

Supplemental Materials

Structure-activity Relationship of Neuroactive Steroids, Midazolam, and Perampanel
Towards Mitigating Tetramine-Triggered Activity in Murine Hippocampal Neuronal
Networks

Shane Antrobus*, Brandon Pressly†, Atefeh Mousavi Nik*, Heike Wulff†, and Isaac N.
Pessah*†

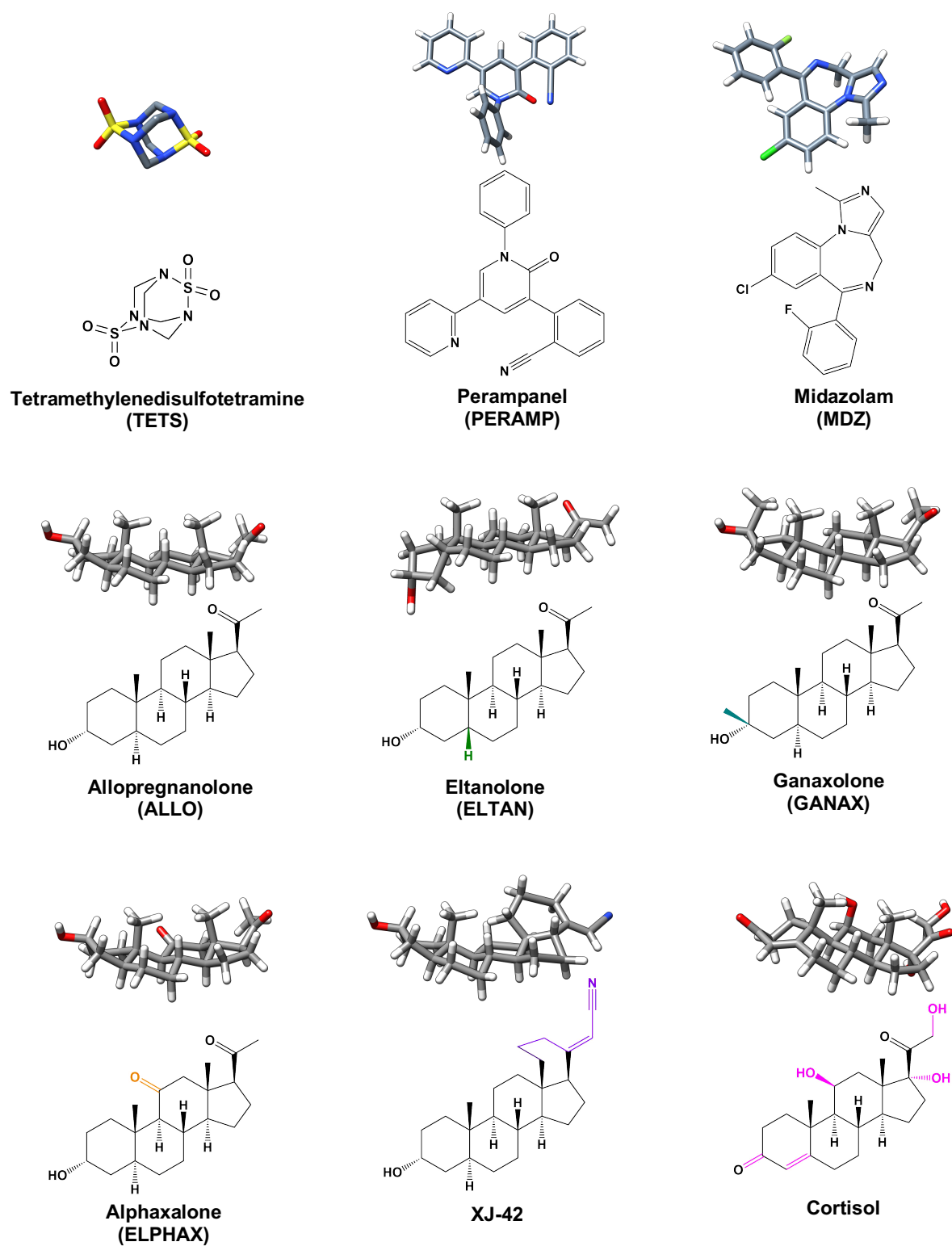
*Department of Molecular Biosciences, School of Veterinary Medicine, †Department
of Pharmacology, School of Medicine, University of California, Davis, CA 95616,
USA

