Cholinergic modulation of GABA$_A$ receptor mediated inhibition in neocortex
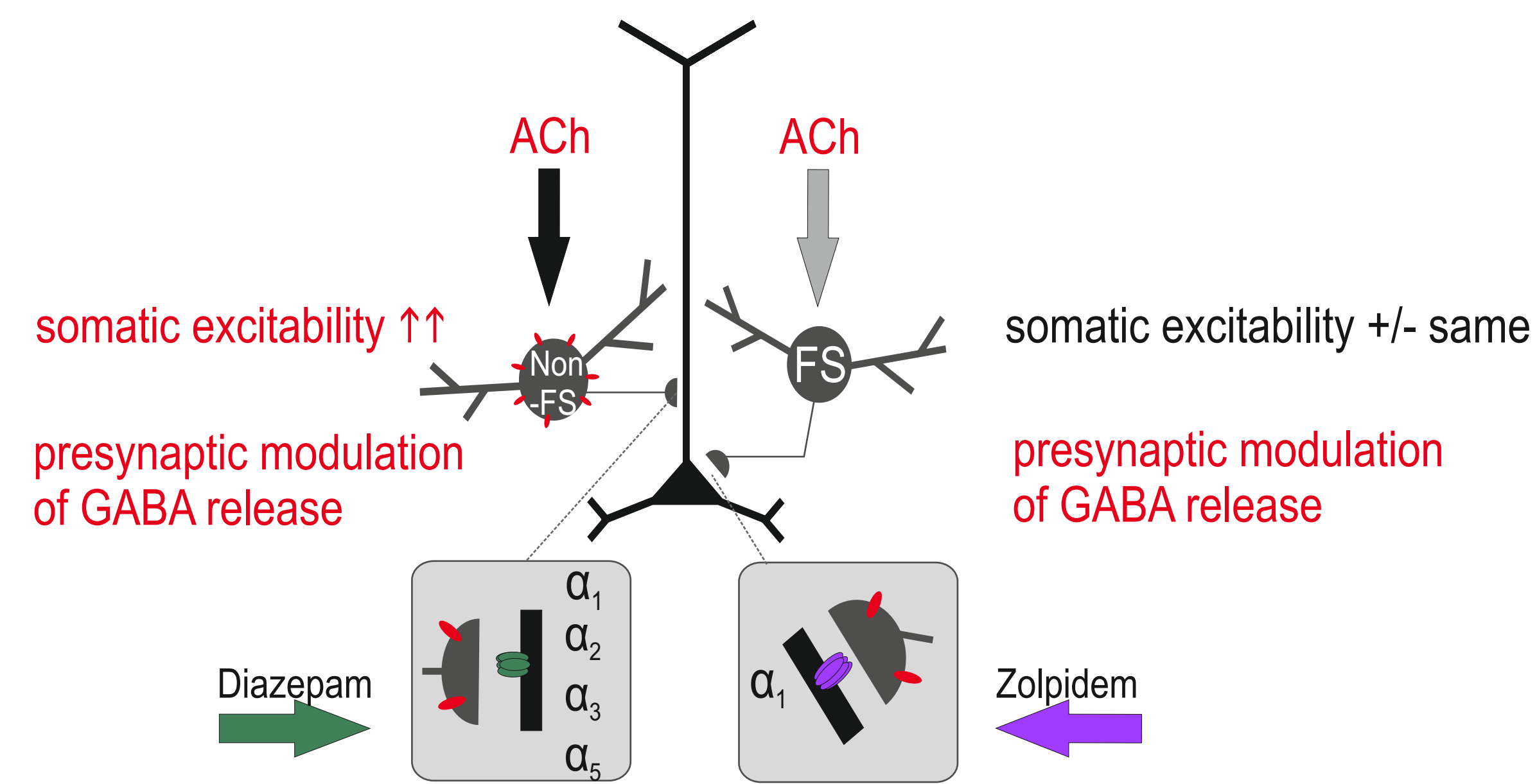
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Central Hypothesis

