

Diazepam and ethanol induce different types of inhibition in the neocortex

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Abstract

Background: Many general anesthetics and sedative-hypnotics exert their clinically desired effects, at least in larger parts, via GABA_A receptors. Neocortical networks express a multitude of GABA_A receptor subtypes, but little is known how drug interactions with different subtypes affects the activity patterns of these networks. Here we investigate the actions of the benzodiazepine diazepam and of ethanol, known to act on largely non-overlapping GABA_A receptor populations. Diazepam exclusively acts on γ -subunit containing synaptic and extrasynaptic GABA_A receptors, whereas ethanol has been found to interact with benzodiazepine-insensitive δ -containing GABA_A receptors. [1,2,3]

Methods: Organotypic slice cultures were prepared from the neocortex of P 3-5 rats. After cultivating them for two weeks in vitro drug effects on spontaneous action potential firing and on inhibitory postsynaptic currents (IPSCs) were determined. Spontaneously active slice cultures displayed oscillations in the local field potential, indicating synchronous network firing. The power spectrum of these oscillations was calculated by fast Fourier transform.

Results: Both diazepam and ethanol depressed spontaneous action potential firing in a dose-dependent manner. Diazepam significantly increased the current decay and charge transfer of the average IPSC. Furthermore, diazepam (30 μ M) significantly enhanced synchronous network firing and led to an increase in power at frequencies between 6 and 15 Hz. Ethanol had no effect on either IPSCs or network oscillations.

Conclusions: Diazepam and ethanol enhance two different types of gabaergic inhibition in neocortical networks. Both drugs act via GABA_A receptors and depress spontaneous activity. Diazepam acts preferentially via synaptic GABA_A receptors, enhancing phasic inhibition. By prolonging IPSC current decay and by phase-locking of principal cells it fosters synchronized network firing. Ethanol does not enhance network oscillations. This could be explained by an action mainly at extrasynaptic GABA_A receptors, which would enhance tonic inhibition without affecting network synchrony. This finding shows that sedative-hypnotic drugs, which exert their clinically desired effects via GABA_A receptors, can have differential effects on neuronal networks.

Summary: Diazepam and ethanol act via largely non-overlapping GABA_A receptor subtypes. Both drugs depress spontaneous action potential firing in cultured neocortical slices. But only diazepam, which modulates phasic inhibition, fosters synchronized network oscillations. This shows that two drugs, although acting both on GABA_A receptors, can affect neuronal networks in different ways.

