THE CLOËTTA PRIZE 2015
IS AWARDED TO

PROFESSOR

FRITJOF HELMCHEN

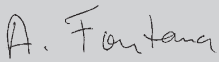
BORN IN 1966 IN TÜBINGEN, GERMANY
PROFESSOR OF NEUROSCIENCES AND
CO-DIRECTOR OF THE BRAIN RESEARCH INSTITUTE
OF THE UNIVERSITY OF ZURICH

FOR HIS OUTSTANDING WORK
IN THE DOMAIN OF NEURONAL NETWORKS

GENEVA, 13 NOVEMBER 2015

IN THE NAME OF THE FOUNDATION BOARD:

THE PRESIDENT



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Prof. Dr. Fritjof Helmchen

BIOGRAPHY

Fritjof Helmchen is Professor of Neuroscience and Co-Director of the Brain Research Institute at the University of Zurich. He was born in 1966 in Tübingen, Germany. He studied physics and the non-clinical part of medicine and received his PhD from the University of Göttingen in 1996 for research that he performed in the department of Bert Sakmann at the Max-Planck Institute for Medical Research in Heidelberg. He then went for 3 years to the Bell Laboratories at Lucent Technologies, New Jersey, where he started to apply two-photon microscopy for in vivo brain imaging as a postdoc with Winfried Denk and David Tank. In 2000 he returned to Heidelberg, where he became a junior group leader at the Max-Planck Institute for Medical Research, further expanding in vivo applications of two-photon calcium imaging. In 2005 he was appointed associate professor at the Medical Faculty of the University of Zurich and in 2013 he was promoted to full professor. Fritjof Helmchen has received several awards, including the Otto-Hahn Medal of the Max-Planck Society, the Emil Du Bois-Reymond Prize of the German Physiological Society in 2003, the Pfizer Research Prize 2011 (with B. Grewe), and most recently an ERC Advanced Grant. His research group is one of very few European labs that so far have received an NIH research grant within the US BRAIN Initiative. He is currently member of the Scientific Advisory Board of the Centre for Integrative Neuroscience, Tübingen, and acts as President of the Betty and David Koetser Foundation of Brain Research, Zurich.

Fritjof Helmchen's research is focused on the advancement and application of optical methods – in particular two-photon microscopy – to study neural activity on the subcellular, cellular, and circuit level. His scientific interest is to understand principles of neural circuit operation and thereby build a bridge in our understanding of brain function from the small scale to the larger scale of brain-wide signal flow. Resolving the heathy brain's working principles he also sees as the basis for examining what goes wrong in various brain diseases. His group has pioneered several microscopy techniques, including 3D two-photon imaging, high-

speed calcium imaging, two-photon microscope miniaturization, and most recently a two-area microscope for the simultaneous measurement of activity in two communicating brain regions. Currently, in most of the ongoing research projects his group studies behaving mice, employing imaging techniques to directly visualize neural activity patterns under relevant behavioral conditions.

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WATCHING BRAIN CELLS IN ACTION: TWO-PHOTON CALCIUM IMAGING OF NEURAL CIRCUIT DYNAMICS

by Fritjof Helmchen

Summary

Brain cells act together to generate the myriad of complex behaviors shown by all creatures while they constantly react and adapt to the challenges in the environment. The networks of excitable neurons in the brain continually generate activity patterns that maintain body functions, process the various sensory inputs, take into account memorized previous experiences, and dynamically coordinate and control muscle activity as needed for specific behaviors. The principles of sensorimotor representations and information processing in neural circuits are still poorly understood, however, largely because in the past methods were scarce for measuring neuronal population activity during behavior. Over the last decade, new opportunities have emerged to investigate neuronal networks in action, most prominently utilizing 2-photon microscopy. Many «activity sensors» are now available, especially fluorescent proteins that have been designed to change their fluorescence emission depending on cell activity. For example, so called «genetically encoded calcium indicators» are now in widespread use. These protein indicators report neuronal activity based on action potential-evoked calcium influx. Long-term expression of genetically-encoded indicators in neuronal populations allows for repeated monitoring of the same cells over weeks and months. In this way both stability and plasticity of functional properties can be investigated longitudinally. Most notably, high-resolution imaging with cellular resolution can be performed in the brain of awake, behaving animals, enabling neuroscientists to «watch» ongoing neuronal network activity while the brain performs a task and to relate network activity patterns to behavioral aspects. In addition, it has become possible to track changes in activity patterns throughout task learning. These advances promise to help reveal principles of neural circuit dynamics underlying sensorimotor processing and learning. In the following, I will summarize our own recent contributions to this rapidly expanding field of neural network imaging, exemplifying the new research opportunities with experimental findings from the mouse brain.

