

# DEVELOPMENT OF AN OPTOGENETIC APPROACH TO INVESTIGATE THE ROLE OF MEDIAL PREFRONTAL CORTEX IN VISUALLY GUIDED BEHAVIOR

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## INTRODUCTION

The rodent medial prefrontal cortex (mPFC) has been suggested as a homologue to the prefrontal cortex of primates, playing an important role in supporting higher cognitive functions such as decision making, memory and learning (Uylings et al). Optogenetic approaches have revolutionised the investigation of neural correlates of behaviour in mice. Hyperpolarising pumps such as ArchT (Han et al) allow us to test whether particular cortical regions are necessary to support the successful completion of rodent psychophysics tasks. We have packaged the GFP-tagged ArchT pump into an AAV1/2 viral vector providing a safe and efficient gene transfer methodology with high neural tissue tropism (Klugmann et al) and effective virus purification with heparin affinity columns. The ability of the Arch-T pump to reduce neuronal excitability and suppress spiking activity during light illumination was confirmed both in vitro and in vivo in acute cortical slices and anaesthetized mice, respectively.

We are working towards optogenetic disruption of visual and prefrontal circuits during visually guided tasks. To this end, we are training mice in a simple discrimination task under head-fixed conditions in a custom designed visually immersive environment, controlled in LabVIEW.

## METHODS

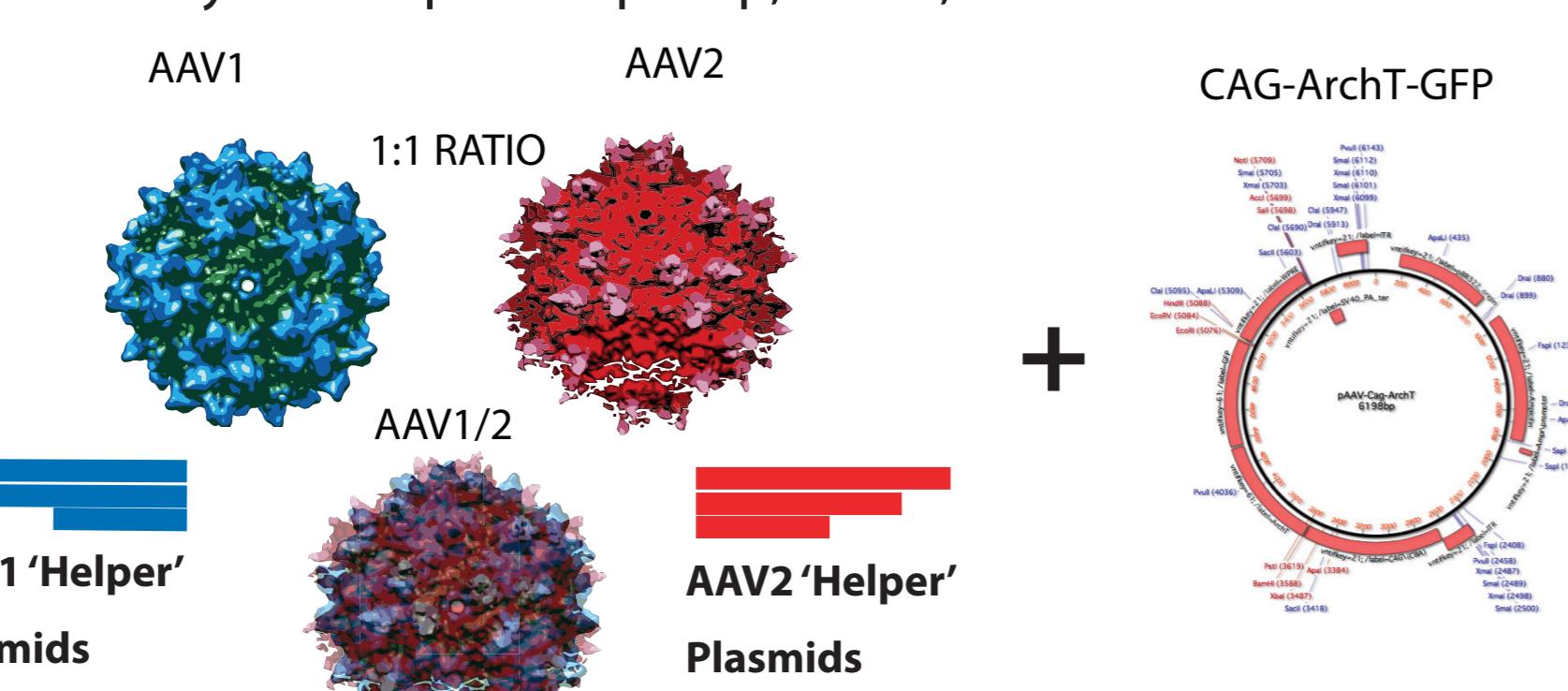
An AAV1/2 mixed serotype virus was constructed to package the Arch-T GFP plasmids (courtesy of Ed Boyden). pFD6, pRV1, pH21 and pArchT were transfected in HEK293 cell cultures using the calcium phosphate transfection method. Following Benzonase endonuclease digestion, viral supernatant was passed through a heparin affinity column and the virus was eluted using a Tris-buffered NaCl gradient. Eluted fractions were concentrated by repeated centrifugation, distributed into 20 µl aliquots and transferred to -80°C for storage.

We used C57BL/6 mice (4-6 weeks) to pressure inject AAV1/2-ArchT under isoflurane anaesthesia into stereotactically determined cortical locations. Following 2 weeks to allow for virus expression, mice were cardiacly perfused and brains fixed in 4% formaldehyde overnight. The following day, 60-80 µm coronal slices were acquired with a Vibratome. We stained the slices with a fluorescent Nissl stain (Neurotrace, 435/455) to provide an anatomical reference for the injections. Confocal microscopy at x10, x40 and x63 magnification was used to determine the expression levels and spread of the virus.

Mice used for functional validation were injected with virus 2-3 weeks prior to recordings. *In-vitro experiments*: recordings were obtained from an acute adult mouse cortical slice preparation. Recordings were at (RT°C) with K-gluconate based internal ( $[Cl^-]_i = 10\text{mM}$ ). Green light illumination was delivered by a wide-field halogen light source with a fluorescent filter. *In-vivo experiments*: extracellular recordings of spontaneous activity were acquired using borosilicate pipettes (1-10MΩ) in mice under isoflurane anaesthesia. 532nm laser stimulation was delivered with a 300µm optical fibre positioned above the injection site on the surface of brain. Following electrophysiology, histological procedures were carried out as described above.

