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Global slowing of network oscillations in mouse neocortex by diazepam

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ABSTRACT

Benzodiazepines have a broad spectrum of clinical applications including sedation, anti-anxiety, and anticonvulsive therapy. At the cellular level, benzodiazepines are allosteric modulators of $GABA_A$ receptors; they increase the efficacy of inhibition in neuronal networks by prolonging the duration of inhibitory postsynaptic potentials. This mechanism of action predicts that benzodiazepines reduce the frequency of inhibition-driven network oscillations, consistent with observations from human and animal EEG. However, most of existing data are restricted to frequency bands below ~30 Hz. Recent data suggest that faster cortical network rhythms are critically involved in several behavioral and cognitive tasks. We therefore analyzed diazepam effects on a large range of cortical network oscillations in freely moving mice, including theta (4–12 Hz), gamma (40–100 Hz) and fast gamma (120–160 Hz) oscillations. We also investigated diazepam effects over the coupling between theta phase and the amplitude fast oscillations. We report that diazepam causes a global slowing of oscillatory activity in all frequency domains. Oscillation power was changed differently for each frequency domain, with characteristic differences between active wakefulness, slow-wave sleep and REM sleep. Cross-frequency coupling strength, in contrast, was mostly unaffected by diazepam. Such state- and frequency-dependent actions of benzodiazepines on cortical network oscillations may be relevant for their specific cognitive effects. They also underline the strong interaction between local network oscillations and global brain states.

1. Introduction

Benzodiazepines, like diazepam (DZ), are positive allosteric modulators of the $GABA_A$ receptor that act by potentiating the agonistic potency of the natural ligand GABA (for a recent review, see Tan et al., 2011). At the cellular and synaptic level, benzodiazepines can enhance the amplitude of inhibitory postsynaptic events, prolong their duration and increase tonic inhibition. At the network level this results in reduced excitability and in characteristic alterations of rhythmic activity patterns. Increased cycle length of inhibition-driven activity reduces the frequency of network oscillations, while increased inhibition efficacy enhances coherence of the multi-neuronal rhythmic activity (Whittington et al., 1996). In line with this, DZ decreases the frequency of hippocampal theta oscillations during REM sleep (Monmaur, 1981), walking (Caudarella et al., 1987) and during active exploratory behavior (Van Lier et al., 2004).

However, mammals express a large variety of different region- and state-dependent network patterns, most of which involve an important role of inhibition (Whittington and Traub, 2003; Mann and Paulsen, 2007). These patterns cover a wide spectrum of frequencies and support different behavioral and cognitive functions (Buzsáki, 2006). Therefore, multiple frequency domains have to be analyzed to assess the effects of benzodiazepines at the network level and relate them to their behavioral or cognitive actions. Moreover, different oscillations can occur simultaneously and can be systematically coupled. For example, multiple fast oscillations coexist in hippocampal CA1 of freely moving rats, with a layer-specific coupling to theta phase (Scheffer-Teixeira et al., 2012; Belluscio et al., 2012). Interactions between different rhythms are related to task-specific cognitive performance both in humans (Axmacher et al.,

Abbreviations: ANOVA, analysis of variance; aWk, active waking state; CFC, cross-frequency coupling; DZ, diazepam; PSD, power spectral density; EEG, electroencephalogram; GABA, gamma-aminobutyric acid; MI, modulation index; NREM, non REM sleep; phREM, phasic REM sleep; qWk, quite waking; REM, rapid eye movement sleep; toREM, tonic REM sleep.

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2010; Canolty and Knight, 2010; Fell and Axmacher, 2011) and animals (Tort et al., 2008, 2009). Moreover, cross-frequency coupling (CFC) also varies with vigilance state across the sleep-wake cycle (Scheffzük et al., 2011; Brankačk et al., 2012). Similar to single oscillation frequencies, synaptic inhibition is also critical for certain forms of CFC (Wulff et al., 2009). Together, these findings suggest that altering GABAergic inhibition could change the characteristics of neuronal oscillations and their coupling.

