GABAergic inhibition shapes frequency adaptation of cortical activity

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Tim S. Heistek¹, Johannes C. Lodder¹, Arjen B. Brussaard¹, Laurens W.J. Bosman¹,² and Huibert D. Mansvelder¹
GABAergic inhibition shapes frequency adaptation of cortical activity in a frequency-dependent manner
¹Department of Integrative Neurophysiology, Center for Neurogenomics and Cognitive Research (CNCR), Neuroscience Campus Amsterdam, VU University, The Netherlands, ²Department of Neuroscience, Erasmus Medical Center, Rotterdam, The Netherlands

Primary sensory cortical areas continuously receive thalamic inputs that arrive at different frequencies depending on the amount sensory activity. The cortical response to repeated sensory stimuli rapidly adapts and different frequencies recruit cortical neuronal networks to different extent. GABAergic inhibition limits the spread of excitation within cortical neuronal networks. However, it is unknown how frequency adaptation of cortical network activity at different frequencies is shaped by GABAergic inhibition. Here, we find that in acute slices of visual cortex area V1 GABAergic inhibition affects frequency adaptation depending on the frequency of activity. Using voltage-sensitive dye imaging, we found that while increasing inhibitory postsynaptic currents (IPSCs) with flunitrazepam dampened the spread of cortical excitation, short-term adaptations to different stimulation frequencies were differentially affected. At high frequencies (40 Hz), facilitation of cortical excitation was no longer transient, but facilitation was sustained. At low frequencies (10 Hz) flunitrazepam decreased a depression of the excitation. In contrast, in mice lacking the GABA_A receptor α1 subunit facilitation was reduced and depression enhanced. These findings suggest that GABAergic inhibition affects cortical excitation at different frequencies differentially, favoring facilitation at higher frequencies of excitation.
Introduction

Sensory information reaches the cortex via convergent input from the thalamus (Reid and Alonso, 1996, Metherate and Cruikshank, 1999, Bruno and Sakmann, 2006). Although the main target of thalamocortical afferents is formed by the glutamatergic spiny stellate cells of layer IV (Wilson and Cragg, 1967, Hubel and Wiesel, 1972, LeVay and Gilbert, 1976), they also form strong connections onto pyramidal cells (Freund et al., 1985) and GABAergic interneurons (Gibson et al., 1999, Swadlow and Gusev, 2001). In addition, several types of GABAergic interneurons control corticocortical neurotransmission (Gupta et al., 2000). Thus, a complex network exists of recurrent excitation balanced by feedforward and feedback inhibition.

The spread of excitatory responses is limited by GABAergic inhibition (Contreras and Llinas, 2001). In the absence of inhibition excitation travels through the cortex in spiral waves (Huang et al., 2004). However GABAergic inhibition is also involved in synchronization of rhythmic activity (Whittington et al., 1995, Tamás et al., 2000, Wendling et al., 2002, Cunningham et al., 2004). Application of zolpidem, routinely used for the treatment of insomnia (Nowell et al., 1997), enhances γ band activity in the hippocampus by strengthening GABAergic inhibition (Palhalmi et al., 2004).

Previous studies indicated that white matter stimulation induces frequency-dependent network adaptations in the sensory cortex (Contreras and Llinas, 2001, Bosman et al., 2005a). Our own work indicates that also changes in inhibition may account for a frequency-dependent response of the sensory cortex to white matter stimulation (Bosman et al., 2005a). In this study, we further investigated the role of GABAergic inhibition in the spread of neuronal excitation at different stimulation frequencies over the visual cortex. Using bidirectional manipulation of GABAergic inhibition we find that inhibition affects facilitation and depression during stimulus trains in a frequency-dependent manner. With increased inhibition, facilitation is more pronounced at high frequencies, while decreasing GABAergic inhibition reduces facilitation. At lower stimulation frequencies depression is reduced with enhanced inhibition, and increased with less inhibition. These effects are most pronounced in the deeper layers of the visual cortex.