### 1 Making a mixed serotype viral vector: The AAV1/2 - ArchT GFP construct.

We used a 1:1 ratio of AAV1 and AAV2 serotype capsid proteins with AAV2 ITRs (Hauck et al) to make a highly neurotrophic viral vector, enabling effective delivery of the proton pump, ArchT, to cortical neurons.



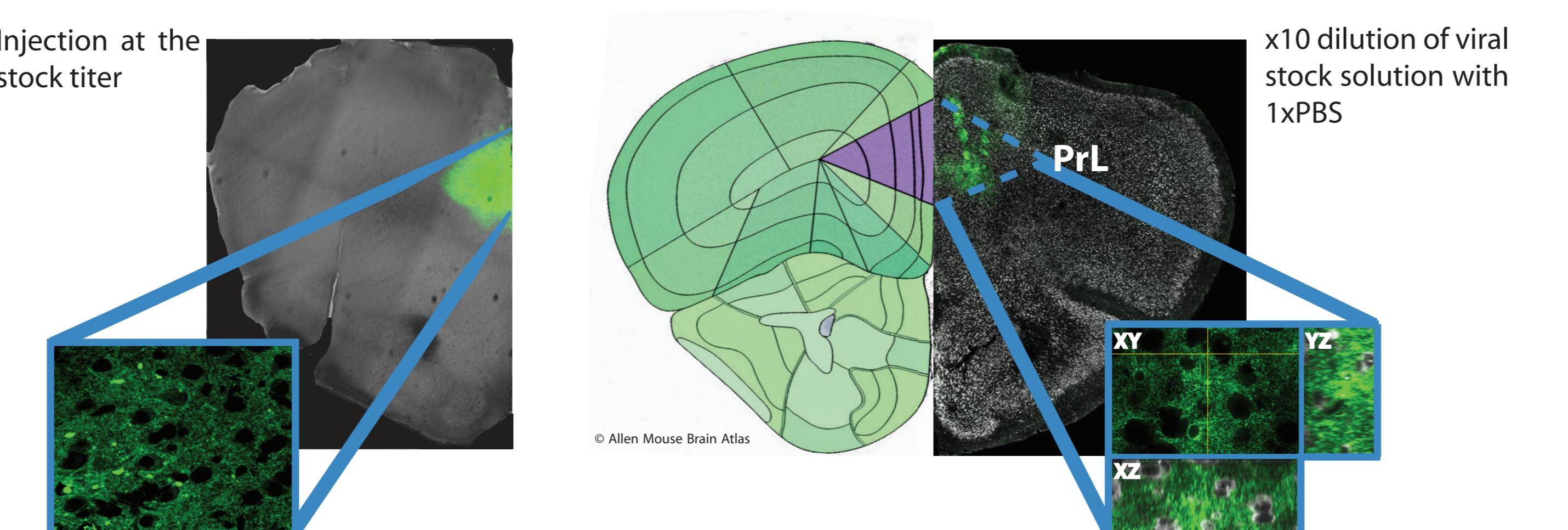
AAV1 and AAV2 are highly homologous serotypes, making them compatible for co-assembly.

AAV2 capsids contain heparan sulphate proteoglycan (HSPG) receptors, allowing the use of heparin affinity columns for virus purification.



