What's in a circuit?

It seems we are in the age of "circuit research". There is hardly any issue of *Nature* or *Science* where there is not a novel circuit described. Curiously, often with very little morphology – it thus misses a "face" and is hard to grasp and remember. In 2011, the famous Göttingen Nobel laureate Bert Sakmann gave a talk at the 10-years ENI-G symposium entitled "Cortical column: if you don't understand the function, do the structure". In fact, this carries the essence of what drove me into neuroanatomy: the realization of deep scientific satisfaction to uncover the wiring of a part of the brain as complex and undoubtedly important as a cortical column. For this study, we have chosen the mouse barrel cortex (Feldmeyer et al., 2013).

So what's in a circuit? Neurons, of course! Well, what kind of neurons? Many different types! But what is a type? Now it is getting difficult. Ask any two scientists what they believe a cell type is and how it should be defined, and you probably get three answers. So, what I am going to elaborate on are three major questions, which drive the experimental work that we are doing at the Institute for Neuroanatomy: (1) what cell types do really exist, (2) how do they wire up to form a functional circuit, and (3) how does this bewildering complexity all come about during development?

What is the definition of a cell type? In the cortex, neurons can be distinguished into excitatory glutamatergic (ca. 80-85%) and inhibitory GABAergic cells (ca. 15-20%). Some scientists say that excitatory cells come in one flavour, i.e. the pyramidal cell (and its small siblings, the star pyramidal and the spiny stellate cell), some scientists say there are over 2000 (!) types (Sorensen et al., 2015). Some scientists say that inhibitory neurons come in (at least) 6 flavours (Staiger et al., 2015) whereas others have claimed that each inhibitory neuron is its own type (Parra et al., 1998). In fact, whereas the experts could not agree on a specific terminology for cell types (Ascoli et al., 2008), at least they agree that GABAergic neurons can be subdivided into 3 broad non-overlapping classes, as defined by neurochemistry (Rudy et al., 2011; Figure 1). We want to contribute to the definition of cell types by quantitatively studying their morphology, electrophysiology, molecular make-up (including neurochemistry) and precise input-output description by using appropriate Cre-driver lines, whole cell recording, biocytin filling, Neurolucida reconstruction, immunostaining and anterograde as well as retrograde tracing (Prönneke et al., 2015). We are part of the Petilla initiative on interneuron nomenclature and the Big Neuron initiative of the Allen Brain Institute.

How do neurons wire up to form a functional circuit? For us, the functional circuit that mediates the conscious perception of touch is at the core of our research. Which cells in which layers connected by how many synapses where on the dendritic tree need to integrate which number of EPSPs (excitatory post-synaptic potentials) and IPSPs (inhibitory post-synaptic potentials) in what temporal sequence to tell the animal that a whisker has touched an object in the outside world? Ideally, we would deflect a whisker of an awake behaving mouse with threshold stimuli and the mouse has to report the presence of such stimuli by licking a water reward spout (Sachidhanandam et al., 2013). This input would drive an immediate early gene, such as c-Fos, to which a sensitive fluorescent reporter is coupled and we could image the cell assembly labelled in a Golgi-like manner, either in a two-photon set-up (that we are just about to start using), or after perfusion and histology, in a confocal laser scanning micro-