Introduction

The functional organization of the brain is hierarchical, ranging from the molecular and cellular level, to the intermediate level of local neuronal circuits, to the brain-wide communication network of different brain regions established by long-range projection pathways. Local neuronal circuits are highly complex, densely connected networks of excitatory and inhibitory neurons, for instance in a region of neocortex or in a particular deep brain nucleus. Their function cannot be understood in isolation as they typically have numerous short- and long-range connections with other brain regions, for receiving input signals and for transmitting output, respectively. A central idea is that specific computational operations are carried out while signals flow through such local networks. Therefore, uncovering the underlying cellular and molecular mechanisms will be crucial for understanding of information processing in the brain (Harris and Mrsic-Flogel, 2013). However, the complexity of neuronal networks – with regard to both anatomy and physiology – cannot be underestimated. The greatness of the research challenge is illustrated by just considering the very different dynamic brain states under anesthetized and awake conditions.

A first, supposedly simple step is the measurement of neuronal network activity under relevant conditions, i.e., in situations, in which the brain is performing a task, when it is «in action». However, the experimental possibilities for such a phenomenological, descriptive approach have been limited in the past. On the macroscopic level, functional magnetic resonance imaging (fMRI) provides a means to inferactivity of particular regions of the human brain in various situations but it is lacking cellular resolution. On the microscopic level, basic properties of individual cell types and molecular events underlying the communication between neurons have been characterized over the past decades with electrophysiology and high-resolution microscopy. However, these experiments were typically carried out in isolated brain tissue slices or under anesthesia. Electrophysiological recordings, although used for long time to study brain cell activity during wakefulness, only can take limited sample measurements of the activity of a few but not all neurons within a local network. Strikingly, to date it was hardly possible to measure the activity of the

same neurons repeatedly over a long time period to examine functional changes of network dynamics, e.g., during learning. Such direct measurement of activity patterns in local neuronal circuits, in particular in the living brain («in vivo») and over long time periods, would also be of high significance to improve our understanding of malfunctions of neuronal networks during brain diseases.

Monitoring neuronal activity with calcium indicators

Since several decades, neuroscientists have utilized the combination of activity-dependent fluorescent indicators and high-resolution microscopy techniques to visualize dynamic excitation patterns of neuronal cell populations. Until the end of last century most indicators were small organic molecules that change their fluorescence properties in response to membrane potential changes or binding to calcium ions (Grienberger and Konnerth, 2012). Fluorescent calcium indicators are particularly attractive because of the extremely high calcium concentration gradient between extracellular and intracellular space that guarantees a large fluorescence change with high signal-to-background ratio each time an action potential elicits a brief calcium influx. Following the development of 2-photon-microscopy around 1990 (Denk et al., 1990) these sophisticated small molecule indicators enabled high-resolution calcium imaging of neurons in the intact brain (Svoboda et al., 1997). The key advantage of 2-photon microscopy is that it is relatively insensitive to light scattering and therefore allows imaging several hundred microns deep in tissue (Helmchen and Denk, 2005). Staining procedures with these synthetic indicators remained challenging, however, and repeated measurements over several days or longer time periods were nearly impossible.

Shortly after of the cloning and widespread distribution of the green fluorescent protein (GFP) (Tsien, 1998) several groups started to develop activity indicators based on GFP variants. The first genetically-encoded calcium indicator «Cameleon» was reported in 1997 (Miyawaki et al., 1997). It is built from two fluorescent proteins (FPs), linked via a calcium-binding protein domain (calmodulin). Binding of calcium to calmodulin triggers a conformational rearrangement that alters the distance and the orientation of the FPs, which in turn leads to an optically detectable

change of the «Förster resonance energy transfer» (FRET) between the FPs (Figure 1). FRET-based calcium indicators have been varied and improved ever since (Mank et al., 2008a; Horikawa et al., 2010). They form

