Altered hippocampal rhythms in GABA$_A$ receptor β_3(N265M) mice

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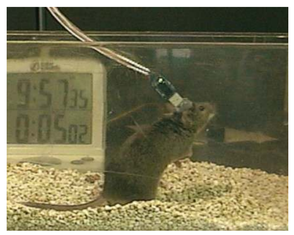
Introduction

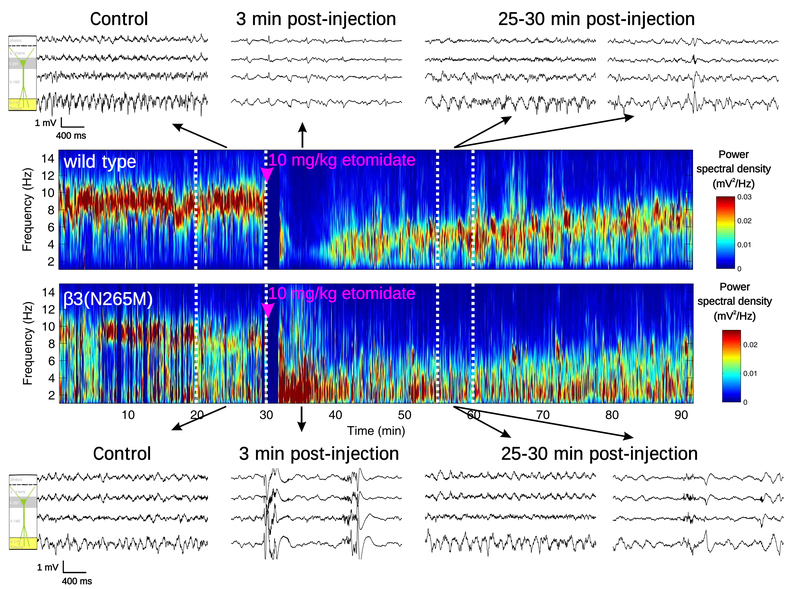
- Hippocampal field potential activity in rodents contains strong θ (4-12 Hz) and γ (40-100 Hz) components
- Both rhythms are nested: γ oscillation amplitude is rhythmically modulated at θ frequency
- GABAergic synapses containing β_3 subunits are abundant in dendritic layers of hippocampus (Sperk et al 1997). There is evidence that they contribute in major ways both to the generation of θ and γ rhythms and to θ/γ nesting:
  - In knockout mice of the β_3 subunit, θ frequency, γ power and the degree of nesting in vivo are reduced (Hentschke et al 2004)
  - GABA$_{A,slow}$, a dendritically located inhibitory current with a τ_{Decay} of ~30-70 ms (Pearce 1993, Banks et al 1998), is largely reduced in β_3 knockouts (Banks et al 2001). As GABA$_{A,slow}$ suppresses fast inhibition in vitro (Banks et al 2000) presumably it mediates nested θ/γ rhythms in vivo
- Here, we used mice harboring a mutation in the β_3 subunit (β_3N265M; Jurd et al, 2003) to elucidate the role of GABAergic synapses containing this subunit in the generation and interplay of θ and γ rhythms. The mutation decreases GABA sensitivity of the receptors (Siegwart et al 2002) and renders the receptors insensitive to the intravenous anesthetic etomidate, leaving GABA$_A$ receptors with β_2 subunits as the sole major target of the anesthetic
- We compared field potential oscillations in area CA1 of wild type (wt) and mutant (β_3-mut) mice under control conditions and after intraperitoneal administration of etomidate. The concentrations were chosen such as to embrace the EC50 of contextual fear conditioning (11 mg/kg, Benkwitz et al 2006)

Methods



- in vivo recordings of hippocampal local field potentials (LFPs) from awake behaving animals
- multielectrode array in CA1 (inter-electrode spacing 200 µm)
- behavioral scoring (grooming, immobility, exploring)
- intraperitoneal injection of 5, 10 or 15 mg/kg etomidate (EC50 of contextual fear conditioning was found to be 11 mg/kg, Benkwitz et al 2006)
- continuous acquisition of LFPs 30 min pre-injection (control) and 60 min post-injection
- analysis of data recorded 20-30 min during the control period and 25-30 min post-injection
- spectral and cross correlation analyses of LFPs