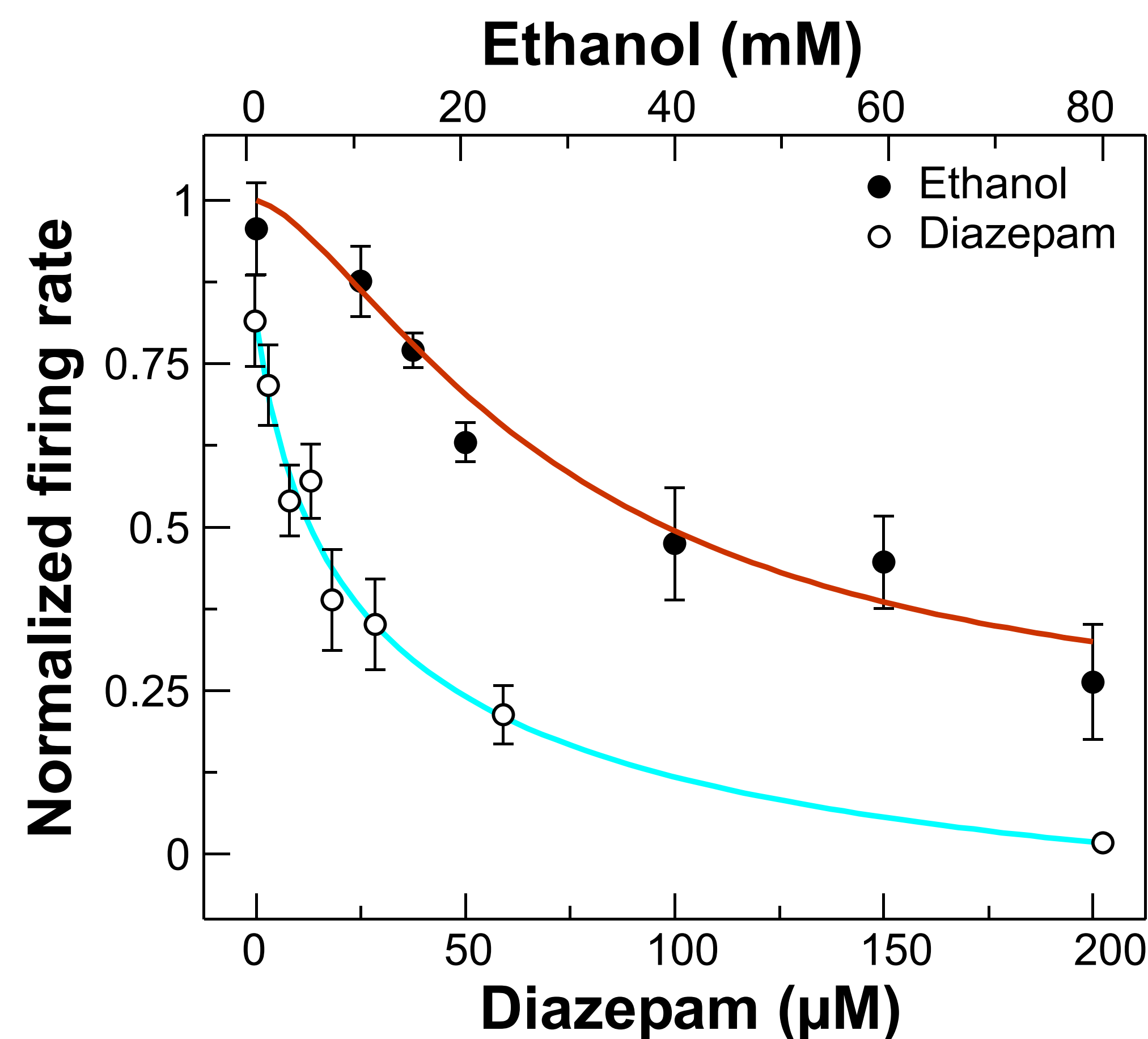


Figure 1: Depression of spontaneous action potential firing by Diazepam and Ethanol

Both drugs dose-dependently depressed spontaneous action potential firing. The EC₅₀ of diazepam was 18.9 μ M, whereas for ethanol it was 38.5mM.

For further experiments diazepam at a concentration of 15 μ M and ethanol at a concentration of 30mM were considered nearly equipotent.