†Corresponding author

Isaac N. Pessah

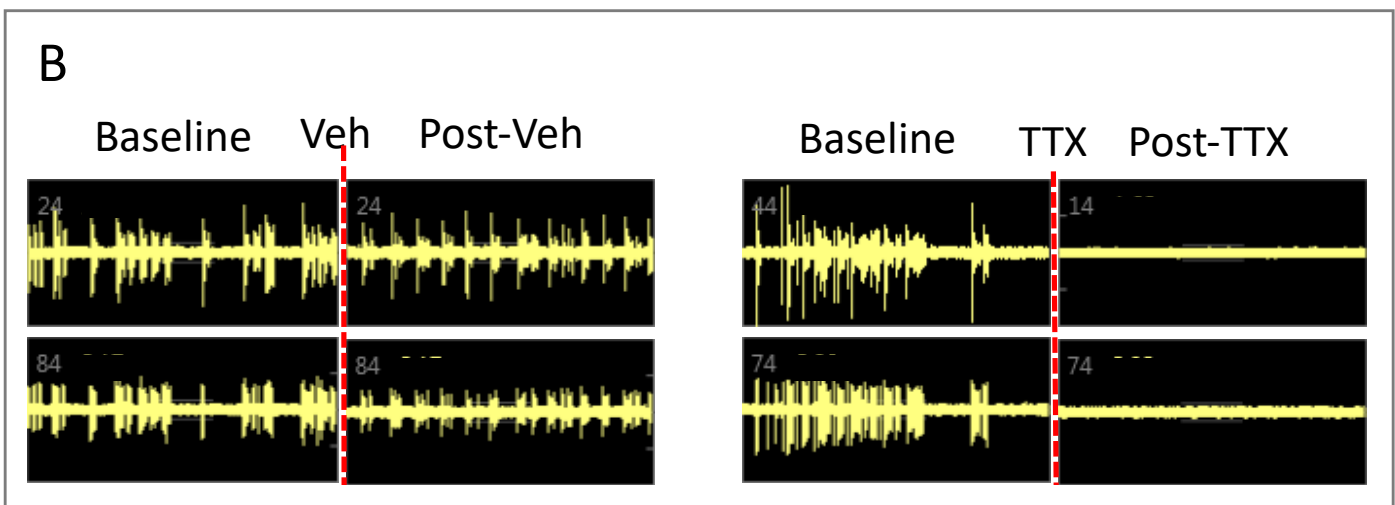
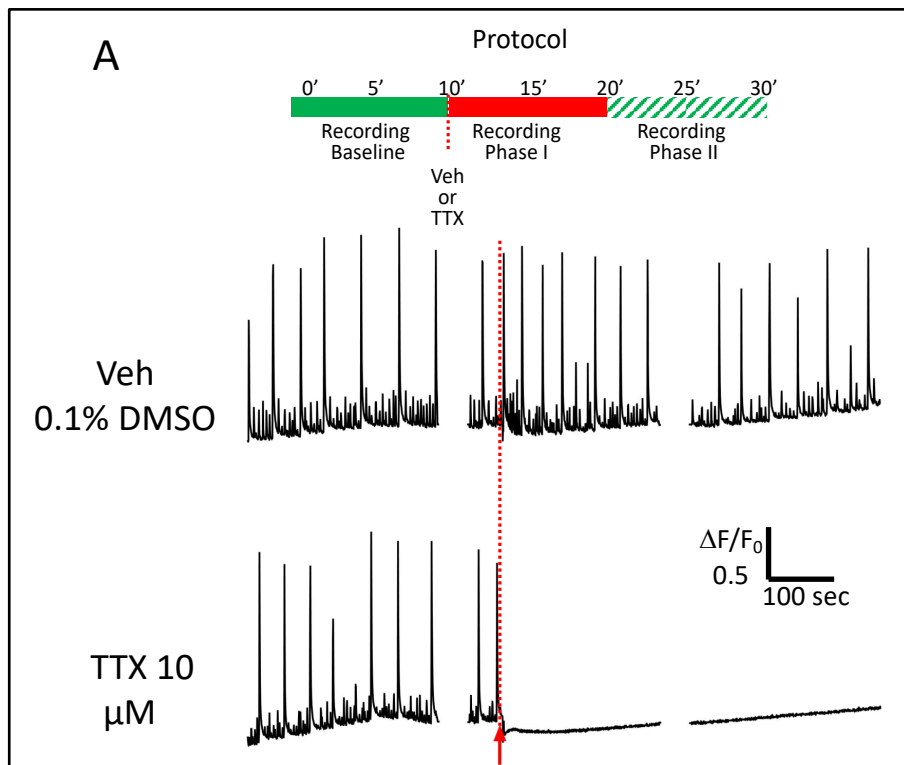
1089 Veterinary Medicine Drive, Davis, CA 95616

