

# Activity Patterns in the Prefrontal Cortex and Hippocampus during and after Awakening from Etomidate Anesthesia

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## ABSTRACT

**Background:** The anesthetic properties of etomidate are largely mediated by  $\gamma$ -aminobutyric acid type A receptors. There is evidence for the existence of  $\gamma$ -aminobutyric acid type A receptor subtypes in the brain, which respond to small concentrations of etomidate. After awakening from anesthesia, these subtypes are expected to cause cognitive dysfunction for a yet unknown period of time. The corresponding patterns of brain electrical activity and the molecular identity of  $\gamma$ -aminobutyric acid type A receptors contributing to these actions remain to be elucidated.

**Methods:** Anesthesia was induced in wild-type and  $\beta 3$ (N265M) knock-in mice by intravenous injection of 10 mg/kg etomidate. Local field potentials were recorded simultaneously in the prefrontal cortex and hippocampus using chronically implanted electrode arrays. Local field potentials were sampled before, during, and after anesthesia.

**Results:** In the prefrontal cortex and hippocampus of wild-type mice, intravenous bolus injection of etomidate evoked isoelectric baselines and subsequent burst suppression. These effects were largely attenuated by the  $\beta 3$ (N265M) mutation. During emergence from anesthesia, power density in the  $\theta$  band (5–15 Hz) transiently increased in the hippocampus of wild types, but not in the mutants, indicating that this action

was caused by the receptors harboring  $\beta 3$  subunits. In both genotypes, etomidate produced a long-lasting ( $> 1$  h after recovery of righting reflexes) decrease in  $\theta$ -peak frequency. Significant slowing of  $\theta$  activity was apparent in the hippocampus and prefrontal cortex.

**Conclusions:** Etomidate-induced patterns of brain activity during deep anesthesia mostly involve actions at  $\beta 3$  containing  $\gamma$ -aminobutyric acid type A receptors. During the post-anesthesia period, altered  $\theta$ -band activity indicates ongoing anesthetic action.

## What We Already Know about This Topic

- ❖ It is speculated that etomidate produces long-lasting effects on cognition by action on  $\gamma$ -aminobutyric acid A ( $GABA_A$ ) receptor subtypes, which proved to be highly sensitive to this anesthetic

## What This Article Tells Us That Is New

- ❖ After awakening from etomidate anesthesia, the long-lasting effects on brain electrical activity were observed in the hippocampus and prefrontal cortex of mice
- ❖ These actions were in part abolished by a mutation of the  $\beta 3$  subunit of the  $GABA_A$  receptor

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THERE is increasing evidence for the existence of  $\gamma$ -aminobutyric acid type A ( $GABA_A$ ) receptors in the brain, which are highly susceptible to general anesthetics.<sup>1–4</sup> It has been shown that at concentrations well below those at which consciousness is lost, anesthetic agents in current clinical use cause sedation and amnesia *via* acting on these supersensitive  $GABA_A$  receptors. For that reason, it seems likely that after termination of general anesthesia, small concentrations of anesthetics impact the brain for a yet unknown period of time, thereby causing a cognitive dysfunction. Reynolds *et al.*<sup>5</sup> provided experimental evidence that anesthetic actions can persist for a long period after terminating anesthesia. These authors report that slow wave sleep in mice was increased for several hours after recovery from etomidate anesthesia. On the molecular level, the anesthetic properties of etomidate are mediated by  $GABA_A$  receptors incorporating a  $\beta$  subunit.<sup>6</sup> Remarkably, etomidate-induced changes in slow wave sleep were absent in

knock-in mice harboring a point mutation in the  $\beta 2$  subunit that rendered GABA<sub>A</sub> receptors containing the mutated subunit insensitive to etomidate.<sup>5</sup> These findings indicate that the ongoing impairment of brain functions after awakening from anesthesia is at least in part mediated by GABA<sub>A</sub> receptors containing the  $\beta 2$  subunit.

Different parts of the brain are not affected to the same degree by general anesthetics. It is well established that the neocortex and hippocampus are some of the most sensitive brain regions.<sup>7,8</sup> The cortical–hippocampal system is the circuitry underlying the formation of declarative memory—our ability to recollect everyday events and factual knowledge.<sup>9</sup> The prefrontal cortex is important for active working memory.<sup>10</sup> Working memory is a basic mechanism, by virtue of which an item of information can be held “on-line” for several seconds and used in the brain. It allows the brain to delay action and gather further sensory information to prepare a behavioral response. Furthermore, working memory facilitates the coordination of multiple neural systems and the integration of relevant information. Synchronized oscillatory activity in the  $\theta$ -frequency band is discussed as a key mechanism for structuring recurrent interactions between neurons in the different brain regions.<sup>11–15</sup> This type of activity seems to be important for hippocampal learning, cortical–hippocampal interactions, and the organization of brain activity during working memory tasks.

Postoperative cognitive dysfunction is unpleasant and can be a problem after outpatient surgery. Furthermore, long-lasting receptor activation is a prime candidate in initiating persistent changes in the brain function. The current study was motivated by the fact that very little is known about how neural activity presents in the brain during and after awakening from general anesthesia. We hypothesized that the prefrontal cortex and hippocampus are subjected to anesthetic action for a considerable period of time after terminating general anesthesia. To test this hypothesis experimentally, we investigated the effects of etomidate in mice, making use of a preparation that allows simultaneous recordings of local field potentials from prefrontal and hippocampal neurons with high temporal and spatial resolution. We further addressed the question as to whether GABA<sub>A</sub> receptors containing  $\beta 3$  subunits contribute to changes in the brain activity caused by etomidate.