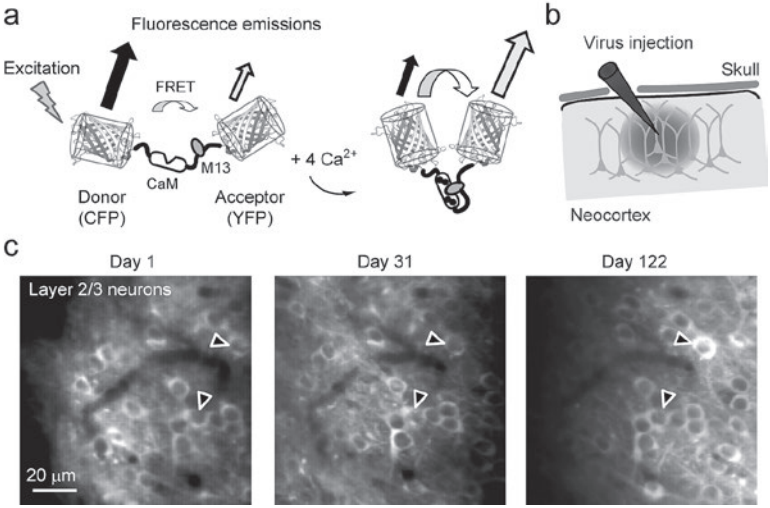

Figure 1: *Expression of genetically encoded calcium indicator in neuronal populations of the mouse neocortex.* ***a:*** *One important class of indicators is based on a distance-dependent energy transfer (so called «Förster resonance energy transfer», FRET) between two coupled fluorescent proteins (here cyan fluorescent protein, CFP, and yellow fluorescent protein, YFP). Binding of calcium ions to the linker-protein calmodulin (CaM) causes interaction with the CaM-binding protein M13, inducing a conformational change that brings the fluorescent proteins closer together. As a consequence, energy transfer from the donor to the acceptor protein increases and the ratio of the fluorescence emission signals at two respective wavelengths changes, indicating calcium influx.* ***b:*** *Virus-mediated expression of genetically encoded calcium indicators. Virus-containing solution (some ten to hundreds nanoliter) are injected into the mouse neocortex through a thin glass pipette. Typically, adeno-associated viruses (AAV) are used, which lead to sufficient expression levels within 2–3 weeks.* ***c:*** *Two-photon microscopic images of neurons in neocortical layer ²/₃ of the mouse brain, taken through a chronic cranial glass window at different time points. The exact same neurons were observed in repeated experiments in anesthetized animals over several months (two examples cells marked). Neurons expressed the calcium indicator YC3.60. Modified from Margolis et al., 2012.*

one important class of protein sensors today. Another class consists of single fluorescent proteins with a built-in calcium-binding domain, so that their fluorescence intensity is modulated depending on the intracel-

lular calcium concentration. The most important members of this group are the so-called GCaMP-proteins, of which a large palette is now available (Nagai et al., 2004; Ohkura et al., 2012; Chen et al., 2013b). The most important features of genetically encoded calcium indicators are their calcium-binding affinity (with typical dissociation constants in the nanomolar range), the dynamic range of attainable fluorescence changes, and their kinetic properties that influence the time course of fluorescence change following calcium influx. For more details, I refer to recent reviews (Grienberger and Konnerth, 2012; Knopfel, 2012).

Unlike classic small-molecule calcium indicators, genetically encoded indicators can be expressed in neurons by various genetic means. The respective genes can be introduced into the organism either by direct gene transfer (e.g. by in utero electroporation in mice), by creating transgenic animals or by using viral vectors. For viral delivery, adeno-associated viruses (AAV) have found widespread application (Dombeck et al., 2007; Lutcke et al., 2010). Appropriate promoters permit neuron-specific expression of indicator genes and selected neuronal subtypes can be targeted using conditional expression methods. Protein concentrations typically increase during the first weeks after virus injection, ideally reaching an expression level that remains stable over several months (Figure 1). As nuclei usually are devoid of indicator protein, cells appear as fluorescent circles in the microscope images. In fact, nuclear fluorescence should remain low as it is an indication of beginning impairment of cell physiology and possibly toxicity, shortening the time window for the experiment.

A critical parameter of genetically encoded calcium indicators is their sensitivity to action-potential evoked calcium signals. Ideally, they should be sensitive enough to report single action potentials in vivo and display little saturation during bursts of action potentials. The sensitivity can be tested in calibration experiments by simultaneous electrical recordings from individual test neurons (Kerr et al., 2005; Grewe et al., 2010; Chen et al., 2013b). Meanwhile, the newest generation of genetically-encoded calcium indicators has reached a sensitivity that is comparable to the best synthetic indicators (Chen et al., 2013b) (Chen et al., 2013a). The combination of high sensitivity and long-term expression makes genetically

encoded calcium indicator the tool of choice for observing and studying neuronal activity patterns in local circuits under various conditions.