Materials and Methods

Tissue preparation

All experimental methods involving animals were approved by the animal welfare committee of our university, as required by Dutch law. We used C57Bl6 mice for voltage sensitive dye experiments and Wistar rats for whole cell recordings. Mice lacking the α1 subunit of the GABA_A receptor were in a mixed 50% C57BL6-50% 129SvEv background, as previously described (Sur et al. 2001). The wild-type control mice were also a cross between C57BL6 and 129SvEv. All animals were between 21 and 26 days old. Prior to the experiment, the animals were decapitated, their brains quickly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF, in mM: 125 NaCl, 25 NaHCO3, 3 KCl, 1.2
NaH$_2$PO$_4$, 2 CaCl$_2$, 2 MgSO$_4$, 10 D(+)-glucose (carboxygenated with 5% CO$_2$ / 95% O$_2$, 304 mosmol, pH 7.4)). Coronal sections (400 μm thick) of the visual cortex were cut using a VT1000S vibratome slicer (Leica, Wetzlar, Germany). Slices were stored at room temperature for up to 8 hours in continuously carboxygenated ACSF.

Voltage-sensitive dye recordings and electrophysiology
Cortical slices were incubated for 30 minutes in dye solution containing 200 μM Di-4-ANEPPS (Invitrogen, Carlsbad, CA) in 2.7% ethanol, 0.13% Cremaphor EL (Sigma, Zwijndrecht, The Netherlands), 48% fetal bovine serum (Sigma), and 48% ACSF and subsequently transferred to the experimental setup, consisting of an upright microscope (Carl Zeiss AG, Oberkochen, Germany) with a 10x WI (0.3 NA) Achromplan objective in combination with an array of 464 photodiodes (Neuroplex II, Red Shirt Imaging, Decator, GA). As a light source, we used a 75 W xenon lamp in combination with a Uniblitz-shutter (Vincent Associates, Rochester, NY). Filter settings were at 510-560 nm for excitation, 580 nm for the dichroic mirror and an emission filter of >590 nm. Images were taken at a full frame rate of 1.6 kHz. Each diode recorded fluorescent signals at an area of 80 x 80 μm. The fluorescent signals are the average of 64 trains of stimuli with subtraction of fluorescent recordings without stimulations. The interval between light exposures with and without stimulations was 5 ms. As we alternated the order of light exposures with and without stimulations the average interval between stimulations was 11 seconds. We recorded 10 and 40 Hz stimulations in different orders followed by 10 and 40 Hz stimulations when we applied flunitrazepam.

Simultaneous with the voltage-sensitive dye recordings, we recorded in all
experiments the local field potential in layer II using a borosilicate glass pipette with a tip resistance of ~1 MΩ. The signal was amplified using a DAM-80 amplifier (World Precision Instruments, Sarasota, FL). The data was stored and analyzed offline using ClampFit (Molecular Devices, Sunnyvale, CA).

Trains of five consecutive stimulations were applied either at either 10 or 40 Hz to the subcortical white matter using a concentric, bipolar electrode with a tip diameter of 25 μm. This provided a focal stimulation deep in the white matter with a pulse width of 100-200 μs. The injected current (100-350 μA) was adjusted to elicit a half-maximal field potential.

Fluorescent data were analyzed using Neuroplex software (Red Shirt Imaging), SigmaPlot (Systat Software, Inc., San Jose, CA) and custom-written LabView VIs (National Instruments, Austin, TX). A signal was considered significant when it had an amplitude more than 4 times the standard deviation of the baseline noise. The moment of passing this threshold was considered the time point of activation for a particular diode. The area of the excitatory wave was calculated as the surface of all active diodes. The decay phase was described using a double exponential fit from which the weighted decay time constant (τ_decay) was derived.

Spontaneous GABAergic IPSCs in layer II/III neurons were recorded in whole-cell mode using an Axopatch 200A amplifier (Axon Instruments, Union City, CA, USA) and borosilicate glass (Harvard Apparatus Ltd, UK) electrodes with tip resistances of 2–5 MOhm. All experiments were performed at 33 °C using ACSF with 20 μM DNQX and 20 μM APV (both from Sigma) to block the ionotropic glutamate receptors. The pipettes were filled with (mM): 135 CsCl, 1 CaCl2, 10 EGTA, 10 Hepes, 2 MgATP, pH 7.2 (with CsOH). Decay time constants were calculated using Whole Cell Program v2.3 (kindly provided by Dr. J. Dempster, Strathclyde University, UK) as described in Bosman et al (2002).