## Materials and Methods

### Animals

Electrophysiologic recordings were carried out on wild-type and  $\beta 3$ (N265M) knock-in mice of both sexes on the same background as described previously (87.5% 129  $\times$  1/SvJ, 12.5% 129/Sv).<sup>16</sup> All experimental and surgical procedures were approved by the Animal Care Committee (Eberhard-Karls-University, Tuebingen, Germany) and were in accordance with the German law on animal experimentation. The generation of  $\beta 3$ (N265M) knock-in mice and their strain background have been described previously in detail.<sup>16</sup> The

behavioral assessment of motor activity and sensitivity to pain did not indicate a difference between wild-type and mutant mice. In addition, wild types and mutants did not differ significantly in body weight ( $33.4 \pm 5.0$  vs.  $36.4 \pm 5.9$  g, respectively). The N265M point mutation causes an almost complete loss of modulation of GABA<sub>A</sub> receptors by etomidate. Furthermore, the sensitivity to  $\gamma$ -aminobutyric acid is decreased slightly.<sup>17</sup>

### Surgical Procedures

For implanting electrodes in the prefrontal cortex and hippocampus, a similar approach was used as described previously for rats.<sup>18,19</sup> Anesthesia was induced and continued till the end of the surgery using isoflurane ( $< 1.5\%$ ). Adequate depth of anesthesia was checked continuously. The body temperature of animals was kept close to 36°C using feedback controlled warming equipment (Fine Science Tools, Heidelberg, Germany). Mice were placed in a stereotaxic device, and craniotomy was performed for implanting electrode arrays. Each animal received two linear arrays of four movable electrodes over the same hemisphere. One array was placed in the prefrontal cortex at a depth of 200  $\mu$ m below the pia, approaching presumably layer II. Another array was inserted through the somatosensory cortex at 900  $\mu$ m below the pia, targeting the hippocampus. The electrode arrays and connecting wires were fixed to the skull using light-curing dental cement (Flowline, Heraeus Kulzer, Hanau, Germany). The wound was cleaned and disinfected with hydrogen peroxide. Open skin was sutured and carefully attached to the implant. After surgery, the animals were kept warm and treated with analgesics (0.02 mg/kg Buprenorphine or Novalgin) and were allowed to recover for at least 14 days before experiments were conducted.

### Electrophysiology

Microelectrode arrays were assembled in our laboratory. Four glass-coated platinum tungsten electrodes (shank diameter: 80  $\mu$ m; diameter of the metal core: 23  $\mu$ m, free tip length: 10  $\mu$ m, and impedance:  $> 1$  M $\Omega$ ; Thomas Recording, Giessen, Germany) were placed inside a 1  $\times$  4 array of polyimide tubing (HV Technologies, Trenton, GA), with a distance between the tips of approximately 300  $\mu$ m. The electrodes were soldered to Teflon-insulated silver wires (Science Products, Hofheim, Germany), which in turn were connected to a microplug (Bürklin, Munich, Germany). They were attached to the screw that allowed moving them in the orthogonal direction. The simultaneous recordings of local field potential signals from both structures were performed using a multichannel extracellular amplifier (MultiChannelSystems, Reutlingen, Germany; gain 5000, sampling rate 20 kHz).

At the end of the experiments, animals were deeply anesthetized with barbiturates and perfused intracardially with 0.1 M phosphate buffer followed by paraformaldehyde (4% in phosphate buffer). The recording sites were marked by electrolytic lesions of the brain tissue, which were identified in the Nissl-stained coronal sections.

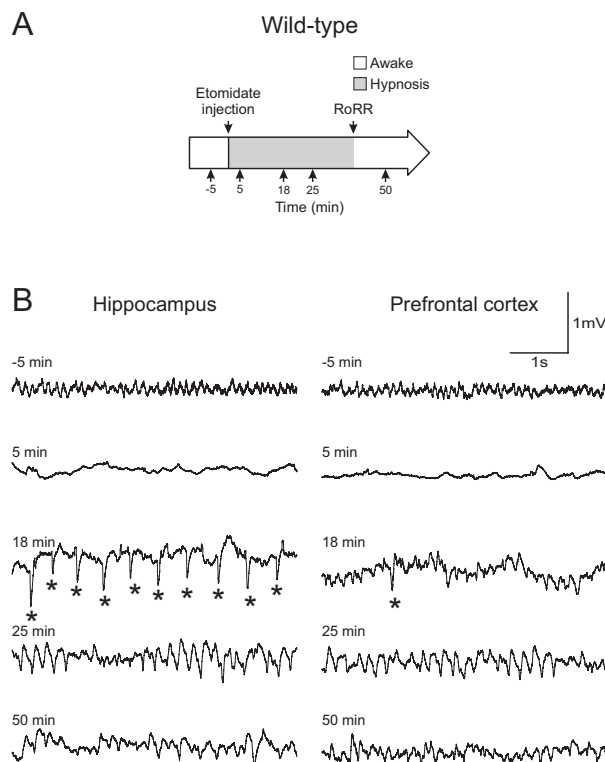
## Drug Administration

We induced deep etomidate anesthesia because we wanted to quantify the differences between mutant and wild-type mice. Because 10 mg/kg etomidate was the minimal dose causing isoelectric brain activity for a few minutes in wild types, it was used in our experiments. After injection, wild-type mice were apneic for a few seconds. For inducing anesthesia, standard solutions of etomidate (B. Braun Melsungen, Germany) were used. In these standard solutions, etomidate was dissolved in the solvent lipofundin (B. Braun). Sham-treated animals were injected with lipofundin. All drugs were administered intravenously through the tail vein. Electrophysiologic recording was started after placing the animal in a head-restraint apparatus. Neuronal activity was monitored for 10 min before etomidate bolus injection. For this predrug condition, no differences in the brain electrical activity were found between wild-type and mutant mice. Awakening from anesthesia was defined as a recovery of the righting reflex. The recordings were discontinued 120 min after etomidate administration. Thereafter, the animals were returned to their home cages.

## Data Analysis

We analyzed the neurophysiologic data by domestic software written in MATLAB version 7.8.0 including the Statistics Toolbox (Mathworks, Natick, MA). For quantifying the coherence between the electrical activity in the prefrontal cortex and hippocampus, the coherence function provided by MATLAB was used. This function takes two time series, computes their power spectra and the cross-spectral density, and returns the quotient of the magnitude of the cross-spectral density and the product of the power spectra. This quotient, a number between 0 and 1, measures the correlation between the two time series as a function of frequency. For analyzing coherent activity, local field potentials were recorded simultaneously in the prefrontal cortex and hippocampus at a temporal resolution of 10 ms. Coherence was integrated in a frequency interval of 0.5–200 Hz.

