Annex 4. Final Report

INDIVIDUAL FELLOWSHIPS

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Marie Curie Actions

IEF – Final Report

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1. Publishable summary report

Illusions can tell us a great deal about how our brain works[1,2]. More than being mere entertainment, their misdirection of neural processing lifts the veil covering the elementary computations that continuously take place when we examine a visual scene. Sensory illusions come in many flavors, but context-dependent optical illusions stand out by their simplicity and prevalence in our daily lives (Fig. 1a-c[3-5]). These illusions are characterized by a dramatically changed appearance or interpretation of a visual stimulus depending on the embedding context. The neural correlate underlying these contextual illusions is the modulation of the neuronal response to part of an image by the visual features surrounding it (Fig. 1d)[6].

Contextual modulations have been extensively described and perturbed in humans (e.g.[7,8]) and primates(e.g.[9-11]), but a full understanding of the circuitry has remained elusive because of technical limits in spatial[12] and temporal resolution[13]. Recently, the arrival of optogenetics has revolutionized neuroscience[14,15]. By use of light gated ion channels and pumps, optogenetics allow us exquisite spatiotemporal control of neural activity. This has suddenly created the opportunity to study the neurobiological mechanisms of contextual modulation at a resolution previously impossible.

The best documented example of contextual modulation in the cerebral cortex is that of surround suppression (Fig. 1e). Typically, neurons in the primary visual cortex demonstrate a rapid decline in response when an increase in stimulus size exceeds an optimal value[16-18]. This process contributes to a relative enhancement of responses to smaller stimuli and could aid in figure-ground segmentation and perceptual pop-out. How center/surround interactions, probes for contextual modulations and proxies to contextual illusions, come about in the cerebral cortex is the main focus of this project proposal.

There are a number of hypotheses for the cause of surround suppression in primary visual cortex (V1) (Fig. 1f). It could arise by (1) relaying suppression in earlier stages of visual processing[19,20], (2a) by intracortical computation either via circuits across layers within the same cortical column, i.e. intralaminar[21] or (2b) via horizontal connections across a larger cortical spread mediated by a subclass of inhibitory interneurons[18] or by (3) long range intercortical communication (ICC) between different brain regions constituting an effective network[11,22]. However, heavy corticogeniculate feedback[23] (1), sluggish and spatially coarse cortical inactivation methods (1, 2a and 3; e.g. ablation[24], pharmacology[21], cooling[11,13] respectively) and undetermined inputs to the specific subclass of interneurons (2b[25]) have prevented corroboration of any of these hypotheses. Furthermore, the strength of the suppression is dependent on the orientation of the surround elements[26,27]. Surrounds which are iso-oriented with respect to the center produce stronger suppression than cross-oriented surrounds. This mechanism could play an important role in figure-ground segregation.

We addressed these questions by recording neural activity in the different layers of V1 cortex in anesthetized mice using laminar electrodes while simultaneously modulating feedback information from higher visual areas to V1 by inhibiting activity in these higher areas using optogenetic intervention.

Our electrophysiological recordings in V1 (Fig. 2a-b) revealed a consistent temporal pattern whereby surround suppression only developed after the initial response (50-150 ms), suggesting recurrent processing. We also observed clear laminar differences (Fig. 2c-d). ***[Objective 1: manuscript submitted to The Journal of Neuroscience, IF 7]***

To test whether intercortical communication is important during this recurrent processing, we used optogenetic interruption of the feedback signals from higher visual areas while recording in V1. We did so by expressing the light-gated cation channel channelrhodopsin-2 (ChR2) in inhibitory neurons of specific higher visual areas, through local viral injections of a Cre-dependent ChR2 vector into GAD2-Cre mice, which express Cre-recombinase in all inhibitory neurons[28]. Within this preparation, illumination of the cortical surface with blue light efficiently activates all transduced GABAergic interneurons. This increase in inhibition silences the injected area of cortex.
Viral injections were targeted using transcranial imaging of the intrinsic signal during retinotopic mapping[29] (Fig. 2e: upper middle and right panel). This allowed us to delineate the borders of V1 and chart the potentially interesting higher visual regions, e.g. lateral areas AL or LM or medial area PM[30,31] (Fig. 2e: upper left panel). Next, we superimposed epi-fluorescence images (eYFP-fused to the virus) and functional retinotopy maps to determine success of our injections (Fig. 2e: lower panel).
Our preliminary optogenetic intervention results bear evidence in favor of feedback signals from higher visual areas to V1 providing the suppressive surround causing a decline in response with increasing stimulus sizes (Fig. 2f: right lower panel). In the absence of cortical feedback (Fig. 2f: right upper panel), mainly from area AL, responses of V1 neurons to large visual stimuli display a release of suppression, while responses to small visual stimuli are retained or even facilitated, with some laminar differences. Data obtained by inactivating area LM and data recorded using single-contact electrodes, yielding perfectly isolated single cell responses, are in agreement with these results. We are currently collecting more data and running more sophisticated analyses to corroborate these preliminary findings. To exclude the possibility that we are directly modulating activity in V1 by aberrant locally transfected neurons, histological sections were made encompassing both the virally expressing higher visual slab of cortex and the V1 recording site marked by coating the electrode with a lipophilic dye (DiI) (Fig. 2f: left panel). These showed an absence of any virally expressing neurons anywhere near the electrode track within V1. ***[Objective 2]***

Although these preliminary data are promising, we will need to do many more experiments and controls, to convincingly conclude that intercortical communication is vital for the surround suppression in V1. Importantly, given a strong dependency of surround suppression on anesthetic state (Fig. 2g) we will need to supplement these results obtained in anesthetized mice with results obtained in the awake and behaving mouse, which is currently our main aim. ***[Objective 3; Objectives 2 & 3: manuscript in preparation]***

Given their cell-specificity, spatial and temporal precision, optogenetic interventions harbor an important and influential instrument to disentangle the basic processing mechanisms of cortical and subcortical functioning. Moreover clinical use in remediating psychiatric dysfunctions will also be an achievable goal in the coming decennium.