Email: inpessah@ucdavis.edu. Telephone: +1-(530)-752-6696



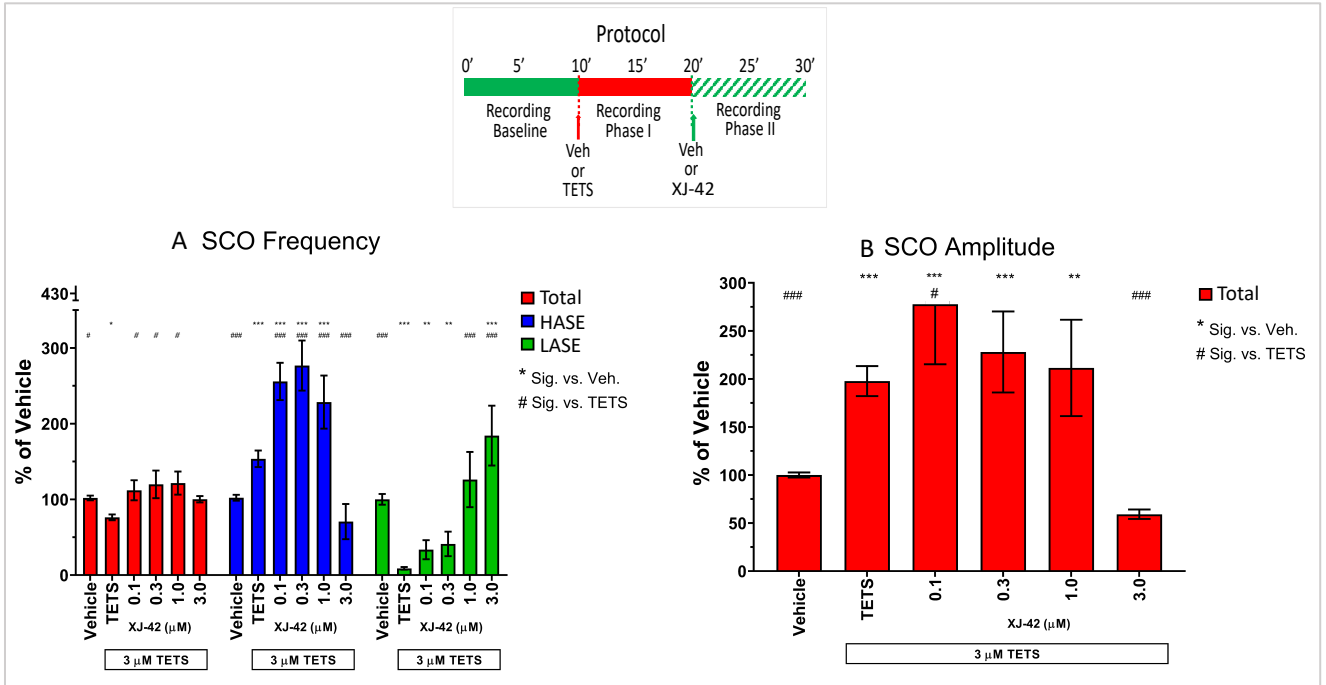
Supplemental Figure 1. Stick 3-D projections and 2-D structures of compounds tested.

Structures we generated with Chimera and ChemDraw. Differences between ALLO and other steroids are highlighted in color.

