Supplementary Materials

Sleep and Diurnal Rest-Activity Rhythm Disturbances in a Mouse Model of Alzheimer's Disease

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Supplementary Tables

Caption Table S1: Mice underwent a pre-treatment evaluation of general fitness and grip strength and weekly assessments throughout dosing with fenobam. Fractions indicate the number of mice exhibiting a phenotype, for example, “No (7/7)” indicates that 7 out of a total of 7mice did not exhibit the listed phenotype and “Yes (1/7)” indicates that 1 mouse out of a total of 7 mice exhibited the listed phenotype.

Caption Table S2: Mice underwent a pre-treatment evaluation of general fitness and grip strength and weekly assessments throughout dosing with CTEP.

Caption Table S3: Activity counts during the first 60 min in the novel environment of the actigraphy chambers were assessed in wild type (WT) and J20 cohorts for the 3 independent experiments. A minimum of 8 mice were tested per cohort. The average age of the mice was 8 months old. Average activity counts are presented ± the standard deviation. 2-Way ANOVA results with genotype and season as between subject variables: interaction $F(2,65)=0.0087$, $p=0.99$; genotype $F(1,65)=1.29$, $p=0.26$; season $F(2,65)=0.087$, $p=0.92$.

Caption Table S4: Total daily average activity counts ± standard deviation were assessed in wild type (WT) and J20 mice in response to vehicle, fenobam and CTEP (2-chloro-4-((2,5-dimethyl-1-(4-(trifluoromethoxy)phenyl)-1H-imidazol-4-yl)ethynyl)pyridine) in 8 month old mice and in response to CTEP in 16-19 month old mice. Fenobam was dosed daily and CTEP was dosed every other day (EOD). 2-way ANOVA fenobam: interaction $F(1,24)=1.14$, $p=0.30$; drug treatment $F(1,24)=0.051$, $p=0.82$; genotype $F(1,24)=9.48$, $p<0.0051$. 2-way ANOVA CTEP (adults): interaction $F(1,43)=0.26$, $p=0.61$; treatment $F(1,43)=0.0048$, $p=0.95$; genotype $F(1,43)=8.29$, $p<0.0062$. 2-way ANOVA CTEP (aged adults): interaction $F(1,25)=0.26$, $p=0.61$; treatment $F(1,25)=0.64$, $p=0.43$; genotype $F(1,25)=4.08$, $p=0.054$. Post-hoc t-tests were calculated if ANOVA results were statistically significant. ND=not determined. Mice for these cohorts were tested in winter and spring.

Caption Table S5: Total daily average activity counts ± standard deviation were assessed in wild type (WT) and J20 mice in response to vehicle, fenobam and CTEP (2-chloro-4-((2,5-dimethyl-1-(4-(trifluoromethoxy)phenyl)-1H-imidazol-4-yl)ethynyl)pyridine) in 8 month old mice and in response to CTEP in 16-19 month old mice. Fenobam was dosed daily and CTEP was dosed every other day (EOD). 2-way ANOVA fenobam: interaction $F(1,24)=0.83$, $p=0.37$; treatment $F(1,24)=2.62$, $p=0.12$; genotype $F(1,24)=4.00$, $p<0.057$. 2-way ANOVA CTEP (adults): interaction $F(1,43)=0.019$, $p=0.89$; treatment $F(1,43)=0.0014$, $p=0.97$; genotype $F(1,43)=0.75$, $p=0.39$. 2-way ANOVA CTEP (aged adults): interaction $F(1,25)=0.10$, $p=0.75$; treatment $F(1,25)=0.0043$, $p=0.95$; genotype $F(1,25)=2.32$, $p=0.14$. Post-hoc t-tests were not calculated as ANOVA results were not statistically significant. Mice for these cohorts were tested in winter and spring.

Caption Table S6: Mixed effect ANOVA analyses of time spent in each vigilance state related to Figure 4. Sleep stages were manually scored from EEG and EMG signals. Percent time spent in each vigilance state in 4, 6-hour time bins are summarized here as marginal mean ± 95% confidence intervals. Results from a mixed-effect ANOVA evaluating main-effects of time (random variable), treatment group (fixed variable), and interactions are shown as F- and *p*-values in each column. Main-effects of time (top) with $p<0.05$ suggest a significant oscillation over the light-dark cycle. Main-effects of treatment group (middle) with $p<0.05$ suggest a significant difference in power among treatment groups. Interaction comparisons (bottom) suggest no significant differences when time and treatment group effects were combined.

Caption Table S7: Multiple comparisons of all interactions (time*group) of vigilance states related to Figure 4. Sleep stages were manually scored from EEG and EMG signals. Percent time spent in each vigilance state (wake, rapid eye movement and non-rapid eye movement sleep) in 4, 6-hour bins are summarized here per treatment group (wild type and J20, with and without CTEP treatment) as marginal mean ± 95% confidence intervals. Results from a post-hoc multiple-comparisons test with a Tukey-Kramer correction, graphically represented in Figure 4. Non-overlapping confidence intervals in each bin within vigilance state suggests statistically significant differences.

Caption Table S8: Mixed model ANOVA statistics of EEG power related to Figure 5. Sleep stages were manually scored from EEG and EMG signals in 4-second epochs. EEG power spectra were calculated for each epoch with fast-Fourier transform and integrated values for frequencies in delta (0.5-4 Hz), theta (5-9 Hz), sigma (10-14 Hz), and gamma (25-100 Hz) ranges were normalized to their sum. Power values from 12, 2-hour time bins are summarized here as marginal mean ± 95% confidence intervals. Results from a mixed-effect ANOVA evaluating main effect of time (random variable), treatment group (fixed variable), and interactions are shown as F- and p-values in each column. Main-effects of time (top) with $p<0.05$ suggest a significant oscillation over the light-dark cycle. Main-effects of treatment group (middle) with $p<0.05$ suggest a significant difference in power among treatment groups. Interaction comparisons (bottom) with $p<0.05$ suggest a significant difference of time and treatment group combined.

Caption Table S9: Mean normalized NREM EEG power values by group and time related to Figure 5. Sleep stages were manually scored from EEG and EMG signals in 4-second epochs. EEG power spectra were calculated for each epoch with fast-Fourier transform and integrated values for frequencies in delta (0.5-4 Hz), theta (5-9 Hz), sigma (10-14 Hz), and gamma (25-100 Hz) ranges were normalized to their sum. Power values from 12, 2-hour time bins are summarized here per treatment group (wild type and J20, with and without CTEP treatment) as marginal mean ± 95% confidence intervals. Results are from a post-hoc multiple-comparisons test with a Tukey-Kramer correction, and graphically represented in figure 5. Non-overlapping confidence intervals in each bin within frequency band suggest statistically significant differences.

Table S1: Neurological Assessment Metrics (Fenobam)						
			WT Vehicle n=7	**J20 Vehicle n=7**	**WT Fenobam n=8**	**J20 Fenobam n=6**
Body Weight (grams) ± SEM		**start**	39.22±1.24	37.58±2.23	39.50±1.37	43.98±2.30
		1 week	37.00±1.05	34.93±2.12	38.00±1.39	42.11±2.36
		2 weeks	35.89±1.15	34.79±2.15	37.13±1.37	41.58±2.49
		3 weeks	34.76±1.34	34.80±1.77	36.16±1.32	41.94±2.67
		4 weeks	34.13±1.19	33.78±1.71	35.91±1.31	40.88±2.70
General Observations			**WT Vehicle**	**J20 Vehicle**	**WT Fenobam**	**J20 Fenobam**
Tremor	Shaking movements	**start**	No (7/7)	No (7/7)	No (8/8)	No (6/6)
		1 week	No (7/7)	No (7/7)	No (8/8)	No (6/6)
		2 weeks	No (7/7)	No (7/7)	No (8/8)	No (6/6)
		3 weeks	No (7/7)	No (7/7)	No (8/8)	No (6/6)
		4 weeks	No (7/7)	No (7/7)	No (8/8)	No (6/6)
Eyelid Closure	Eyelids closed (cataracts)	**start**	No (7/7)	No (7/7)	No (6/7)	No (6/6)
		1 week	No (7/7)	No (7/7)	No (6/7)	No (6/6)
		2 weeks	No (6/7)	No (7/7)	No (6/7) Yes (1/7)	No (6/6) Yes (1/7)
		3 weeks	No (6/7)	No (7/7)	No (6/7) Yes (1/7)	No (6/6) Yes (1/7)
		4 weeks	No (6/7)	No (7/7)	No (6/7) Yes (1/7)	No (6/6) Yes (1/7)
Piloerection	Involuntary erection or bristling of hairs	**start**	No (7/7)	No (7/7)	No (8/8)	No (6/6)
		1 week	No (7/7)	No (7/7)	No (8/8)	No (6/6)
		2 weeks	No (7/7)	No (7/7)	No (8/8)	No (5/6)
		3 weeks	No (7/7)	No (7/7)	No (8/8)	No (6/6)
		4 weeks	No (6/7)	No (7/7)	No (8/8)	No (6/6)
Lacrimation	Secretion of tears	**start**	No (7/7)	No (7/7)	No (8/8)	No (6/6)
		1 week	No (7/7)	No (7/7)	No (8/8)	No (6/6)
		2 weeks	No (7/7)	No (7/7)	No (8/8)	No (6/6)
		3 weeks	No (7/7)	No (7/7)	No (8/8)	No (6/6)
		4 weeks	No (7/7)	No (7/7)	No (8/8)	No (6/6)
Salivation	Secreting saliva	**start**	No (7/7)	No (7/7)	No (8/8)	No (6/6)
		1 week	No (7/7)	No (7/7)	No (8/8)	No (6/6)
		2 weeks	No (7/7)	No (7/7)	No (8/8)	No (6/6)
		3 weeks	No (7/7)	No (7/7)	No (8/8)	No (6/6)
		4 weeks	No (7/7)	No (7/7)	No (8/8)	No (6/6)
Dirty Coat	Coat is dirty	**start**	No (7/7)	No (7/7)	No (8/8)	No (6/6)
		1 week	No (7/7)	No (7/7)	No (8/8)	No (6/6)
		2 weeks	No (7/7)	No (7/7)	No (8/8)	No (6/6)
		3 weeks	No (7/7)	No (7/7)	No (8/8)	No (6/6)
		4 weeks	No (7/7)	No (7/7)	No (8/8)	No (6/6)
Grip Strength	Mouse allowed to grip cage feed bin	**start**	2.1±0.3	1.6±0.2	1.5±0.2	1.3±0.2
		1 week	1.1±0.1	1.4±0.2	1.4±0.2	1.2±0.2
		2 weeks	1.6±0.2	1.1±0.1	1.5±0.2	1.7±0.2

