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A novel network of multipolar bursting interneurons in the neocortex

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GABAergic interneurons can phase the output of principal cells giving rise to oscillatory activity in different frequency bands. Using transgenic mice in which subsets of interneurons are labeled with the *in vivo* marker EGFP, we describe a new subtype of GABAergic interneuron, the multipolar bursting cell (MB cell) in layer 2/3 of the mouse neocortex. In the first part of the study (1) we described MB cells at the cellular level and studied the cell properties with respect to electrophysiological characteristics, morphology, postsynaptic target distribution and expression of neurochemical markers. In the second part of the study (2) we analyzed the synaptic connectivity and the different forms of synaptic plasticity exhibited by MB cells. In the third part of the study (3) we examined the functions of MB cells at the network level. The main findings can be summarized as follows:

1.) MB cells are parvalbumin (PV)-positive but differ from the fast-spiking (FS) multipolar cells, the predominant cortical PV-positive interneurons, in their morphological, neurochemical and physiological properties. Thus, in comparison with FS cells, MB cells have a different firing pattern of action potentials upon current injection, they have wider axonal and dendritic projections and they are dendritic inhibitory cells in contrast to FS cells, that exhibit a perisomatic or axo-axonic target distribution. In addition to the

slow Ca^{2+} buffer PV, MB cells express the fast Ca^{2+} buffer calbindin (CB). Thus, MB cells form a so far undescribed distinct and homogenous interneuron population.

2.) Whole cell recordings from connected cell pairs showed that MB cells are reciprocally connected with layer 2/3 pyramidal cells and are coupled with each other by chemical and electrical synapses. Notably, MB cells innervate FS cells but not vice versa. Both MB to MB cell as well as MB to pyramidal cell synapses exhibit pronounced paired pulse facilitation (PPF). More thorough characterization of PPF in MB to pyramidal cell terminals revealed that Ca^{2+} buffer saturation, a novel mechanism of short-term synaptic enhancement, underlies PPF in these terminals. Ca^{2+} buffer saturation was proposed to be one of the mechanisms of synaptic facilitation. However, whether this mechanism operates under native conditions remained unclear. Here we show that saturation of the endogenous fast Ca^{2+} buffer CB plays a major role in PPF at CB containing synapses. Paired recordings from synaptically connected MB cells and pyramidal cells revealed that dialysis increased the amplitude of the first response and decreased PPF. Loading the presynaptic terminals with BAPTA or CB rescued the effect of the CB washout. The effects of different extracellular Ca^{2+} concentrations and of EGTA indicated that PPF in CB containing terminals depended on Ca^{2+} influx rather than on the initial release probability. To strengthen the causal role of CB for this form of PPF, we extended the study to the facilitating excitatory mossy fiber - CA3 pyramidal cell synapse, known to also express CB. Indeed, Ca^{2+} buffer saturation was found to be the mechanism of PPF at this synapse as well. Finally, experiments in CB knockout mice confirmed our hypothesis that saturation of endogenous Ca^{2+} buffers is a novel basic presynaptic mechanism for activity-dependent control of synaptic gain.

3.) Application of carbachol selectively induced synchronized theta frequency oscillations in MB cells, but not in FS cells. Synchrony required both gap junction coupling and GABAergic chemical transmission between MB cells, but not excitatory glutamatergic input. Most importantly, the MB cell network was capable of shaping the activity of layer 2/3 pyramidal cells. Hence, MB cells form a distinct inhibitory network, which upon cholinergic drive can generate rhythmic and synchronous theta frequency activity providing temporal coordination of principal cell output.

In summary, using a multidisciplinary approach – transgenic techniques, patch-clamp recording, immunocytochemistry and electronmicroscopy – we identified a novel cell type in the neocortex, which we characterized at the cellular and system level.