Diazepam

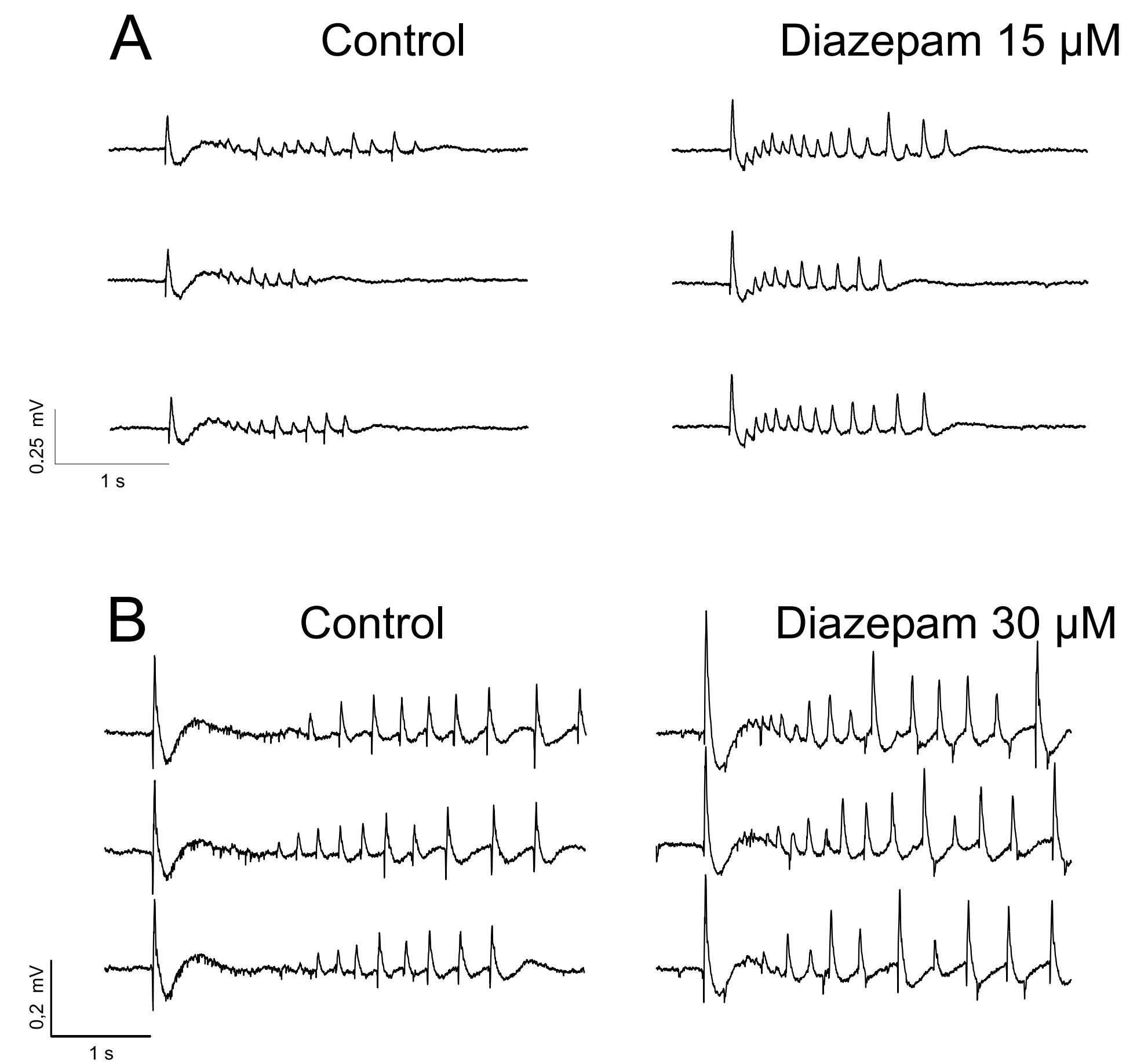


Figure 2: Diazepam fosters oscillations in the local field potentials (A and B) while ethanol fails to do so (C and D)

Episodes taken from extracellular recordings of the local field potential (LFP). LFPs exhibit a characteristic time structure: at the onset a biphasic signal occurs in the LFP, exhibiting a large positive and a smaller negative peak. In the following seconds neurons discharge at lower rates while the LFP is close to baseline, indicating the presence of uncorrelated action potential activity. Towards the end of a burst of action potentials an oscillatory component in the LFP developed, arising from progressive synchronization of neuronal activity. During this phase, action potentials were time locked to the rising phase of the oscillations.