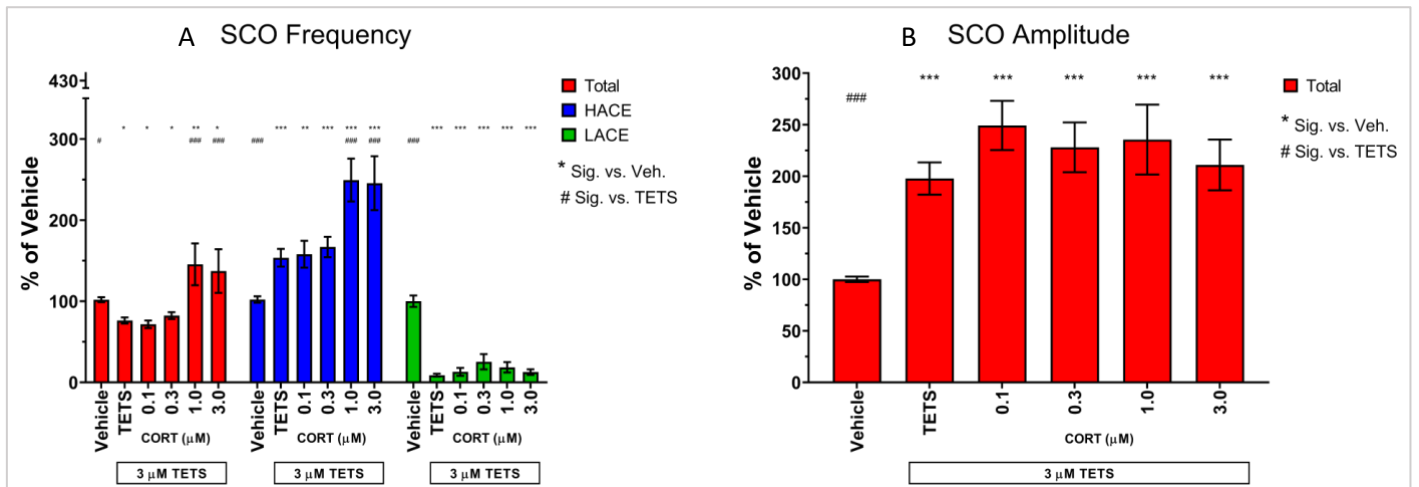


Supplemental Figure 2. TTX blocks all SCO activity by blocking Na⁺ channels. (A)

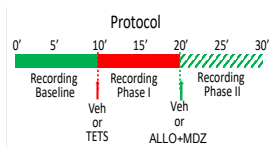
Inhibition of Na⁺ channel current with addition of tetrodotoxin (TTX, 10 μM) blocked both LASE and HASE components of SCO activity measured in cultured hippocampal neurons. Vehicle (Veh, 0.01% DMSO) or TTX were added to separate wells where indicated by the red arrow. Traces shown are representative of 4-6 duplicate wells measured at 14 DIV. **(B)** Inhibition of Na⁺ channel current with addition of tetrodotoxin (TTX, 10 μM; dashed line) blocks all electrical spike activity measured from hippocampal neurons at 14 DIV cultured on microelectrode arrays. Vehicle (0.01% DMSO) had no influences on ESA parameters, whereas TTX stopped all ESA activity. Traces shown are representative 100-sec recordings from two electrodes immediately before and 1 min after addition of either Veh or TTX. TTX blocked basal ESA activity measured from all exposed wells/electrodes. Numbers on traces represent electrode positions.



Supplemental Figure 3. Synthetic NAS XJ-42 exacerbates TETS-triggered HASE patterns at concentrations having no influence on LASE or Total SCO activity. Upper panel shows the experimental protocol for recording SCO during baseline and Phase I and II post-addition of TETS (3.0 μ M) and subsequent addition of 0.1-3.0 μ M XJ-42. Only Phase II responses were analyzed in this study. Total SCO frequency and amplitude (**A** and **B**, red bars), and HASE (**A**, blue bars) and LASE (**A**, green bars) frequencies are normalized to Vehicle control (0.01% DMSO) and statistically compared to Vehicle (*) or TETS (#) using ANOVA with Tukey post-hoc correction. Mean \pm S.E.M. n=14-15 wells measured on 3 independent culture days. *, # p<0.05; **, ## p<0.01; ***, ### p<0.001. Summary statistics are reported in Table 2.

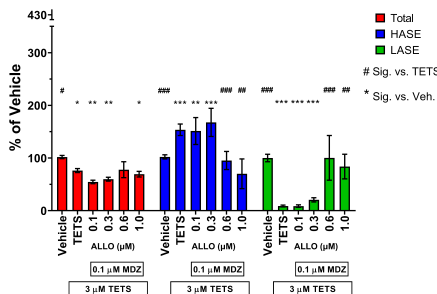


Supplemental Figure 4. Cortisol does not attenuate TETS-triggered SCO patterns, rather it potentiates HASE. Total SCO frequency and amplitude (**A and B**, red bars), and HASE (**A**, blue bars) and LASE (**A**, green bars) frequencies are normalized to Vehicle control (0.01% DMSO) and statistically compared to Vehicle (*) or TETS (#) using ANOVA with Tukey post-hoc correction. Mean \pm S.E.M. n=14-15 wells measured on 3 independent culture days. *, # $p \leq 0.05$; **, and ##, $p \leq 0.01$; ***, ### $p \leq 0.001$.

