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Cortical 1-containing $GABA_A$ receptors mediate inhibition during high network activity

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Abstract

Background: $GABA_A$ receptors are the targets of clinically important drugs including many general anesthetics and benzodiazepines. There is evidence that these agents induce sedation via 1 subunit containing $GABA_A$ receptors on cortical glutamatergic neurons. [1] Although $GABA_A$ receptors harboring the 1 subunit define the most abundant receptor subtype in the cerebral cortex, little is known about their specific function in controlling neuronal activity. Here we address this issue by making use of the 1-preferring modulator zolpidem and 1 (H101R) knock-in mutant mice, in which 1-subunit containing receptors are insensitive to zolpidem. [2]

Methods: Neocortical slice cultures were prepared from 3 days old wild type and 1(H101R) mutant mice. After two weeks in vitro, cultured neurons displayed spontaneous action potential firing. Microelectrodes were positioned in the tissue and neuronal activity was recorded extracellularly. In the absence of magnesium ions from the bathing solution, the firing patterns were characterized by a periodic alternation of long-lasting (1-10 seconds) action potential bursts, from which the average discharge rates (ADR) were calculated. Furthermore, the bursts were aligned in time and peri-event time histograms of action potentials were constructed (t0 = burst start). From these, average spike counts in specific phases during bursts were computed. For statistical comparison Hedges' d including 95% confidence interval was used.

Results: The firing patterns of neurons in slice cultures prepared from wild type and 1(H101R) mutant mice did not differ under drug-free conditions. Zolpidem (1.5 12.5µM) caused a concentration-dependent decrease of ADR. This parameter did not differ between wild type and 1(H101R) mutant slices. However, striking differences between wild types and 1(H101R) mutant slices were found during burst activity. Under drug-free conditions, intra-burst discharge rates were highest at the onset of bursting and then gradually declined. Zolpidem (1.5 and 3µM, n=10 and 17) significantly reduced action potential firing within the first 40ms of a burst in wild type slices, but not in slices from 1(H101R) mutant mice (n=9 and 22).

Conclusions: Zolpidem induced a marked depression of discharge rates only at the onset of spontaneous bursting, i.e. during intense action potential firing in wild type, but not in mutant slices. Therefore we conclude that 1-containing $GABA_A$ receptors in the neocortex mediate inhibition predominantly during phases of high network activity. This is consistent with their localization postsynaptic to fast spiking GABAergic interneurons, fire at very high rates in the initial phase of bursts. [3]

Material and Methods

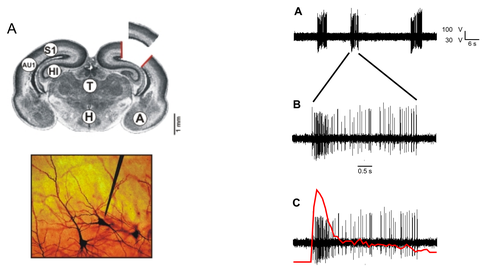

Figure 1: Neocortical slice cultures were prepared from 2- to 4- day- old wildtype and the zolpidem insensitive 1(H101R)$GABA_A$ receptor mutant mice. The neuronal network was characterized by spontaneous neuronal activity after two weeks in vitro. This network activity showed typical transient periods of high excitation with action potentials arranged to bursts. Recordings were performed by means of extracellular electrodes. The activity phases were isolated and grouped into time segments (bins) of 5 milliseconds. The number of action potentials per bin was calculated under control conditions and after zolpidem application. The effect size (Hedges' d) with 95%-confidence interval was used for statistical quantification.

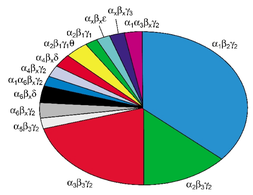

Figure 2: Distribution of $GABA_A$ receptor subtypes in the CNS
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$GABA_A$ receptor subtypes in the brain: a paradigm for CNS drug discovery?

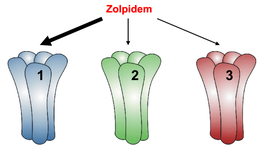


Figure 3a: The selective drug zolpidem acts preferentially via, but not exclusively via 1 containing $GABA_A$ receptor subtypes.

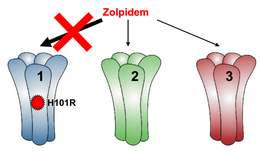


Figure 3b: The zolpidem effect at 1 containing $GABA_A$ receptors is abolished by the 1(H101R) mutation. The resulting effect of zolpidem is due to other subtypes.