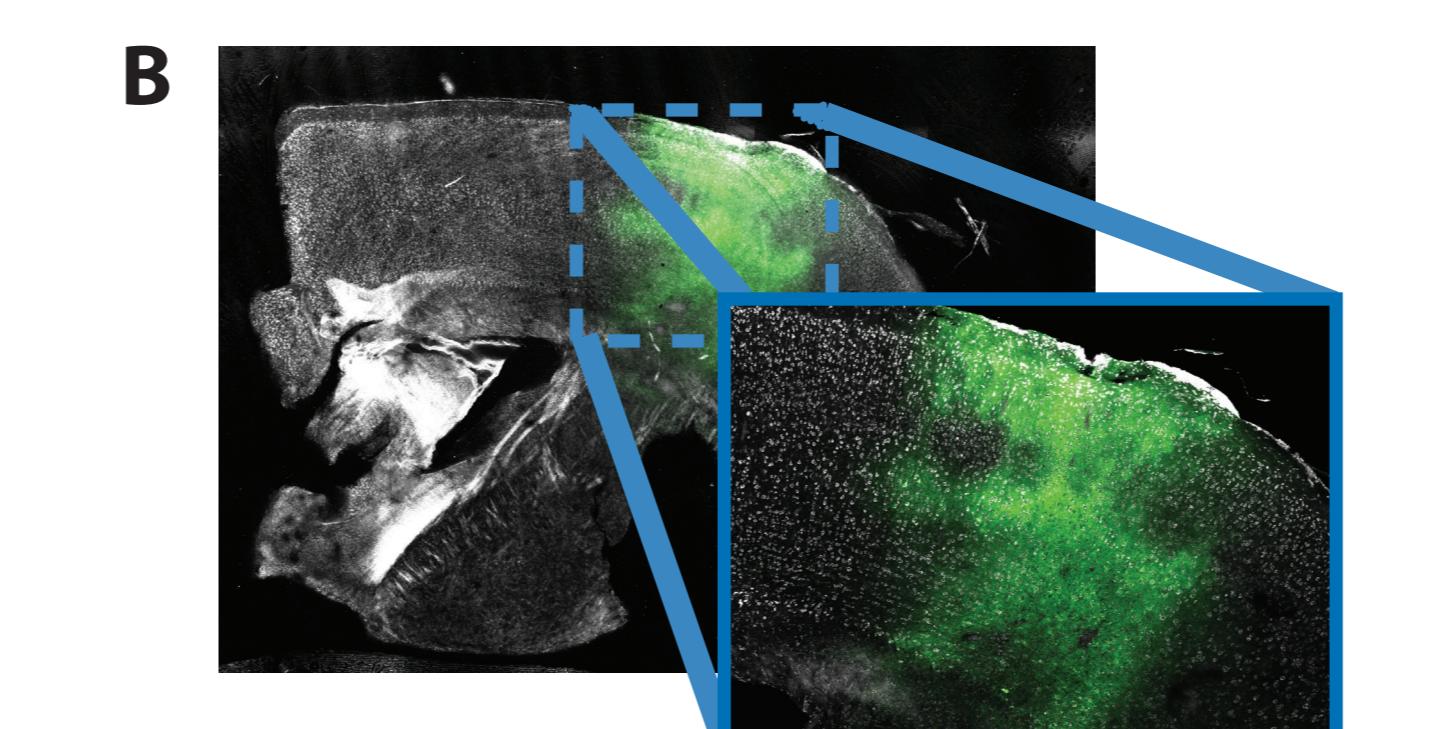
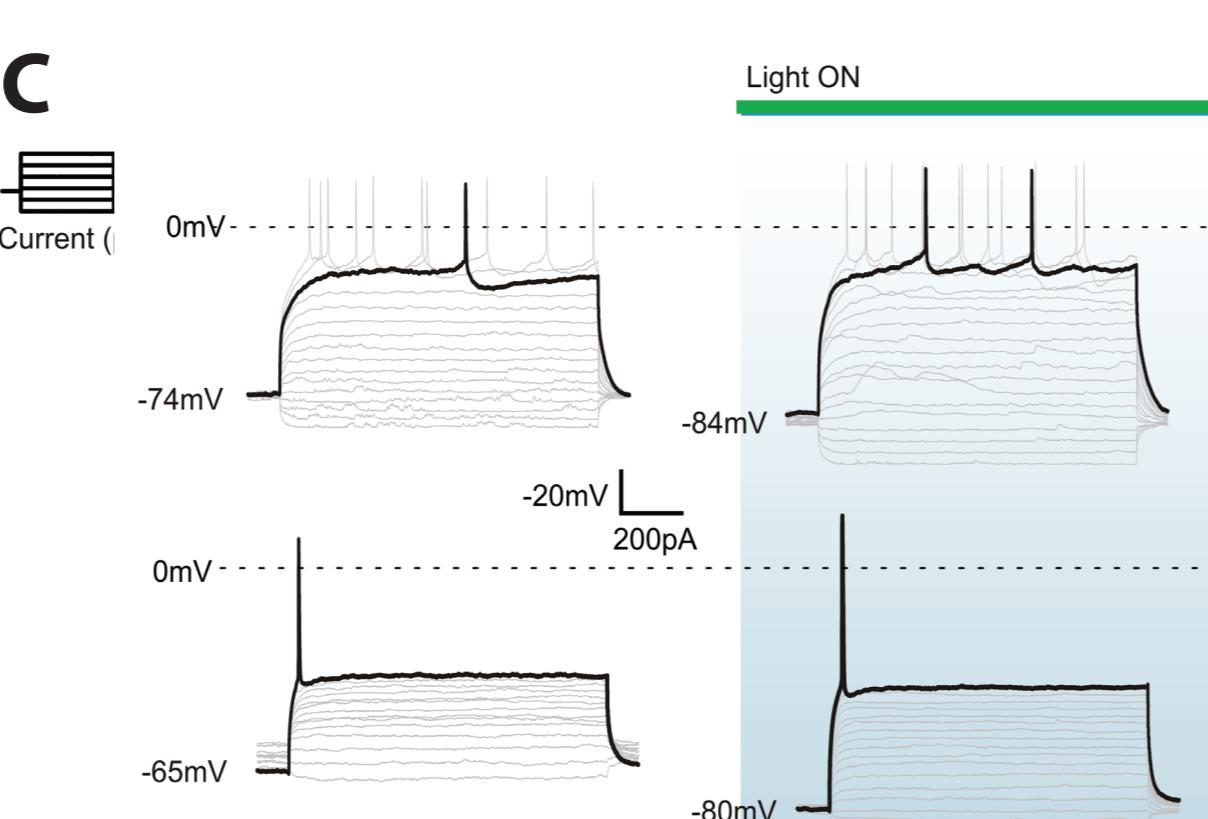
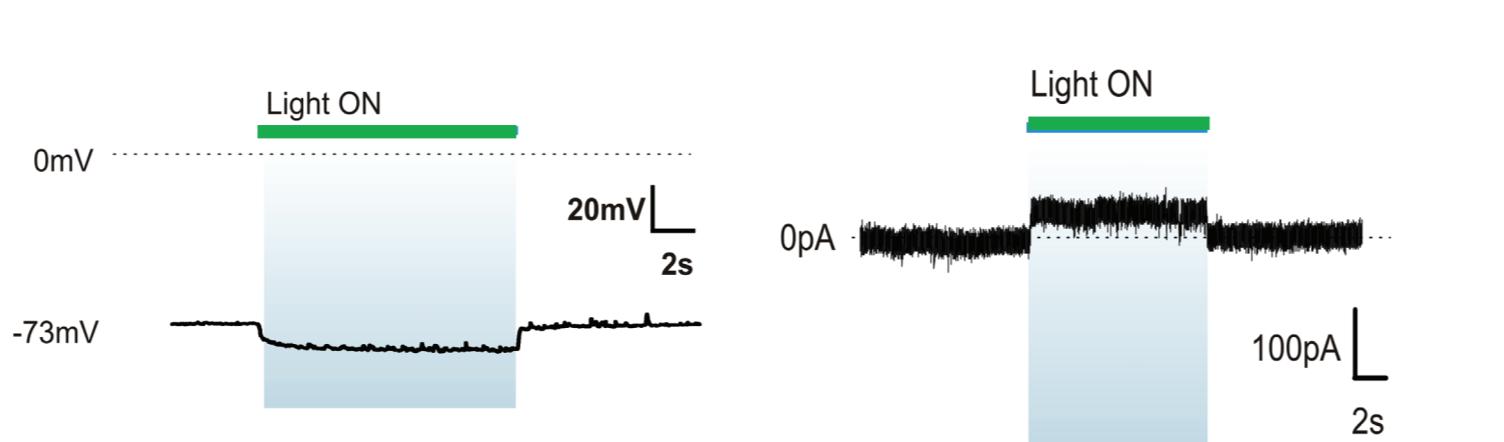
### 2 Injections of AAV1-2 ArchT in mPFC to test tissue tropism and expression levels

Injections of AAV1/2-ArchT into V1 and Prelimbic Cortex (PrL) of the mPFC at different concentrations showed reliable fluorescence in the intended regions. Note the prelimbic site refers to mouse 5 of the in-vivo validation experiments. Confocal microscopy images acquired at x10, x63 magnification.