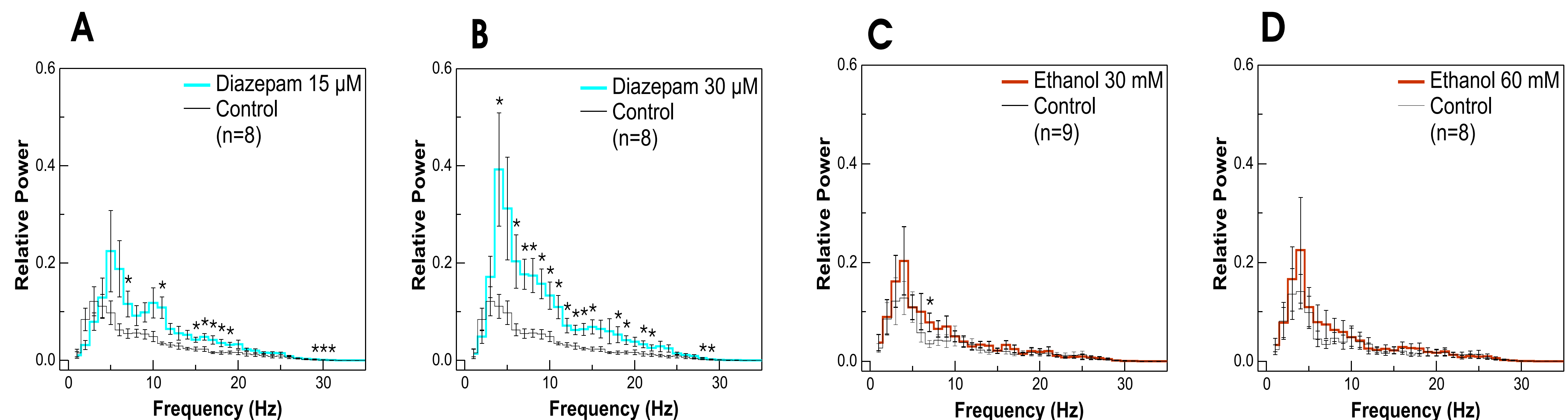


Figure 3: Power spectra from the local field potential. Diazepam enhances oscillations over a broad frequency range while ethanol does not

Diazepam in a concentration of 15 μ M significantly increased the power at 7, 11, 14 and 28-30Hz (ANOVA, $p < 0.05$) compared to control condition (fig 3A). This synchronizing effect was even more pronounced at a concentration of 30 μ M (fig. 3B), where the frequencies at 4, 6-15, 18, 19, 21, 22, 28 and 29Hz were significantly enhanced (ANOVA, $p < 0.05$).

Ethanol on the other hand did not lead to pronounced changes in oscillatory activity. At a bath concentration of 30mM it only augmented the power at a frequency of 7Hz to a statistically significant degree (fig. 3C). No significant difference between control condition and in the presence of 60mM Ethanol could be observed at any frequency (fig 3D).

For computation of power-density spectra from the LFP episodes of ongoing activity (EOAs) of fixed length were cut from the continuous LFP recording. Power density between zero and 30 Hz in the EOAs was then determined by Fast Fourier Transform using Matlab 6.5. An average spectrum for each 180 s recording was obtained by averaging the spectra of the individual EOAs. The mean spectrum was then calculated from all the spectra obtained under the same experimental condition. All spectra were normalized to the total power in their spectrum under control conditions.