	wire while investigator gently pulls backwards horizontally: 0 = no grip, 1 = slight grip, 2 = moderate grip, 3 = active grip, 4 = unusually effective grip	**3 weeks**	1.7±0.2	1.4±0.2	1.6±0.3	1.3±0.2
		4 weeks	1.6±0.3	1.3±0.2	1.4±0.2	1.5±0.2
Reflexes			**WT Vehicle**	**J20 Vehicle**	**WT Fenobam**	**J20 Fenobam**
Righting Reflex	Hold mouse by tail and turn onto back. Failure to right their body into an upright position within 5 seconds is scored as No.	**start**	Yes (7/7)	Yes (7/7)	Yes (8/8)	Yes (6/6)
		1 week	Yes (7/7)	Yes (7/7)	Yes (8/8)	Yes (6/6)
		2 weeks	Yes (7/7)	Yes (7/7)	Yes (8/8)	Yes (6/6)
		3 weeks	Yes (7/7)	Yes (7/7)	Yes (8/8)	Yes (6/6)
		4 weeks	Yes (7/7)	Yes (7/7)	Yes (8/8)	Yes (6/6)
Pinna Reflex	While the mouse is gently restrained, touch each ear canal lightly with the tip of a metal spatula. Monitor for ear retraction or head movement. Yes = movement in both ears.	**start**	Yes (6/7)	Yes (6/7)	Yes (7/8)	Yes (5/6)
		1 week	Yes (7/7)	Yes (4/7)	Yes (6/8)	Yes (5/6)
		2 weeks	Yes (7/7)	Yes (7/7)	Yes (8/8)	Yes (5/6)
		3 weeks	Yes (7/7)	Yes (7/7)	Yes (8/8)	Yes (6/6)
		4 weeks	Yes (7/7)	Yes (7/7)	Yes (8/8)	Yes (5/6)
Eyeblink Response	When a cotton swab is moved toward an open eye, the mouse blinks. Yes = at least one eye blinks.	**start**	Yes (6/7)	Yes (4/7)	Yes (7/8)	Yes (5/6)
		1 week	Yes (5/7)	Yes (2/7)	Yes (4/8)	Yes (1/6)
		2 weeks	Yes (7/7)	Yes (7/7)	Yes (7/8)	Yes (6/6)
		3 weeks	Yes (7/7)	Yes (4/7)	Yes (5/8)	Yes (1/6)
		4 weeks	Yes (7/7)	Yes (4/7)	Yes (7/8)	Yes (6/6)
Observations in Arena			**WT Vehicle**	**J20 Vehicle**	**WT Fenobam**	**J20 Fenobam**
		start	2 (7/7)	2 (7/7)	2 (/8/8)	2 (/6/6)

Body Posture	In home cage: 0 = no movement, totally flattened posture; 1 = signs of some ataxia, slightly flattened posture; 2 = elevated posture	**1 week**	2 (7/7)	2 (7/7)	2 (/8/8)	2 (/6/6)
		2 weeks	2 (7/7)	2 (7/7)	2 (/8/8)	2 (/6/6)
		3 weeks	2 (7/7)	2 (7/7)	2 (/8/8)	2 (/6/6)
		4 weeks	2 (7/7)	2 (7/7)	2 (/8/8)	2 (/6/6)
Transfer Arousal	After transfer to arena: 0 = coma, 1 = slight, 2 = freeze & move, 3 = normal (momentary stop/freeze allowed), 4 = swift, 5 = maniac	**start**	3 (7/7)	3 (7/7)	3 (8/8)	3 (6/6)
		1 week	3 (7/7)	3 (7/7)	3 (8/8)	3 (6/6)
		2 weeks	3 (7/7)	3 (7/7)	3 (8/8)	3 (6/6)
		3 weeks	3 (7/7)	3 (7/7)	3 (8/8)	3 (6/6)
		4 weeks	3 (7/7)	3 (7/7)	3 (8/8)	3 (6/6)
Tail Elevation	After transfer to arena: 0 = dragging, 1 = rattling, 2 = normal	**start**	2 (7/7)	2 (7/7)	2 (/8/8)	2 (/6/6)
		1 week	2 (7/7)	2 (7/7)	2 (/8/8)	2 (/6/6)
		2 weeks	2 (7/7)	2 (7/7)	2 (/8/8)	2 (/6/6)
		3 weeks	2 (7/7)	2 (7/7)	2 (/8/8)	2 (/6/6)
		4 weeks	2 (7/7)	2 (7/7)	2 (/8/8)	2 (/6/6)

Table S2: Neurological Assessment Metrics (CTEP)						
			WT Vehicle n=14	**J20 Vehicle n=9**	**WT CTEP n=12**	**J20 CTEP n=12**
Body Weight (grams) ± SEM		**start**	37.18±1.50	33.42±2.44	36.59±0.67	32.13±1.64
		1 week	36.67±1.42	32.57±2.41	35.90±0.64	30.94±1.51
		2 weeks	36.53±1.42	32.52±2.26	35.47±0.55	31.41±1.47
		3 weeks	36.66±1.69	32.37±2.14	35.48±0.74	31.73±1.41
		4 weeks	36.12±1.59	32.25±2.13	34.99±0.59	31.88±1.52
General Observations			**WT Vehicle**	**J20 Vehicle**	**WT CTEP**	**J20 CTEP**
Tremors	Shaking movements	**start**	No (14/14)	No (9/9)	No (12/12)	No (12/12)
		1 week	No (14/14)	No (9/9)	No (12/12)	No (12/12)
		2 weeks	No (14/14)	No (9/9)	No (12/12)	No (12/12)
		3 weeks	No (14/14)	No (9/9)	No (12/12)	No (12/12)
		4 weeks	No (14/14)	No (9/9)	No (12/12)	No (12/12)
Eyelid Closure	Eyelids closed (cataracts)	**start**	No (14/14) Yes (1/14)	No (9/9) Yes (1/9)	No (11/12)	No (12/12)
		1 week	No (14/14) Yes (1/14)	No (9/9) Yes (1/9)	No (11/12)	No (12/12)
		2 weeks	No (14/14) Yes (1/14)	No (9/9) Yes (1/9)	No (11/12)	No (12/12)
		3 weeks	No (14/14) Yes (1/14)	No (9/9) Yes (1/9)	No (11/12)	No (12/12)
		4 weeks	No (14/14) Yes (1/14)	No (9/9) Yes (1/9)	No (11/12)	No (12/12)
Piloerection	Involuntary erection or bristling of hairs	**start**	No (14/14)	No (9/9)	No (11/12)	No (12/12)
		1 week	No (14/14)	No (9/9)	No (11/12)	No (12/12)
		2 weeks	No (14/14)	No (9/9)	No (12/12)	No (12/12)
		3 weeks	No (14/14)	No (8/9)	No (12/12)	No (12/12)
		4 weeks	No (14/14)	No (9/9)	No (12/12)	No (12/12)
Lacrimation	Secretion of tears	**start**	No (14/14)	No (9/9)	No (12/12)	No (12/12)
		1 week	No (14/14)	No (9/9)	No (12/12)	No (12/12)
		2 weeks	No (14/14)	No (9/9)	No (12/12)	No (12/12)
		3 weeks	No (14/14)	No (9/9)	No (12/12)	No (12/12)
		4 weeks	No (14/14)	No (9/9)	No (12/12)	No (12/12)
Salivation	Secreting saliva	**start**	No (14/14)	No (9/9)	No (12/12)	No (12/12)
		1 week	No (14/14)	No (9/9)	No (12/12)	No (12/12)
		2 weeks	No (14/14)	No (9/9)	No (12/12)	No (12/12)
		3 weeks	No (14/14)	No (9/9)	No (12/12)	No (12/12)
		4 weeks	No (14/14)	No (9/9)	No (12/12)	No (12/12)
Dirty Coat	Coat is dirty	**start**	No (14/14)	No (9/9)	No (11/12)	No (12/12)
		1 week	No (14/14)	No (9/9)	No (12/12)	No (12/12)
		2 weeks	No (14/14)	No (9/9)	No (12/12)	No (12/12)
		3 weeks	No (14/14)	No (9/9)	No (12/12)	No (12/12)
		4 weeks	No (14/14)	No (9/9)	No (12/12)	No (12/12)
Grip Strength	Mouse allowed to grip cage feed bin wire while	**start**	1.9±0.2	1.4±0.2	1.6±0.2	1.5±0.2
		1 week	1.4±0.1	1.4±0.2	1.5±0.2	1.6±0.3
		2 weeks	1.3±0.1	1.1±0.1	1.3±0.2	1.3±0.2