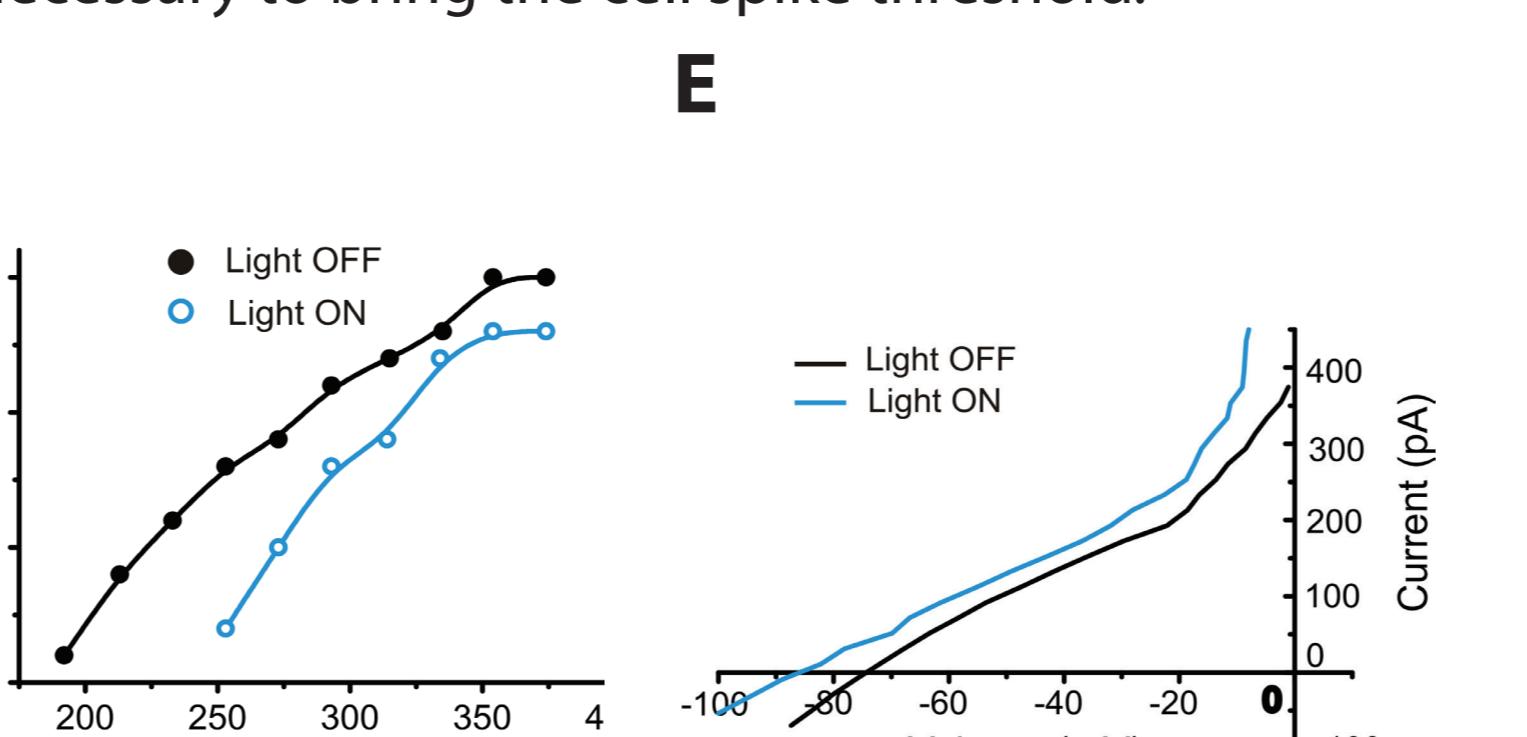


### 3 In vitro validation of AAV1/2 ArchT: Whole cell recording in cortical slices

A Whole cell recordings confirm the hyperpolarising effect of ArchT proton pump on infected cells when activated by green light.



Activation of ArchT increases the amount of current necessary to bring the cell spike threshold.



- A. Representative current clamp and voltage clamp ( $V_h = -70\text{mV}$ ) recording showing membrane hyperpolarisation and current induced by light.
- B. Expression levels of ArchT in the cortical slice from which the recordings in A were obtained.
- C. Voltage response to somatic current injection on application of light. In the light ON condition, a larger number of current steps was required to bring the cell to threshold.
- D. Frequency-Current Relationship. Note, the rightward shift in the curve (less excitable) and the increased current required to reach threshold.
- E. Current-Voltage (I-V) relationship on application of light. This demonstrates the more hyperpolarised reversal potential in the Light ON condition.

## CONCLUSIONS

AAV1/2 mixed capsid viruses provide an efficient package for delivery of ArchT, achieving high levels of expression in cortical tissue.

Successful suppression of neural activity in infected regions can be seen within 2-3 weeks of virus injection.

Some infected neurons show significant changes in spontaneous firing rate after light illumination.