To quantify the effects of etomidate during the period the animals emerged from anesthesia, we first computed power spectra of the local field potential traces at a time resolution of 10 ms and calculated the integral of power spectrum density within  $\theta$  bandwidth.  $\theta$  Band was defined as the frequency spectrum between 5 and 15 Hz. In a following step, we averaged the integrals at 1-min intervals and normalized the numbers to the mean integral of the power in the predrug recording. To estimate the time course of  $\theta$  power recovery, a fourth order polynomial was fitted to the data. To assess the effects of the drug on the  $\theta$  rhythm in both drug and sham-injected mice, the nonparametric Kruskal–Wallace test was applied. In all other cases, the two-sided *t* test was used. Data are expressed as mean  $\pm$  SD. In some cases, the sample sizes were different because a small portion of the data was contaminated with artifacts and, therefore, had to be excluded from further analysis. *P* values  $< 0.05$  were considered significant.

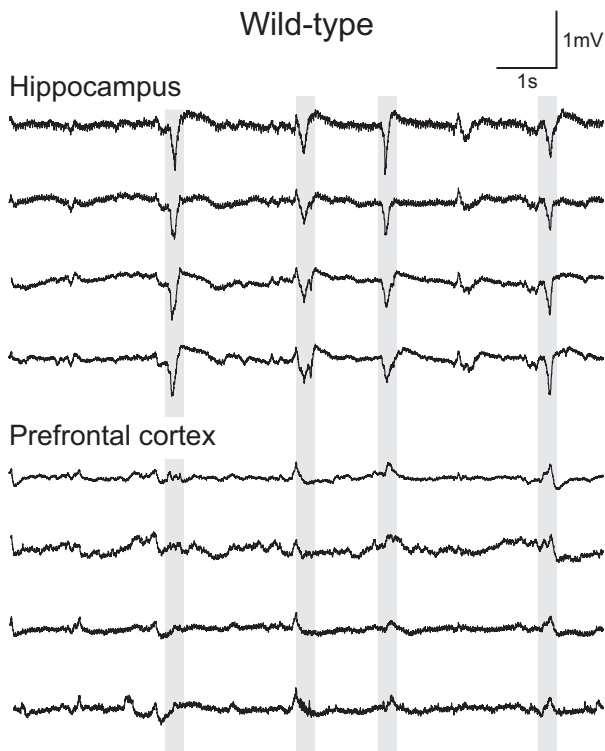


**Fig. 1.** Etomidate-induced modulation of electrical activity monitored simultaneously in the prefrontal cortex and hippocampus of wild-type mice. (A) Duration of loss of righting reflex, a common measure for a hypnotic state, caused by bolus injection of 10 mg/kg etomidate. The points in time at which the anesthetic was provided and when recovery of righting reflexes (RoRR) occurred are indicated by arrows. Field potential traces monitored at 5, 18, and 25 min after etomidate administration reflect brain activity during anesthetic-induced hypnosis. (B) Local field potential traces as recorded before and after etomidate injection. Isoelectric activity (5 min) is followed by typical burst suppression patterns (18 min). Single bursts are marked by asterisks.

## Results

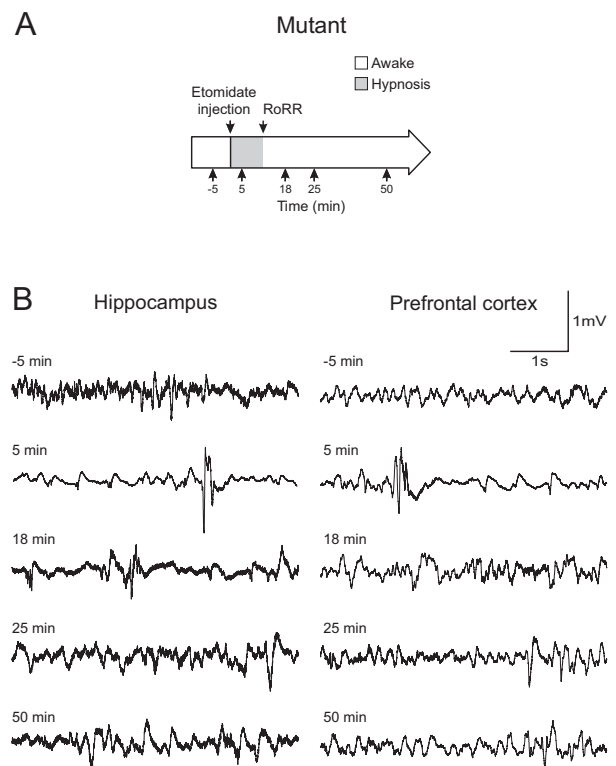
### Neuronal Activity during Etomidate Anesthesia

The current study investigates the role of  $\beta 3$  subunit containing GABA<sub>A</sub> receptors in mediating anesthetic-induced changes in the spontaneous neuronal activity of the prefrontal cortex and hippocampus. We have shown previously that the bolus injection of 10 mg/kg etomidate causes the loss of the righting reflex, a standard measure of hypnosis in rodents, for approximately 40 min in wild types and for approximately 10 min in  $\beta 3$  knock-in mice.<sup>16</sup> We expected that within these time windows, the activity of cortical neurons should be significantly altered by the anesthetic. In wild-type mice, 10 mg/kg etomidate dramatically changed the ongoing activity monitored simultaneously in the prefrontal cortex and hippocampus. The results from a typical recording are shown in figure 1. Within the first 5 min after injection, isoelectric baselines were visible, indicating almost complete neuronal depression. Thereafter, the burst suppression patterns developed. Bursts appeared highly synchro-



**Fig. 2.** Burst suppression activity recorded simultaneously with four electrodes in the hippocampus and four electrodes in the prefrontal cortex. The distance between two recording sites in the hippocampus and prefrontal cortex, respectively, was 300  $\mu\text{m}$ . To facilitate easy comparison between different electrodes, hippocampal spikes displaying large amplitudes are marked by vertical bars. The traces show that spontaneous electrical brain activity is highly regular and synchronized between prefrontal cortex and hippocampus.