	investigator gently pulls backwards horizontally: 0= no grip, 1= slight grip 2 = moderate grip, 3 = active grip, 4 = unusually effective grip	**3 weeks**	1.3±0.1	1.0±0.0	1.3±0.1	1.4±0.2
		4 weeks	1.2±0.1	1.0±0.0	1.3±0.1	1.1±0.1
Reflexes			**WT Vehicle**	**J20 Vehicle**	**WT CTEP**	**J20 CTEP**
Righting Reflex	Hold mouse by tail and turn onto back. Failure to right their body into an upright position within 5 seconds is scored as No.	**start**	Yes (14/14)	Yes (9/9)	Yes (12/12)	Yes (12/12)
		1 week	Yes (14/14)	Yes (9/9)	Yes (12/12)	Yes (12/12)
		2 weeks	Yes (14/14)	Yes (9/9)	Yes (12/12)	Yes (12/12)
		3 weeks	Yes (14/14)	Yes (9/9)	Yes (12/12)	Yes (12/12)
		4 weeks	Yes (14/14)	Yes (9/9)	Yes (12/12)	Yes (12/12)
Pinna Reflex	While the mouse is gently restrained, touch each ear canal lightly with the tip of a metal spatula. Monitor for ear retraction or head movement. Yes = movement in both ears.	**start**	Yes (13/14)	Yes (8/9)	Yes (10/12)	Yes (11/12)
		1 week	Yes (13/14)	Yes (6/8)	Yes (11/12)	Yes (11/11)
		2 weeks	Yes (14/14)	Yes (9/9)	Yes (11/12)	Yes (12/12)
		3 weeks	Yes (14/14)	Yes (9/9)	Yes (12/12)	Yes (12/12)
		4 weeks	Yes (12/14)	Yes (7/9)	Yes (10/12)	Yes (10/12)
Eyeblink Response	When a cotton swab is moved toward an open eye, the mouse blinks. Yes = at least one eye blinks.	**start**	Yes (12/14)	Yes (7/9)	Yes (10/12)	Yes (9/12)
		1 week	Yes (11/14)	Yes (7/9)	Yes (10/12)	Yes (5/11)
		2 weeks	Yes (9/14)	Yes (5/9)	Yes (10/12)	Yes (6/12)
		3 weeks	Yes (13/14)	Yes (8/9)	Yes (12/12)	Yes (11/12)
		4 weeks	Yes (14/14)	Yes (8/9)	Yes (12/12)	Yes (10/12)
Observations in Arena			**WT Vehicle**	**J20 Vehicle**	**WT CTEP**	**J20 CTEP**
Body Posture	In home cage: 0 = no movement, totally flattened posture; 1 = signs of some ataxia, slightly flattened posture; 2 = elevated posture	**start**	2 (14/14)	2 (8/9) 1 (1/9)	2 (12/12)	2 (12/12)
		1 week	2 (14/14)	2 (9/9)	2 (12/12)	2 (12/12)
		2 weeks	2 (14/14)	2 (9/9)	2 (12/12)	2 (12/12)
		3 weeks	2 (14/14)	2 (9/9)	2 (12/12)	2 (12/12)
		4 weeks	2 (14/14)	2 (9/9)	2 (12/12)	2 (12/12)
Transfer Arousal	After transfer to arena: 0 = coma, 1 = slight, 2 = freeze & move, 3 = normal (momentary stop/freeze allowed), 4 = swift, 5 = maniac	**start**	3 (14/14)	3 (9/9)	3 (12/12)	3 (12/12)
		1 week	3 (14/14)	3 (9/9)	3 (12/12)	3 (12/12)
		2 weeks	3 (14/14)	3 (9/9)	3 (12/12)	3 (12/12)
		3 weeks	3 (14/14)	3 (9/9)	3 (12/12)	3 (12/12)
		4 weeks	3 (14/14)	3 (9/9)	3 (12/12)	3 (12/12)
Tail Elevation	After transfer to arena: 0 = dragging,1 = rattling, 2 = normal	**start**	2 (14/14)	2 (7/9) 0 (2/9)	2 (12/12)	2 (12/12)
		1 week	2 (14/14)	2 (9/9)	2 (12/12)	2 (12/12)
		2 weeks	2 (14/14)	2 (9/9)	2 (12/12)	2 (12/12)
		3 weeks	2 (14/14)	2 (9/9)	2 (12/12)	2 (12/12)
		4 weeks	2 (14/14)	2 (9/9)	2 (12/12)	2 (12/12)

Table S3: Activity Counts During Habituation to the Actigraphy Chambers				
Experiment	**WT**		**J20**	
	N	**Activity Counts**	**N**	**Activity Counts**
A: fall	15	737±122	13	818±73
B: winter	12	763±64	11	832±61
C: spring	12	767±71	8	860±38

Table S4: Peak Acrophase in Response to $mGluR_5$ Inhibitor Treatment					
Experiment	**WT**		**J20**		**T-Test (*p*)**
	N	**Peak Acrophase (min)**	**N**	**Peak Acrophase (min)**	
Vehicle (daily)	7	912±84	7	1,050±87	0.011
Fenobam (daily)	8	940±84	6	1,007±97	0.19
Vehicle (EOD)	14	1,002±96	9	1,108±137	0.04
CTEP (EOD)	12	1,020±104	12	1,094±94	0.079
Vehicle (EOD) (aged mice)	7	942±117	7	1,064±116	ND
CTEP (EOD) (aged mice)	9	1,005±156	6	1,078±106	ND

Table S5: Activity Levels in Response to $mGluR_5$ Treatment						
	WT			**J20**		
Experiment	**N**	**Age (days)**	**Activity Counts**	**N**	**Age (days)**	**Activity Counts**
Vehicle (daily)	7	256±11	3,039±911	7	254±3	3,938±880
Fenobam (daily)	8	252±3	3,821±723	6	258±12	4,156±715
Vehicle (EOD)	14	278±9	4,010±784	9	276±11	4,306±1,958
CTEP (EOD)	12	277±8	3,939±924	12	275±8	4,347±1,752
Vehicle (EOD)	7	508±38	3,769±1,082	7	504±39	4,209±771
CTEP (EOD)	9	507±42	3,626±1,080	6	545±58	4,304±898

Table S6: Mixed-Model ANOVA Statistics of Vigilance states			
	Wake	**NREM**	**REM**
Time	$p < 0.01$	$p < 0.001$	$p < 0.001$
F(3,152)	79.27	134.47	51.18
T1	33.49±1.79%	58.2±1.55%	6.79±0.3%
T2	29.35±1.79%	60.25±1.54%	7.88±0.3%
T3	48.83±1.79%	44.41±1.55%	3.86±0.3%
T4	58.14±1.79%	35.54±1.55%	2.37±0.3%
Group	$p<0.03$	$p<0.01$	$p=0.21$
F(3, 152)	4.33	11.07	1.37
WT/vehicle	40.18±1.92%	53.11±1.65%	6.15±0.32
WT/CTEP	46.64±1.66%	45.88±1.4%	5.69±0.33
J20/vehicle	40.51±1.66%	48.0±1.43%	4.87±0.28
J20/CTEP	42.47±1.91%	51.08±1.66%	4.17±0.28
Interaction (Time*group)	$p = 0.71$	$p = 0.92$	$p = 0.21$
F(9,152)	0.7	0.42	1.37
*Group means±95CI.			

