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NORMAL SLEEP HOMEOSTASIS AND LACK OF EPILEPSY PHENOTYPE IN $GABA_A$ RECEPTOR $\alpha3$ SUBUNIT-KNOCKOUT MICE

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Abstract—Thalamo-cortical networks generate specific patterns of oscillations during distinct vigilance states and epilepsy, well characterized by electroencephalography (EEG). Oscillations depend on recurrent synaptic loops, which are controlled by GABAergic transmission. In particular, $GABA_A$ receptors containing the $\alpha3$ subunit are expressed predominantly in cortical layer VI and thalamic reticular nucleus (nRT) and regulate the activity and firing pattern of neurons in relay nuclei. Therefore, ablation of these receptors by gene targeting might profoundly affect thalamo-cortical oscillations. Here, we investigated the role of $\alpha3$-$GABA_A$ receptors in regulating vigilance states and seizure activity by analyzing chronic EEG recordings in $\alpha3$ subunit-knockout ($\alpha3$-KO) mice. The presence of postsynaptic $\alpha3$-$GABA_A$ receptors/gephyrin clusters in the nRT and $GABA_A$-mediated synaptic currents in acute thalamic slices was also examined.

EEG spectral analysis showed no difference between genotypes during non rapid-eye movement (NREM) sleep or at waking–NREM sleep transitions. EEG power in the spindle frequency range (10–15 Hz) was significantly lower at NREM–REM sleep transitions in mutant compared with wild-type mice. Enhancement of sleep pressure by 6 h sleep deprivation did not reveal any differences in the regulation of EEG activities between genotypes. Finally, the waking EEG showed a slightly larger power in the 11–13-Hz band in $\alpha3$-KO mice. However, neither behavior nor the waking EEG showed alterations suggestive of absence seizures. Furthermore, $\alpha3$-KO mice did not differ in seizure susceptibility in a model of temporal lobe epilepsy. Strikingly, despite the disruption of postsynaptic gephyrin clusters, whole-cell patch clamp recordings revealed intact inhibitory synaptic transmission in the nRT of $\alpha3$-KO mice. These findings show that the lack of $\alpha3$-$GABA_A$ receptors is extensively compensated for to preserve the integrity of thalamo-cortical function in physiological and pathophysiological situations.

Key words: thalamo-cortical network, EEG rhythms, spectral analysis, gephyrin, spike-wave discharges, sleep deprivation.

Changes in brain electrical activity during vigilance states and epilepsy are evidenced by complex rhythms in the electroencephalogram (EEG). Frequency, amplitude and modulation of neuronal oscillations are determined by the firing patterns and connectivity of specific neuronal networks (Llinas and Steriade, 2006). In particular, the functional coupling of the thalamus and cerebral cortex plays a strategic role in the emergence of behaviorally relevant rhythmic activities (Steriade et al., 1993) and hypersynchronization leading to seizures (Timofeev and Steriade, 2004). The function of thalamo-cortical circuits depends critically on reciprocal synaptic loops between thalamic relay nuclei, the thalamic reticular nucleus (nRT) and the neocortex (Jones, 2002; Pinault, 2004). These reciprocal excitatory and inhibitory connections, as well as inputs to this network, give rise to specific oscillatory activities that underlie EEG rhythms (Domich et al., 1986; Steriade et al., 1986; Steriade, 2003).

The nRT, exclusively composed of GABAergic neurons, plays a pivotal role in oscillatory activities by providing a powerful and widespread inhibitory tone onto thalamic relay nuclei (Huguenard and Prince, 1994b; Cox et al., 1996). The activity of nRT neurons is, in turn, modulated by afferent fibers from several brain regions including thalamic nuclei, brainstem and basal nuclei, and the most powerful input connections arise from cortical layer VI (Liu and Jones, 1999). Importantly, nRT neurons are interconnected via GABAergic synapses (Jones, 2002). $GABA_A$ receptor-mediated currents in both the nRT and relay thalamic nuclei are critical in modulating neuronal firing patterns in thalamo-cortical circuits (von Krosigk et al., 1993; Cox et al., 1997).

