurons or a decrease in number of Cox4 +ve pyramidal neurons.

Future work:
A further stain (e.g. Nissl) needs to be integrated into the study to obtain a ratio of cells expressing Cox4 to cells not expressing Cox4.

Conclusion

- We showed a decrease in COX4 expression in those suffering CVD based dementia when compared to controls, suggesting a link between vascular pathology and metabolic expression.
- Quantity of Layer V Cox4 positive neurons in post stroke cases may indicate executive dysfunction.

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References: [1]Foster V et al. Pyramidal neurons of the prefrontal cortex in post-stroke, vascular and other ageing-related dementias. Brain 2014; 137(Pt 9):2509-21