Acetylcholine (ACh) has differential effects on GABAergic inhibition:
- somatic excitation of some non-fast-spiking interneuron types
- presynaptic modulation of GABA release (decrease: muscarinic, increase: nicotinic)
Our central hypothesis states that **cholinergic activation of neocortex should change the quality of GABAergic inhibition by altering the relative contribution of the diverse GABA$_A$ receptor subtypes to net inhibition**

Aim

Quantification of cholinergic effects on

- inhibitory postsynaptic currents in neocortical neurons
- the efficacy and activity-shaping influence of GABA$_A$ receptor modulators (diazepam, zolpidem) differing in their GABA$_A$ receptor subtype selectivity

Conclusion

Cholinergic status
- ACh modulates spontaneous activity in cortical networks in vitro in multiple ways:
  - increase of intracortical IPSC frequency -> overall increase of interneuronal excitability
  - depression of LFP peak amplitudes
  - increase of up-state frequency

Interaction: GABA$_A$R modulators x cholinergic status
- enhancement of GABAergic currents leads to depression of AP activity during up-states
- impact of GABA$_A$R modulators on some aspects of activity depends on the cholinergic status
- this relationship is at least in part mediated by the α$_1$ subunit

Conclusion: the cholinergic status impacts GABAergic inhibition in neocortex
-> cholinergic activation weakens inhibition via GABA$_A$ receptors with α$_2$, α$_3$ or α$_5$ subunits, but less so via GABA$_A$ receptors with the α1 subunit

-> we speculate that ACh may strongly suppress IPSCs of dendrite-targeting interneurons mediated by GABA$_A$ receptors with α$_2$, α$_3$ or α$_5$ subunits

Acknowledgements

Methods

Electrophysiology
- organotypic cultures of mouse somatosensory cortex from wild type mice and α$_1$(H101R) mice (GABA$_A$ receptors containing the α$_1$ subunit in the latter are insensitive to benzodiazepines; Rudolph et al Nature 401:796-800, 1999)
- all recordings performed at 34°C
- whole cell voltage clamp recordings were performed with glutamatergic receptors blocked; amplitudes, decay times, median charge per IPSC and charge/time carried by IPSCs were assessed
- in spontaneously active networks (glutamatergic signalling intact), phases of activity (up states) were determined from local field potentials (LFP); peri-event time histograms (PETH) of action potentials (AP) were constructed around the beginning of up-states
- all drugs applied via bath perfusion
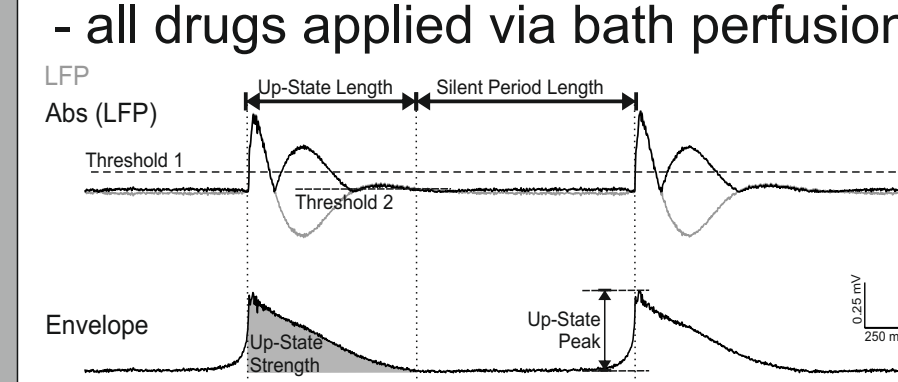
Drug conditions
1) Modulation of cholinergic tonus
   ACh+: acetylcholine (1µm) & neostigmine (1µm)
   ACh-: atropine (1µm) and mecamylamine (3µm)
2) GABA$_A$ receptor 'modulation profile'
diazepam in wild type: GABA$_A$R containing α$_{1-3}$ & α$_5$ subunits
diazepam in α$_1$(H101R): GABA$_A$R containing α$_{2-3}$ & α$_5$ subunits
zolpidem in wild type: GABA$_A$R containing α$_1$ subunits