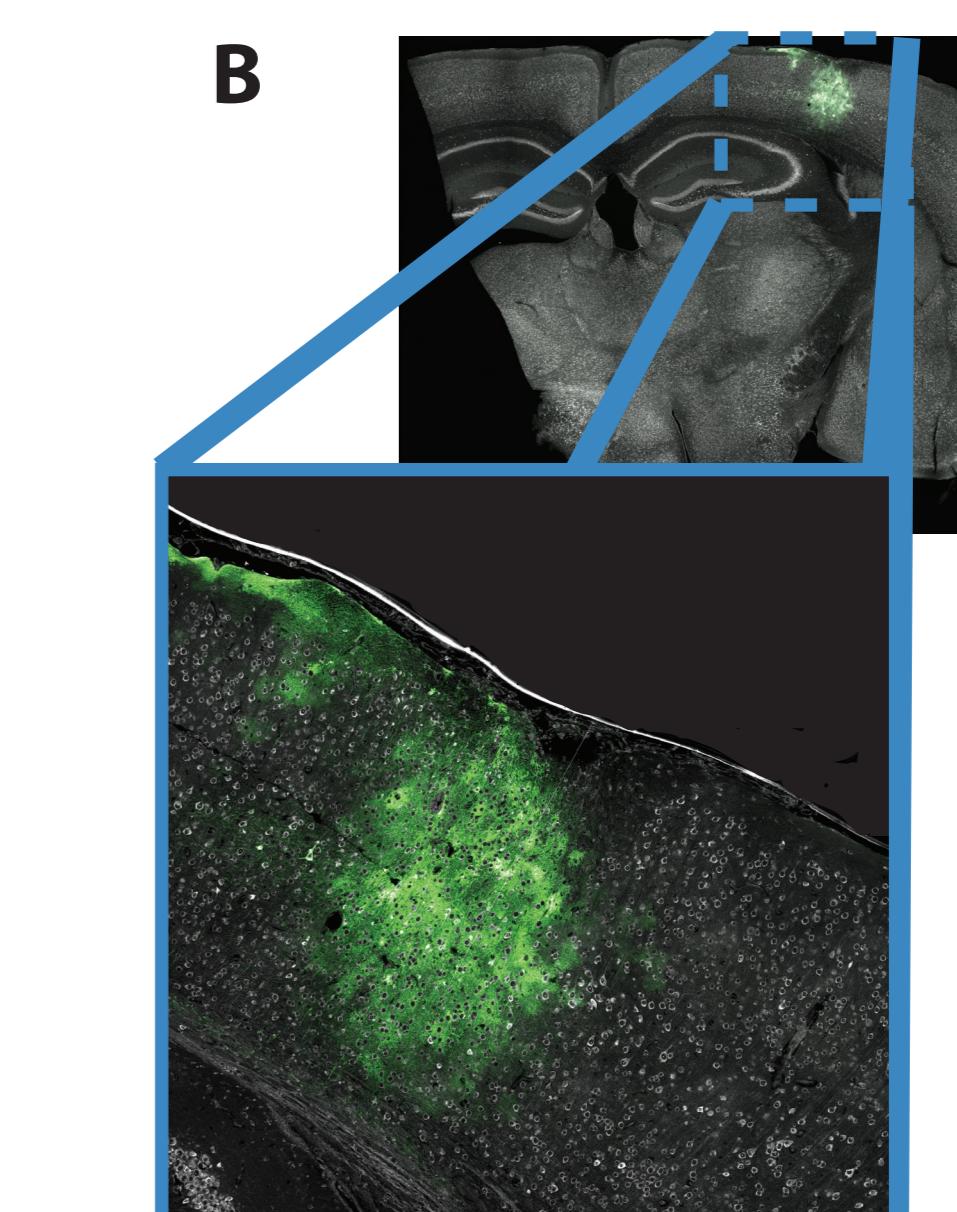
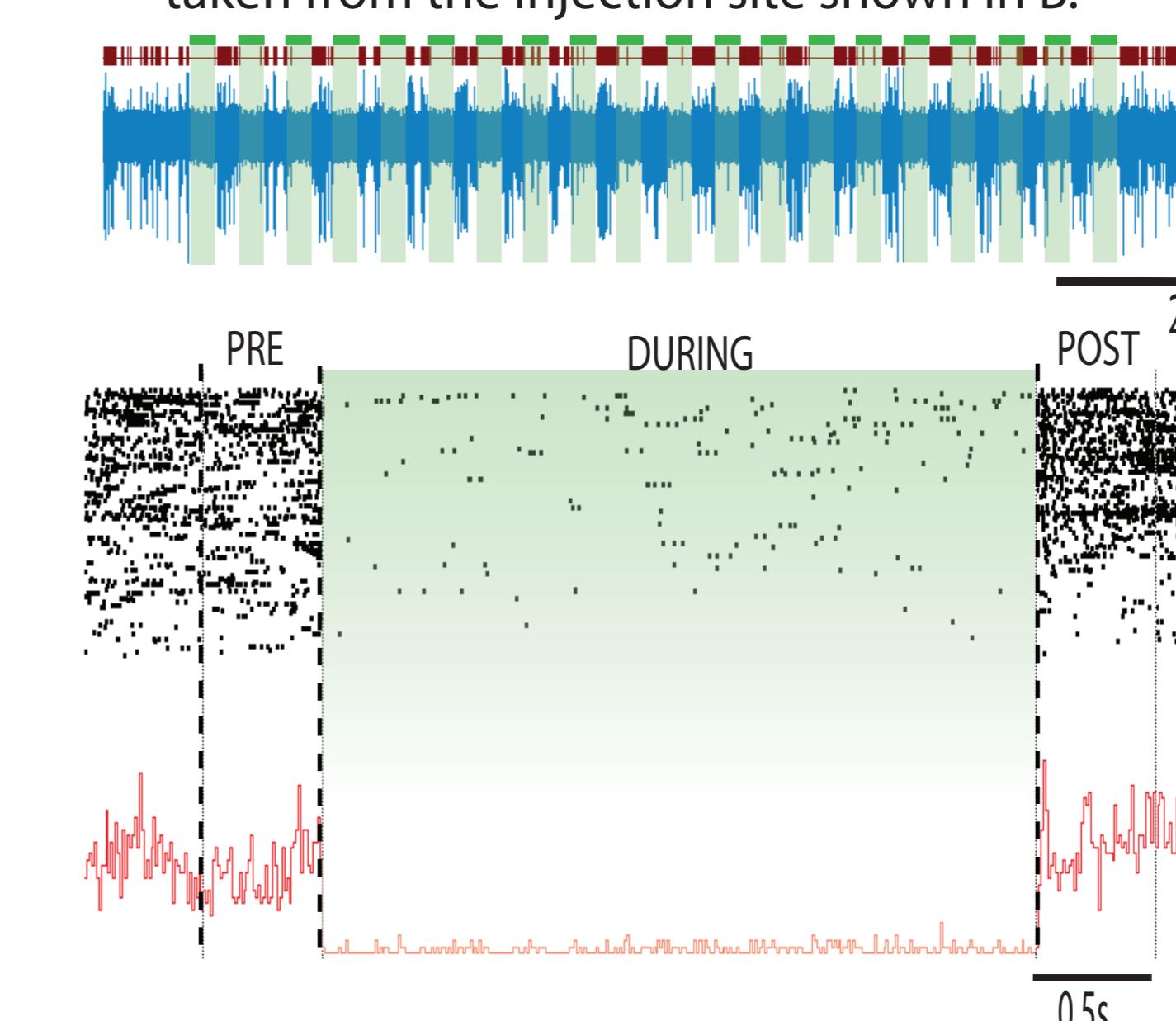
- REFERENCES:
1. Uylings et al, Beh. Brain Res., 146:1-2, 2003
  2. Han et al, Front Syst Neurosci, 5:18, 2011
  3. Klugmann et al, Mol. Cell. Neurosci, 28:2, 2005
  4. Hauck et al, Mol. Ther, 7, 2003