Here we investigated the effects of DZ on the EEG of freely behaving mice during different vigilance states. Power spectra were analyzed for three frequency domains: theta (4–12 Hz), gamma (40–100 Hz) and fast gamma (120–160 Hz) oscillations. In addition, coupling between theta and fast oscillation patterns was analyzed before and after DZ administration. We found that DZ induces a global slowing of EEG oscillations in all frequency domains independent of behavioral state whereas power changes induced by DZ depend on the behavioral state and differ between frequency domains: theta power decreased only in active waking, gamma power increased in active waking but decreased during REM sleep, whereas fast gamma power decreased in all behavioral states. Despite of the power changes and the shift in frequency, DZ left the strength of interactions between simultaneous oscillations (CFC) largely intact. Our data therefore show a general slowing of cortical network oscillations by benzodiazepines, which occurs with altered power content and preserved CFC.

2. Methods and materials

2.1. Ethics statement

This study was carried out in accordance with guidelines of the European Science Foundation (Use of Animals in Research, 2001), the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals (Guide for the Care and Use of Laboratory Animals, 1996) and has been approved by the Governmental Supervisory Panel on Animal Experiments of Baden Württemberg at Karlsruhe (35-9185.81/G-30/08). All efforts were made to minimize animal suffering and to reduce the number of animals used. Due to the behavioral aspect of the study, alternatives to in vivo techniques were not available.

2.2. Animal care and housing conditions

Male C57BL/6N mice were purchased at 28 or 45 days of age from Charles River (Sulzfeld, Germany). For a minimum of two weeks they were housed in groups of four to five inside a ventilated Scantainer (Scanbur BK A/S Denmark) on an inverted 12/12-h light/dark cycle with light on between 8:00 p.m. and 8:00 a.m. Animals had free access to water and food. After electrode implantation, mice were housed individually throughout the experiment.

2.3. Animal preparation

Ten male C57BL/6N mice were anesthetized with isoflurane in medical oxygen (4% isoflurane for induction, 1.5–2.5% for maintenance, flow rate: 1 l per min). Anesthetized animals were placed in a stereotactic apparatus with a custom-made inhalation tube. For analgesia, 0.1 mg/kg of buprenorphin was injected subcutaneously prior to and 8 h after surgery. After exposure of the skull bone, two stainless steel watch screws were permanently fixed in the skull. One screw was used for EEG recording and placed over the left lateral parietal association cortex (2 mm posterior of bregma, 1.5 mm lateral to the midline). In mice this neocortical region covers the dorsal hippocampus permitting reliable recording of the theta rhythm with comparable amplitudes among different animals. A second screw over the cerebellum served as ground and reference electrode. The impedance of the epidural screw electrodes was 7.1 kOhm at 100 Hz (range: 6.8–7.6) and 3.0 kOhm at 1 kHz (range: 3.0–3.1). Two pairs of varnish-insulated nichrome wires (100 μm, glued together) cut at an angle of 45° were implanted into the right hippocampal CA1 area and a single nichrome wire into the ventral hippocampus for a different set of experiments. Due to the limited number of recording channels, we omitted parallel recording of EMG. For staging of vigilance state we used data from three-dimensional accelerometry (see below).

2.4. Electrophysiology and recording of behavior

One week after surgery, experiments began with a 2-h session in a Phenotyper home cage (Noldus Information Technology, Wageningen, Netherlands) measuring 30 cm × 30 cm with free access to food and water. EEG recordings were performed with a miniaturized data logger (Neurologger 2A), an advanced version of the device previously described (Vyssotski et al., 2006, 2009) with the reference connected to ground. The input impedance of all recording channels including reference was larger than 30 MOhm. Four channels of EEG signals were bandpass-filtered (1–700 Hz, −3 dB, attenuation −6 dB/octave) and digitized by an on-board A/D-converter (6400 Hz per channel) after amplification at ×1000 (input range of ±1 mV). Samples were stored in the on-board 512 MB memory at a rate of 1600 Hz. The dimensions of the neurologger were 23 × 15 × 13 mm and the total weight was 3.6 g with two batteries (Renata ZA 10; Itingen, Switzerland). This weight includes the neurologger itself (1.4 g), the add-on accelerometer/infrared synchronization board (0.4 g), batteries (0.6 g), battery holders as well as protective casing. The head implant (electrodes, wires, contacts and dental acrylic) had an additional weight of 0.7 g. Special care was taken to habituate the mice to the recording chamber and weight of data logger. The movement of the mice was not visibly altered (for details, see Brankačk et al., 2010). Prior to the first full-length recording of 10 h, all animals went through 3 habituation sessions. These were performed during the light period and contained epochs of data logger recordings with increasing duration from 2–3 h to 4 h in the third night. After each trial, the phenotyper cage was carefully wiped with 70% EtOH to remove all odor traces of previous animals, the cage bedding was removed and stored in a clean box so that it could be used again for the same subject. At the end of the experiment, data was downloaded onto a personal computer for further analysis. Animal behavior was continuously recorded by the video tracking system Ethovision XT 7.1 (Noldus Information Technology, Wageningen, Netherlands).