Long-term calcium imaging of the same neurons

Repeated monitoring of the activity of individual neurons in local microcircuits over long time periods (weeks to month) is now possible with 2-photon-microscopy. In this approach, often termed «chronic imaging», a small piece of the skull is surgically removed and replaced by a permanent glass window (Holtmaat et al., 2009). The virus injection can also be made during this procedure. Additionally, a small head-post is usually secured to the skull, to enable fixation of the animal's head under the 2-photon microscope (Lutcke et al., 2010). Typically, water-immersion objectives with 16x- to 40x-magnification and a high numerical aperture are used, allowing simultaneous imaging of ten to hundreds of cells within 100-500 mm field-of view. The vasculature system provides ideal landmarks for repeatedly finding the same cells during chronic experiments with the same animal.

Long-term chronic imaging was first used to study morphological changes in vivo, for example changes of synaptic structures following plasticity protocols (Grutzendler et al., 2002; Trachtenberg et al., 2002). Genetically encoded calcium indicators now in addition enable long-term tracking of functional properties of neurons. Of special interest are the study of stability and plasticity of responses to sensory stimuli (such as visual or tactile stimuli) as well as the correlation of cellular activity with processing of sensory stimuli or with movement patterns during behavior.

In a first chronic 2-photon imaging study using a genetically encoded calcium indicator, the same neurons in mouse visual cortex neurons were imaged repeatedly during anesthesia (Mank et al., 2008b). Orientation turning of the same neurons was found to remain stable over the entire experiment period (2–3 weeks), showing for the first time directly that fundamental properties of receptive fields of cortical neurons remain stable over longer time periods. In our own study, we measured neuronal responses in the primary somatosensory cortex (S1) of mouse – in the area corresponding to the facial whiskers, the so-called «barrel cortex»

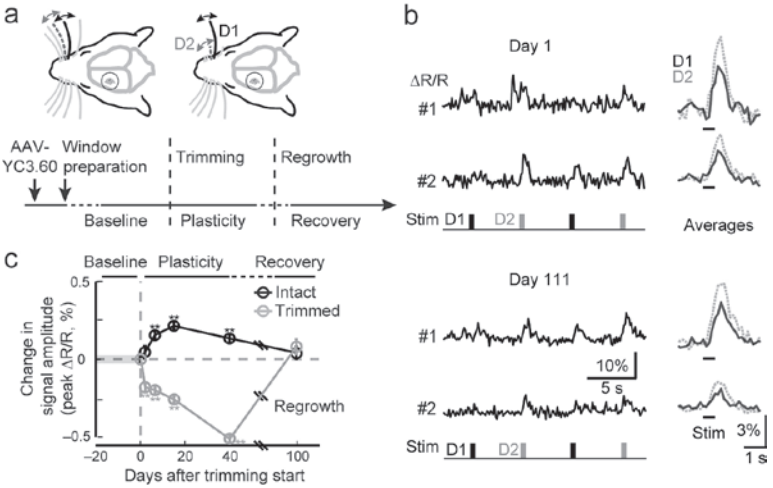

Figure 2: *Chronic imaging of sensory-evoked neuronal activity and reorganization of neuronal activity following sensory deprivation.* ***a:*** *Experimental protocol for studying plasticity of neuronal network activity induced by unilateral trimming of all but one whisker in primary somatosensory cortex of the mouse using YC3.60.* ***b:*** *Example calcium transients of two cortical neurons (those indicated in Fig. 1), evoked by mechanical deflection of the D1 and D2 whisker, respectively (left, raw signals; right, averaged traces). The same cells were measured before, during, and after whisker trimming. Calcium signals shown were measured on day 1 (top) and day 111 (bottom).* ***c:*** *Changes in signal amplitude (mean of all neurons) for the spared (non-trimmed) and the neighboring trimmed whisker over the entire time course of the experiment. Modified from Margolis et al., 2012.*