Ethanol

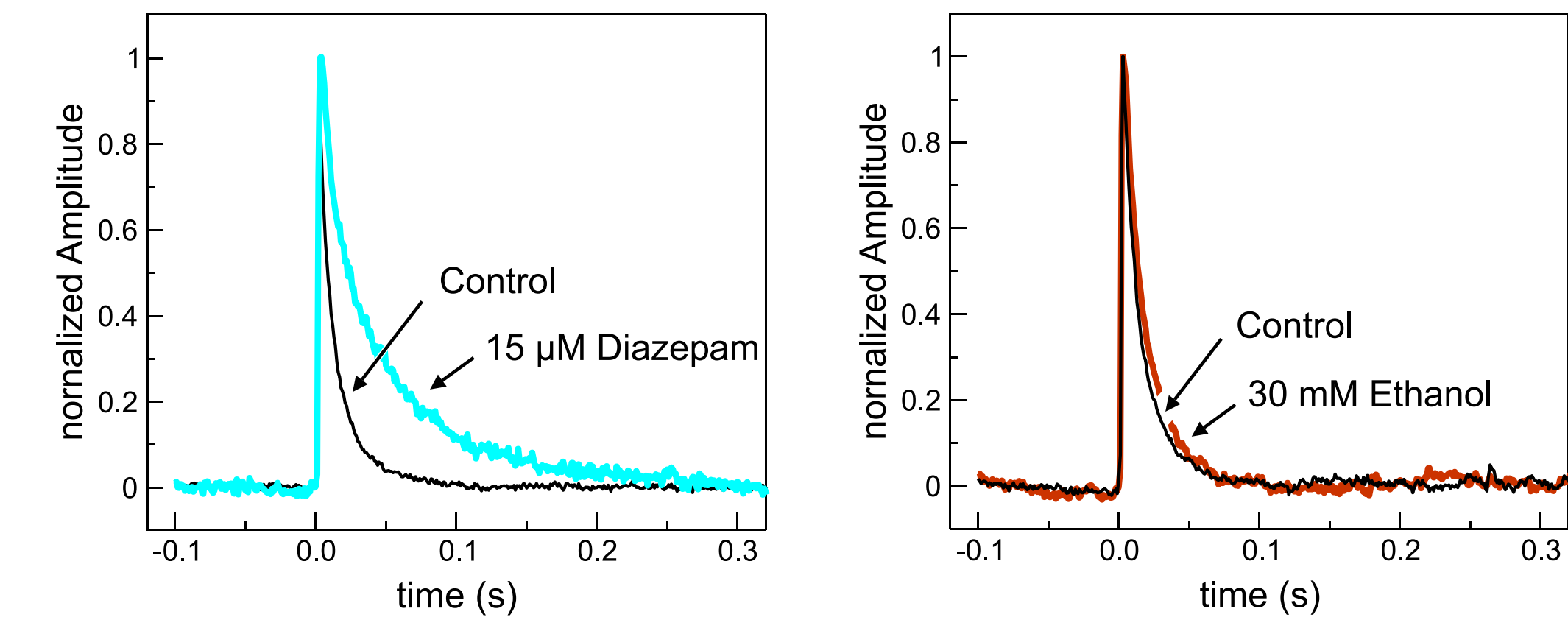
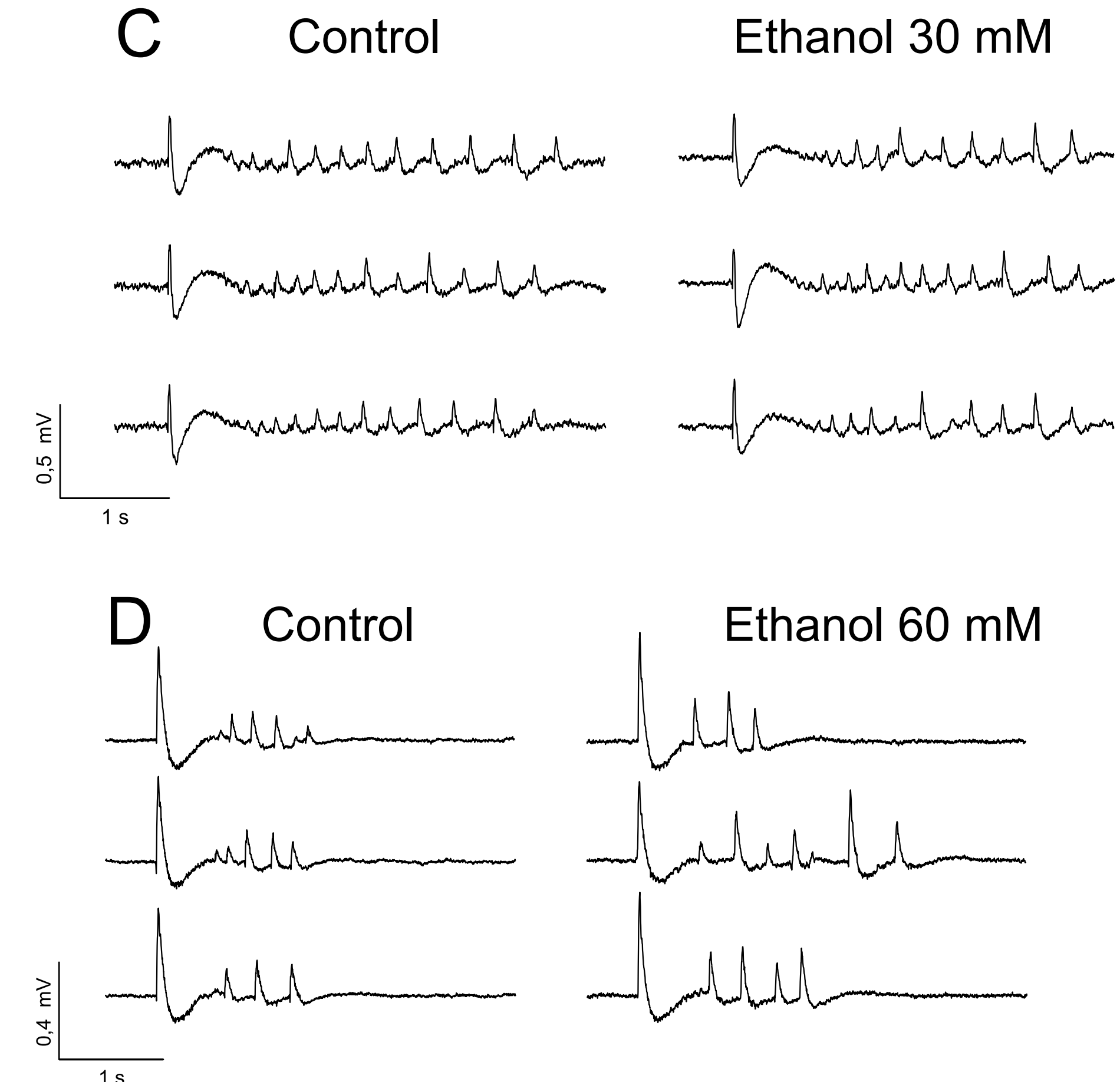


