

# Cortical fosGFP Expression Reveals Broad Receptive Field Excitatory Neurons Targeted by POM

Jean-Sébastien Jouhanneau,<sup>1,2</sup> Leiron Ferrarese,<sup>1,2</sup> Luc Estebanez,<sup>1,2</sup> Nick J. Audette,<sup>3</sup> Michael Brecht,<sup>2,4</sup> Alison L. Barth,<sup>3</sup> and James F.A. Poulet<sup>1,2,\*</sup>

<sup>1</sup>Department of Neuroscience, Max Delbrück Center for Molecular Medicine (MDC), Berlin-Buch, Robert-Rössle-Str. 10, 13092 Berlin, Germany

<sup>2</sup>Cluster of Excellence NeuroCure, Neuroscience Research Center, Charité-Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany

<sup>3</sup>Department of Biological Sciences and Center for the Neural Basis of Cognition, Carnegie Mellon University, Pittsburgh, PA 15213, USA

<sup>4</sup>Bernstein Center for Computational Neuroscience (BCCN), Humboldt University Berlin, Philippstrasse 13, 10115 Berlin, Germany

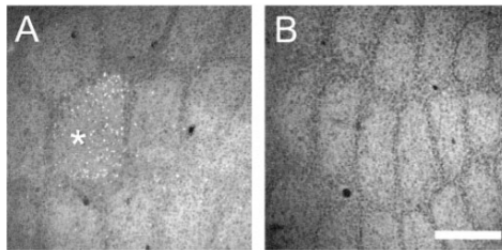
\*Correspondence: [james.poulet@mdc-berlin.de](mailto:james.poulet@mdc-berlin.de)

<http://dx.doi.org/10.1016/j.neuron.2014.10.014>

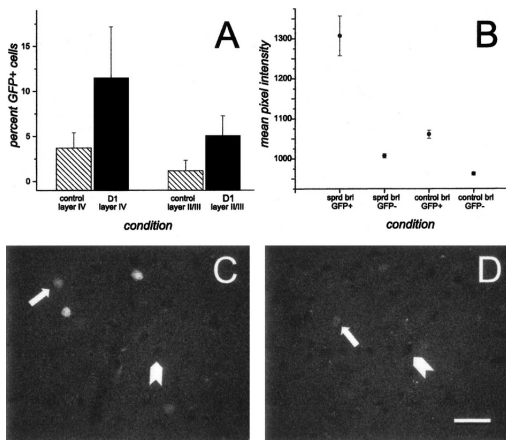
November 28, 2014

# Introduction

- ▶ c-fos is a transcription factor, its expression is correlated with learning
- ▶ fosGFP fusion protein made that is driven by c-fos promoter - made a transgenic mouse to check out activity-dependent changes in the brain in vivo



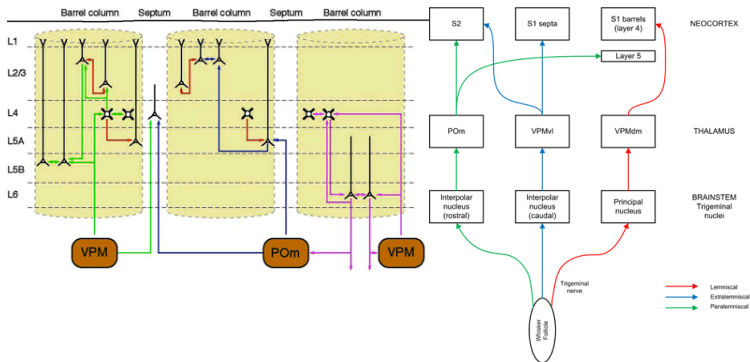
expression only in barrel with spared whisker (all other whiskers plucked)  
(Barth et al, 2004)



Sensory stimulation increases both the number and intensity of fosGFP cells in barrel cortex. **A**, Quantitation of the number of fosGFP cells in control versus the spared whisker barrel in layer IV and layer II–III. **B**, Average pixel intensity of labeled cells increases after single-whisker stimulation. Error bars in **A** and **B** represent SE. **C**, Example of labeled nuclei (arrow) and unlabeled cells (chevron) from layer II–III of D1-only cortex. **D**, The same as **C** but in contralateral unplucked cortex from the same animal. Scale bar, 20  $\mu$ m (Barth et al, 2004)