Table S7: Multiple Comparisons of all Interactions (Time*Group) Related to Figure 4				
	Wake			
	WT		**J20**	
Time bin	**Veh**	**CTEP**	**Veh**	**CTEP**
1	28.3±3.8%	38.2±3.3%	34.8±3.3%	32.6±3.8%
2	25.2±3.8%	34.2±3.3%	29.1±3.3%	28.6±3.8%
3	50.6±3.8%	54.3±3.3%	42.6±3.3%	47.8±3.8%
4	56.6±3.8%	59.9±3.3%	55.2±3.3%	60.8±3.8%
	NREM			
	WT		**J20**	
	Veh	**CTEP**	**Veh**	**CTEP**
1	62.9±3.3%	54.9±2.9%	55.8±2.8%	59.1±3.3%
2	65.7±3.3%	56.5±2.9%	56.3±2.8%	62.4±3.3%
3	45.2±3.3%	39.4±2.9%	45.7±2.8%	47.3±3.3%
4	39.8±3.3%	32.7±2.9%	32.7±2.8%	35.5±3.3%
	REM			
	WT		**J20**	
	Veh	**CTEP**	**Veh**	**CTEP**
1	8.0±0.6%	6.1±0.5%	5.8±0.5%	7.2±0.6%
2	9.0±0.6%	7.8±0.5%	5.3±0.5%	8.9±0.6%
3	4.2±0.6%	3.1±0.5%	3.2±0.5%	4.9±0.6%
4	3.3±0.6%	2.4±0.5%	1.9±0.5%	1.7±0.6%

Table S8: Mixed Model ANOVA Statistics of EEG Power Related to Figure 5				
Stats	**Delta**	**Theta**	**Sigma**	**Gamma**
Time	$p<0.001$	$p<0.05$	$p<0.001$	$p=0.09$
F(11, 446462)	3.92	1.47	4.323	1.77
Time Bin*	**Delta**	**Theta**	**Sigma**	**Gamma**
1	0.63±0.00066	0.193±0.00039	0.106±0.00032	0.071±0.00032
2	0.612±0.00064	0.199±0.00038	0.112±0.00031	0.076±0.00031
3	0.599±0.00063	0.204±0.00037	0.119±0.0003	0.077±0.0003
4	0.578±0.00062	0.209±0.00037	0.126±0.0003	0.086±0.0003
5	0.569±0.00063	0.207±0.00038	0.126±0.00031	0.097±0.00031
6	0.575±0.00065	0.21±0.00039	0.128±0.00032	0.087±0.00031
7	0.564±0.00068	0.216±0.00041	0.129±0.00034	0.091±0.00033
8	0.579±0.00072	0.21±0.00044	0.122±0.00035	0.089±0.00035
9	0.587±0.00083	0.211±0.0005	0.118±0.00041	0.081±0.00041
10	0.589±0.00078	0.213±0.00048	0.118±0.00039	0.081±0.00038
11	0.602±0.00077	0.206±0.00047	0.111±0.00038	0.082±0.00037
12	0.625±0.00099	0.201±0.0006	0.101±0.00049	0.072±0.00048
Group	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$
F(3, 446462)	16.55	28.94	32.83	14.05
WT/vehicle	0.624±0.00043	0.186±0.00026	0.096±0.00021	0.094±0.00021
WT/CTEP	0.605±0.0004	0.2079±0.00024	0.124±0.0002	0.638±0.0002
J20/vehicle	0.554±0.00039	0.219±0.00024	0.136±0.00019	0.09±0.00019
J20/CTEP	0.587±0.00045	0.214±0.00027	0.117±0.00022	0.083±0.00022
Interaction (time*group)	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$
F(33, 446462)	178.31	92.58	103.42	287.44

*Records were divided into 2-hour bins and EEG power of NREM epochs within each bin were grouped. Bins = means ± 95% CI.

Table S9: Multiple Comparison of Normalized NREM EEG Power Values (Time*Group) Related to Figure 5

Delta (0.5-4 Hz)					Theta (5-9 Hz)			
WT		J20			WT		J20	
Vehicle	CTEP	Vehicle	CTEP	Time bin	Vehicle	CTEP	Vehicle	CTEP
0.638±0.0013	0.642±0.0013	0.601±0.0013	0.632±0.0014	1	0.186±0.0008	0.194±0.0007	0.201±0.0008	0.193±0.0008
0.634±0.0014	0.620±0.0012	0.578±0.0012	0.615±0.0014	2	0.190±0.0008	0.201±0.0008	0.209±0.0006	0.199±0.0008
0.622±0.0012	0.610±0.0012	0.565±0.0012	0.603±0.0014	3	0.192±0.0007	0.206±0.0007	0.214±0.0007	0.204±0.0008
0.582±0.0013	0.594±0.0012	0.549±0.0012	0.590±0.0013	4	0.199±0.0007	0.212±0.0007	0.219±0.0007	0.208±0.0008
0.562±0.0013	0.600±0.0012	0.539±0.0012	0.578±0.0013	5	0.185±0.0007	0.207±0.0007	0.224±0.0007	0.213±0.0008
0.595±0.0013	0.593±0.0012	0.533±0.0013	0.577±0.0013	6	0.189±0.0008	0.211±0.0007	0.226±0.0008	0.214±0.0008
0.590±0.0014	0.577±0.0015	0.527±0.0012	0.560±0.0014	7	0.199±0.0008	0.218±0.0008	0.229±0.0007	0.221±0.0008
0.658±0.0015	0.590±0.0014	0.519±0.0014	0.548±0.0015	8	0.166±0.0009	0.212±0.0009	0.233±0.0007	0.229±0.0009
0.635±0.0018	0.602±0.0015	0.535±0.0016	0.577±0.0018	9	0.186±0.001	0.210±0.0009	0.231±0.0009	0.218±0.0011
0.617±0.0017	0.597±0.0014	0.550±0.0015	0.591±0.0016	10	0.199±0.001	0.212±0.0008	0.225±0.0009	0.215±0.0009
0.637±0.0017	0.608±0.0015	0.573±0.0014	0.589±0.0015	11	0.184±0.001	0.208±0.0009	0.215±0.0008	0.219±0.0009
0.714±0.0016	0.623±0.0020	0.578±0.0018	0.585±0.0025	12	0.160±0.0009	0.204±0.001	0.213±0.0011	0.230±0.0015

Sigma (10-15 Hz)					Gamma (25-100 Hz)			
WT		J20			WT		J20	
Vehicle	CTEP	Vehicle	CTEP	Time bin	Vehicle	CTEP	Vehicle	CTEP
0.099±0.0006	0.107±0.0006	0.119±0.0006	0.102±0.0007	1	0.077±0.0006	0.056±0.0006	0.079±0.0006	0.073±0.0006
0.091±0.0007	0.118±0.0006	0.130±0.0005	0.110±0.0006	2	0.085±0.0007	0.060±0.0006	0.083±0.0005	0.076±0.0006
0.103±0.0006	0.122±0.0005	0.136±0.0005	0.116±0.0006	3	0.083±0.0006	0.063±0.0005	0.086±0.0005	0.077±0.0006
0.110±0.0006	0.130±0.0005	0.141±0.0005	0.123±0.0006	4	0.109±0.0006	0.065±0.0005	0.090±0.0005	0.079±0.0006
0.103±0.0006	0.128±0.0006	0.146±0.0005	0.126±0.0006	5	0.150±0.0006	0.065±0.0005	0.092±0.0005	0.083±0.0006
0.108±0.0007	0.130±0.0006	0.147±0.0006	0.128±0.0006	6	0.107±0.0007	0.066±0.0006	0.094±0.0006	0.081±0.0006
0.100±0.0007	0.137±0.0007	0.149±0.0005	0.131±0.0006	7	0.111±0.0007	0.068±0.0007	0.096±0.0005	0.087±0.0007
0.074±0.0007	0.135±0.0006	0.148±0.0006	0.131±0.0007	8	0.102±0.0007	0.064±0.0006	0.099±0.0006	0.092±0.0007
0.096±0.0009	0.124±0.0007	0.139±0.0007	0.114±0.0009	9	0.083±0.0009	0.064±0.0007	0.096±0.0007	0.090±0.0006
0.107±0.0008	0.124±0.0006	0.132±0.0007	0.108±0.0008	10	0.077±0.0008	0.067±0.0006	0.093±0.0007	0.085±0.0007
0.093±0.0008	0.118±0.0007	0.124±0.0007	0.107±0.0007	11	0.086±0.0008	0.066±0.0007	0.088±0.0006	0.086±0.0007
0.071±0.0007	0.110±0.0009	0.121±0.0008	0.103±0.0012	12	0.056±0.0007	0.063±0.0009	0.089±0.0009	0.082±0.0012

Text S1

Upon consultation with pharmaceutical industry experts on dosing with $mGluR_5$ inhibitors, we were advised that: (1) drug properties can differ dependent on the source of the compound, i.e. they prefer to use in-house prepared compounds versus commercially synthesized drugs; (2) differences in pharmacokinetics usually parallel differences in the physical property and/or solubility of the compound, (3) it may be advantageous to use a suspending agent such as HPMC; and (4) some of them have had great difficulty in reproducing pharmacokinetic results using published solvents and protocols. Thus, we modified the drug preparation protocol (*Method 2*) and tested the dose dependency (0.1, 0.5 and 2 mg/kg) of CTEP pharmacokinetics by oral gavage in WT mice (2-months old) with blood plasma collected 2.25 hours after dosing. Due to material transfer agreement issues, we continued to use a commercial source of CTEP. We found a clear dose response in drug levels with a single dose of CTEP by oral gavage [0.1 mg/kg: 4.2±2.2 ng/mL; 0.5 mg/kg: 30±1.3 ng/mL; 2 mg/kg: 128±19 ng/mL]. Thus, *Method 2* dosing was used for Figure 5 (aged mice). However, after 25 days chronic dosing with CTEP every other day (EOD) in aged WT and J20 cohorts, pharmacokinetic analysis of samples collected 1 hr after the last CTEP dose indicated 17.8±10.2 ng/mL CTEP in WT plasma (n=10), 14.3±21.9 ng/mL in WT brain (n=11), 12.9±8.8 ng/mL in J20 plasma (n=8), and 9.5±5.3 ng/mL in J20 brain (n=8). All were 6-23-fold lower than expected.