	ACh+	ACh-
diazepam wild type	α$_{1235}$	α$_{1235}$
diazepam α$_1$(H101R)	α$_{235}$	α$_{235}$
zolpidem wild type	α$_1$	α$_1$

Standardized mean differences
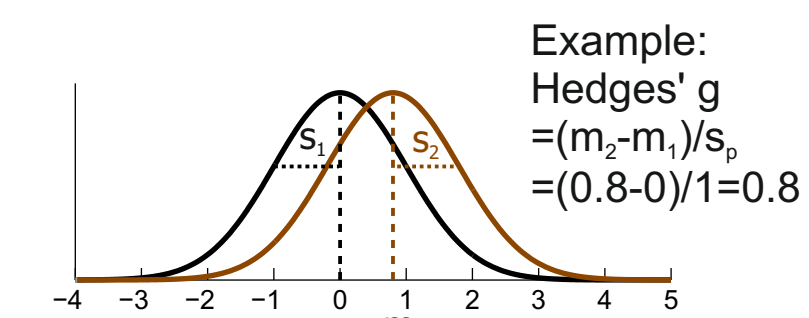- *Hedges' g* (two samples): mean difference divided by pooled standard deviation (see illustration below)
- M_D/S_D (two samples): mean difference divided by std of difference score
- g_Ψ (>2 samples & contrasts): standardized mean difference of a contrast
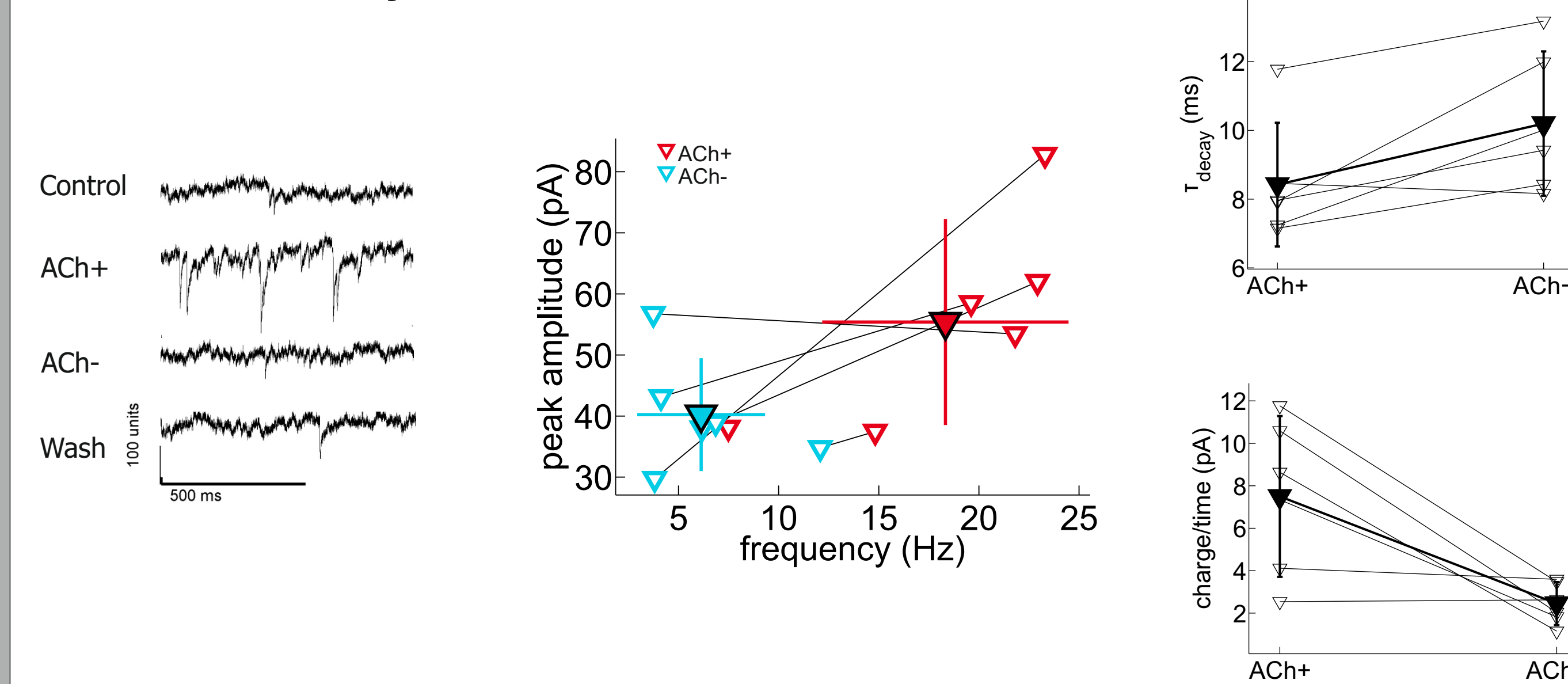
Correlational measure
- *partial eta squared* (η_p^2): proportion of variance due to effect (after removal of all non-effect sources of variability), range 0 (no effect) to 1. For more detailed information on effect size statistics see Hentschke & Stüttgen, Eur. J. Neurosci. (in press) and the 'Measures of effect size toolbox' at http://www.mathworks.com/matlabcentral/fileexchange/32398-measures-of-effect-size-toolbox)