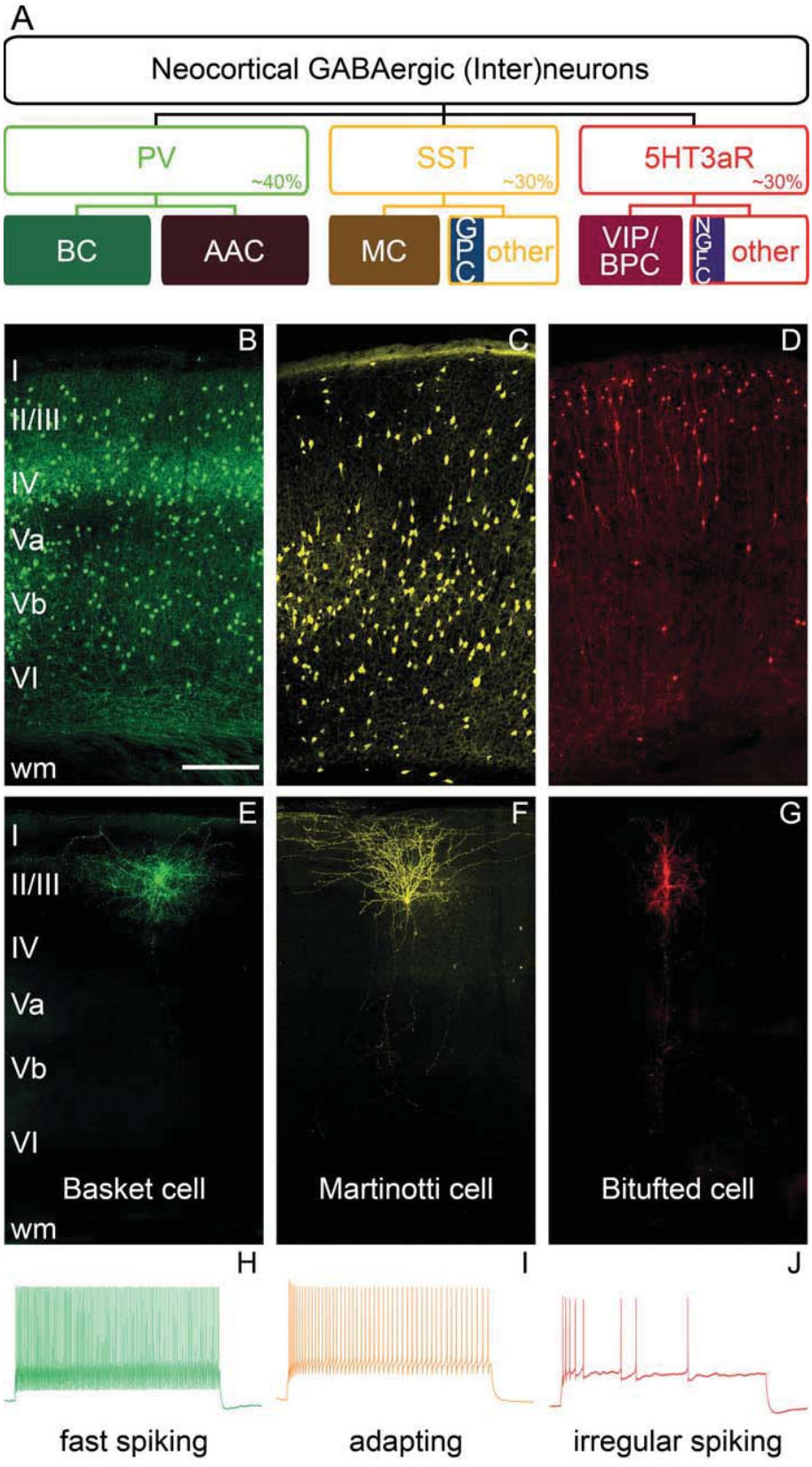
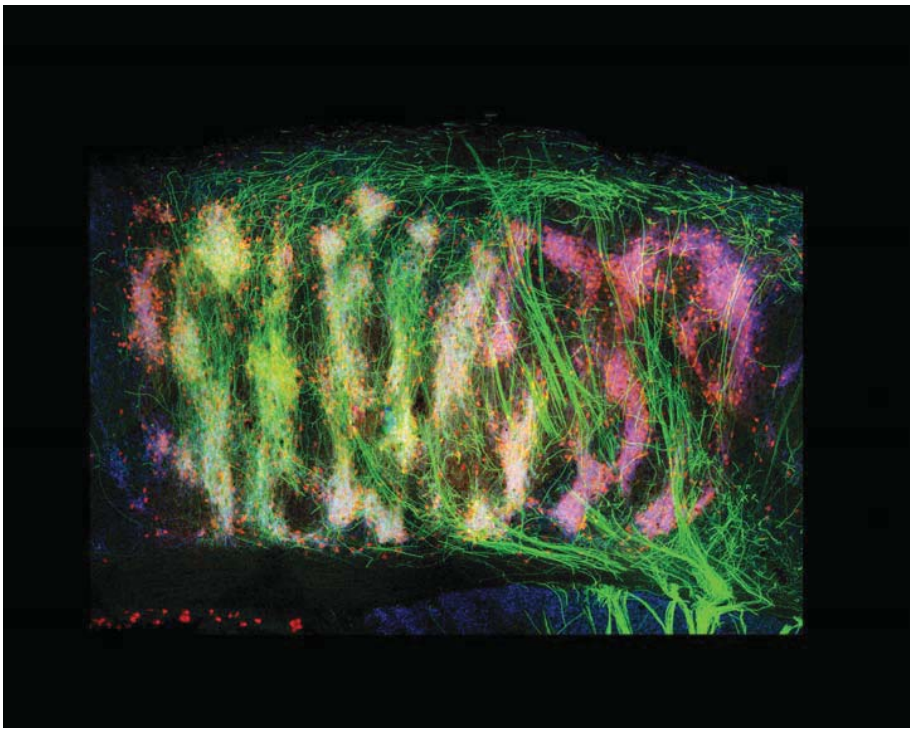