nized across adjacent recording electrodes (fig. 2). Burst frequency increased from approximately 1 Hz, as measured 5 min after etomidate injection, up to 10 Hz, approximately 20 min later. Etomidate-induced burst suppression closely resembled the activity patterns evoked by thiopental or propofol in the neocortex of cats.<sup>20</sup> Approximately 20–30 min after the administration of etomidate, burst amplitudes gradually declined. In  $\beta 3$ -mutant mice, episodes of isoelectric baselines were never observed on etomidate administration. Instead, burst suppression activity immediately became established after injection of the anesthetic (fig. 3, traces at 5 min). The burst frequency was close to 1 Hz shortly after etomidate application and increased up to 5 Hz approximately 15 min later. To quantify the strength and duration of synchronized brain activity during the period of burst suppression, we computed the coherence in the local field potential between the prefrontal cortex and the hippocampus. Figure 4A shows a representative example of how coherence in the frequency spectrum between 0.5 and 200 Hz developed over 60 min after drug administration in wild-type and mutant mice. To quantify the duration of coherent activity, we integrated the data in figure 4A between 1 and 100 Hz and extracted the duration by setting the threshold as



**Fig. 3.** The  $\beta 3(\text{N265M})$  mutation largely attenuates the effects of etomidate on brain electrical activity observed in wild-type mice. (A) In  $\beta 3(\text{N265M})$  mice, hypnosis lasts less than 10 min, compared with approximately 40 min in wild types. (B) Local field potential recordings before and after etomidate injection show that in mutant mice, bolus injection of 10 mg/kg etomidate does not evoke isoelectric brain activity. RoRR = recovery of righting reflexes.

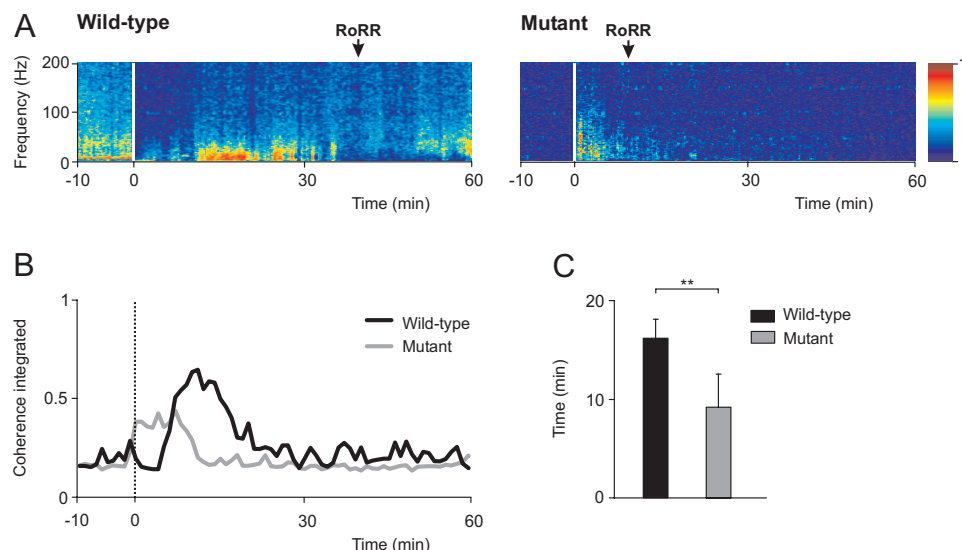
the upper confidence interval of coherence before drug administration (fig. 4B). In wild types, etomidate-induced increase in coherence lasted significantly longer than in mutants ( $16.20 \pm 1.95$  [ $n = 19$ ] and  $9.19 \pm 3.36$  [ $n = 25$ ], respectively,  $P < 0.01$ ) as indicated in figure 4C.

Figures 5A and B display spectrograms computed from representative local field potential recordings in wild-type and mutant mice. In wild-type mice, power density was almost completely depressed for a period of several minutes after drug injection in all frequency bands (fig. 5C). In the mutant, this effect was less pronounced in the hippocampus and almost completely absent in the prefrontal cortex.

### Recovery from Deep Etomidate Anesthesia

To provide a quantitative measure of recovery from deep anesthesia, we plotted the averaged time course of the power in the  $\theta$  band after etomidate bolus injection (fig. 6). Data were normalized to the control recordings carried out before drug administration.  $\theta$  Power dropped immediately on etomidate administration. In wild-type mice, the activity reached the level of the predrug condition in  $17.5 \pm 12.8$  and  $17.1 \pm 8.23$  min in both hippocampus ( $n = 26$ ) and prefrontal cortex ( $n = 19$ ), respectively. In mutants, it only took  $2.97 \pm 2.8$  min in the hippocampus ( $n = 30$ ) and





**Fig. 4.** Synchronization of the brain activity between the hippocampus and the prefrontal cortex during etomidate anesthesia. (A) Spectrograms of coherent activity between 0.5 and 200 Hz. (B) Integrated coherent activity between 1 and 100 Hz. In wild types, synchronized activity was low immediately after the injection of etomidate. Coherent activity increased after a delay of 4 min, reaching a maximum when burst suppression patterns were apparent in the hippocampus and prefrontal cortex. In mutants, injection of etomidate immediately increased coherent activity. (C) In wild types, coherent activity lasted significantly longer than in mutants (\*\*  $P < 0.01$ ). RoRR = recovery of righting reflexes.

$3.55 \pm 2.03$  min in the prefrontal cortex ( $n = 25$ ) to reach control level. The difference between wild types and mutants was statistically significant (hippocampus and prefrontal cortex:  $P < 0.01$ ). In both genotypes, the power returned to values exceeding the control level. This overshoot was expected because at moderate concentrations causing hypnosis but not surgical anesthesia, general anesthetics increase the power in all frequency bands except the  $\gamma$  range. In summary, the dissimilar anesthetic actions observed in wild-type and mutant mice strongly suggest that the modulation of  $\beta 3$  subunit containing GABA<sub>A</sub> receptors is required for producing deep anesthesia. Because in mutant mice, anesthetic depth is largely reduced, recovery is much faster.