(Petersen, 2007) – and used tactile stimulation by mechanically moving the whiskers (Margolis et al., 2012). In these experiments, we applied the FRET-indicator Yellow Cameleon 3.60 (YC3.60). Through an implanted cranial window, sensory-evoked activities of S1 neurons in cortical layer $^{2}/_{3}$ were repeatedly measured under anesthesia, first during a baseline period with all whiskers intact, then after all whiskers except for one were trimmed (and re-trimmed over a few weeks), and finally following regrowth of the whiskers (Figure 2). During baseline, activity levels in the neuronal population were quite heterogeneous but the activity distribution across the population remained stable over time (Figure 2). Following trimming, we compared stimulus-evoked calcium signals for the trimmed whiskers and the non-trimmed (spared) whisker. Whisker trimming is a

classical, so-called deprivation protocol that reduces sensory input and thereby induces plastic changes in the brain, especially in the affected neocortical regions. The responses of S1 neurons to stimulation of the spared whisker versus the neighboring trimmed whiskers were monitored for several weeks, which was the first time individual neurons could be followed over long time after induction of plasticity. The results showed that responses evoked by stimulation of the trimmed whisker were generally reduced, whereas stimulation of the spared whisker on average evoked stronger responses, indicating an enhanced representation of this whisker in the cortex (Figure 2). However, changes in responsiveness for the spared whisker were not homogenous across the cell population. While initially low-responsive cells became more active, the activity of formerly high-responders tended to decrease. Further analysis of such redistribution of neuronal population activity, e.g., following changes in sensory inputs or in mouse models of brain diseases like Alzheimer's disease or stroke, may help to reveal principles of functional reorganization of neuronal networks during adaptation and regeneration (Lutcke et al., 2013a; Margolis et al., 2014).

Activity of neocortical projection pathways during behavior

Activity patterns in neuronal networks eventually need to be interpreted within a relevant behavioral context. For experiments in awake, behaving animals, genetically encoded calcium indicators are especially beneficial, not least because they enable repeated calcium-imaging experiments to be performed following the time-consuming habituation of an animal to the experimental setup and the behavioral task training. A prime experimental approach is to habituate mice to tolerate head restraining by fixation of the head with the implanted head-post so that stable imaging can be performed with a 2-photon microscope. The animal can either sit snuggly in a card board tube or can even run on a treadmill, constructed for example from an air-supported free-floating Styrofoam ball (Dombeck et al., 2007). In this setting, the animal can be trained to perform in various sensory detection or discrimination tasks as well as to engage in locomotion behavior. From our and others experience, calcium imaging of individual neurons is still possible even when the animal is running fast on a treadmill, albeit clearly motion artifacts sometimes need to be corrected for.

In our own work, we have established a texture discrimination task, in which the mouse has to discriminate with its whiskers the perceived coarseness of sandpaper panels presented to its whiskers in the dark (Chen et al., 2013a). Using a classic «go/nogo» paradigm, the mouse has to learn to report the detection of a target texture (e.g. the roughest sandpaper) by licking at a water spout, from which it receives a water drop as reward (Figure 3). Mice are motivated to perform this task by keeping them thirsty before they enter the experimental session and they can learn this task within a few days to a week. We first aimed to study in well-trained, expert mice the relationship between neuronal activity in layer $^{2}/_{3}$ of S1 cortex and the whisker touches of the sandpaper textures. We were particularly interested in disentangling different subpopulations within layer $^{2}/_{3}$, since this area is known to give rise to very different long-range projections, in particular to the secondary somatosensory area (S2) and the primary motor cortex area (M1) (Aronoff et al., 2010). We therefore used retrograde tracing techniques to label S2-projecting and M1-projecting neurons in S1, respectively, which turn out to represent fairly separate, non-overlapping, but spatially mingled neuronal pools (Figure 3).