These integrated recurrent synaptic loops enable the synchronized neuronal activity underlying major EEG rhythms, notably delta waves, spindles, and the cortical slow oscillation, which define non-rapid-eye movement (NREM) sleep (Steriade, 2006). In contrast, abnormal activity of the thalamo-cortical network can lead for example, to the onset of spike-wave discharges that are EEG hallmarks of absence seizure episodes (for review, Steriade, 2003, 2005). Numerous studies have demonstrated the importance of nRT neurons in network desynchronization,

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Abbreviations: ACSF, artificial cerebrospinal fluid; EEG, electroencephalogram; EMG, electromyogram; IR, infrared; KO, knockout; NREM, non-rapid-eye movement; nRT, reticular nucleus of the thalamus; REM, rapid-eye movement; SD, sleep deprivation; sIPSCs, spontaneous inhibitory postsynaptic currents; SWA, slow-wave activity; TLE, temporal lobe epilepsy; TPMPA, 1,2,5,6-tetrahydropyridin-4-yl)-methylphosphinic acid; VIAAT, vesicular amino acid transporter; WT, wild-type.

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which prevents widespread activity and subsequent hypersynchrony leading to seizures (Steriade et al., 1993; von Krosigk et al., 1993; Huguenard and Prince, 1994a; Huntsman et al., 1999; Sohal et al., 2000, 2003; Sohal and Huguenard, 2003).

The functional and pharmacological properties of $GABA_A$ receptors depend on their subunit composition. A large family of constituent subunits (α1–6, β1–3, γ1–3, δ, ρ1–3, θ, π, ε) allows the assembly of a variety of $GABA_A$ receptor subtypes (Rudolph and Mohler, 2006). Strikingly, the nRT mainly expresses $GABA_A$ receptors containing the α3 subunit (α3-$GABA_A$ receptors) (Wisden et al., 1988, 1992; Fritschy and Mohler, 1995; Pirker et al., 2000; Studer et al., 2006) and this receptor subtype is also abundant in layer VI of the neocortex. Although when assayed in whole brain this subtype represents a minor subpopulation of $GABA_A$ receptors (10–15%), it is predominant in several neuronal networks (i.e. arousal activating systems as well as sleep-promoting circuitries) that play a key role in the generation and maintenance of the sleep–wake cycle (Gao et al., 1993, 1995; Fritschy and Mohler, 1995; Rodriguez-Pallares et al., 2001; Jones, 2005).

In α3-knockout (KO) mice, there is no detectable change in the expression of other α subunit variants (Yee et al., 2005) and no replacement of α3-$GABA_A$ receptors in the nRT by another subtype is apparent (Studer et al., 2006). Therefore, we hypothesized that changes in neuronal activity in the nRT and neocortical layer VI due to lack of α3-$GABA_A$ receptors may alter thalamo-cortical activity and thereby result in a sleep phenotype or enhanced susceptibility to epileptic seizures. A recent study has shown that genetically epilepsy-prone rats, displaying abnormal thalamic synchronization, exhibit a specific loss of α3-$GABA_A$ receptors in the nRT (Liu et al., 2007). Here, we performed chronic EEG recordings in freely moving wild-type (WT) and α3-KO mice to investigate alterations in sleep and wakefulness and test for the presence of spike-and-wave discharges. Next, we studied the response of α3-KO mice to sleep deprivation (SD), a well-established method to enhance sleep pressure and thereby uncover differences in sleep regulation. Finally, the susceptibility of α3-KO mice to experimentally-induced recurrent focal seizures was investigated in a model of temporal lobe epilepsy (TLE). To further assess potential alterations of inhibitory synaptic transmission in the nRT, we performed whole-cell patch clamp recordings on thalamic slices obtained from juvenile α3-KO mice and investigated gephyrin and α3-$GABA_A$ receptor clustering at postsynaptic sites in the nRT using immunofluorescence staining.