Figure 1 (left): Classification of three major neurochemically-defined GABAergic neuronal populations and their basic morphological and electrophysiological properties in the barrel cortex.
(A) Parcellation of neocortical GABAergic neurons based on molecular markers (BC, basket cell; AAC, axo-axonic cell; MC, Martinotti cells; GPC, GABAergic projection cell; BPC, bipolar-bitufted cell; NGFC, neurogliaform cell). (B) Low-magnification image of a PV-Cre section (PV, parvalbumin; green), which makes it clear that PV cells (that should be mostly basket cells in this stain; see Figure 2E) have a preferential localization in layers IV and Vb. (C) Low-magnification image of a SSTcre section (yellow), showing that SST cells (that should be mostly Martinotti cells in this stain; see Fig. 2F) have a preferential localization in layers V and VI. (D) Low-magnification image of a VIP-Cre section (VIP, vasoactive intestinal peptide; red), demonstrating that VIP cells (that should be mostly bipolar/bitufted cells in this stain; see Fig. 2G) have a preferential location in layer II/III. Roman numerals indicate cortical layers; scale bar, 200 µm. (E-G)Photoreconstructions, all in layer II/III, of a PV-expressing, fast-spiking basket cell (E), a somatostatin-expressing, adapting Martinotti cell (F), and a VIP-expressing irregular-spiking bitufted cell (G). Note the varicosities of the basket cell in L I, which are dendritic and not axonal. Also note that the axonal arbor of the Martinotti cell in L I is truncated due to restrictions of figure size but extends over more than 2 mm tangentially below the pia. (H-J) One of the typical action potential firing patterns upon strong depolarizing current injections via whole cell patch clamp electrodes during continuous fast-spiking (H), continuous adapting (I), and irregular-spiking (J).

Figure 2 (below): Lemniscal thalamic fibers in the primary somatosensory "barrel" cortex of the reeler mouse.
The ventral posteromedial nucleus of the thalamus was stereotactically injected with a GFP expressing viral vector (AAV2/6 eGFP). Thus, thalamocortical axons (TCAs) anterogradely transported GFP (green fibers). TCAs first run up to the pial surface, reverse, and form terminal fields at different (vertical) levels of the cortex. TdTomato is expressed under the layer IV-specific Scnn1a promoter. The red neurons represent ectopic layer IV neurons in the disorganized reeler cortex. Interestingly, the terminal fields of TCAs overlap with the ectopic clusters of layer IV neurons, indicating that the thalamus in the disorganized cortex still targets its native input compartment (i.e. layer IV neurons). Moreover, lemniscal synapses, visualized by immunostaining for vGluT2 (blue staining), are concentrated in these overlapping spots.



scope (where we are producing very large high-resolution-at-large-field-of-view images; Figure 2). Since this circuit visualization method is not established yet, we will do it the hard way: two-photon-targeted patch clamp recordings of single neurons of interest while the animals are receiving whisker stimuli. Let's see whether we can get it to work until end of 2017!
How does this all come about during development? Well, "adult" scientists often escape into "development" when they are scared about the complexity of a fully grown brain because they hope development will present them with versions that can be understood more easily. What a big illusion! Basically, the molecular complexity and the fast pace of moment-to-moment changes is as formidable a task to dissect and understand as any other scientific question in the adult nervous system. But certainly a nice aspect of development is its in-build function, i.e. to build a functional adult nervous system. Thus, we started to work on reeler mutant mice which were thought to have an inverted cortex due to a defect in reelin secretion by Cajal-Retzius cells (D'Arcangelo, 2014). However, we found that the cortex of reeler mice is completely scrambled and no layers are left. This is highly surprising, considering that (apart from the strong motor phenotype coming from the cerebellar hypoplasia) these animals survive well, get old, can learn, and are highly plastic (Pielecka-Fortuna et al., 2015). Moreover, cortical columns do form, thus implying that the local circuitry as well as the long-range connections are preserved (Wagener et al., 2016; Figure 2). How this massive plasticity is achieved during development is one of our main future questions.

Prof. Dr. Jochen Staiger

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