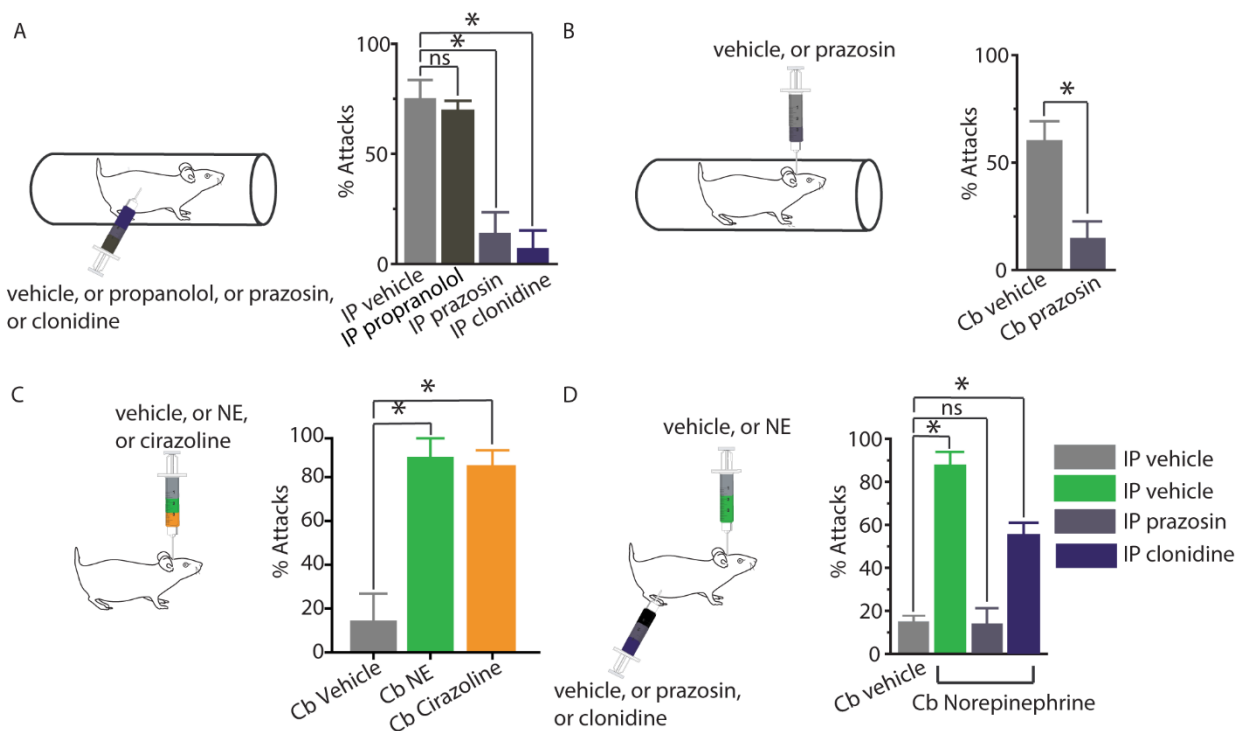


Dissecting the mechanism by which stress induces motor attacks in episodic ataxias

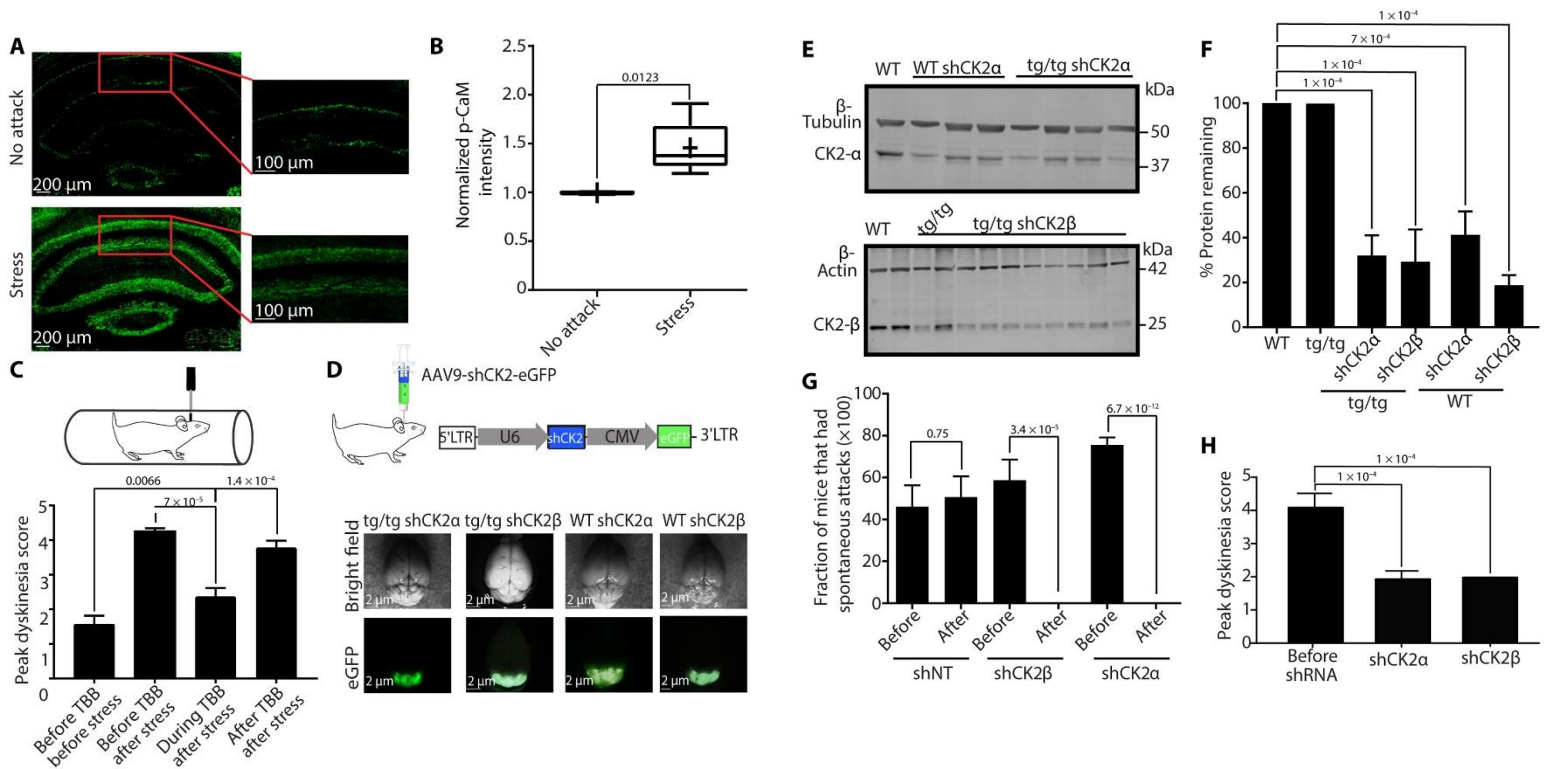
Stress is the most common trigger among episodic neurologic disorders. In episodic ataxia type 2 (EA2), physical or emotional stress causes episodes of severe motor dysfunction that manifest as ataxia and dystonia. We used the tottering (tg/tg) mouse, a faithful animal model of EA2, to dissect the mechanisms underlying stress-induced motor attacks.