Figure S1: Mouse longevity of wild type (WT) and J20 littermates through 9 months of age. J20 mice (orange; n=114) exhibited a 40% premature death rate by 3 months of age compared to 0% premature deaths in WT mice (blue; n=123). Data for male mice is shown. Females exhibited a similar profile through 3 months of age (data not shown because females were not housed for the extended period of time shown for the males).

Figure S2: Representative EEG traces for sleep vigilance states. (A) Wakefulness is typically characterized by high-EMG amplitude. (B and C) Manual score of sleep necessitates a low EMG amplitude, where NREM is dominated by a slow, large amplitude delta power (1-4 Hz), while REM sleep contains lower amplitude waveforms with largely theta band (6-9 Hz) activity.

Figure S3: J20 mice exhibit decreased habituation. Habituation during the first 2 hours in the actigraphy chambers was assessed in 3 separate cohorts of wild type (WT) (blue) and J20 (orange) 8-month old mice (A=fall, B=winter, C=spring). Total activity counts (binned in 1 minute increments) were averaged for cohorts and plotted on the y-axis versus the first 120 minute time period on the x-axis. (A) Cohort consists of WT (n=15) and J20 (n=13). (B) Cohort consists of WT (n=12) and J20 (n=11). (C) Cohort consists of (n=12) and J20 (n=8).

Figure S4: Dosing scheme and behavioral battery for wild type (WT) and J20 mice. The timeline for testing and dosing included a pre-treatment neurobehavioral screen at 8-9 months of age with follow-up neurobehavioral screens at 7, 14, 21 and 28 days dosing. Fenobam was dosed daily and CTEP every other day. Rotarod testing was conducted during weeks 2 & 4 of dosing, actigraphy during week 3 of dosing, and passive avoidance on dosing days 28-30. Tissue was collected on dosing day 30. All procedures were performed during the light cycle.

Figure S5: Diurnal activity levels in wild type (WT) and J20 mice in response to CTEP (2-chloro-4-((2,5-dimethyl-1-(4-(trifluoromethoxy)phenyl)-1H-imidazol-4-yl)ethynyl)pyridine). Activity counts were assessed in 16-19-month old WT and J20 mice after chronic treatment with vehicle or CTEP. Total activity counts (binned in 1 minute increments) were averaged over 7 days of readings for cohorts and plotted on the y-axis versus a 24 hour time period (in minutes). Time zero is “Lights On”. Cohorts consist of WT mice treated with vehicle (n=7, blue), J20 treated with vehicle (n=7, orange), WT treated with CTEP (n=9, red), and J20 treated with CTEP (n=6, green). (A) WT mice

treated with vehicle (blue) versus CTEP (red). (B) J20 mice treated with vehicle (orange) versus CTEP (green). (C) Vehicle-treated WT (blue) versus J20 (orange). (D) CTEP-treated WT (red) versus J20 (green).

Figure S6: Dosing scheme for wild type (WT) and J20 mice for the electroencephalography (EEG) study. Mice (11-2 months old) were implanted with electrodes as described in the Methods on Day 1. After 3 days recovery from surgery (Day 4), mice received vehicle or CTEP treatment every other day (EOD). EEG recordings were collected from Day 8-12. Dosing continued EOD throughout the EEG recordings.

Figure S7: Amyloid-beta (Aβ) levels in response to metabotropic glutamate receptor 5 ($mGluR_5$) inhibition. $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ levels were quantitated in (A) blood plasma and (B) brain (right cortex) from 8.5-10-month old WT and J20 mice by ELISA after chronic treatment with fenobam or CTEP (2-chloro-4-((2,5-dimethyl-1-(4-(trifluoromethoxy)phenyl)-1H-imidazol-4-yl)ethynyl)pyridine). Optical densities at 450 nm were converted to pmol/L, corrected for dilution factors, and plotted versus treatment/genotype conditions. Fenobam cohorts consisted of wild type (WT) mice treated with vehicle daily (V) (n=6), WT mice treated with fenobam daily (F) (n=7), J20 mice treated with vehicle daily (n=7), and J20 mice treated with fenobam daily (n=7). CTEP cohorts consisted of WT mice treated with vehicle every other day (EOD) (n=14), WT mice treated with CTEP EOD (n=12), J20 mice treated with vehicle EOD (n=9), and J20 mice treated with CTEP EOD (n=12). Error bars indicate standard error of the mean (SEM).

2-way ANOVA results:

<u>plasma/Aβ40/fenobam</u>: interaction p=0.032, $F(1,23)=5.2$; drug treatment p=0.15, $F(1,23)=2.2$; genotype, $p<0.0001$, $F(1,23)=34$

plasma/Aβ40/CTEP: interaction p=0.86, F(1,43)=0.03; drug treatment p=0.58, F(1,43)=0.30; genotype, p<0.0001, F(1,43)=153

cortex/Aβ40/fenobam: interaction p=0.51, F(1,23)=0.45; drug treatment p=0.52, F(1,23)=0.42; genotype, p=0.0002, F(1,23)=19.83

cortex/Aβ40/CTEP: interaction p=0.44, F(1,43)=0.59; drug treatment p=0.45, F(1,43)=0.57; genotype, p=0.0037, F(1,43)=9.43

plasma/Aβ42/fenobam: interaction p=0.31, F(1,23)=1.08; drug treatment p=0.61, F(1,23)=0.27; genotype, p<0.0001, F(1,23)=108.6

plasma/Aβ42/CTEP: interaction p=0.77, F(1,43)=0.084; drug treatment p=0.79, F(1,43)=0.072; genotype, p<0.0001, F(1,43)=193.8

cortex/Aβ42/fenobam: interaction p=0.82, F(1,23)=0.052; drug treatment p=0.82, F(1,23)=0.055; genotype, p=0.0007, F(1,23)=15.24

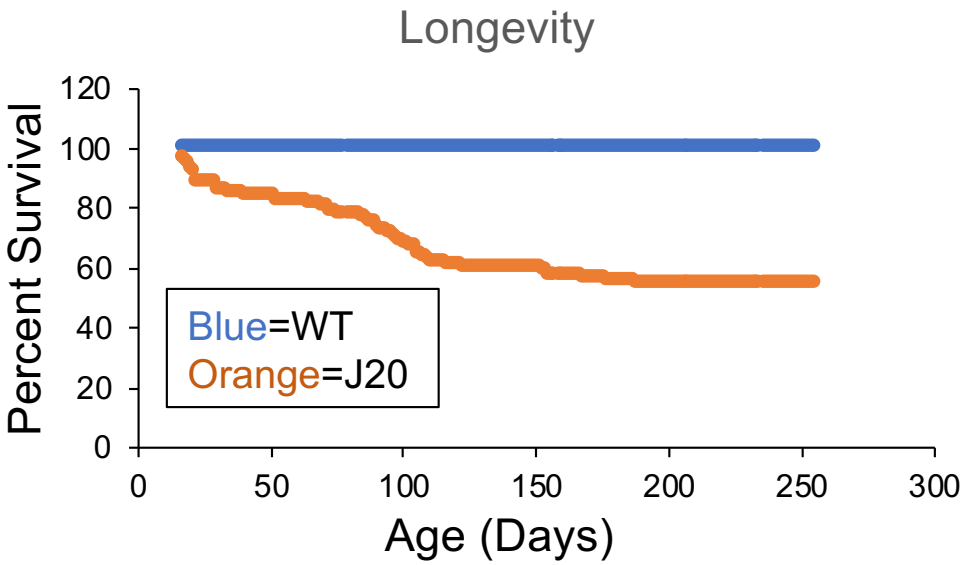
cortex/Aβ42/CTEP: interaction p=0.47, F(1,43)=0.54; drug treatment p=0.47, F(1,43)=0.54; genotype, p=0.0004, F(1,43)=14.90

Figure S8: Learning and memory in response to metabotropic glutamate receptor 5 ($mGluR_5$) inhibition. Learning and memory was assessed by passive avoidance testing. Mice were (A) trained with a 2-second 0.6 mA footshock on the dark side of a light-dark chamber and tested for learning and memory (B) 6 hours, (C) 24 hours, and (D) 48 hours later by latency time to enter the dark side. Neither genotype (WT = wild type; J20 = Alzheimer's mice) nor treatment (veh = vehicle, fen = fenobam, CTEP = 2-chloro-4-((2,5-dimethyl-1-(4-(trifluoromethoxy)phenyl)-1H-imidazol-4-yl)ethynyl)pyridine) significantly affected learning & memory in the passive avoidance task.