2.5. Drug preparation

Diazepam (Sigma–Aldrich) was prepared in a 10% (2-Hydroxypropyl)-β-cyclodextrin solution (Sigma–Aldrich). The same solvent was used as vehicle in control experiments. Diazepam was injected intraperitoneally in doses of 1, 2, or 4 mg/kg (Straub et al., 2010) in a random order with inter-injection intervals of at least 48 h. The injected volume was 10 ml/kg. Each injection was followed by data acquisition for a period of 10 h, starting 30 min after injection of drug or vehicle.

2.6. Data analysis

Continuous EEG recordings of 10 h duration from the circadian quiet phase (from 9:00 p.m. to 7:00 a.m.) of ten male animals were used for analysis. Data was imported into a MATLAB-based program (The Mathworks Inc., Natick, MA) using both built-in- and custom-written routines. Visual classification of REM-sleep, non REM-sleep (NREM), quiet waking (qWk) and active wakefulness (aWk) was based on: 1) three-dimensional accelerometer activity (aWk distinguished by prominent signals); 2) the amount of high amplitude-low frequency delta activity in the neocortex (characteristic for NREM); 3) regular theta (4–12 Hz) oscillations (indicative of REM or aWk). For a detailed description of behavioral staging, see Brankačk et al. (2010). Waking states with movement were reliably and automatically detected by crossing a threshold in the summed integral of all three accelerometer dimensions. The remaining time corresponded to NREM sleep, drowsiness or immobile waking. Manual scoring in two animals revealed that less than 1% of the time spent in immobility consisted of qWk while most of the immobility corresponded to drowsiness and NREM sleep. For analyzing the sleep-wake cycle we therefore did not distinguish waking immobility as a separate state in the present study. REM sleep is heterogeneous and can be divided into tonic and phasic REM (Sakai et al., 1973), the latter characterized by brief periods of increased theta amplitude and frequency, ponto-geniculo-occipital spikes, muscle twitches, increased eye movements and vegetative arousal (Montgomery et al., 2008). Phasic REM was detected with a MATLAB program based on methods adapted from Mizuseki et al. (2011) and covered less than 3% of total REM duration (for detailed analysis of phasic versus tonic REM, see Brankačk et al., 2012). In addition, latency from vehicle or drug application to the first occurrence of NREM and REM sleep was identified. Baseline data on aWk, tonic and phasic REM in the absence of diazepam have been described elsewhere (Brankačk et al., 2012).

Power spectral density (PSD) estimation was done by means of the Welch periodogram method using the built-in MATLAB function “pwelch” from the Signal Processing Toolbox. We employed 50% overlapping Hamming windows with a length of 4 s. To estimate “peak frequency” in the gamma and fast gamma range we first removed a $1/f$ fit from the PSD, and then smoothed the remaining PSD using a 20-Hz moving average. For an illustration of this procedure see Supplementary Fig. 1. Band power was defined as the area under the curve of the corresponding frequency domain.

The stability of theta oscillations was estimated by the width of the peak observed in the PSD (see Fig. 3B). Power values between 0 and 30 Hz were fitted by a Gaussian curve. For this procedure, we only took into account values above a minimal level chosen to provide the best fit (usually 20 or 30% of peak theta power). Theta peak width was then defined as the standard deviation of the Gaussian fit.

For general estimation of DZ effects on oscillation frequency, we calculated the mean frequency in the 0–20 Hz and 20–160 Hz ranges, respectively (see Fig. 5). Power values were transformed into probabilities Pj by dividing the power of each