Statistical analysis
We used two classes of measures of effect size (MES), including 95% confidence intervals (CI), as primary statistics:

Example: Hedges' g $=(m_2-m_1)/s_p$ $=(0.8-0)/1=0.8$
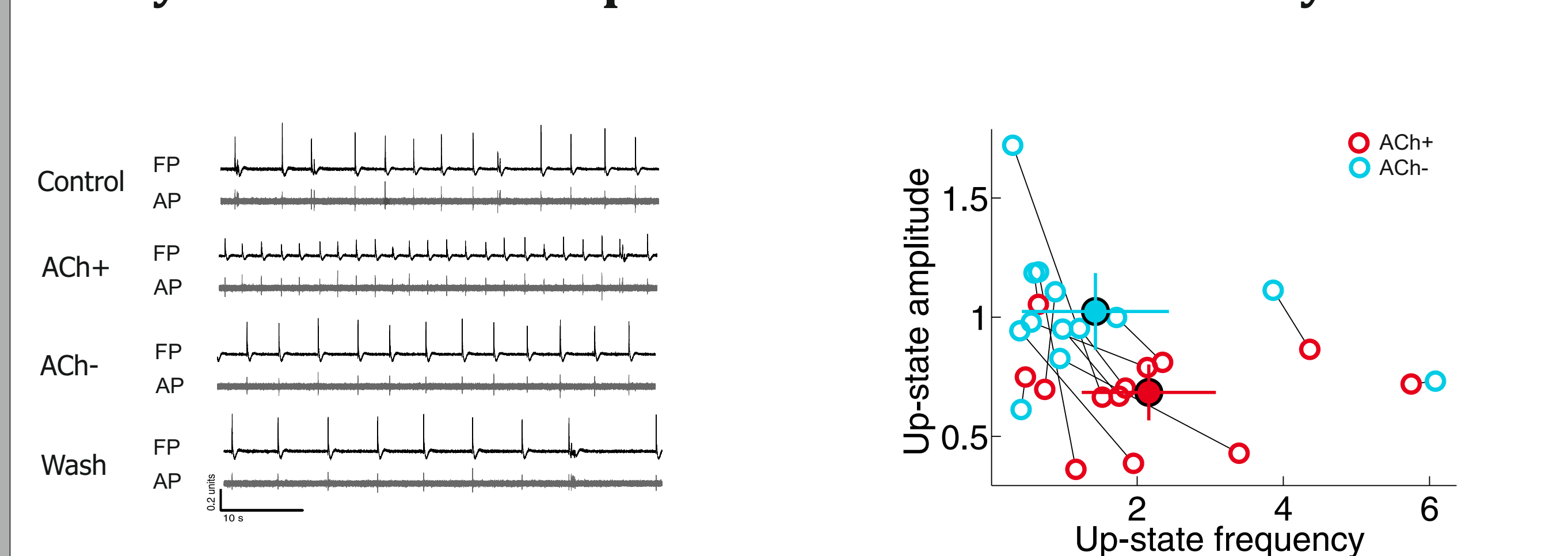
Results
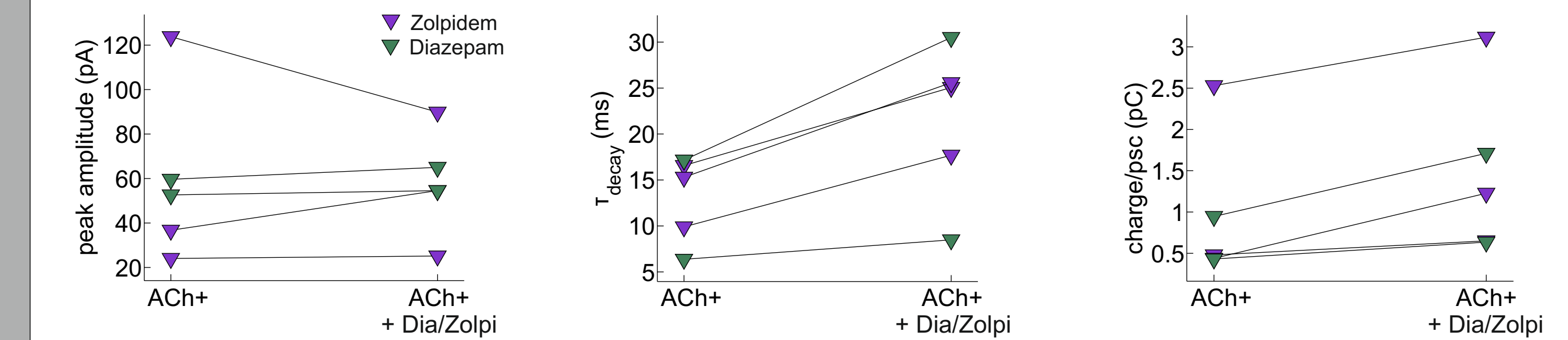
Effects of acetylcholine on IPSCs