### Acknowledgements

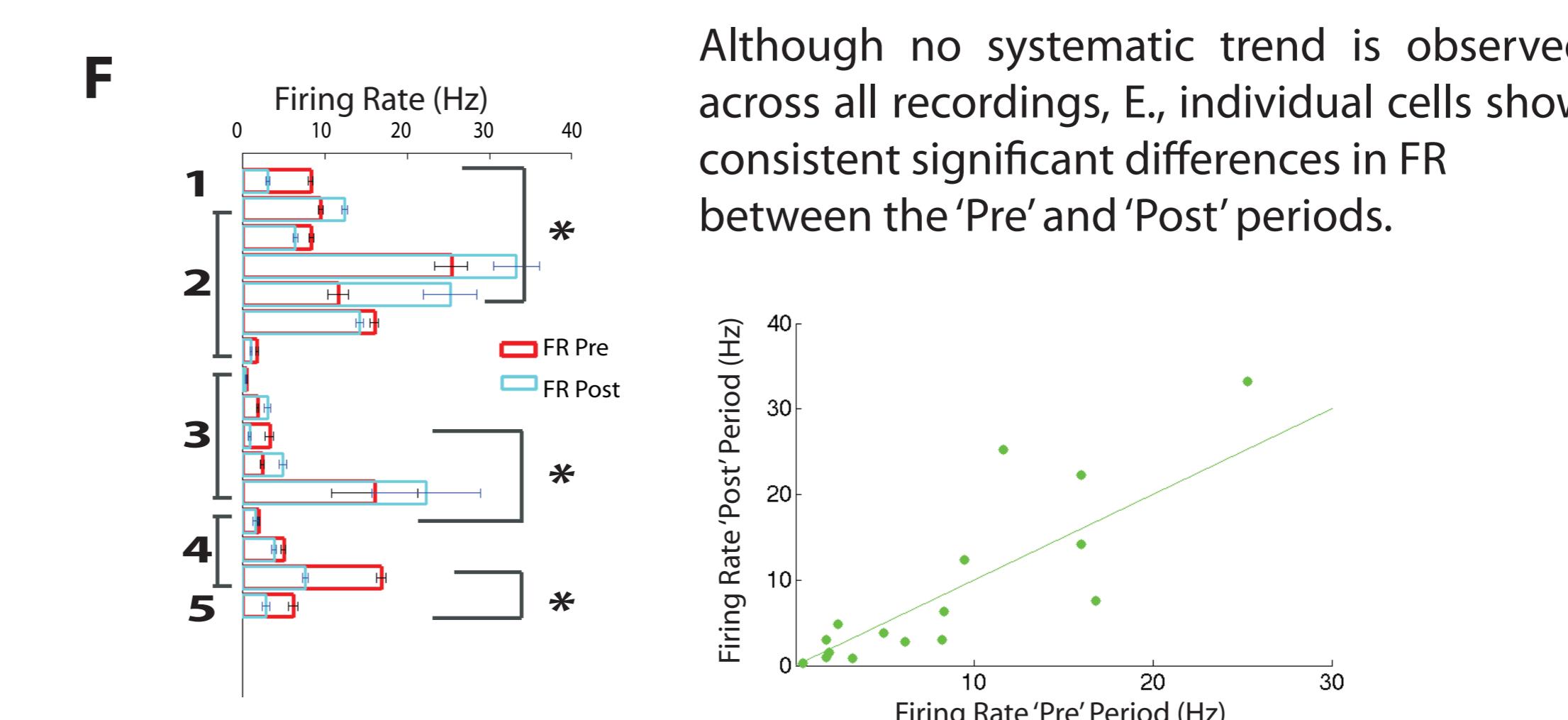
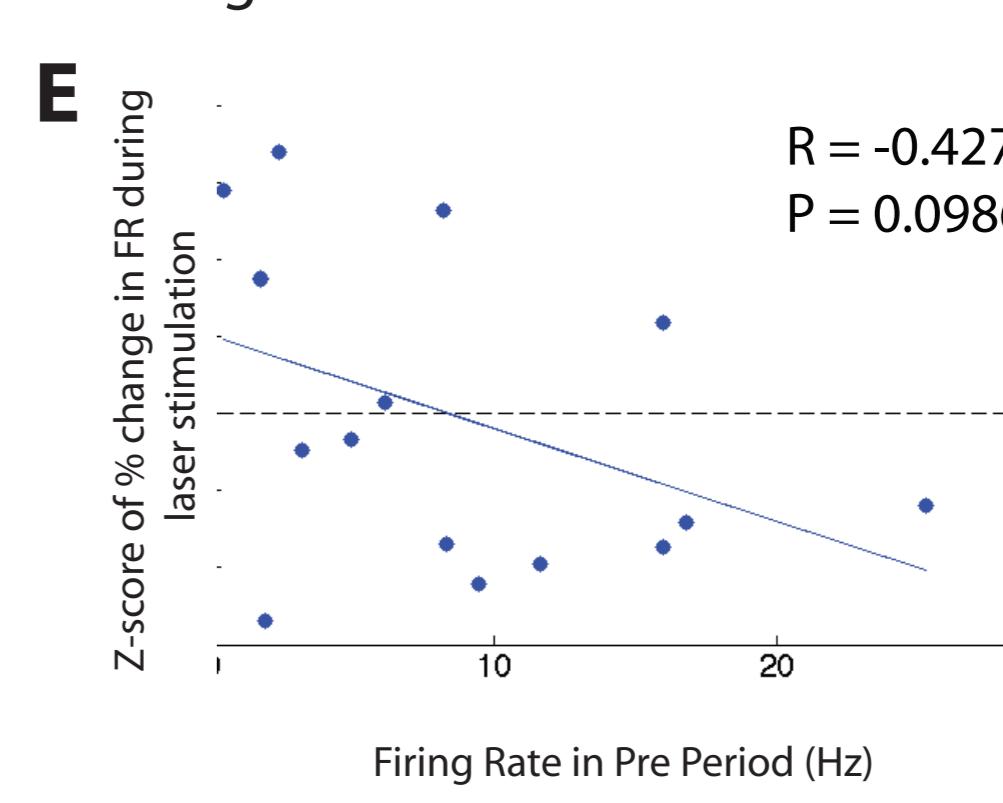
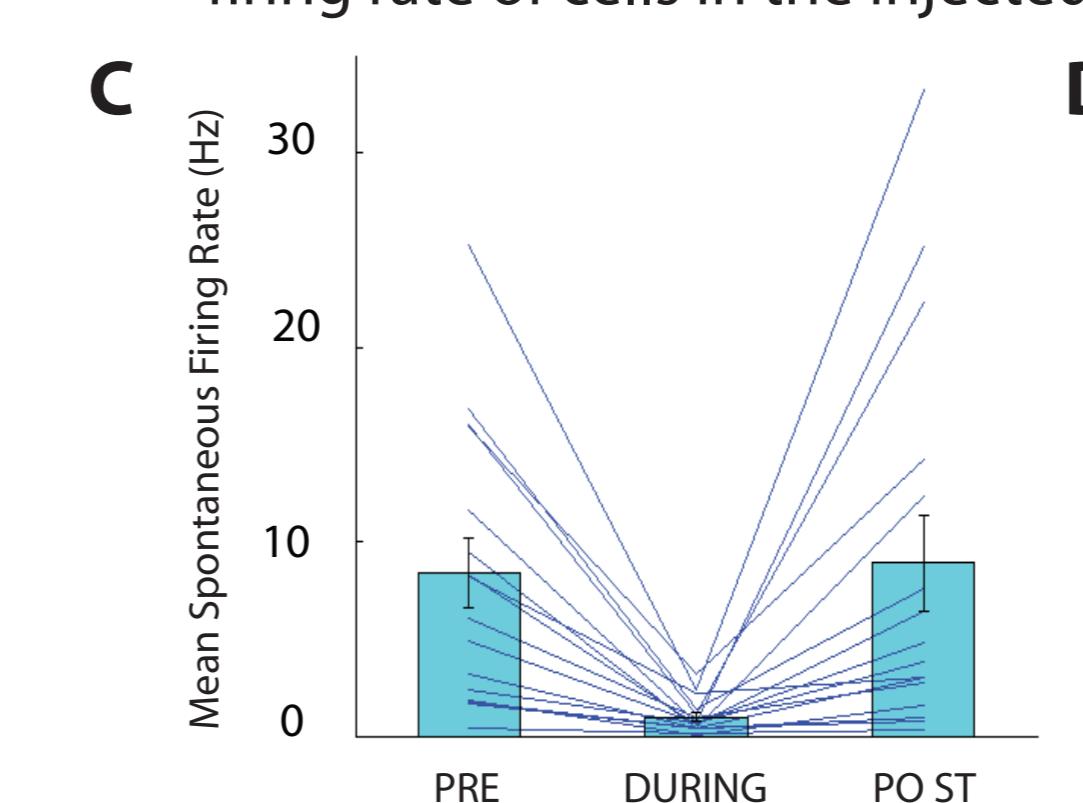
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### 4 In vivo validation of AAV1/2 ArchT: Pipette recordings in anaesthetised mice