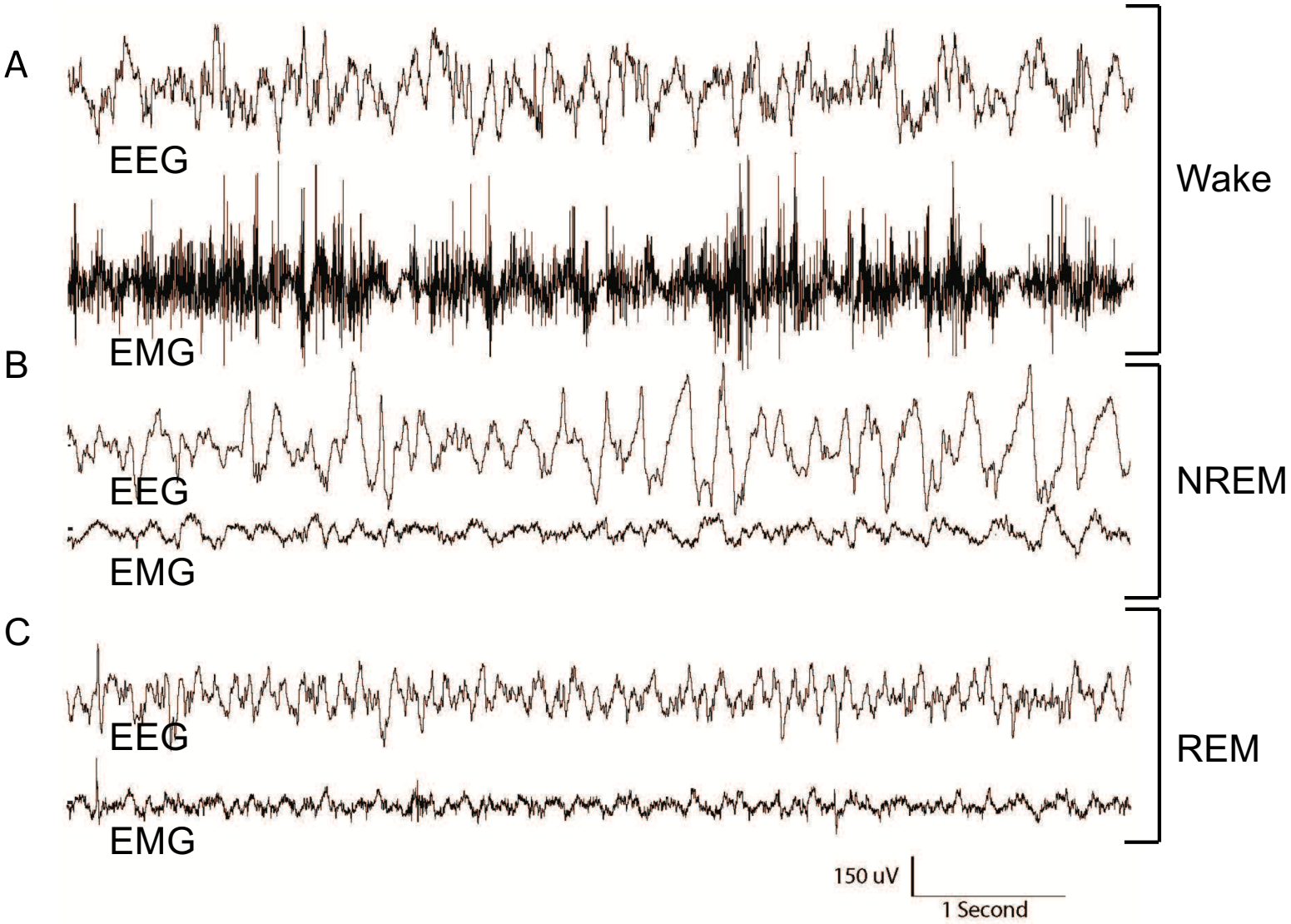
Figure S9: Motor coordination in response to metabotropic glutamate receptor 5 ($mGluR_5$) inhibition. Motor coordination was assessed on the rotarod (A) pretreatment, (B) after 9 days treatment with veh (vehicle), Fen (fenobam) or CTEP (2-chloro-4-((2,5-dimethyl-1-(4-(trifluoromethoxy)phenyl)-1H-imidazol-4-yl)ethynyl)pyridine), and (C) after 23 days treatment with veh, Fen or CTEP. Six trials or series were run for each experiment with 4 trials on Day 1 and 2 trials on Day 2. Treatment did not impair motor coordination. All treatment groups exhibited increased motor learning with an increased number of trials during the pretreatment testing. All treatment groups maintained the maximal motor coordination ability attained during the pretreatment testing in subsequent testing after both 9 and 23 days of treatment.

Figure S10: CTEP (2-chloro-4-((2,5-dimethyl-1-(4-(trifluoromethoxy)phenyl)-1H-imidazol-4-yl)ethynyl)pyridine) levels in blood plasma and brain after chronic dosing. CTEP levels were quantitated in blood plasma and brain cortical tissue from wild type (WT) (n=12) and J20 (n=11) mice (9-months old) after chronic drug dosing at 2 mg/kg by *Method 1*.