The statistical tests that were used are mentioned in the text. Throughout this manuscript, differences were considered to be significant at p values of 0.05 or less.

Results

Firing frequencies of inputs to the visual cortex depend on the amount of sensory activity and range from 5 Hz to 500 Hz (Funke and Worgotter, 1997). To investigate whether the amount of neuronal activity depends on the frequency of white matter stimulation in mouse visual cortex, we recorded neuronal network activity in acute, coronal slices of the mouse V1 area and stimulated afferent and efferent fibers in the white matter below layer VI. Changes in the membrane potential were visualized using the voltage-sensitive dye Di-4-ANEPPS (Tominaga et al., 2000), and fluorescence was monitored by a 464-channel photodiode array at 1.6 kHz. The fluorescent signal corresponds to changes in membrane potential and resembled the change in local field potential (Figure 5.1A-C). Fluorescence changes were stable for the entire recording period. Changes in fluorescence showed a typical pattern of spread, starting close to the stimulation site and then spreading in apical
Figure 5.2 Frequency-dependent spreading of excitation signal. (A) Photodiode recording of a voltage-sensitive dye loaded cortex slice upon electrical stimulation in the white matter. Recordings before stimulation and of the peak signal following five consecutive stimuli are shown. Stimulations were given at either 10 (top) or 40 Hz (bottom). Color scale bar: ΔF/F from 0% (blue) to 0.008% (red). (B) Averaged traces of 6 diodes in different layers following 10 Hz or 40 Hz stimulation. (C) The peak values of the consecutive responses showed a clear decrease with 10 Hz stimulation (p < 0.05, ANOVA). With 40 Hz stimulation, the peak values increased with consecutive stimulations (p < 0.05, ANOVA). Shown are the averaged peak responses, normalized to the first response per layer (n=9). (D) Number of diodes reaching the threshold normalized to the first peak. (E) The peak values of the consecutive responses of stimulation trains at increasing frequencies. Shown are the averaged peak responses, normalized to the first response (n=4). Error bars show S.E.M.
and lateral direction. Fluorescence changes were in the same range as reported by Tominaga et al (2000).

Next, we applied pulse trains of five consecutive stimuli at frequencies between 10 and 40 Hz (Figure 5.2). With stimulus trains of 10 Hz, the spread of activity decreased with subsequent stimuli. The area of the excitatory wave decreased gradually from 0.84 ± 0.18 mm² to 0.55 ± 0.15 mm². In contrast, with 40 Hz the spread of excitation showed a transient increase, which at the end of the stimulus train was back at the level of the first stimulation (Figure 5.2D). During 40 Hz stimulation trains the excitation area increased from 0.77 ± 0.16 mm² to 0.94 ± 0.16 mm² at the 2nd peak and then slowly decreases.

In addition to changes in response area the amplitude of fluorescence change showed facilitation or depression during stimulus trains (Figure 5.2C, E). At 10 Hz stimulation, the strength of excitation in response to trains of pulses showed a similar profile in all layers of the visual cortex (Figure 5.2C). In superficial layers II/III the depression in the strength of excitation was larger than in the deeper layers V and VI (layers II/III, 61% ±3 of first response at the 5th pulse; Layer IV, 72% ±3, Layers V/VI 81% ±6 at 5th pulse). Step-wise increases in stimulation frequency showed that at 10 and 15 Hz the responses showed depression or little change during the stimulus train (Figure 5.2E). At 20, 30 and 40 Hz the response showed facilitation (Figure 5.2E). At 40 Hz stimulation, the facilitation of fluorescence change differed between layers, but the response was increased in all layers at the second stimulus (layer V/VI 118% ± 7%, IV 115% ± 6% and II/III 111% ± 6%
relative to the first response). In superficial layers II/III facilitation was transient and the response to the fifth pulse had a smaller amplitude than the response to the first pulse of the train (82% ± 4%). In deeper layers V and VI, facilitation was more sustained during the entire stimulus train (amplitude of fifth response 121% ± 4%; Figure 5.2C). Thus, trains of inputs at a frequency at 40 Hz showed a transiently facilitating spread of cortical excitation, whereas during a train at 10 Hz the responses were depressing. This suggests that plasticity of cortical neuronal network excitation in response to inputs arriving from the white matter is frequency-dependent, and that high frequency inputs engage a larger part of the network.