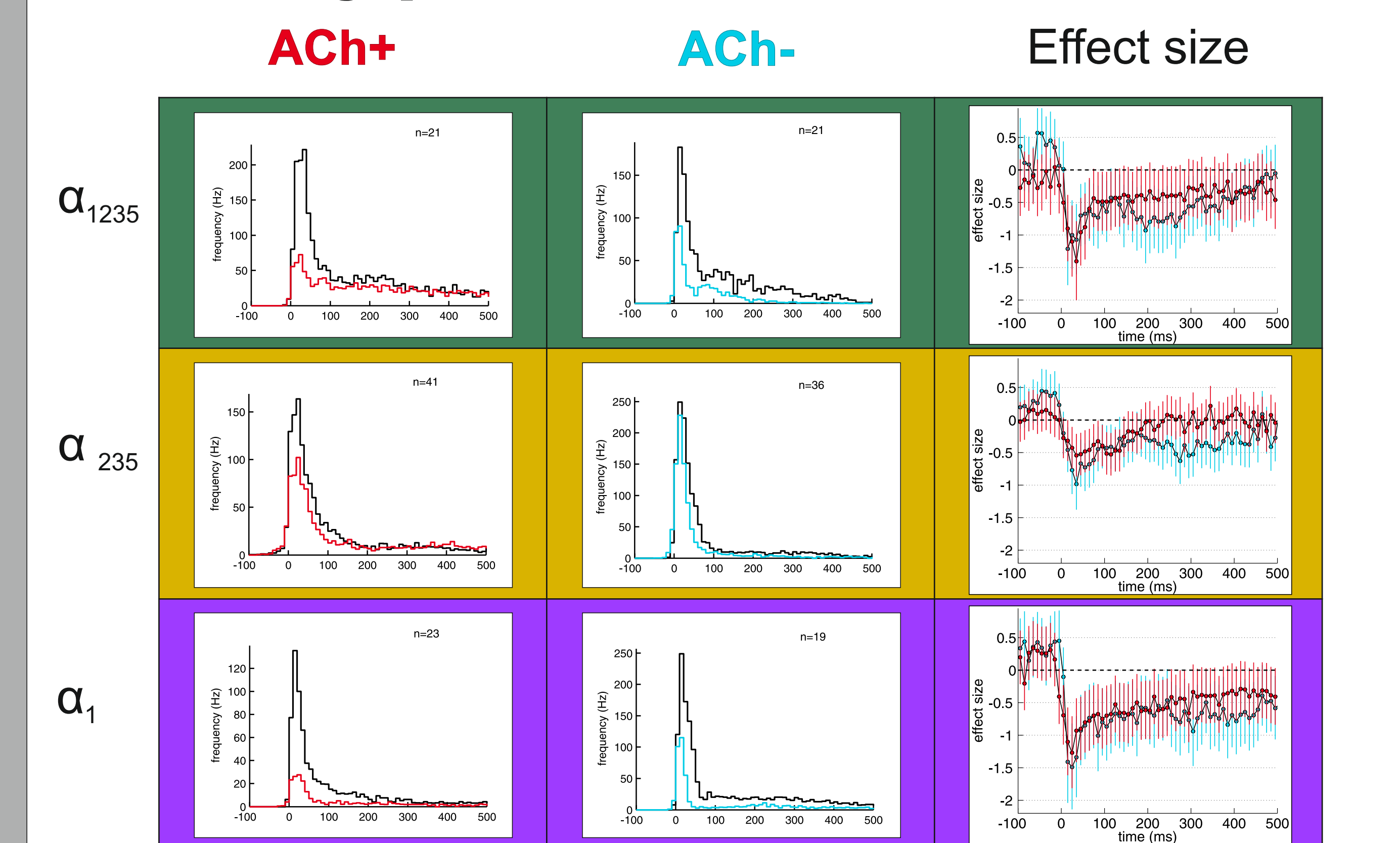### Acetylcholine affects spontaneous network activity