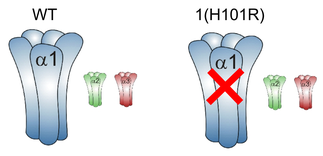
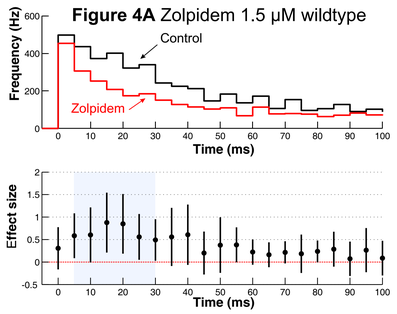
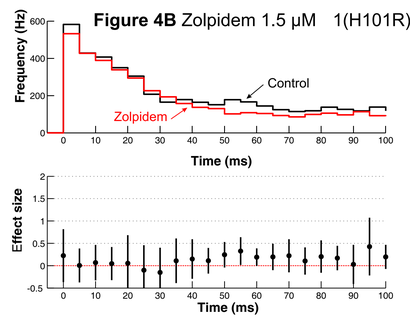
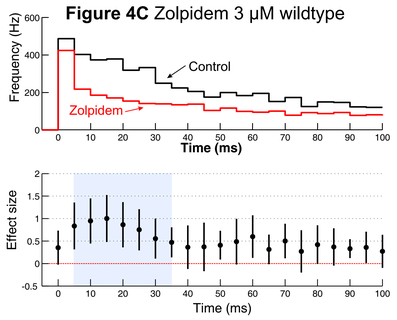


Figure 3c: By comparing the effects of zolpidem in wildtype and 1(H101R) mutant mice the impact of 1 containing $GABA_A$ receptors for the inhibition in the neocortex can be estimated.

Results







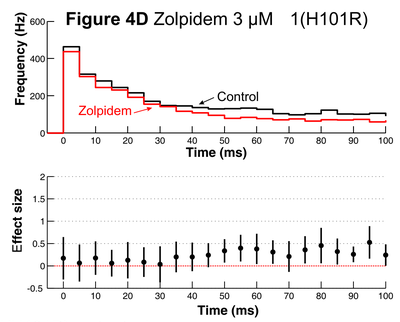


Figure 4: Effect of zolpidem (1.5 and 3 µM) in neocortical slices of wildtype (C57bl6) and 1(H101R) mutant mice.
The upper part of each figure illustrates the frequency of action potentials in bins of 5 ms within the first 100 ms of action potentials arranged in bursts. The black line represents the activity under control conditions, the red line the effect of zolpidem.
Zolpidem causes a concentration dependent inhibition which starts almost immediately after the beginning of a burst in wildtype slices (4A and C) but not in the 1(H101R) mutant (B and D).
The lower part of the figures illustrates the effect of zolpidem as Hedges' d with 95%- confidence interval. Zolpidem leads to a significant inhibition in wildtype slices within the time period of 5 to 35 ms of a burst, marked by grey shading. There is no significant inhibition in 1(H101R) mutant slices.

Comparison of WT and 1(H101R) slices under drug-free conditions

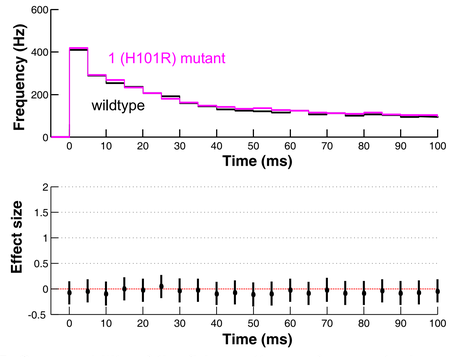


Figure 5: Comparability of the firing patterns of organotypic cortical slice cultures of wildtype and 1(H101R) mutant mice

The upper part of the figure illustrates the frequency of action potentials in bins of 5 ms within the first 100 ms of action potentials arranged in bursts. The black line represents the activity of wildtype, the pink of of 1(H101R) mutant mice slices under control conditions.
The effect size (Hedges' d) with 95%-confidence interval is displayed in the lower part of the figure. There is no difference between wildtype and 1(H101R) mutant slices within the first 100 ms of action potentials arranged in bursts.

Conclusion

Although $GABA_A$ receptors containing 1-subunits are quite common in the cerebral cortex, they are activated only during phases of high neuronal activity.

References

1. Zeller A et al., Mol Pharmacol 2008, 73:282-291
2. Crestani F et al., Brit J Pharm 2000, 131: 1251-1254
3. Kawaguchi Y, J Neuroscience 2001, 21(18): 7261-7272

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