The amount and spread of cortical activation in response to white matter inputs depends on interneuron activity in the cortex. However, it is unknown to what extent the frequency-dependence of activity in the visual cortex depends on GABAergic inhibition. We tested the effect of strengthening of GABAergic inhibition on frequency-dependent adaptation during trains of stimulation using the benzodiazepine flunitrazepam, an allosteric GABA<sub>A</sub> receptor agonist. Bath application of flunitrazepam (500 nM) altered the properties of spontaneous GABAergic inhibitory post-synaptic currents (sIPSCs) in whole-cell recordings from rat layer II/III pyramidal neurons (Figure 5.3). Flunitrazepam increased the decay time constant of sIPSCs from 7.77 ± 1.21 ms to 12.53 ± 1.58 ms (p=0.009, n=7) (Figure 5.3B, D).

As flunitrazepam substantially increased IPSC decay kinetics, we expected that the spreading of activity in response to white matter stimulation in mouse visual cortex would be reduced in the presence of flunitrazepam. Indeed, bath application of 500 nM flunitrazepam decreased cortical activity in response to white matter stimulation, observed by a decrease both in fluorescence (72 ± 4%, p=0.01, paired t-test, n=9) and in the area of excitation (from 0.90 ± 0.06 mm<sup>2</sup> to 0.72 ± 0.11 mm<sup>2</sup>, paired t-test, p=0.02) at the first pulse of the stimulus train (Figure 5.4A-B). In control experiments where flunitrazepam was not applied fluorescence responses were not altered during the entire recording (95 ± 3%, p>0.05, n=9; data not shown).

The propagation speed in both apical and lateral directions was not affected by flunitrazepam application (data not shown). We next examined whether enhancing GABAergic inhibition by flunitrazepam would differentially affect frequency-dependent facilitation and depression of cortical activity at 10 Hz and at 40 Hz stimulus trains. Flunitrazepam did not affect the depression of the spread of activity observed at 10 Hz stimulation (Figure 5.4C). In contrast, with 40 Hz stimulation trains the facilitation of the spread of activity was prolonged (Figure 5.4C, p<0.05, 2-way repeated measures ANOVA). Compared to control conditions where 40 Hz stimulation resulted in a transient facilitation of spread of excitation, now in the presence of flunitrazepam facilitation of the spread of excitation was sustained throughout the stimulus train.

Strengthening GABAergic inhibition by flunitrazepam differentially affected short-term plasticity in different layers of the visual cortex. The strength of excitation in response to trains of pulses, reflected by the amount of change in fluorescence, showed a different profile in different layers of the visual cortex. In the deep layers of the visual cortex, layers IV, V, VI, flunitrazepam diminished depression during 10 Hz stimulation (p<0.05, 2-way repeated
Figure 5.4 Flunitrazepam changes the spatiotemporal patterns of cortical excitation waves. (A) Fluorescence before stimulation (left) and peak fluorescence after stimulation before (middle) and after bath application of flunitrazepam (500 nM, right). Color scale bar: ΔF/F from 0% (blue) to 0.008% (red). (B) Number of diodes reaching the threshold after stimulation in a 5 pulse train at 10 or 40 Hz. Data are normalized to the first peak in control situation. (C) Number of diodes reaching the threshold normalized to the first peak. (D) The peak values of the consecutive responses of 10 and 40 Hz stimulation trains in different cortical layers. Shown are the averaged peak responses, normalized to the first response of control per layer (n=9). Error bars show S.E.M.
Figure 5.5 The spatiotemporal patterns of cortical excitation waves are altered in α1-/- mice. (A) Averaged traces of 6 diodes in different layers following 10 Hz or 40 Hz stimulation in both α1+/+ and α1-/- mice. (B) The peak values of the consecutive responses of 10 and 40 Hz stimulation trains in different cortical layers. Shown are the averaged peak responses, normalized to the first response of control per layer (α1+/+ n=5, α1-/− n=6). Error bars show S.E.M.
measures ANOVA, Figure 5.4D). In contrast, in superficial layers II/III flunitrazepam did not affect depression. At 40 Hz stimulation, the amount of excitation showed prolonged facilitation in layers IV, V and VI in the presence of flunitrazepam (p<0.05, 2-way repeated measures ANOVA, Figure 5.4D). Again, in superficial layers II/III increasing GABAergic IPSC decay kinetics did not affect plasticity.