Results

Increase of GABAergic currents by GABA$_A$-receptor modulators

Acetylcholine diminishes the impact of GABA$_A$R modulators on APs during up-states

Interaction of the cholinergic status with the effects of GABA$_A$R modulators

AP frequency	up-state length	up-state peak
$\eta_p^2 = 0.13$ [0.04 0.23] (F = 11.88, p = 1.58*10^{-05})	$\eta_p^2 = 0.006$ (F = 0.50, p = 0.61)	$\eta_p^2 = 0.13$ [0.04 0.22] (F = 11.43, p = 2.35*10^{-06})
Main effect 1 GABA$_A$Rs	Main effect 1 GABA$_A$Rs	
$\eta_p^2 = 1.64*10^{-04}$ (F = 0.03, p = 0.87)	$\eta_p^2 = 0.02$ (F = 3.25, p = 0.07)	$\eta_p^2 = 0.01$ (F = 1.62, p = 0.20)
Main effect 2 ACh+/-	Main effect 2 ACh+/-	
$\eta_p^2 = 0.002$ (F = 0.13, p = 0.88)	$\eta_p^2 = 0.05$ (F = 3.80, p = 0.02)	$\eta_p^2 = 0.04$ (F = 2.83, p = 0.06)
Interaction	Interaction	
$g_\Psi = 0.19$ [-0.56 0.95] (F = 0.25, p = 0.62)	$g_\Psi = 0.85$ [0.09 1.61] (F = 4.89, p = 0.03)	$g_\Psi = 0.87$ [0.11 1.63] (F = 5.09, p = 0.03)
Interaction contrast α$_{235}$ vs. α$_1$	Interaction contrast α$_{235}$ vs. α$_1$	