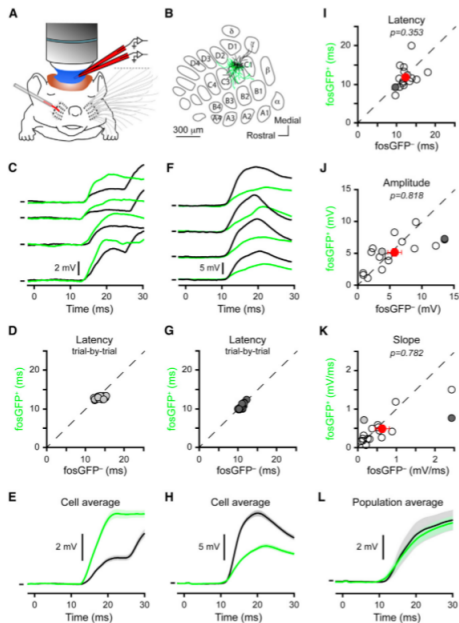
# this study

- ▶ how is subcortical input differentially distributed across excitatory neurons - could these differences cause response heterogeneity in superficial layers?
  - ▶ at least exists differences among layers
  - ▶ posteromedial nucleus (POm) prefers L5A and L1
  - ▶ ventral posteromedial nucleus (VPM) afferents terminate in L5B and L4



Going to use fosGFP as a marker to distinguish among excitatory neurons

# fosGFP<sup>+</sup> and fosGFP<sup>-</sup> have similar early synaptic responses



**Figure 1. Single Principal Whisker Stimulation Triggers a Similar Early Synaptic Response in fosGFP<sup>+</sup> and fosGFP<sup>-</sup> Neurons**

(A) Schematic of piezo-driven glass rod (shaded gray) deflecting a single principal whisker (C2, bold red) and two-photon targeted dual whole-cell recordings in the C2 barrel column.

(B) Partial reconstruction within the barrel map of a fosGFP<sup>+</sup> (green) fosGFP<sup>-</sup> (black) cell pair confirms C2 targeting.

(C) Four single trial responses to piezo-driven C2 whisker deflection.  $V_m$  mark fosGFP<sup>+</sup>/fosGFP<sup>-</sup> (mV) from top to bottom: -63.5/-58.3; -63.6/-57.0; -60.8/-58.7; -62.4/-56.8.

(D) Trial-by-trial measurements of latency from the pair in (C) ( $n = 27$  trials) shows no differences in latency.

(E) Averaged subthreshold response to piezo stimulation for the pair of cells shown in (C). SEM is shown in shaded color around the mean.  $V_m$  mark fosGFP<sup>+</sup>/fosGFP<sup>-</sup> (mV): -61.9/-56.2.

(F) Four single trial responses to piezo-driven C2 whisker deflection from the reconstructed pair in (B).  $V_m$  mark fosGFP<sup>+</sup>/fosGFP<sup>-</sup> (mV) from top to bottom: -64.9/-60.7; -65.3/-64.1; -64.6/-60.3; -63.9/-61.7.

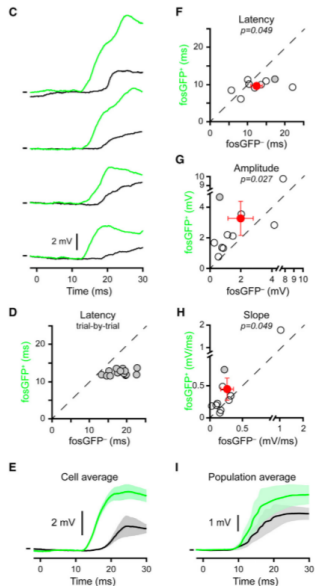
(G) Trial-by-trial measurements of latency for the pair shown in (F) show no differences in the latency of the fosGFP<sup>+</sup> neuron compared to the fosGFP<sup>-</sup> ( $n = 20$  trials).

(H) Averaged subthreshold response to piezo stimulation to the pair of cells in (F). SEM is represented in shaded color around the mean.  $V_m$  mark fosGFP<sup>+</sup>/fosGFP<sup>-</sup> (mV): -65.3/-62.7.