I. Etomidate-induced, rhythmic field potential 'spikes' in β_3-mut mice immediately post-injection



II. γ oscillations are stronger in wild types than in β_3-mut; etomidate depresses γ and θ rhythms in both genotypes

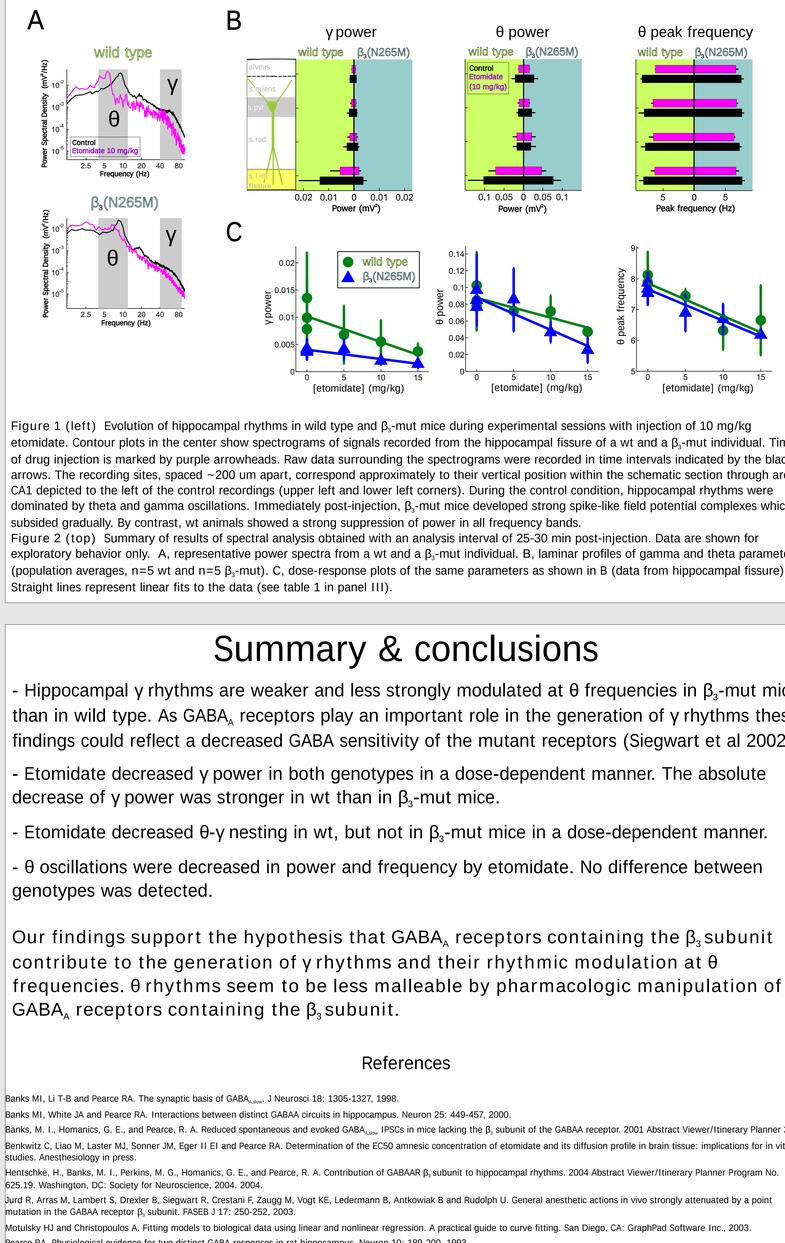


Figure 1 (left) Evolution of hippocampal rhythms in wild type and β_3-mut mice during experimental sessions with injection of 10 mg/kg etomidate. Contour plots in the center show spectrograms of signals recorded from the hippocampal fissure of a wt and a β_3-mut individual. Time of drug injection is marked by purple arrowheads. Raw data surrounding the spectrograms were recorded in time intervals indicated by the black arrows. The recording sites, spaced ~200 um apart, correspond approximately to their vertical position within the schematic section through area CA1 depicted to the left of the control recordings (upper left and lower left corners). During the control condition, hippocampal rhythms were dominated by theta and gamma oscillations. Immediately post-injection, β_3-mut mice developed strong spike-like field potential complexes which subsided gradually. By contrast, wt animals showed a strong suppression of power in all frequency bands.
Figure 2 (top) Summary of results of spectral analysis obtained with an analysis interval of 25-30 min post-injection. Data are shown for exploratory behavior only. A, representative power spectra from a wt and a β_3-mut individual. B, laminar profiles of gamma and theta parameters (population averages, n=5 wt and n=5 β_3-mut). C, dose-response plots of the same parameters as shown in B (data from hippocampal fissure). Straight lines represent linear fits to the data (see table 1 in panel III).

III. θ–γ 'nesting' is weak and not affected by etomidate in β_3-mut mice

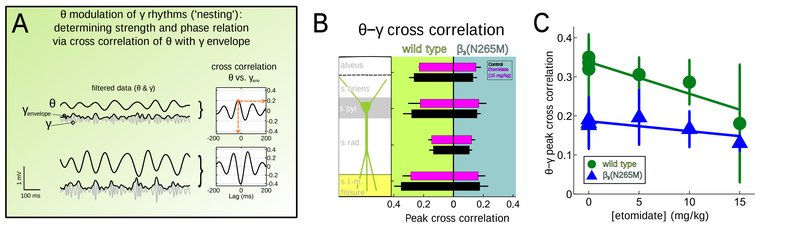


Figure 3 Analysis of θ modulation of γ rhythms under control conditions and with etomidate (25-30 min post-injection interval). A, the envelope of γ was cross correlated with the q signal from the same electrode. The cross correlation functions were normalized (range of values [-1 1]) and thus independent of the amplitudes of the correlated signals. The magnitude of the central peak was extracted as a measure of the degree of θ -γ nesting. B, laminar profile of θ-γ nesting (population averages from same animals as in figure 2). C, dose-response curve of nesting (data from hippocampal fissure).