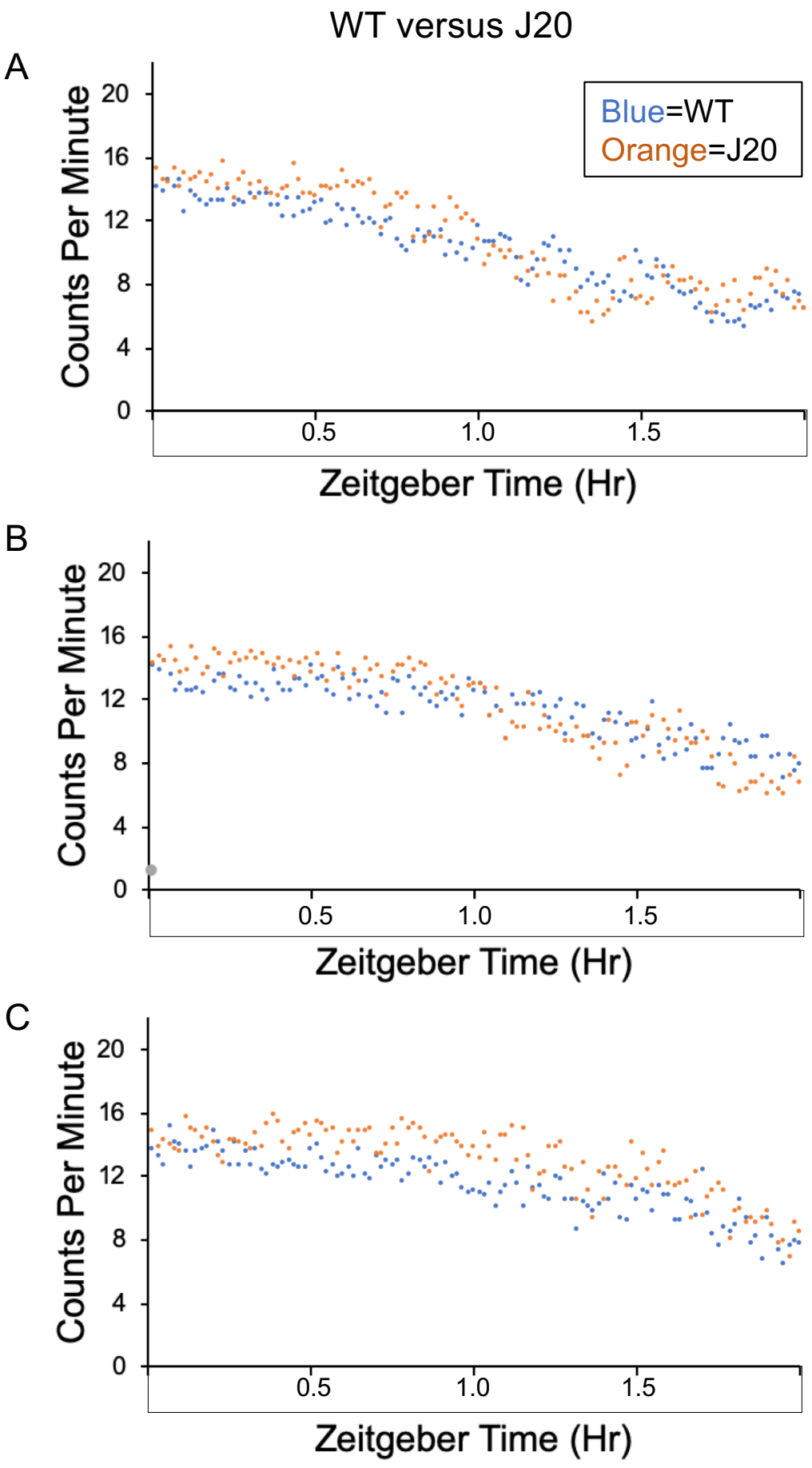
Supplementary Figure 1



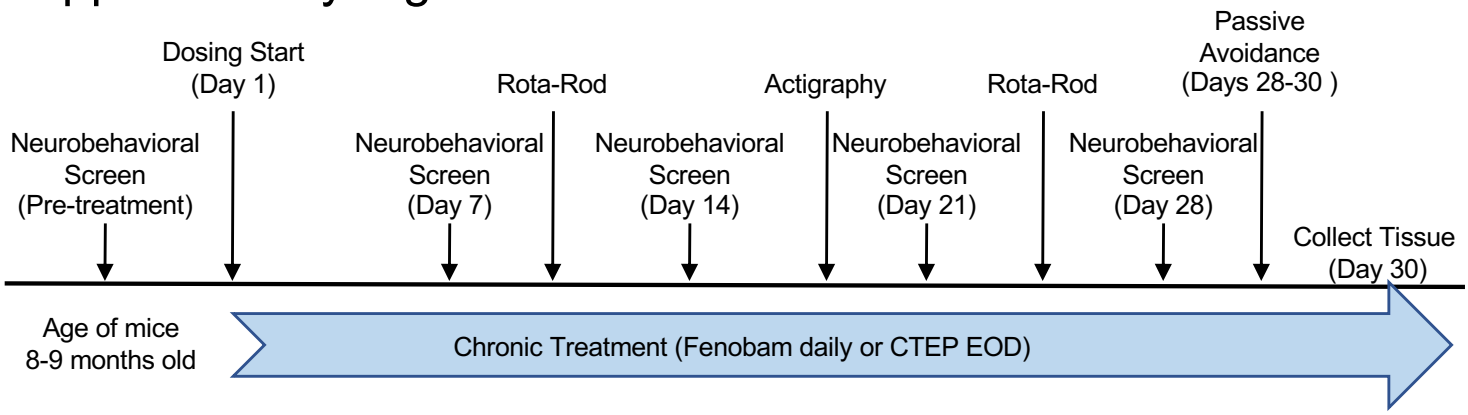
Supplementary Figure 2



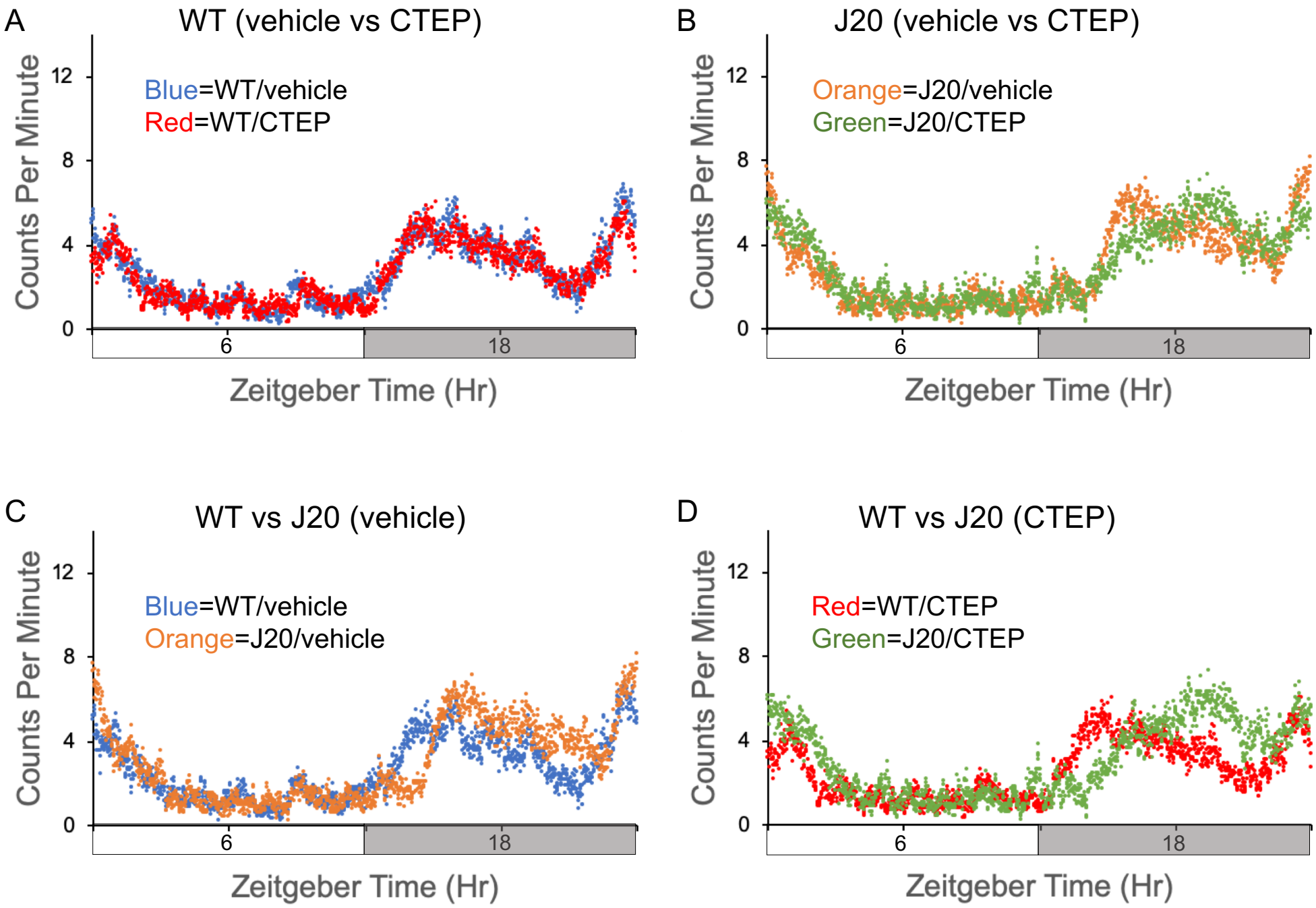
Supplementary Figure 3



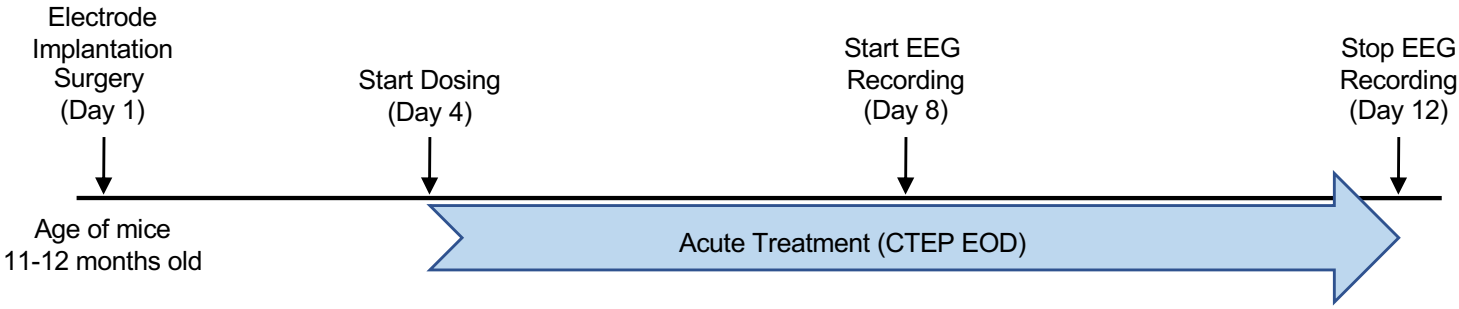
Supplementary Figure 4



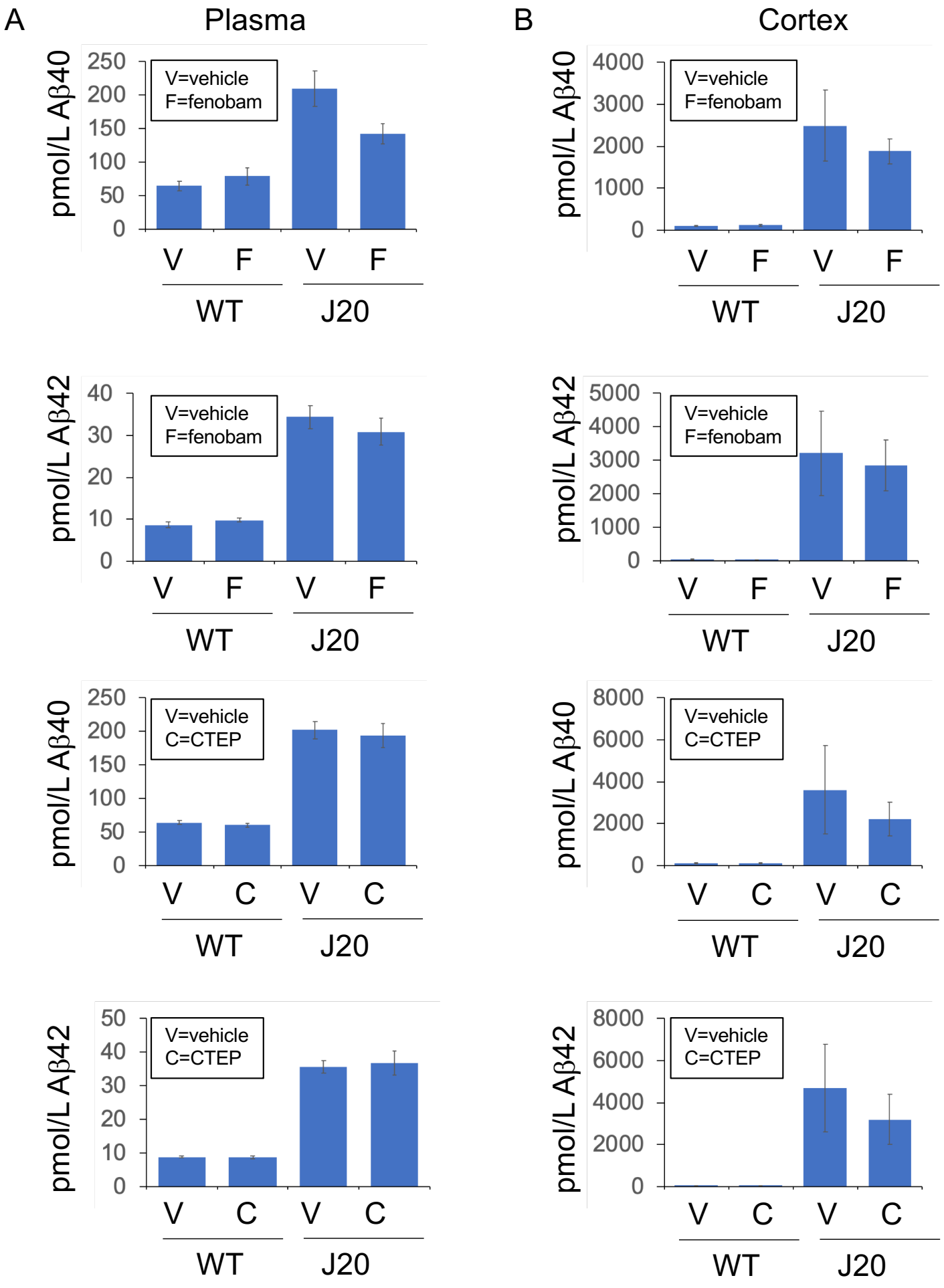
Supplementary Figure 5



