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CHAPTER 15

Role of synaptic inhibition in spatiotemporal patterning of cortical activity

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Abstract: Developmental upregulation of the $GABA_A$ receptor α1 subunit causes a faster decay of GABAergic inhibitory postsynaptic currents (IPSCs) in the visual cortex around the time of eye opening. In α1 deficient mice, a juvenile type of $GABA_A$ receptors is retained during maturation. As a result the decay time of the IPSCs is longer in α1-/- mice than in WT mice during the whole life span of the mice. Hence they form a valuable mouse model for studies on cellular aspects of neuronal network functioning. Using voltage sensitive dye imaging methods, we monitored the spatiotemporal excitation patterning in visual cortex slices upon local stimulation of the network. We found that in the α1-/- mice, the ability of the network to fire synchronously at γ-frequencies (20–50 Hz) is diminished. This finding indicates that early onset of GABA synapse maturation is required for the normal neuronal network function in the maturating visual cortex.

Introduction

GABA synapse maturation and oscillations

GABA synapses during neonatal development display a remarkable functional maturation around the time of eye opening, since a robust shortening in the τ_{decay} of IPSCs occurs during early postnatal development. This change affects the ionic postsynaptic current density at individual synapses on neocortical cells, and thus may be regarded as an important regulator of the efficacy of synaptic inhibition around that time. In order to assess the functional significance of this developmental property of the neocortex beyond the level of individual synapses, we tested to what extent long range gamma band oscillatory neuronal network activity was affected in a mutant mouse model, in which the normal maturation of the GABA synapse was retained. For these experiments we used the α1-/- mice generated by Sur et al. (2001), and previously tested by us (Bosman et al., 2002; Heinen et al., 2003, 2004).

The rationale to test the effect of GABA synapse maturation on gamma band oscillations is as follows. Upon conscious perception of sensory stimuli, cortical networks fire in synchrony at γ-frequencies (20–50 Hz). These γ-oscillations, which have been demonstrated by EEG studies on humans and animals, can be evoked in tissue slices of, e.g., hippocampus and cortex (Fisahn et al., 1998). In the cortex, it has been shown that gap junctions, in concert with fast glutamatergic signalling, stimulate the postsynaptic neurons, whereas GABAergic synapses prevent irregular firing (Whittington et al., 1995; Traub et al., 1996, 2000, 2003; Fuchs et al., 2001; Hormuzdi et al., 2001). Moreover, pharmacological enhancement of GABAergic IPSCs has been shown

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to reduce the ability of the network to fire at γ-frequencies (Tamás et al., 2000; Bartos et al., 2002). Hence we hypothesized that the prolonged GABAergic IPSCs in the juvenile brain might have a significant effect on the occurrence of γ-oscillations.

We performed voltage sensitive dye imaging experiments of WT versus α1-/- neocortical tissue slices. The network behavior in these slices was activated using electrical stimulation at defined frequencies and was monitored by following the spatiotemporal kinetics of the neuronal network responses. We expected to see robust effects of genetically altered $GABA_A$ receptor kinetics on the spatiotemporal dynamics in the neocortex, given the fact that frequency dependent spatial organization of neocortex activation was previously reported to be sculpted by local inhibitory network processing (Contreras and Llinás, 2001).

Results

Voltage sensitive dye imaging in neocortex

We made photodiode recordings of postnatal day 21–28 visual cortex slices loaded with voltage-sensitive dye (RH 414) during electrical stimulation of the white matter. As shown previously (Contreras and Llinás, 2001), optical responses in these experiments are primarily generated by synaptic activation. Electrical stimulation of the white matter underlying the cortical mantle excites both cortical afferent (primarily consisting of cortico-cortical and thalamo-cortical fibers) and efferent fibers. Thus, optical responses may result from antidromic or orthodromic (monosynaptic and/or polysynaptic) activation. On occasion such electrical stimuli may also directly activate cells in layer 6 and the basal dendrites of layer 5 cells.