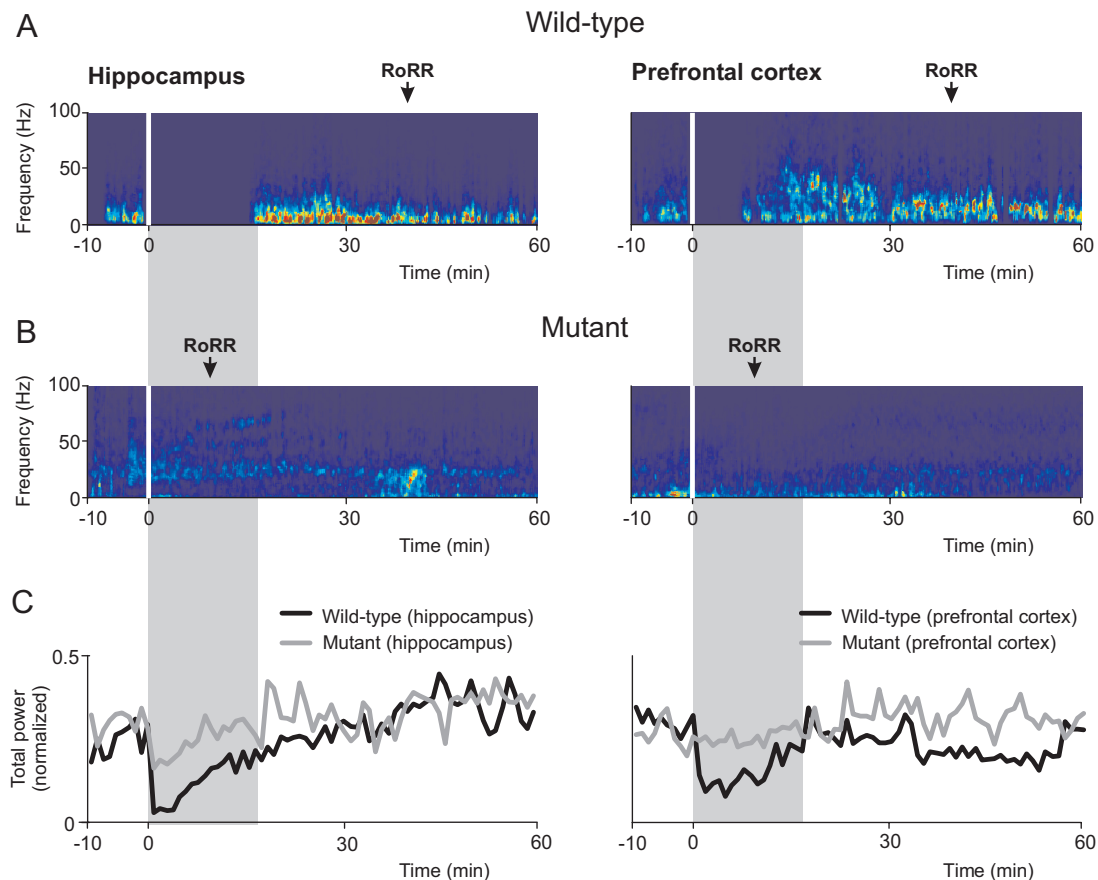
#### ***$\theta$ -Band Activity during Emergence from Anesthesia***

We quantified the changes in  $\theta$  power after etomidate bolus injection, including the time period of emergence from anesthesia (fig. 7). In wild-type mice, enhancement of  $\theta$  power was observed 40–60 min after etomidate injection in the hippocampal recordings, immediately after the recovery of righting reflexes (fig. 7A). In  $\beta 3$  mutants,  $\theta$  power in the hippocampus returned to the baseline level within a few minutes on injecting the anesthetic and remained almost constant for the rest of the recording. To see whether the difference between the actions of etomidate in wild-type and mutant mice was statistically significant, we compared the integrated  $\theta$  activity between 40–60 min and 80–100 min after injection, respectively. The first time window corresponded to the very early phase of emergence from anesthesia, soon after righting reflexes had returned. In the second time window, recovery from anesthesia had closely approached predrug conditions. Hippocampal  $\theta$  power was significantly different between wild-type ( $n = 26$ ) and mutant mice

( $n = 30$ ) in the first ( $P < 0.01$ ) but not the second ( $P > 0.05$ ) time window. The recordings in sham-injected animals of both genotypes in both structures (wild type:  $n = 14$  in the prefrontal cortex,  $n = 16$  in the hippocampus; mutant:  $n = 11$  in the prefrontal cortex,  $n = 16$  in the hippocampus) did not show enhanced  $\theta$  activity 40–60 min after injection. In summary, these results indicate that etomidate-induced augmentation of hippocampal  $\theta$  oscillations is time locked to the early phase of emergence from anesthesia and is mediated *via*  $\beta 3$  subunit containing GABA<sub>A</sub> receptors.

We further analyzed the effects of etomidate on  $\theta$  activity in the prefrontal cortex of mice of both genotypes (wild types:  $n = 19$ ; mutants:  $n = 25$ ). In this part of the brain, the anesthetic did not boost  $\theta$  power, and a difference between wild-type and mutant animals was not observed (fig. 7B). Thus, etomidate-induced enhancement of  $\theta$  oscillations was selective for the hippocampus.

