GABAergic PARVALBUMIN-EXPRESSING INTERNEURONS UNDERLIE DISTINCT LOCAL EEG MICROSTRUCTURE DURING DIFFERENT STATES OF UNCONSCIOUSNESS IN RATS

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ABSTRACT

Background: The GABAergic mechanism is an important target for the action of anesthetics and the promotion of sleep. We investigated the changes in hippocampal and reticulo-thalamic nucleus (RT) GABAergic parvalbumin (PV)-expressing interneurons as possible underlying mechanisms of the different local cortical and hippocampal EEG microstructures during NREM sleep compared with anesthesia-induced unconsciousness by two anesthetics with different main mechanisms of action.

Methods: Twenty adult male Wistar rats were implanted for sleep recordings. After 3 hours of sleep recording, half of the rats were anesthetized with ketamine/diazepam (100 mg/kg, i.p.) and the other half with propofol (100 mg/kg, i.p.). We recorded EEGs of the motor cortex and hippocampus during the one-hour stable surgical phase of both anesthetics. The EEG microstructures of the motor cortex and hippocampus in local NREM sleep were compared with their EEG microstructures during 30 minutes of unconsciousness induced by a given anesthetic. At the end of each recording under stable anesthesia, rats were sacrificed for further PV and postsynaptic density protein 95 (PSD-95) immunohistochemistry.

Results: All three states of unconsciousness differed in the motor cortical and hippocampal EEG microstructures (**Fig. 1 left**; **Fig. 2**; $\chi 2 \geq 9.46; p \leq 0.01$). While the lower hippocampal gamma amplitude was the common feature of all three states of unconsciousness ($z \geq -3.44; p \leq 0.05$), the hippocampal delta amplitude was higher than in the motor cortex ($z \geq -3.38; p \leq 0.04$) during both anesthesia-induced unconsciousness. In contrast to NREM sleep and propofol anesthesia, where hippocampal theta amplitude was augmented, it was attenuated ($z \geq -3.48; p \leq 0.01$) during ketamine anesthesia. In addition, the hippocampal sigma and beta amplitude were attenuated relative to the motor cortex ($z \geq -2.96; p \leq 10\text{-}3$) only during propofol anesthesia. During propofol-induced unconsciousness, attenuated delta and augmented sigma/beta amplitudes (**Fig. 1 right; Fig. 2**; $z \geq -4.13; p \leq 0.01$) were the globally expressed difference, whereas increased gamma amplitude ($z=2.35; p=0.02$) was the only difference at the motor-cortical level compared to NREM sleep. During ketamine/diazepam-induced unconsciousness, attenuated theta and increased beta/gamma amplitudes ($z \geq -5.53; p \leq 10\text{-}4$) were the globally expressed difference from NREM sleep. Both anesthesia-induced unconsciousness expressed globally as increased beta amplitude ($z \geq -4.13; p \leq 10\text{-}3$) and increased motor-cortical gamma amplitude ($z \geq -4.20; p \leq 0.02$) compared to NREM sleep (**Fig. 1 right**). Moreover, while the motor cortical/hippocampal theta synchronization increased during propofol anesthesia vs. NREM and ketamine/diazepam anesthesia, beta and gamma synchronization decreased during ketamine/diazepam anesthesia vs. NREM and propofol anesthesia (**Fig. 3**). In contrast to propofol anesthesia, there was significant suppression of PV expression in the hippocampus (**Fig. 4**; $z \leq -2.71; p \leq .01$) and RT (**Fig. 5**) during ketamine/diazepam anesthesia in all rats, but suppression of PV expression was followed by an inhibitory/excitatory imbalance only in the hippocampus (**Fig. 6**).

Conclusions: Although anesthesia and sleep share many neurobiological features, they are distinct states in terms of local EEG microstructure and underlying GABAergic and molecular substrate in the local neuronal networks of brain structures important for unconsciousness and EEG rhythm formation.

RESULTS

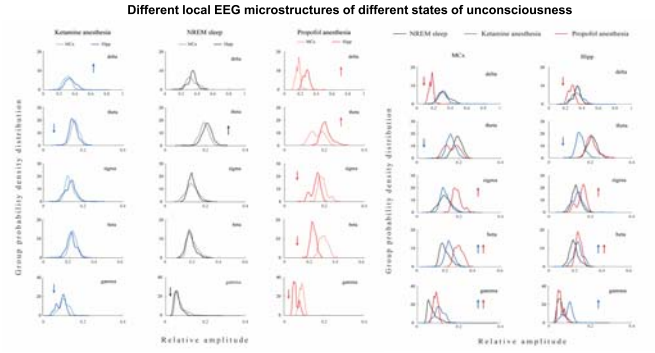

Fig. 1. Topography of EEG microstructure during distinct states of unconsciousness (left) and their comparison during NREM vs. both anesthsia-induced unconsciousness (right). The group probability density distributions (PDE)/30 min of the relative amplitudes of all conventional EEG frequency bands of all rats belonging to corresponding experimelnal group in the motor cortex (MCx) compared to the hippocampus (Hipp) for each state of unconsciousness. Arrows up and down show statistically significant higher and lower amplitude at $p \leq 0.05$.