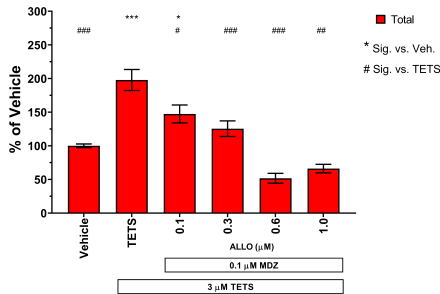


0.1 μ M MDZ

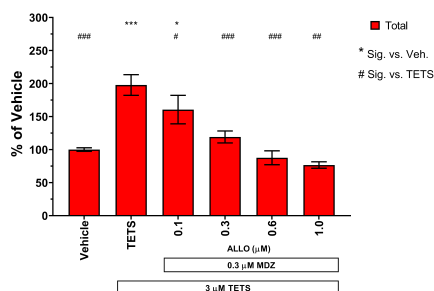
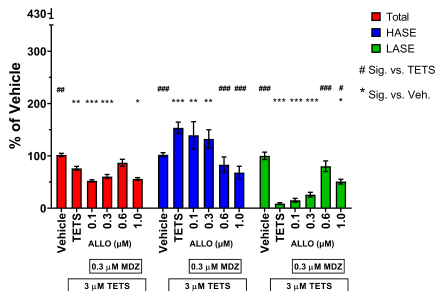
SCO Frequency



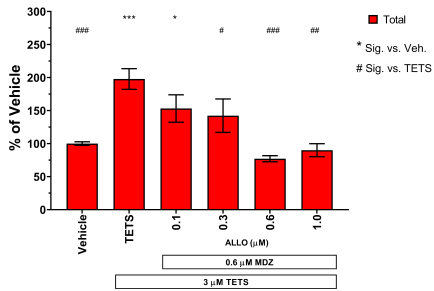
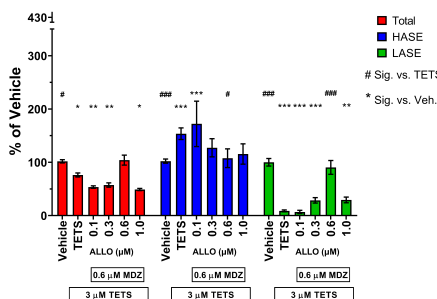
SCO Amplitude



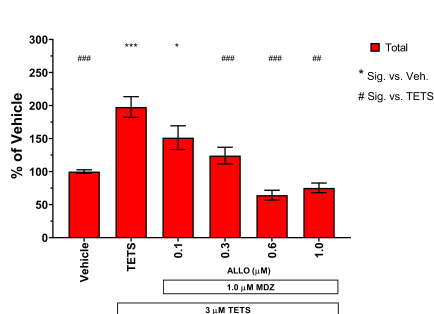
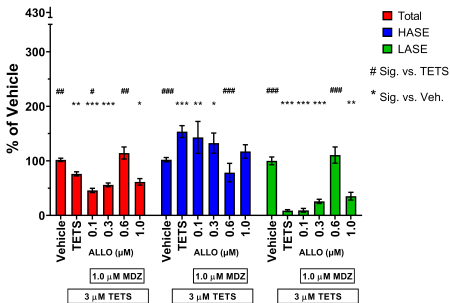
0.3 μ M MDZ



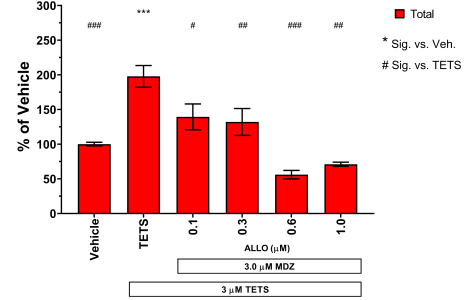
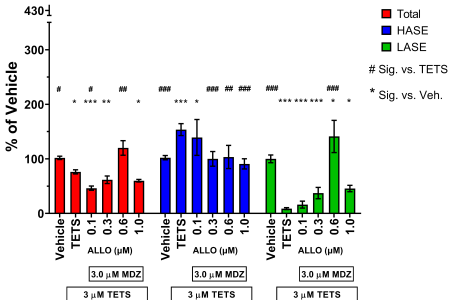
0.6 μ M MDZ



