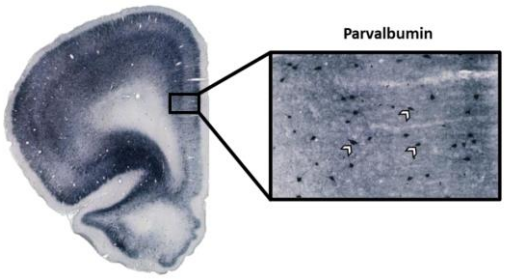
METHODOLOGY

We conduct analysis for a range of different neuronal and inflammatory cell types.
The procedure below briefly illustrates the protocol for conventional staining of parvalbumin (PV) interneurons in the prefrontal cortex (PFC) in free floating sections followed by staining using the avidin-biotin complex (ABC) method for detection.



We also offer fluorescent analysis for individual and co-localization studies. Further information of markers and analysis available are available on request.

Sample preparation

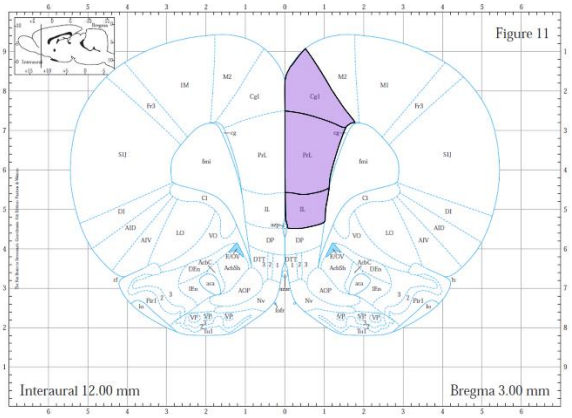
Following behavioural experiments animals are sacrificed and transcardially perfused with 1M phosphate buffered saline (PBS), post-fixed with 4% PFA for 24-36 hours and then transferred into 30% sucrose (~48 hours). Samples are flash frozen using iso-pentane and stored at -80°C until sectioning. 30µm slices of the PFC are cut using a cryostat (Leica CM1950). One in eight serial sections are stored in cryoprotectant at -20°C (30% glycerol, 30% ethylene glycol, 30% dH2O, 10% 1M PBS).

Parvalbumin Immunohistochemistry

Sections are washed in 1x PBS three times for five minutes each. For PV, sections underwent heat-induced antigen retrieval in sodium citrate buffer (0.294% (w/v) sodium citrate, 0.07% (v/v) tween-20, 500ml distilled water, pH adjusted to 6.0) for 30 minutes at 80°C. Unless otherwise stated, samples are washed twice in 1x PBS between each step. Samples are incubated in hydrogen peroxide solution (1.5% H_2O_2, Sigma; 0.4% Triton x-100, Sigma; 10% methanol, 88.1% 1x PBS) for 30 minutes at room temperature (RT). Samples are incubated in protein block (5% normal horse serum, Vector Laboratories; 0.6% triton x-100, Sigma; 94.4% 1x PBS) for one hour. Then, without washing, incubated in parvalbumin (1:5000; 24 hours; 235, Swant) at 4°C. On day 2, PV samples are incubated with secondary antibody (PV: horse anti-mouse; 1:200; BA-2000-15, Vector Laboratories) for 2 hours. PV, sections are incubated with the VECTASTAIN Elite ABC-HRP kit (PK-4000, Vector Laboratories) for 45 minutes and visualised with DAB substrate kit (SK-4100, Vector Laboratories). When a sufficient colour change has been observed, samples are moved into distilled water to stop the peroxidase reaction. Sections are mounted onto Superfrost slides and assigned randomised codes. Slides are then dehydrated in increasing ethanol concentrations (70%, 90% and 100%, 5 minutes each) and washed in Histoclear (National Diagnostics) for 5 minutes. Coverslips are applied with DPX mounting media (Sigma).

Image analysis

Images are acquired on a 3D-Histech Pannoramic-250 microscope slide-scanner using a 20x / 0.80 Plan Apochromat objective (Zeiss). Snapshots of the slide scans are taken using the CaseViewer software (3D-Histech). For PV images, the region of interest (PFC) is delineated, and the number of cell detections manually counted within the CaseViewer software. Density is calculated by dividing the number of positive cell detections by the area of the region of interest (**Error! Reference source not found.**).



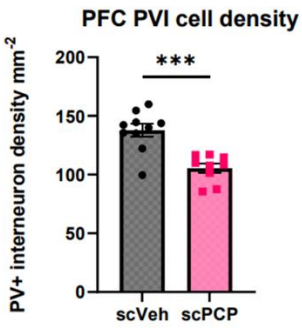


Figure 1: Prefontal cortex and its regions (cingulate cortex, prelimbic cortex and infralimbic cortex). PV cell density (cells/mm^2) is reduced in the PFC in scPCP treated animals.