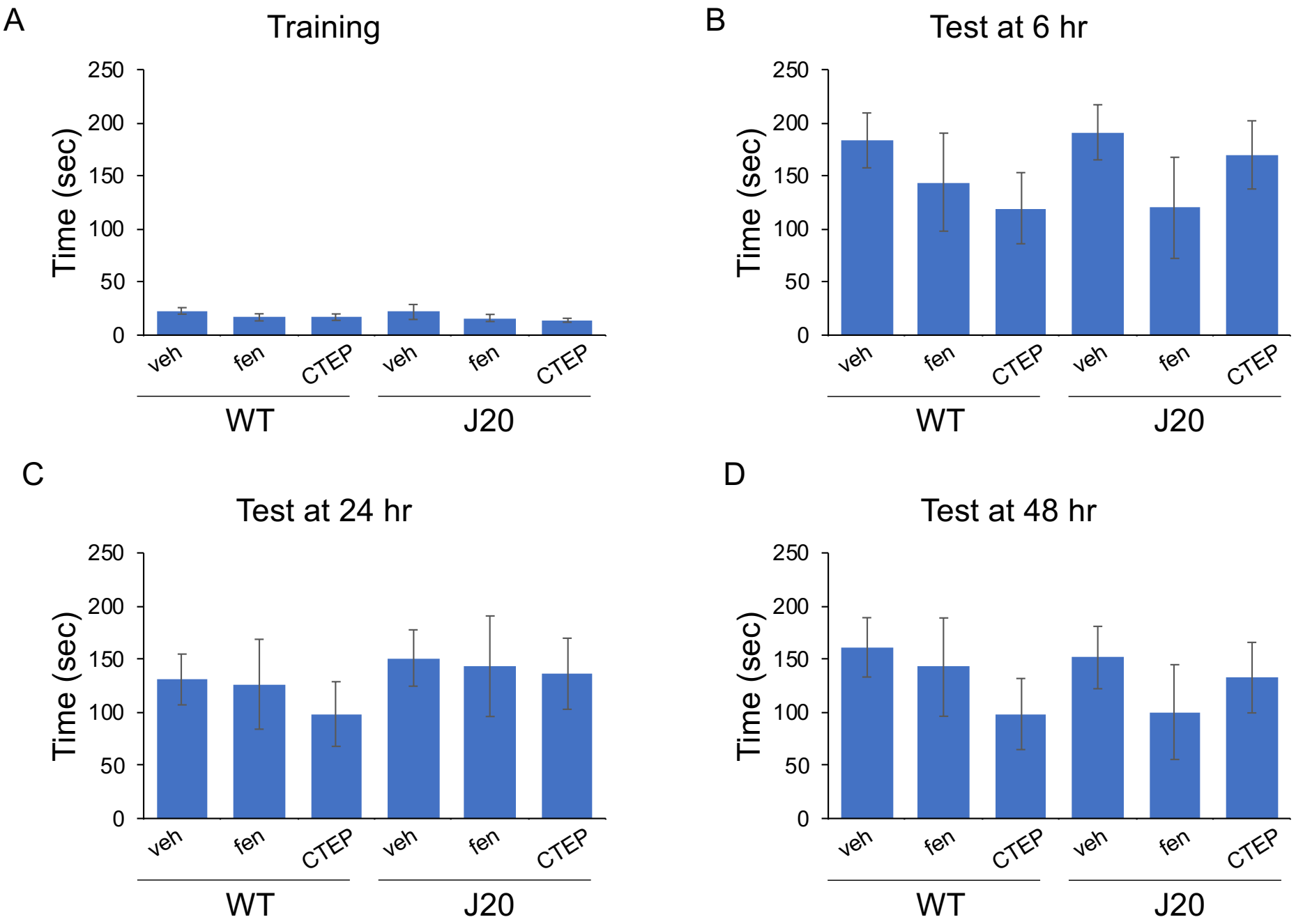
Supplementary Figure 6



Supplementary Figure 7

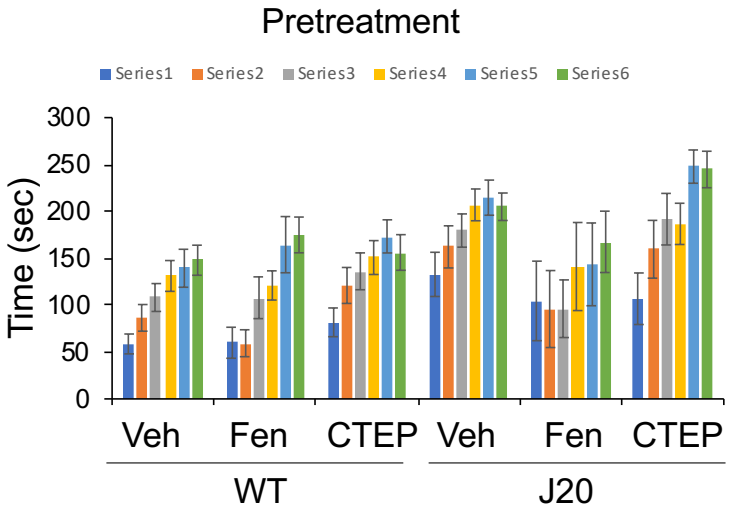


Supplementary Figure 8



Supplementary Figure 9

A



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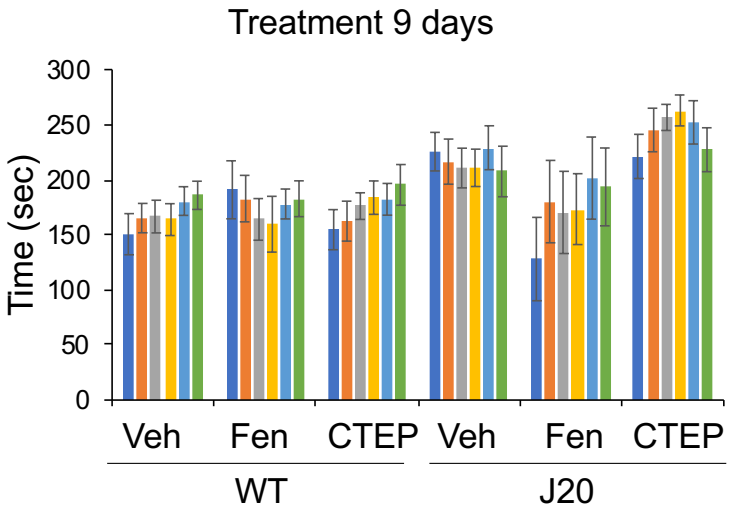
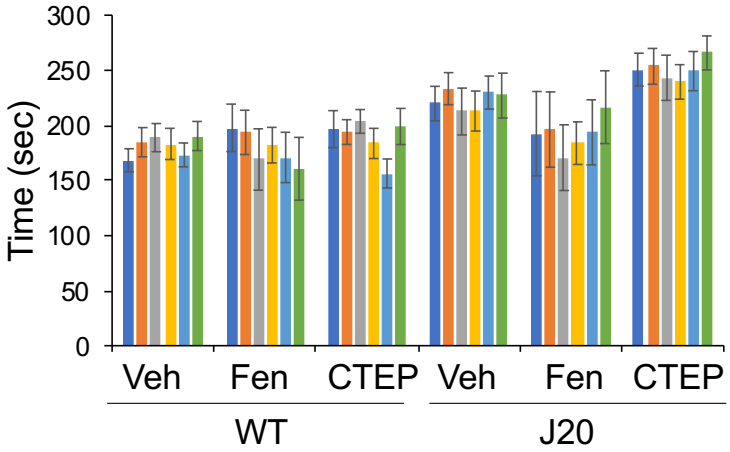


C



Supplementary Figure 10