In our experiments, a concentric electrode delivered stimuli (1 ms in duration) to the white matter, just beneath layer VI. This caused a robust excitation that spread over the visual cortex towards layer I. This signal was measured as a change in the fluorescence of the voltage sensitive dye (Fig. 1A, example recording). The fluorescence signal corresponded to the change in field potential (Fig. 1B, example recording, see legend for details).

We gave pulse trains of 5 stimuli (each 1 ms in duration) at either 10 or 40 Hz. All data obtained were the averages of 64 recordings, obtained with repetition time of 10 s. We analyzed the optical signal (i.e., network excitation) along an imaginary line from the stimulation site to the apex. This "apical spread" of this excitation in the cortex slices, as judged by the amplitude of the first peak (i.e., maximal amplitude after first stimulus), was fairly uniform. During the 10 Hz pulse trains of 5 stimuli,

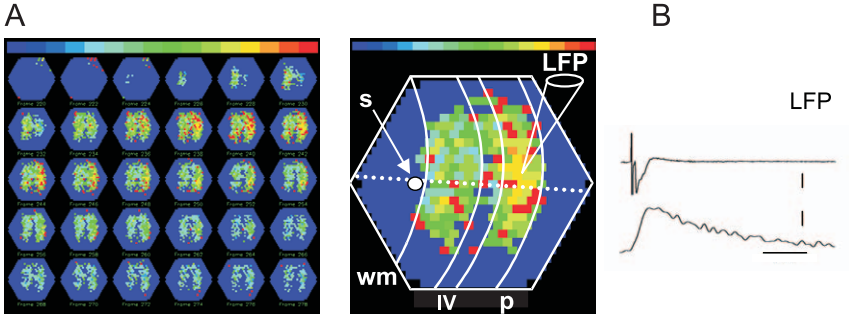

Fig. 1. Voltage-sensitive dye imaging: frequency-dependent spread of excitation signal in apical direction. (A) Photodiode recording of a visual cortex slice loaded with voltage-sensitive dye. Left: time lapse recording of the changes in fluorescence upon electrical stimulation. The stimulation was given to the white matter between the second and the third frame, after which the signal can be seen spreading towards the apex. The interval between each frame is 0.614 ms, shown here is every second frame starting in the left upper corner with frame "220." Electrical stimulation occurred between frame 222 and 224. Maximal activation of an excitation wave was typically reached within 10 ms and the optical signal had a clear decay after 20 frames (i.e., 24 ms or longer). One frame (242 at second row, fifth image) is enlarged (right) to show the spatial profile in detail. (B) Comparison of field potential recording (in apical area of the cortex; upper graph) and the optical signal (lower graph). Calibration: 20 ms vs. 1 mV and 1% $\Delta F/F$.