Cerebellar adrenergic signaling is required for stress-induced attacks



(A) Compared to vehicle, systemic injection of the α_1 adrenoreceptor antagonist prazosin at 0.5 mg/kg or α_2 adrenoreceptor agonist clonidine at 0.05 mg/kg before subjecting *tottering* mice to restraint stress decreases the frequency with which restraint induces attacks. Injection of the β -adrenoreceptor antagonist propranolol at 5 mg/kg does not alter the frequency of stress-induced attacks. Vehicle $N = 6$ mice, propranolol $N = 6$ mice, prazosin $N = 6$ mice, and clonidine $N = 6$ mice, in each, repeated 5 to 10 times. (B) Pretreatment with intracerebellar (Cb) infusion of 1.5 μ g of prazosin decreases frequency of stress-induced attacks compared to pretreatment with vehicle. Vehicle $N = 3$ mice and prazosin $N = 3$ mice, in each, repeated four to seven times. (C) The frequency of attacks following intracerebellar (Cb) infusion of 10 μ g of NE or 2.5 μ g of the α_1 agonist cirazoline is significantly higher than that following vehicle infusion. Cb vehicle $N = 6$ mice, Cb NE $N = 5$, and Cb cirazoline $N = 5$ mice, in each, repeated five to nine times. (D) Systemically injecting *tottering* mice with the α_1 antagonist prazosin at 0.5 mg/kg decreases the frequency of attacks following intracerebellar infusions of 10 μ g of NE (green) compared to pretreatment with either vehicle (gray) or 0.8 μ g of the α_2 agonist clonidine (dark blue). Vehicle $N = 2$ mice, prazosin $N = 2$ mice, and clonidine $N = 3$ mice, in each, repeated four to nine times. Data represent means $\pm$ SEM. One-way analysis of variance (ANOVA) with Tukey's multiple comparisons test.

AAV mediated Knock Down of CK2 in the cerebellum prevents stress attacks



(A) Representative images of cerebella from a tottering mouse that was undergoing stress attack and a tottering mouse that was not undergoing attack at the time of sacrifice. (B) Normalized p-CaM levels from Purkinje cell somas of mice undergoing stress attacks compared to mice not undergoing attacks. Stress, N = 6; no attack, N = 5. One-sample t test. (C) The frequency of stress-induced attacks is decreased during the chronic perfusion of 20 mM CK2 inhibitor (TBB) into the cerebellum of tottering mice. One-way ANOVA with Dunnett's multiple comparisons test. (D) Top: Schematic of the AAV9 construct used to drive shCK2 in vivo. Bottom: Representative whole brains of tottering and WT mice injected with shCK2 β or shCK2 α . Photo credit: Ariel Vitenzon. (E) Top: Cropped immunoblot from the cerebellum of a WT mouse and three WT and four tottering mice injected with shCK2 α , showing bands at 45 kDa for CK2 α and 50 kDa for β -tubulin. Bottom: Cropped immunoblot from the cerebellum of WT noninjected tottering and 10 tottering mice injected with shCK2 β , showing bands at 25 kDa for CK2 β and at $\sim$ 42 kDa for β -actin. (F) Quantification of the above blots showing average KD of CK2 α and CK2 β in tottering cerebellum (N = 8 for CK2 α and N = 10 for CK2 β) and WT cerebellum (N = 3 for CK2 α and N = 4 for CK2 β). Error bars represent SD. One-way ANOVA with Tukey's multiple comparisons test. (G) Frequency of spontaneous stress attacks in tottering mice injected with shNT, shCK2 β , and shCK2 α before and after shRNA expression. Error bars represent SEM. N = 7 shNT, N = 8 shCK2 α , and N = 8 shCK2 β . Student's t test. (H) Average peak dyskinesia scores of tottering mice before and after injection of shCK2 α and shCK2 β , 40 min after restraint stress. Error bars represent SEM. Baseline: N = 10; shCK2 α : N = 10; shCK2 β : N = 4. One-way ANOVA with Dunnett's multiple comparisons test.