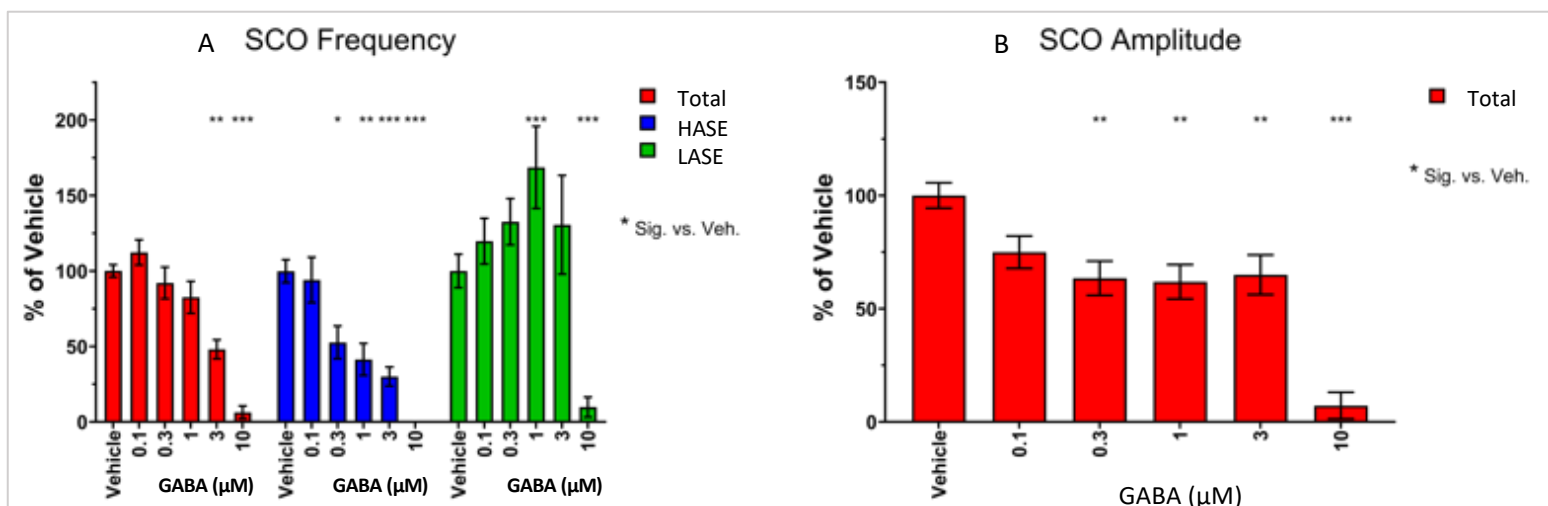
1.0 μ M MDZ



3.0 μ M MDZ



Supplemental Figure 5. Concentration-effect relationships of ALLO (0.1-1.0 μ M) + MDZ (0.1-3.0 μ M) toward modifying TETS-triggered SCO patterns. Total SCO frequency and amplitude (**A** and **B**, red bars), and HASE (**A**, blue bars) and LASE (**A**, green bars) frequencies were normalized to Vehicle control (0.01% DMSO) and statistically compared to Vehicle (*) or TETS (#) using ANOVA with Tukey post-hoc correction. Mean + S.E.M. n=14-15 wells measured on 3 independent culture days. *, # p<0.05; **, ##, p<0.01; ***, ### p<0.001.



Supplemental Figure 6. Exposure of neuronal networks to GABA diminishes HASE and enhances LASE SCO patterns. Concentration-effect relationships of GABA (0.1-10 μM) toward modifying SCO patterns in hippocampal neurons. Total SCO frequency and amplitude (**A and B**, red bars), and HASE (**A**, blue bars) and LASE (**A**, green bars) frequencies were normalized to vehicle control (assay buffer) and statistically compared to GABA using ANOVA with Tukey post-hoc correction. Mean ± S.E.M. n=10 wells per GABA concentration measured on 2 independent culture days. *, $p \leq 0.05$; ** and ***, $p \leq 0.01$; ***, $p \leq 0.001$.