A Recording during laser excitation and PSTH taken from the injection site shown in B.



Cells with higher firing rates tend to show a higher percentage suppression during laser stimulation.



Although no systematic trend is observed across all recordings, E., individual cells show consistent significant differences in FR between the 'Pre' and 'Post' periods.

A. Representative trace of extracellular recording in cortex of an anaesthetised mouse. Peristimulus time histogram of the recording in A showing reliable firing rate(FR) suppression on stimulation with green light.

B. Injection site in mouse number 2. Confocal microscopy images acquired at x10 and x40 (inset) magnification showing ArchT-GFP on a grayscale Nissl background.

C. Mean spontaneous FR over 0.5s 'Pre', 'During', 0.5s 'Post' laser stimulation. Bars show average over all recorded cells. Lines show results from individual cells. (n=16)

D. Change in FR relative to FR in 'Pre' period. Dots show mean over trials for individual cells. (n=16).

E. Magnitude of light-evoked suppression of activity as a function of individual mean firing rate in 'Pre' period. Correlation coefficient (R) and p-value are indicated.

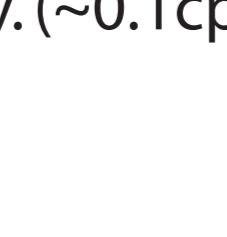
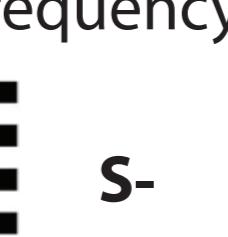
F. Comparison of spontaneous FR in the 'Pre' and 'Post' periods. Left subpanel shows superimposed average FR, 'Pre' (red) and 'Post' (blue) with s.e.m. error bars for each cell. Right subpanel indicates the relationship between the means for each individual cell.

(\* P<0.05, two tailed Student's T-test) Cells (n=16) are grouped by animal (n=5).