As we observed an increased facilitation with enhanced inhibition we tested whether facilitation is affected in mice lacking the GABA_A receptor α1 subunit, the most abundant α subunit in most brain regions (Fritschy et al., 1994, Heinen et al., 2004). There is little to no up-regulation of other α subunits in visual cortex area V1 of these animals (Bosman et al., 2005b). Short term plasticity of the amplitude of the fluorescent signal was affected in α1-/- mice (α1+/+ n=5, α1-/- n=6; Figure 5.5A-C). Depression at 10 Hz was larger in α1-/- mice in the deeper layers IV, V and VI compared to α1+/+ mice (p<0.05, 2-way ANOVA, Figure 5.5C), while there was no difference in layers II/III. During 40 Hz stimulation, facilitation was decreased in the α1-/- compared to α1+/+ mice in all layers (p<0.05, 2-way ANOVA, Figure 5.5C). These data suggest confirm that GABAergic inhibition affects short-term plasticity of cortical excitation in a frequency-dependent manner. Increasing inhibition by flunitrazepam enhances facilitation at higher frequencies of activation and decreases depression at lower frequencies, while knocking out the most abundant GABA_A receptor α subunit reduces facilitation and enhances depression. Thus, GABAergic inhibition shapes frequency adaptation of cortical activity in a frequency-dependent manner.

Discussion

We addressed the question whether GABAergic inhibition differentially affects the recruitment of cortical neuronal networks at different frequencies. We found that this is the case and that manipulating GABAergic inhibition differentially affects short-term plasticity of cortical excitation in superficial and deep layers in the visual cortex.

In the visual cortex, stimulation of the white matter resulted in a wave of excitation that spread from the deep layers to the superficial layers of the visual cortex, as well as laterally within the layers, in line with previous findings (Yuste et al., 1997, Contreras and Llinas, 2001). Overall, the excitation was most prominent in the layers V/VI and II/III, and relatively weak in layer IV. The activity spread in lateral direction over a width of more than 1600 μm in mice, similar to what was found in guinea pig visual cortex (Contreras and Llinas, 2001). However these values are more than double of the lateral spread of activity in the visual cortex of ferrets (Nelson and Katz, 1995).

The cortical response to sensory stimuli, including visual stimuli, rapidly adapts (Ohzawa et al., 1982), which may serve to maximize the range of sensory input strengths that can be processed in the cortex (Wainwright, 1999, Brenner et al., 2000). One of the processes that could play a role in this frequency adaptation is short-term depression of thalamocortical synapses upon repeated stimulation, as observed in vivo (Chung et al., 2002). Synaptic depression of corticocortical synapses may further augment this
adaptation (Abbott et al., 1997). However, during high frequency activity of the cortical neuronal network, different cell types show distinct adaptation of synaptic input (Reyes et al., 1998). We observed a facilitation of cortical excitation at frequencies above 20 Hz inputs and a depression of cortical excitation at 10 Hz, while 15 Hz stimulation gives no adaptation at all.

Given the multitude of non-linear neuronal interactions during ongoing activity, it is not straight forward to predict how GABAergic inhibition affects processing of trains of inputs at different frequencies. We find that increasing inhibition prolongs facilitation of the spread of excitation at high frequency inputs, while decreasing inhibition shortens facilitation. This suggests that in addition to plasticity at thalamo-cortical synapses, the inhibitory neuronal network within the visual cortex plays a role in frequency adaptation of the visual cortex neuronal network and, as a consequence, of sensory processing.