Finally, we compared the peak frequency in the  $\theta$  band before and after recovery from etomidate anesthesia. For this purpose, we averaged the peak frequencies in the  $\theta$  range for 20 min immediately before etomidate injection and 40–60 min and 80–100 min after etomidate injection. Under predrug conditions, no difference was found between genotypes and between the prefrontal cortex and the hippocampus. Etomidate reduced the peak frequency in the hippocampus and prefrontal cortex to a similar extent (fig. 8). The differences between genotypes were not observed. In wild types, the peak frequency had changed after injection from  $6.97 \pm 0.6$  Hz to  $6.22 \pm 0.44$  Hz and from  $6.83 \pm 0.58$  Hz to  $6.39 \pm 0.48$  Hz in the hippocampus ( $n = 26$ ) and prefrontal cortex ( $n = 19$ ), respectively. This effect reached statistical significance in both structures ( $P < 0.01$ ). In mutants, the initial peak frequency decreased from  $7.04 \pm 0.6$  to  $6.21 \pm 0.4$  in the hippocampus ( $n = 30$ ) and from  $6.83 \pm 0.59$  to  $6.36 \pm 0.54$  in the prefrontal cortex



**Fig. 5.** (A) Spectrograms calculated from etomidate-induced electrical brain activity monitored in wild-type (A) and  $\beta 3$ (N265M) mice (B). The data in the *left column* are obtained from the recordings in the hippocampus and the data on the *right* from the prefrontal cortex. The time point of etomidate administration is indicated by the *vertical white bars*. In the wild type, etomidate strongly reduced the power in all frequency bands between 0.5 and 35 Hz in the hippocampus and prefrontal cortex. This effect is less pronounced in the mutant. (C) Total power before and after etomidate injection in the wild-type and the  $\beta 3$ (N265M) mutants. RoRR = recovery of righting reflexes.

( $n = 25$ ). This reduction was also statistically significant in both structures ( $P < 0.01$ ), suggesting that this effect is not mediated by  $\beta 3$  subunit containing GABA<sub>A</sub> receptors.

## Discussion

### Major Findings of the Study

We studied the effects of etomidate in wild-type and  $\beta 3$  mutant mice during and after awakening from anesthesia. The anesthesia-related patterns of brain electrical activity were attenuated by the mutation as indicated by the absence of isoelectric activity (figs. 3 and 5) and faster recovery of  $\theta$ -band power (fig. 6) in mutant mice. These findings are in accordance with behavioral studies, showing that the duration of loss of righting reflex is considerably shorter in mutants.<sup>16</sup>

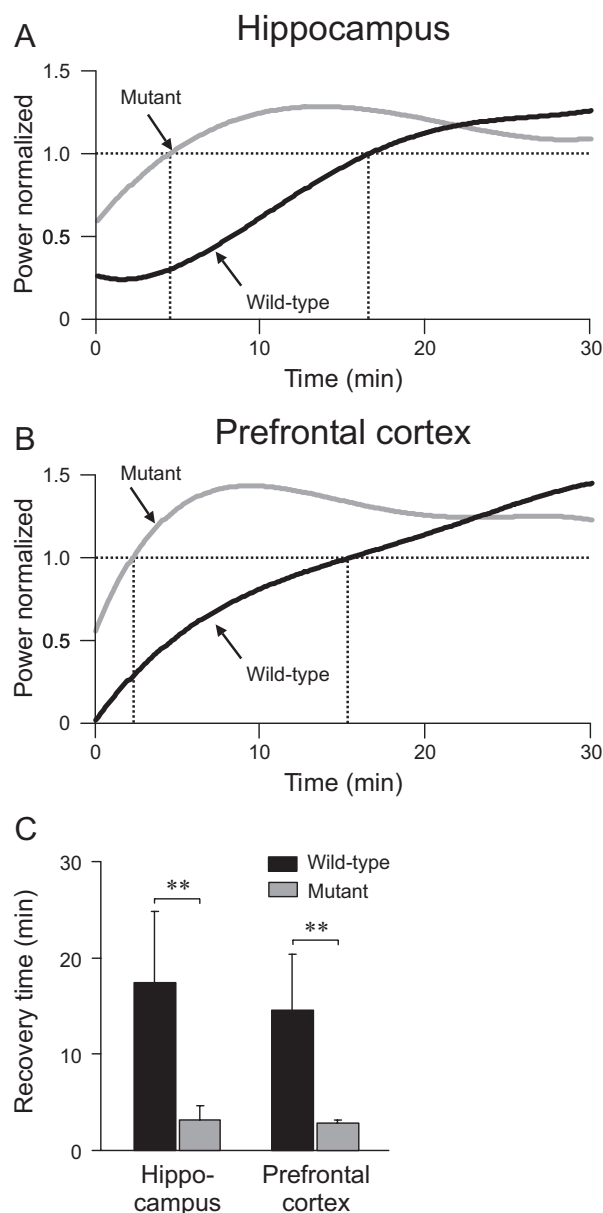
Soon on emergence from anesthesia, as indicated by the reappearance of righting reflexes, the power in the  $\theta$  band rapidly increased in the hippocampus (fig. 7A). During the next hour,  $\theta$  power gradually declined. We speculate that the latter effect is linked to a decline in the brain concentration of etomidate, caused by metabolism and elimination of the drug. Furthermore, etomidate-induced enhancement of hippocampal  $\theta$

power was abolished by the  $\beta 3$  mutation, indicating the involvement of GABA<sub>A</sub> receptors containing the  $\beta 3$  subunit. In summary, these findings are consistent with the hypothesis that the transient enhancement of hippocampal  $\theta$  power during the postanesthesia period is caused by the modulation of hippocampal GABA<sub>A</sub> receptors by small concentrations of etomidate.

In addition, we observed that after awakening from anesthesia, the peak frequency in the  $\theta$  band was significantly shifted to lower values. This was found in the prefrontal cortex and in the hippocampus. The underlying mechanisms remain to be elucidated in further studies, because this effect was not sensitive to the mutation, and no recovery was observed during the recording period. Longer recordings during the postanesthesia period are required to determine whether this action persists over days or weeks.

### Effects of Etomidate in the Hippocampus and Prefrontal Cortex during Anesthesia

Intravenous bolus injection of etomidate in wild-type mice rapidly produced isoelectric activity and subsequent burst suppression in the prefrontal cortex and hippocampus. Only



**Fig. 6.** Polynomial fits of the time course of  $\theta$ -band power after bolus injection of etomidate in the hippocampus (A) and prefrontal cortex (B). The traces represent grand averages. Corresponding raw data are shown in figures 7A and B. (C) In the  $\beta 3$ (N26 5M) mutants,  $\theta$  power reaches baseline levels within 3 min after etomidate administration. In wild types, this takes more than 15 min. The difference between wild types and mutants is statistically significant (\*\*  $P < 0.01$ ).