Figure 4: Diazepam, but not ethanol, prolongs IPSC kinetics

A typical averaged inhibitory postsynaptic current (IPSC) from a single 180 second recording is shown. The current decay of the IPSCs is clearly prolonged in the presence of 15 μ M diazepam compared to control condition (fig 4A). In contrast, ethanol in a concentration of 30mM does not affect the inhibitory postsynaptic current (fig 4B).

Whole-cell patch clamp recordings were performed at room temperature. To block glutamatergic currents and network activity CNQX and AP5 (50 μ M each) were added to the extracellular medium. Neurons were held at -70mV. IPSCs were sampled at 10 kHz with the Clampex 9.0 software for a recording period of 180 s. Data were analyzed offline using self-written programs in OriginPro7. Spontaneous events were counted using an automated event detection algorithm. All automatically detected events were individually checked. Decay phases were best fit by two exponential components, using a biexponential equation in the form $I(t) = A_f \exp(-t/\tau_{fast}) + A_s \exp(-t/\tau_{slow}) + C$, where $I(t)$ is the current amplitude at any given time t , C is the baseline current, τ_{fast} and τ_{slow} are the fast and slow time constants of current decay, respectively, and A_f and A_s are the estimated fast and slow intercepts of the components at time zero.

Conclusions

Diazepam and ethanol enhance two different types of GABAergic inhibition in neocortical networks. Both drugs act via GABA_A receptors and depress spontaneous activity. Diazepam acts preferentially via synaptic GABA_A receptors, enhancing phasic inhibition. By prolonging IPSC current decay and by phase-locking of principal cells it fosters synchronized network firing. Ethanol does not enhance network oscillations. This could be explained by an action mainly at extrasynaptic GABA_A receptors, which would enhance tonic inhibition without affecting network synchrony. This finding shows that sedative-hypnotic drugs, which exert their clinically desired effects via GABA_A receptors, can have differential effects on neuronal networks.

References

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