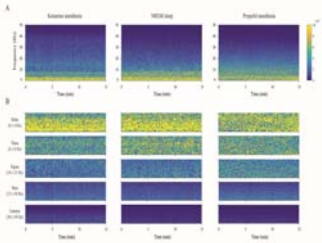

Fig. 2. Hippocampal spectrograms during different states of unconsciousness.
The individual examples of the total hippocampal spectrograms (0-50 Hz frequency range) during 15 min of each state of unconsciousness (A) with their spectrograms for each frequency band (B). The color bar is the same for all spectrograms.

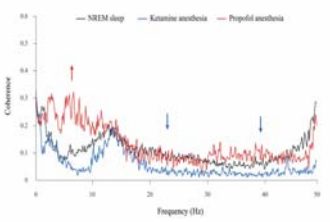

Fig. 3. Cortico-hippocampal coherence spectra during different states of unconsciousness. Mean coherence spectra for NREM (n=12), ketamin/diazepam (n=6) and propofol anesthesia (n=6). Arrows up/down show statistically significant increase or decrease in cortico-hippocampal coherence within the specific EEG frequency band at $p \leq 0.05$

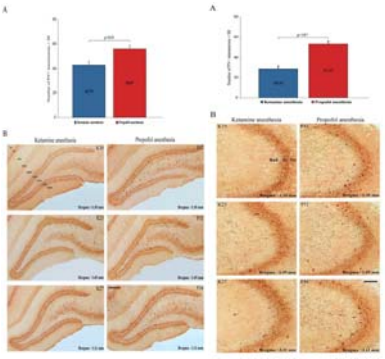

Fig. 4. Suppression of PV+ interneurons in the hippocampal DG (left) and CA3 (right) during ketamine/diazepam anesthesia. (A) Mean number of PV+ interneurons in the DG and CA3 in ketamine/diazepam (n=9) and propofol anesthesia (n=10). **(B)** Typical individual examples of PV supression within the DG and CA3. Scale is 200 μm.

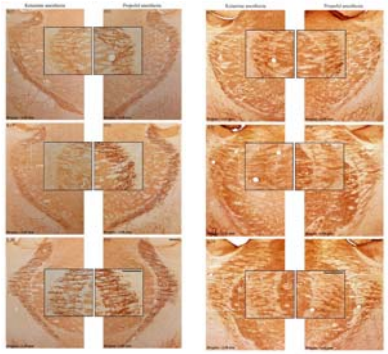

Fig. 5. Suppression of PV+ interneurons and PSD-95 expression in the reticulothalamic nucleus (RT). Typical individual examples of suppression of PV+ interneurons in the RT during ketamine/diazepam vs. propofol-induced unconsciousness (left), ut with no differences in PSD-95 expression during both anesthesia induced unconsciousness (right).
Scale is 200 μm and 100 μm.

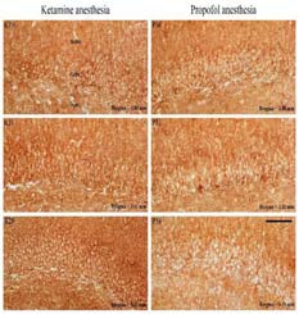

Fig. 6. PSD-95 expression in the DG of hippocampus. Individual examples of the increased PSD-95 expression in the suprapyramidal granule cell layer of DG during PV suppression in the ketamine/diazepam -induced unconsciousness. Scale is 50 μm

CONCLUSIONS

1. Both states of anesthesia-induced unconsciousness are expressed by globally increased beta and cortically increased gamma amplitude vs. NREM.
2. The hallmark of ketamine/diazepam induced unconsciousness is globally attenuated theta and increased hippocampal gamma amplitude.
3. The hallmark of propofol-induced unconsciousness is globally attenuated delta and augmented sigma amplitude.
4. Suppression of PV+ interneurons in the hippocampus and RT during ketamine/diazepam–induced unconsciousness might be an additional underlying mechanism of different local and global EEG microstructures compared to NREM and propofol-induced unconsciousness.
5. Our study suggests that NMDA blockade is prefentially mediated on GABAergic interneurons in the hippocampus and RT during ketamine/diazepam unconsciousness.
6. While suppression of PV+ interneurons in the hippocampus increased PSD-95 expression, it did not lead to an imbalance between inhibition and excitation at RT during ketamine/diazepam-induced uncosciousness (no alteration of RT local excitation), indicating that excitation was already equally abolished by both anesthetics.
7. Although anesthesia and sleep share many neurobiological features, they are distinct states in terms of local EEG microstructure and underlying GABAergic and molecular substrate in the local neuronal networks of brain structures important for unconsciousness and EEG rhythm formation.
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