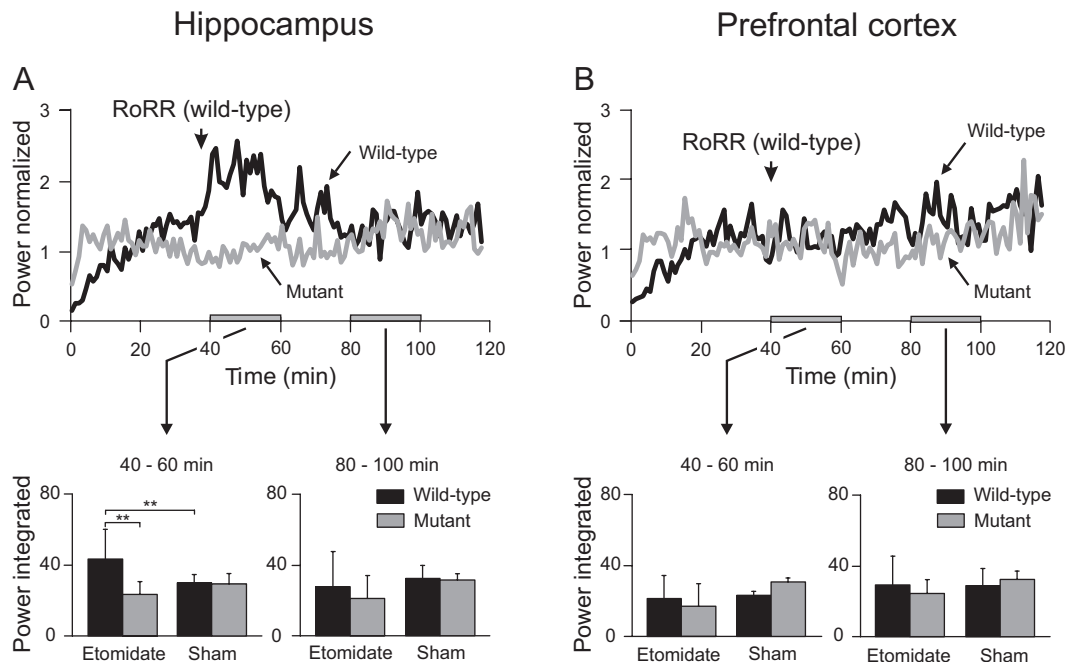
if burst suppression patterns were apparent, neuronal activity was highly synchronized in the hippocampus and prefrontal cortex. In a pioneering study, MacIver *et al.*<sup>21</sup> compared the effects of thiopental in the neocortex and hippocampus. Similar to our findings with etomidate, these authors report that during burst suppression, (1) cortical burst events were synchronized with events in the hippocampus, (2) the peaks of hippocampal and neocortical events did not show a stable phase relationship (fig. 2), and (3) synchrony was markedly reduced in the late stages of burst suppression (fig. 3). The

same authors report an uncoupling of hippocampal and neocortical activity at high anesthetic concentrations. A related phenomenon was observed in our recordings because after injection of etomidate, burst suppression activity appeared earlier in the prefrontal cortex when compared with the hippocampus (fig. 5).

On the molecular level, etomidate predominantly acts *via* GABA<sub>A</sub> receptors incorporating  $\beta 2$  or  $\beta 3$  subunits.<sup>6,22,23</sup> Moreover, the anesthetic properties of etomidate have been investigated in knock-in mice, in which GABA<sub>A</sub> receptors containing the  $\beta 2$  or the  $\beta 3$  subunit were rendered insensitive to etomidate by a point mutation.<sup>5,16</sup> In  $\beta 2$  knock-in mice, a significant reduction in the loss of motor reflexes in response to intravenously applied etomidate was reported.<sup>5</sup> However, in the same genotype, the changes in electroencephalographic activity that were caused by etomidate did not differ from those observed in wild-type mice. This finding provided indirect evidence that etomidate alters electroencephalographic activity predominantly *via*  $\beta 3$  subunit containing GABA<sub>A</sub> receptors. Our results are in good accordance with this suggestion. The effects of etomidate on the electrical activity of the cortical neurons clearly differed between  $\beta 3$  mutant and wild-type mice. Most importantly, etomidate failed to evoke isoelectric baselines in  $\beta 3$  mutants, indicating that deep anesthesia was not achieved in these animals. Conversely, the current study shows that further molecular targets contribute to etomidate anesthesia, because the burst suppression patterns were not completely abolished in  $\beta 3$  knock-in animals (fig. 3).

#### ***$\theta$ -Band Power in the Hippocampus Increases Transiently after Awakening from Anesthesia***

$\theta$  Oscillations in the hippocampus arise from the synchronized activity of a large population of neurons, causing fluctuations of extracellular field potentials in the  $\theta$ -frequency band.<sup>24</sup> There is extensive evidence for a causal link between the hippocampal  $\theta$  rhythm and the mnemonic functions in rodents.<sup>24</sup> At subhypnotic concentrations, etomidate suppresses hippocampus-dependent learning tasks such as contextual fear conditioning and spatial learning.<sup>2</sup> Cheng *et al.*<sup>2</sup> showed that these actions are largely produced by the  $\alpha 5$  subunit. The GABA<sub>A</sub> receptors incorporating this subunit are densely packed in the dendrites of hippocampal pyramidal cells, mediating a tonic conductance.<sup>25</sup> Furthermore, GABA<sub>A</sub> receptors harboring  $\alpha 5$  subunits contribute to a slow form of synaptic inhibition in the hippocampal CA1 pyramidal cells (GABA<sub>A,slow</sub>).<sup>26</sup> The presence of GABA<sub>A,slow</sub> has also been demonstrated in the neocortical pyramidal cells.<sup>27</sup> By virtue of its dendritic localization and kinetic properties, which closely match those of *N*-methyl-D-aspartate receptor-mediated excitation, GABA<sub>A,slow</sub> controls synaptic plasticity and memory formation. In a recent study, Dai *et al.*<sup>4</sup> have shown that GABA<sub>A,slow</sub> is potentiated by the amnesic concentrations of etomidate, leaving synaptic inhibition at the soma almost unaffected. These findings strongly suggest that impairment of hippocampal learning by etomidate involves