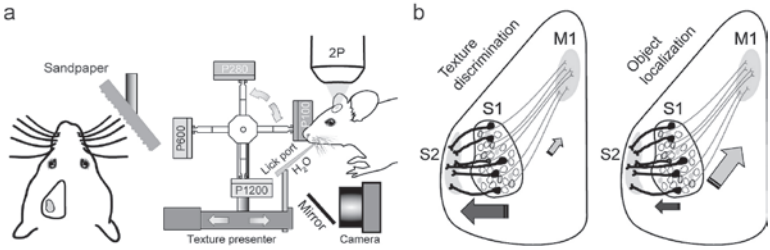

Figure 3: *Calcium imaging of in defined neocortical populations in awake, behaving mice.* ***a:*** *Setup for two-photon (2P) imaging of S1 layer $^{2}/_{3}$ neurons in head-fixed mice performing in a texture discrimination task, in which they had to judge the roughness of sandpapers presented to their facial whiskers. Mice were trained to lick when presented with a target texture (the coarse sandpaper, P100), receiving a water reward for correct «hit» trials) and to withhold licking when presented with one of three non-rewarded, «non-target» textures of increasingly smoother grades (P280, P600, P1200; «correct rejections»). Misses on go trials were not rewarded, and false alarms on no-go trials were punished with a time-out period.* ***b:*** *We discriminated neuronal pools in S1 with different projection targets (either S2 or M1) using retrograde staining techniques. Our results demonstrated that the S1àS2 projections were prominently involved in the texture discrimination task, whereas S1àM1 projection neurons were better in a different, object localization task. Thus, signal flow across neocortical regions is dependent on the specific learned behavior. Modified from Chen et al., 2013a.*

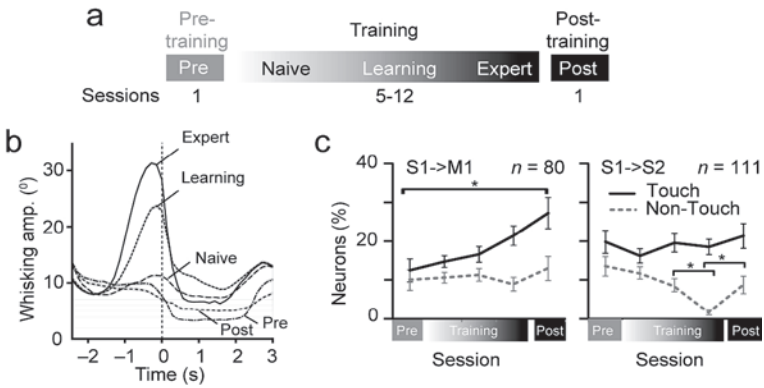

Figure 4: *Learning-related changes in behavior and neuronal activity.* ***a:*** *Experimental time course for chronic imaging during learning of the texture discrimination task. As control layer 2/3 neuronal activity was measured before and after training with passive texture presentations («Pre» and «Post»). The training period was divided into «Naïve», «Learning», and «Expert» phases according to the animal's performance.* ***b:*** *Time course of whisking amplitude aligned to first touch across different training periods. Mice developed a characteristic anticipatory whisking during training. c: Distribution of active neurons across sessions for M1- and S2-projecting neurons (n=7 mice). The number of touch-related S1àM1 projections neurons increased during learning. Although the mean fraction of S1àS2 projecting neurons remained constant, further experiments analysis showed that this pool underwent large reorganization. Moreover, neurons that displayed activity not related to touch were suppressed specifically in expert sessions. Modified from Chen et al., 2015.*

Using 2-photon calcium imaging in S1 while the mouse performed the task, we found that S1àS2 neurons are particularly involved in the texture discrimination task, displaying a high fraction of neurons that are activated during touch and possess higher discrimination power compared to S1àM1 neurons (Chen et al., 2013a). Notably, a different picture emerged when mice were trained in a different task, where they had to judge the localization (rather than the textural quality) of an object with their whiskers. Here, S1àM1 neurons apparently were more prominently involved and encoded object location to a comparably higher degree, in line with other studies (Huber et al., 2012) (Xu et al., 2012). We concluded from this study that the information flow between cortical regions, the strength and content conveyed along distinct cortico-cortical pathways, depends on the particular behavior (Figure 3), presumably adjusting according to the computational needs to solve the task.

Learning-related changes in neuronal pathway activity

The findings described in the previous section indicate that neuronal network activity largely depends on the environmental situation and the behavioral circumstances. This means that neocortical signal flow will need to adjust when an animal encounters a new situation, when it learns how to solve a new problem, in order to adequately process sensory information. Chronic 2-photon calcium imaging is perfectly suited to study such modifications in neural circuit dynamics that are associated with learning. In follow-up study we therefore investigated how the activity patterns in the two distinct S1àS2 and S1àM1 pathways adapt during learning of the texture discrimination task (Chen et al., 2015). First of all, we found that mice typically develop a behavioral strategy that apparently helps them to solve the task: over time they showed increased «active whisking» (back-and-forth movements of whiskers) in anticipation of the arriving sandpaper panel (auditory cues indicated the arrival time) (Figure 4). We could show that this active whisking strategy enhances texture-related whisker bending features and thereby improves performance in the task.