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Figures

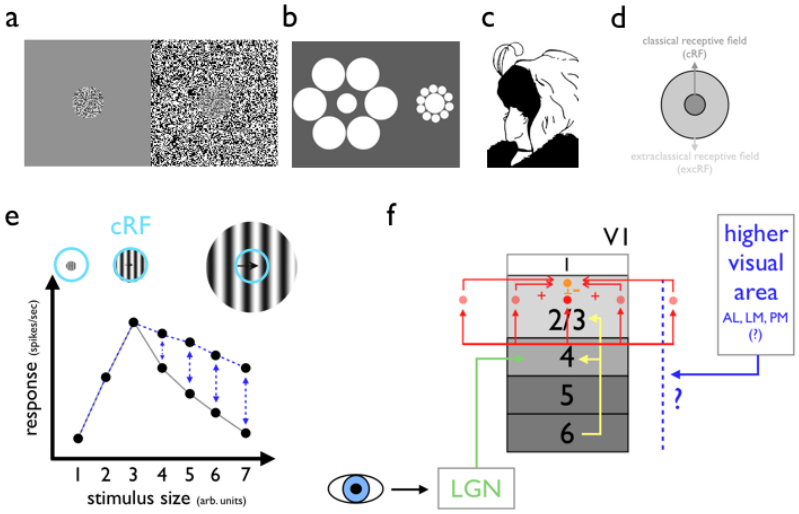

Fig. 1. Perceptual and neuronal accounts of contextual modulations. (*a-c*) Experimental and daily life examples of contextual illusions: changes in perceived (*a*) contrast, Chubb illusion, (*b*) size, Ebbinghaus illusion and (*c*) categorical class membership, multi-interpretable figure old vs yound woman. (*d*) Basic neuronal correlate in which stimulation of the surround (excRF) modulates stimulation within the cRF. (*e*) Surround suppression mechanism (gray lines) and schematized representation of release from surround suppression (striped blue lines) based on our preliminary findings. (*f*) Four potential explanations for surround suppression (each indicated by a different color group). In our proposal we tested the validity of feedback connections (blue schema).

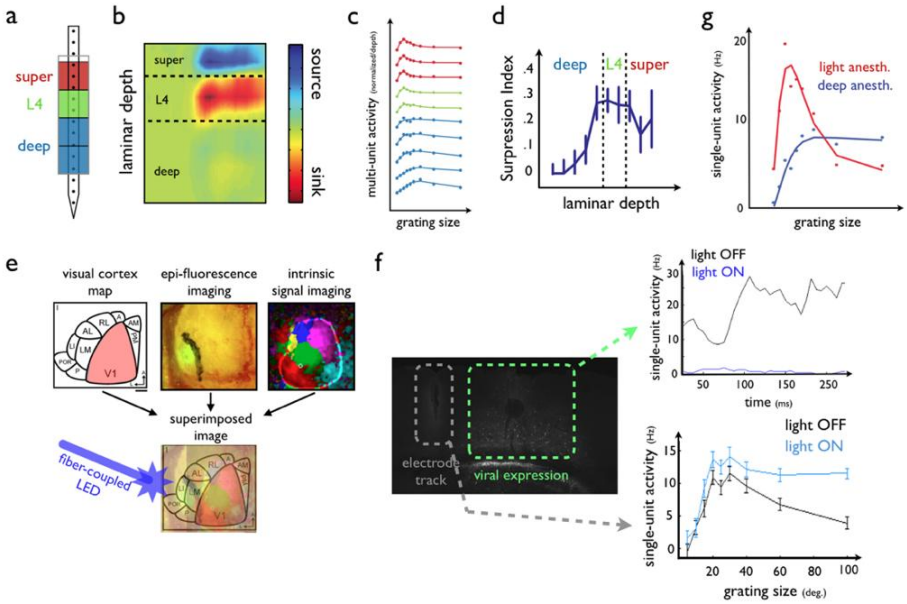

Fig. 2. Preliminary results providing evidence in favor of intercortical communication during surround suppression. (*a-d*) Results from recordings with linear multi-contact electrodes: schematic of the approximate distribution of contact points wrt V1 layers (*a*); current-source density analyses were performed in order to align contact positions across sessions and animals, i.e. initial sink in L4 (*b*); laminar difference in surround suppression (*c* and *d*). (*e*) viral injections of ChR2 were restricted to higher visual areas, guided by epi-fluorescence and transcranial intrinsic signal imaging. Superimposing maps allows to verify the spatial specificity of this local viral approach. (*f*) Preliminary results clearly showing cortical inactivation in the virally transfected patch (green box and upper right panel) and a functional release from inhibition in V1 caused in an indirect manner (gray box and lower right panel). (*g*) The strength of the surround suppression dependent on the depth of anesthesia.