EXPERIMENTAL PROCEDURES

Animals

Mice lacking the $GABA_A$ receptor α3 subunit (α3-KO) and their WT controls were maintained on either 129X1/SvJ or C57Bl/6J background (see Yee et al., 2005 for characterization) and genotyped by PCR analysis of tail biopsies. Mice were housed individually with *ad libitum* access to food and water. The animal facility was maintained on a 12-h light/dark cycle (light on at 9 am; ~30 lux), at a constant ambient temperature (22–24 °C) and 50% relative humidity. All experimental procedures were carried out in accordance with the European Communities' Council Directive of 24 November 1986 (86/609/EEC) and were approved by the Cantonal Veterinary Office of Zurich or the Stanford University Institutional Animal Care and Use Committee. The minimum number of animals necessary to obtain statistically reliable data was utilized. Every effort was made to minimize animal suffering.

Sleep and motor activity recordings

Surgery. A first group of adult 129X1/SvJ mice (male) was used for surgery (11–13 weeks old at surgery; α3-KO: n=12, 35.8±2.0 g; WT: n=11, 31.2±1.4 g). For EEG recording, mice were implanted epidurally under deep anesthesia (ketamine 100 mg/kg–xylazine 20 mg/kg, 10 ml/kg, i.p.). Gold-plated miniature screws (diameter 0.9 mm) were positioned on the right hemisphere above the frontal cortex (1.5 mm anterior to bregma and 2 mm lateral to the midline) and the parietal cortex (2 mm posterior to bregma and 3 mm lateral to the midline). A reference electrode was placed above the cerebellum (2 mm posterior to lambda, on the midline). Electrodes were connected to stainless steel wires and fixed to the skull with dental cement. Two gold wires (diameter 0.2 mm) were inserted bilaterally in the neck muscles to record the electromyogram (EMG). After 3 weeks' recovery, the mice were adapted for at least 3 days to the recording conditions.

EEG recording. Continuous EEG-EMG recordings were obtained throughout 48 h. A 24-h baseline recording was followed by 6 h SD starting at light onset, and the subsequent 18 h recovery. SD was performed by introducing a variety of objects (e.g. nesting material, pieces of wood) into the cage, as well as by gently tapping on the cage whenever a mouse appeared to be drowsy (Tobler et al., 1997). The mice were under constant observation and motor activity was continuously recorded by an infrared (IR) sensor placed above the cage during the two experimental days.

Data acquisition and analysis. The EEG and EMG signals were amplified (amplification factor approx. 2000), conditioned by analog filters (high-pass filter: −3 dB at 0.016 Hz; low-pass filter: −3 dB at 40 Hz, less than −35 dB at 128 Hz.) sampled with 256 Hz, digitally filtered (EEG: low-pass FIR filter 25 Hz; EMG: band-pass FIR 20–50 Hz) and stored with a resolution of 128 Hz. EEG power spectra were computed for consecutive 4-s epochs by a fast Fourier transform routine within the frequency range of 0.25–25 Hz. Between 0.25 and 5 Hz, the 0.25 Hz bins were added to yield 0.5 Hz bins, and between 5.25 and 25 Hz to yield 1 Hz bins.

Based on the raw parietal and frontal EEG, the corresponding slow-wave activity (SWA), as well as the raw and integrated EMG, three vigilance states were visually scored for 4-s epochs as NREM sleep, rapid-eye movement (REM) sleep and waking (Tobler et al., 1997). Epochs containing artifacts were identified and excluded from spectral analysis (% of recording time: α3-KO: 7.5±1.7; WT: 6.4±2.3%). Data analysis was carried out using the MATLAB software package (The Math Works, Inc., Natick, MA, USA).

Motor activity. Motor activity was also recorded in a second group of mice (~12 weeks old; male; n=9 α3-KO mice; n=10 WT). These mice did not undergo EEG-EMG surgery and were littermates of the mice included in the sleep experiment. After at least 10 days' adaptation, motor activity was recorded continuously for 10 days via an IR sensor placed above the cage. Activity counts were stored in 1-min epochs (Tobler et al., 1996) and 10-day mean activity profiles were computed (Stanford Software Systems, Chronobiology Kit, Stanford, CA, USA). Rest was defined as the amount of 1-min epochs where activity counts equaled zero.