	γ power	θ power	θ frequency	θ-γ cross corr
slope WT (imm/expl)	<0.005/0.71	<0.05/0.067	<0.001/<0.001	<0.001/<0.01
slope MUT (imm/expl)	<0.001/<0.01	<0.001/<0.005	<0.01/<0.001	0.11/0.24
slope WT vs. MUT (imm/expl)	0.27/0.49	0.50/0.67	0.72/0.99	<0.05/0.27
offset WT vs. MUT (imm/expl)	<0.005/<0.01	0.99/0.99	0.96/0.61	<0.001/<0.001

Table 1 Summary of linear regression analysis. Dose-response curves in figures 2 and 3 were fitted to a linear model (y=a+b*x). Significance of the slopes of the resulting fits and the differences between the genotypes (in terms of slopes and constant terms) was computed via F-tests (Motulsky & Christopolous 2003). The table lists the resulting p-values for data acquired during immobile (imm) and exploring (expl) behavior. Note the highly significant difference of θ-γ nesting between wild types and β_3-mut, as well as a lack of dependence of nesting on etomidate concentration in β_3-mut.

Summary & conclusions

- Hippocampal γ rhythms are weaker and less strongly modulated at θ frequencies in β_3-mut mice than in wild type. As GABA$_A$ receptors play an important role in the generation of γ rhythms these findings could reflect a decreased GABA sensitivity of the mutant receptors (Siegwart et al 2002)
- Etomidate decreased γ power in both genotypes in a dose-dependent manner. The absolute decrease of γ power was stronger in wt than in β_3-mut mice.
- Etomidate decreased θ-γ nesting in wt, but not in β_3-mut mice in a dose-dependent manner.
- θ oscillations were decreased in power and frequency by etomidate. No difference between genotypes was detected.

Our findings support the hypothesis that GABA$_A$ receptors containing the β_3 subunit contribute to the generation of γ rhythms and their rhythmic modulation at θ frequencies. θ rhythms seem to be less malleable by pharmacologic manipulation of GABA$_A$ receptors containing the β_3 subunit.

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