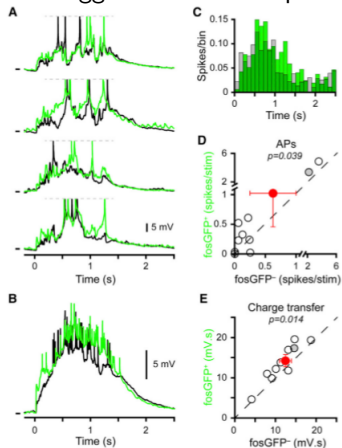
(I-K) FosGFP<sup>+</sup> and fosGFP<sup>-</sup> neurons show no significant differences in the (I) latency, (J) amplitude, and (K) onset slope of the early synaptic response to brief deflection of the principal whisker ( $n = 17$  pairs). Light gray and dark gray circles correspond to example neurons in (C) and (F), respectively. Red filled circles with error bars show mean  $\pm$  SEM.

(L) Population average of the synaptic response to principal whisker stimulation to principal whisker stimulation in neighboring fosGFP<sup>+</sup> and fosGFP<sup>-</sup> neurons ( $n = 17$  pairs). Shaded background shows the SEM of the baseline-subtracted synaptic responses.  $V_m$  mark fosGFP<sup>+</sup>/fosGFP<sup>-</sup> (mV): -61.4/-61.0.

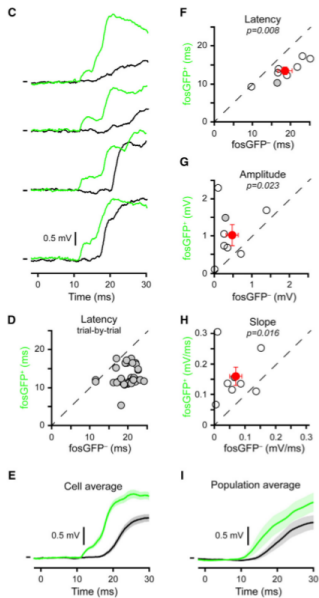
# fosGFP<sup>+</sup> respond with shorter latency and larger amplitude to multi-whisker stimulation



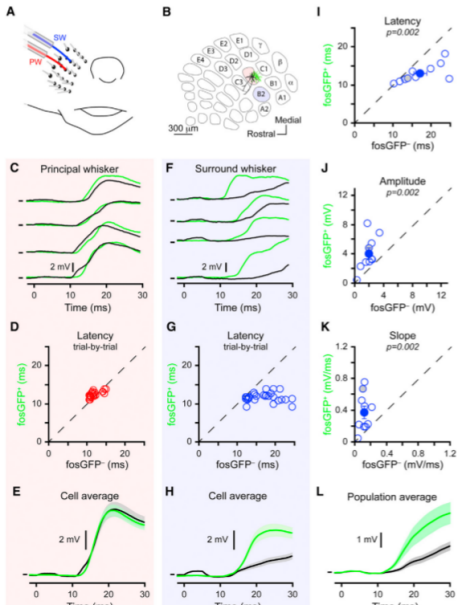
also triggers more action potentials



# fosGFP+ respond more to surround multi-whisker stimulation



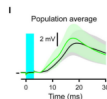
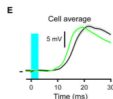
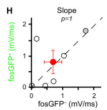
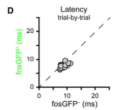
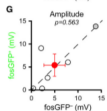
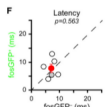
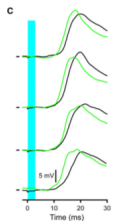
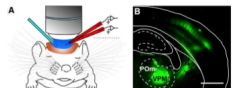
# fosGFP<sup>+</sup> respond more to single surround whisker stimulation too



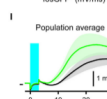
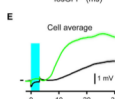
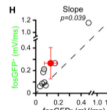
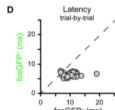
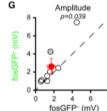
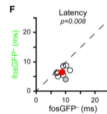
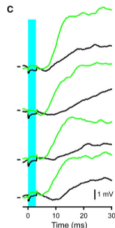
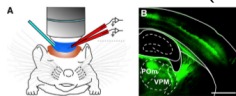


# fosGFP+ receives earlier and larger amplitude synaptic responses from POm vs VPM stim

## VPM stimulation (sharply tuned)



## POm stimulation (broadly tuned)



# Conclusion

- ▶ VPM neurons project to barrel column center, POm neurons to septal regions between barrels, but fosGFP+ neurons did not show distinct clustering in septal regions
- ▶ VPM could also contribute to fosGFP+ cells differentially, projecting from different barrels
- ▶ fosGFP+ seems to be assembled by input competition
  - ▶ cells with broader inputs upregulate c-fos transcription factor