When comparing the activity of identified S1àS2 and S1àM1 projection neurons in S1, we found different alterations during the learning phase (Chen et al., 2015). We observed an increase in the mean fraction of touch-related active M1-projecting neurons (Figure 4), as a result of recruitment of further neurons. This result indicates a robust representation of touch events in the S1àM1 neuronal pool and a strengthening of transmission of this sensory representation to frontal motor areas. In contrast, the mean fraction of S2-projecting neurons remained constant but a more detailed analysis of the distribution of active neurons revealed that a larger reorganization happened in this S1àS2 pool, with some neurons becoming silent and others being newly recruited (Figure 4). In addition, the ability to discriminate between the behaviorally relevant trial types («Hit» for the target texture, «Correct reject» for the non-target texture) increased during the learning phase particularly strongly in neurons of the S1àS2 pool. Moreover, in this pool we observed a suppression of non-touch related active neurons only when mice were engaged in performing the task (Figure 4). In conclusion, we propose that these changes in neu-

ronal pathway activity relate to procedural learning, in which a percept becomes linked to an action (here licking). The modifications in S1 presumably serve to both route specific information to downstream areas and refine sensory representations in specific pathways that are relevant for executing behavior. Apart from elucidating signal processing in the sensory cortex, these experiments highlight the most significant potential of genetically encoded calcium indicators, namely the possibility to reveal changes in neuronal dynamics over long time periods during learning.

Conclusions and outlook

The development of genetically encoded calcium indicators and their combination with 2-photon microscopy have opened many new possibilities to study neuronal network activity, especially under behaviorally relevant conditions when the examined networks are in action. These new experimental opportunities may provide fundamental insights into the operation of local microcircuits and they can also help to bridge the levels of single-cell integration and of macroscopic network activity in connected brain regions. Further development of protein-based indicators is currently ongoing and additional improved indicators, not only for calcium but also for membrane potential can be expected soon. Besides virus injections, other means of inducing calcium indicator expression are emerging. For example, new transgenic mouse lines have been generated, which allow to induce expression of the best available genetically encoded calcium indicator, if needed only during adulthood and in a cell-type specific manner (Madisen et al., 2015). In addition to discriminating distinct pools of neurons with different projection patterns, as in our studies, further dissection of the different neuronal subtypes is an important goal. In this respect, functional characterization of the diverse subtypes of inhibitory interneurons, which presumably are highly relevant for local signal processing, is of particular importance (Kepecs and Fishell, 2014). Finally, calcium indicators are also suitable for studying the activity of non-neuronal cells, e.g., astrocytes and microglial cells, for which it is still poorly understood how they interact with their neighboring neurons.

Imaging methods also experience continual further development. One major goal is to improve the temporal resolution of fluorescence mea-

surements, in order to infer action potential patterns in neuronal network with even higher temporal precision (Grewe et al., 2010; Lutcke et al., 2013b). Minimization or compensation of motion artifacts, especially in awake, behaving animals, will be crucial to achieve this goal. Moreover, imaging of ever-larger neuronal populations (and at greater imaging depths) is desirable to obtain an as-complete-as-possible view of neuronal network dynamics. Currently, the modest state of research allows functional imaging of a few hundred neurons, which is obviously a tiny fraction of the total number of neurons presumably involved in a particular behavior (in one cubic millimeter there are on the order of 100 000 neurons). Within the next few years, it seems feasible to achieve in vivo imaging of several thousand cells, albeit probably at relatively low acquisition rates. Such experiments should give us further insights into the principle features of network activity patterns. Furthermore, recently developed methods for visualizing deeper brain structures with miniaturized endoscopic optics, even in freely behaving animals, will be further optimized.

Finally, in future studies we plan to move beyond purely descriptive studies in order to attain a deeper understanding of neuronal circuit function. New anatomical methods promise to reveal further and further structural details of the connectivity in neuronal networks (Denk et al., 2012; Oh et al., 2014), which should allow us to correlate structural aspects of neuronal wiring with functional microcircuit operation. Combining functional imaging with novel optogenetic (Fenno et al., 2011) or chemogenetic (Sternson and Roth, 2014) methods that allow for specific manipulations such as activating or silencing a particular neuronal pathway using light or specially-designed drugs, should help to move beyond a purely correlative description and elucidate causal relations between network activity and behavior.

Acknowledgements

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