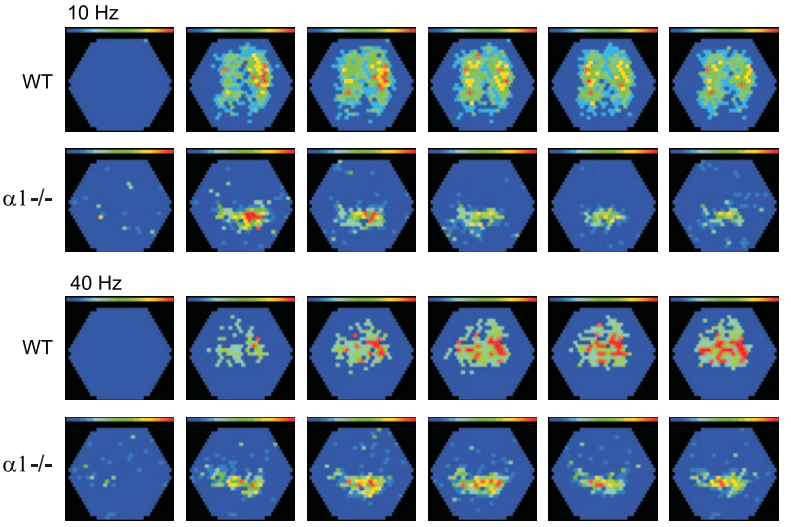

Fig. 2. Voltage-sensitive dye imaging: frequency-dependent horizontal spread of excitation signal. Photodiode recording of a voltage-sensitive dye loaded cortex slice upon electrical stimulation in the white matter. Recordings before stimulation (left) and of the peak (i.e., maximal amplitude) optical signal following each one of five stimuli are shown. Stimulations were given at either 10 or 40 Hz. Shown here are averages of 64 stimulations, taken with a repetition interval of 10 s.

the signal diminished (Fig. 2, $n \geq 7$). This phenomenon was more pronounced in the α1-/- slices than in the WT slices. On the opposite, during the 40 Hz trains, the signal increased (Fig. 2). This was unambiguous in the WT slices. In the α1-/- slices, the signal increased initially, but then falls below control levels.

Thus, with the use of a photodiode array we recorded the spread of an excitation in the visual cortex. In WT slices, the consecutive excitations diminished during a 10 Hz pulse train of 5 stimuli, but clearly increased at 40 Hz. Given the decay of the optical signal shown in Fig. 1B, this was to be expected. Apparently, the cortical network is able to recruit an increasing number of postsynaptic elements during the 40 Hz pulse train. The α1-/- slices also showed a decrease in signal at 10 Hz, even stronger than the WT slices. However, in contrast to the WT slices, the neuronal network in slices from mutant mice could not sustain adequate excitation amplitudes during the 40 Hz pulse train. Thus, although, like the WT, mutants show an initial increase in the amplitude of the signal, this rapidly deteriorated.

Next, it is noteworthy to mention that the size of the spatial area of the excitation is also affected in these recordings (Fig. 2). When a diode recorded a optical signal with an amplitude of more than four times the prestimulation baseline noise, it may be marked as an "active" diode. Since each diode records the optical signal of a certain area within the slice (in our setup, approximately 40 μm^2), the number of active diodes is a measure of the excited area. The WT showed a larger excitation area than the α1-/- slices (Fig. 2), both at 10 and at 40 Hz.

Discussion

Functional significance

When the WT slices were stimulated at 10 Hz, the consecutive excitation waves showed a decline, as can be observed from the amplitude of the optical signal. In contrast, when stimulated at 40 Hz, it showed an increase. This indicates that in the cortical network summation of excitation occurs during a 40 Hz pulse train, but not at a lower frequency. In the α1-/- mice, the 40 Hz stimulation did not show this temporal summation, which indicates that the deletion of α1 may have interfered with the ability to fire at γ-like frequencies. Therefore at this point it seems justified to conclude that functional deletion of α1 at a crucial

moment of the development (i.e., before the time of eye opening) affects neuronal network processing later on, via altering the local inhibitory microcircuitry.

Why is correct timing of the GABA-synapse dependent oscillatory activity important for the maturation of the neocortex? Before eye opening, input via the retina is mainly spontaneous and random (Shatz, 1996; Katz, 1999). If one accepts the idea that through the Hebbian postulate of fire-together-wire-together, neuronal networks may be strengthened by the synchronous γ-oscillations (see for instance Bailey et al. (2000)), we would argue that in neocortex slow decaying IPSCs are there to inhibit γ-oscillations at an age at which they have no meaning, i.e., before eye opening. In contrast after eye opening *y*-band oscillations may positively affect the consolidation of appropriate synapses, thereby contributing to the fine-tuning of the correct wiring diagram of both the inhibitory as well as the excitatory neuronal network of the visual cortex.

Abbreviations

IPSCs	inhibitory postsynaptic currents
GABA	γ-aminobutyric acid

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