**Fig. 7.**  $\theta$ -Band power during recovery from etomidate anesthesia in the hippocampus (A) and prefrontal cortex (B) of wild-type and  $\beta 3$ (N265M) mutant mice. Bar plots indicate  $\theta$  power 40–60 min and 80–100 min after etomidate injection. The first time window matches the period shortly after righting reflexes reappeared in wild-type animals. Therefore, it coincides with an early phase of emergence from anesthesia. During this period, etomidate caused a significant increase in  $\theta$  power in the hippocampus but not in the prefrontal cortex. This action is sensitive to the  $\beta 3$ (N265M) mutation. It was absent in sham-injected animals of both genotypes (\*\*  $P < 0.01$ ). RoRR = recovery of righting reflexes.

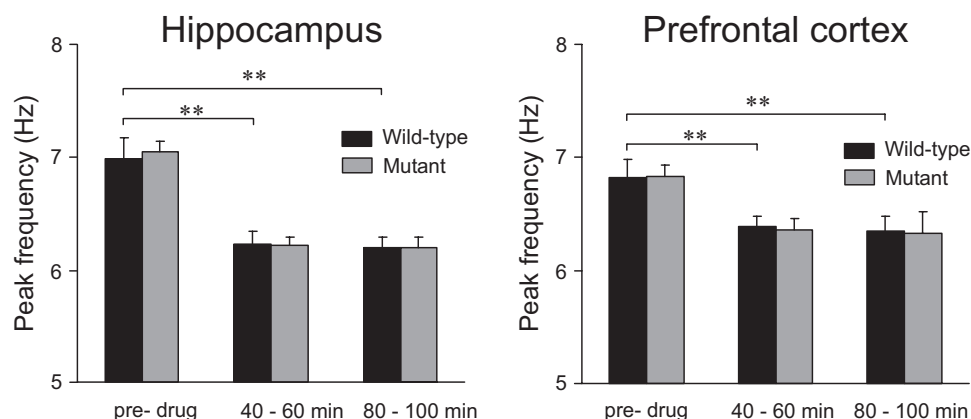
to a large degree GABA<sub>A,slow</sub>. It seems possible that GABA<sub>A,slow</sub> contributes to the hippocampal and neocortical  $\theta$  rhythms.<sup>28–30</sup> Accordingly, we found that subhypnotic concentrations of etomidate slowed  $\theta$  oscillations. This effect was observed in both the hippocampus and prefrontal cortex. Although the function of  $\theta$  activity in the prefrontal cortex is less clear than in the hippocampus, there is evidence for a role during working memory tasks.<sup>13</sup>

Because etomidate enhanced  $\theta$ -band power only in the hippocampus and the  $\alpha 5$  subunit of the GABA<sub>A</sub> receptor is mostly expressed in the hippocampus, we speculate that etomidate-induced amplification of  $\theta$ -band power is mediated

by GABA<sub>A</sub> receptors harboring the  $\alpha 5$  subunit. Our finding that etomidate-induced enhancement of  $\theta$  power was absent in  $\beta 3$  knock-in mice suggests that  $\beta 3$  subunit containing receptors are involved.

#### Slowing of $\theta$ Activity Is Observed in the Prefrontal Cortex and Hippocampus

Etomidate caused a slowing of  $\theta$  activity in the hippocampus and prefrontal cortex. In striking contrast to the enhancement of hippocampal  $\theta$  power, which only transiently appeared during emergence from anesthesia, etomidate-induced slowing of  $\theta$ -peak frequency did not show signs of



**Fig. 8.** Effects of etomidate on the peak frequency in the  $\theta$  band as observed after emergence from anesthesia. Etomidate produced a significant slowing of  $\theta$  activity in both the hippocampus and the prefrontal cortex (\*\*  $P < 0.01$ ). This action was not sensitive to the  $\beta 3$ (N265M) mutation.



recovery. However, the recording sessions were terminated 1 h after reappearance of righting reflexes. The duration of recording was restricted to this time window to avoid considerable discomfort for animals, which were head fixed during all experiments.

We observed that etomidate slowed  $\theta$  activity in wild types and mutants to almost the same extent, indicating that this action does not depend on  $\beta 3$ -containing GABA<sub>A</sub> receptors. Because etomidate acts predominantly *via* receptors harboring the  $\beta 2$  or  $\beta 3$  subunit, slowing of  $\theta$  activity is most likely linked to the  $\beta 2$  subunit.

Reynolds *et al.*<sup>5</sup> showed that GABA<sub>A</sub> receptors containing the  $\beta 2$  subunit contribute to sedation during the postanesthesia period. These authors demonstrated that after awakening from 10 mg/kg intravenous etomidate, a dose that also was applied in the current study, the levels of slow wave sleep were largely increased in the wild-type but not in the mutant mice carrying the N265S mutation in the  $\beta 2$  subunit of the GABA<sub>A</sub> receptor. In their study, enhancement of slow wave sleep was quantified for up to 3 h after mice regained the righting reflex. During this time, no recovery was observed. These findings raise the possibility that (1) the enhancement of slow wave sleep and (2) the decrease in brain  $\theta$  activity are linked to the same molecular target, as both involve the  $\beta 2$  subunit and recovery is not observed within hours after terminating etomidate anesthesia. The neuronal substrates mediating these long-lasting actions are unknown. However, the changes in sleep regulation reported by Reynolds and coworkers<sup>5</sup> are pointing to the neuronal pathways of sleep and arousal, which have been shown to be subject to anesthetic modulation<sup>31</sup> and also regulate cortical  $\theta$  activity.<sup>29</sup> A subcortical mechanism of action could explain why the amount by which  $\theta$ -peak frequency shifted toward smaller values was found to be almost identical in the prefrontal cortex and hippocampus.

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