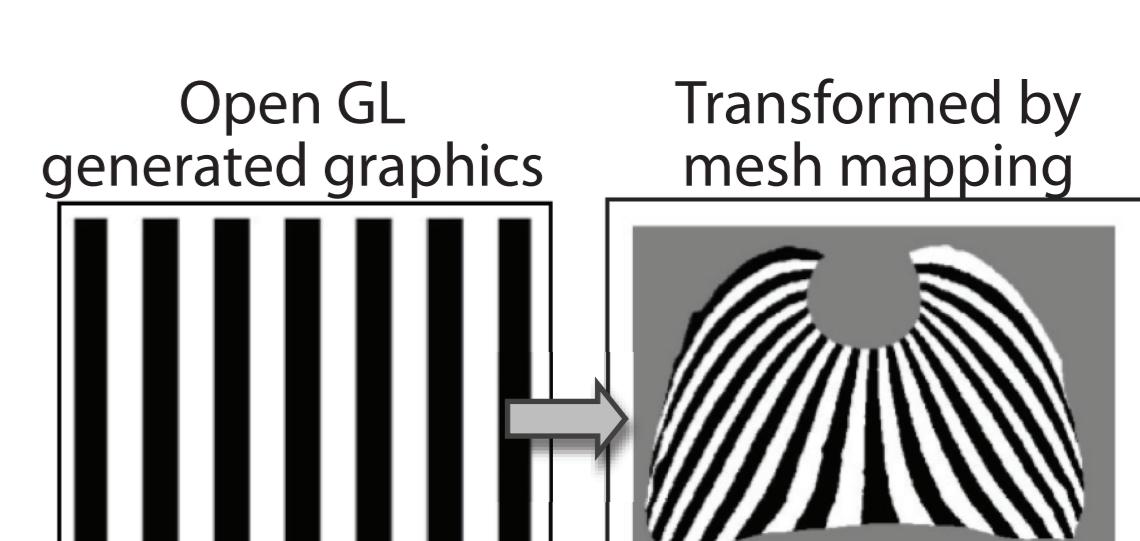
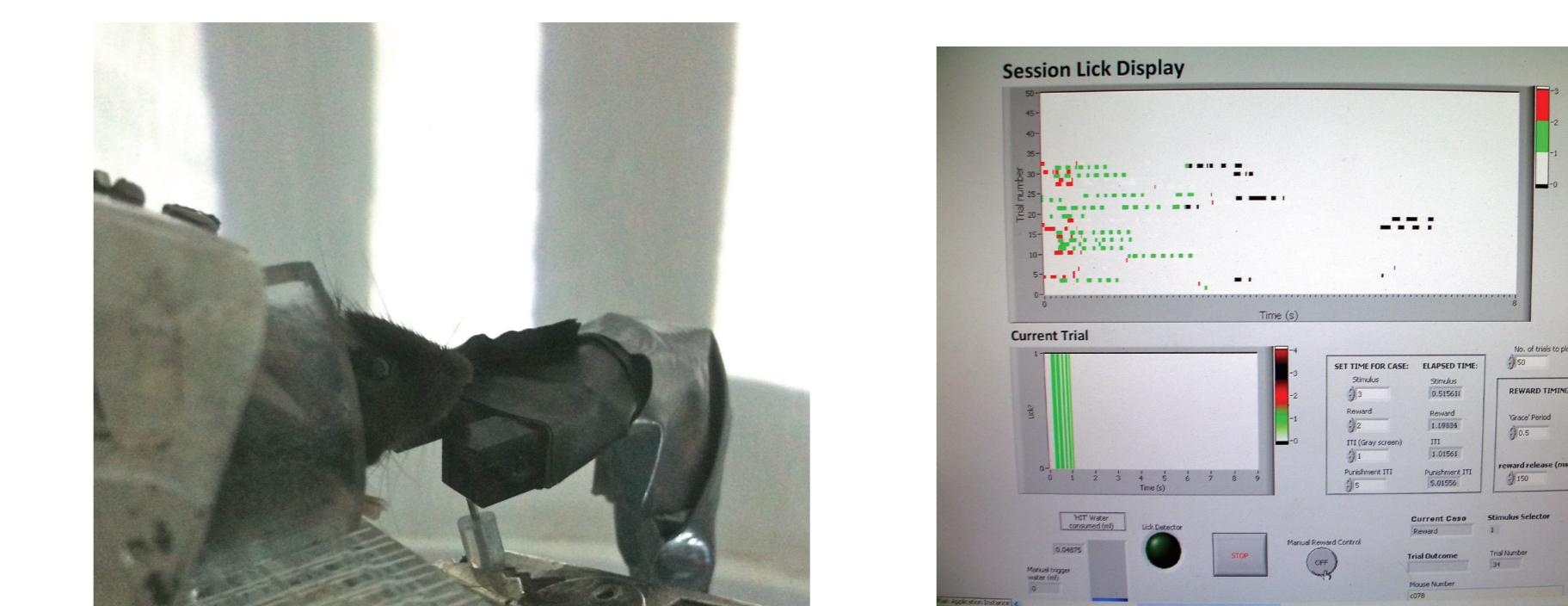
### 5 TOWARDS INVESTIGATING VISUALLY GUIDED BEHAVIOR

CURRENTLY...

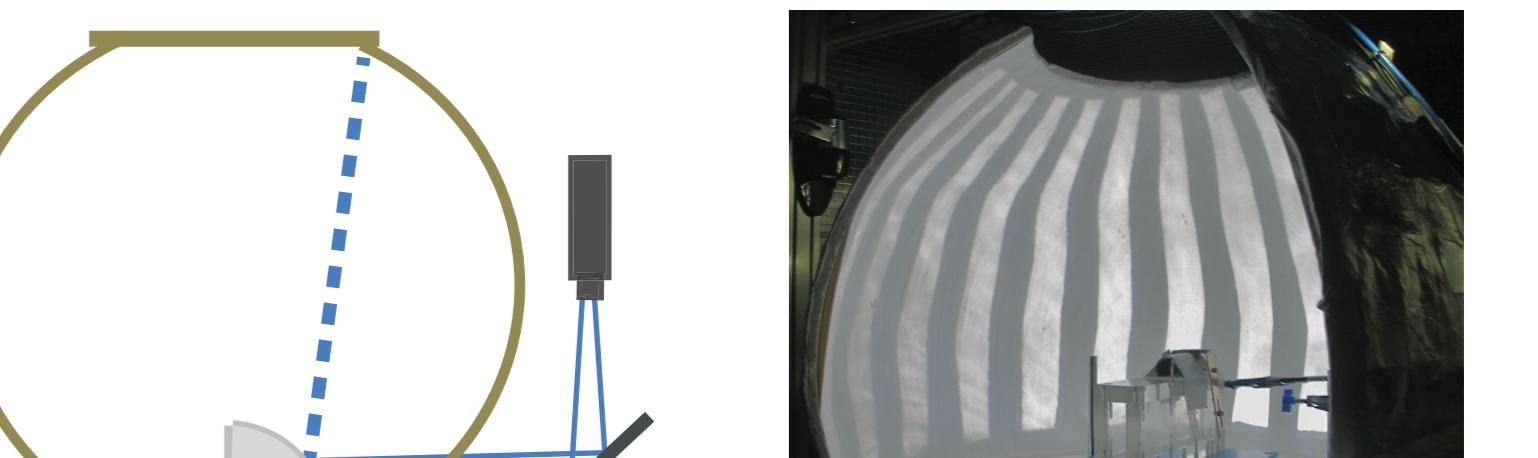
#### A EASY DISCRIMINATION: Vertical and horizontal gratings at optimal spatial frequency. (~0.1cpd).



Interactive LabVIEW User Interface



Transformed by mesh mapping



We are currently training animals in a simple discrimination task using full screen orthogonal gratings stimuli. The stimuli are coded in OpenGL and coordinate-mapped onto a surround screen to appear undistorted from the central viewing point the mouse assumes.

We are using a head-fixed Go-NoGo Task approach, with the mouse receiving a water reward for licks during the S+ stimulus. The task structure and input/output event registration is coordinated by a customised LabVIEW program with a user-friendly interface.

We plan to perturb this behavior with optical fibre light delivery to PrL.

FUTURE...

#### B DELAYED MATCH TO SAMPLE

