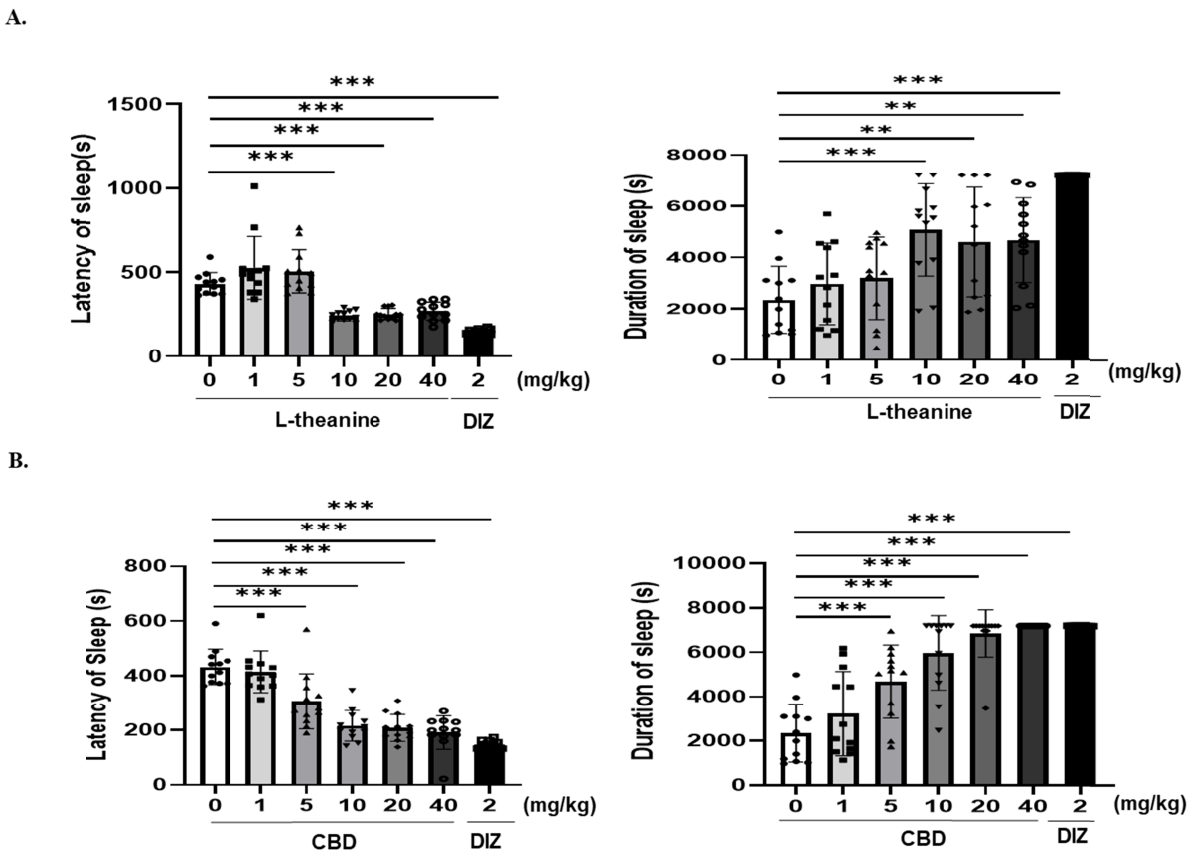
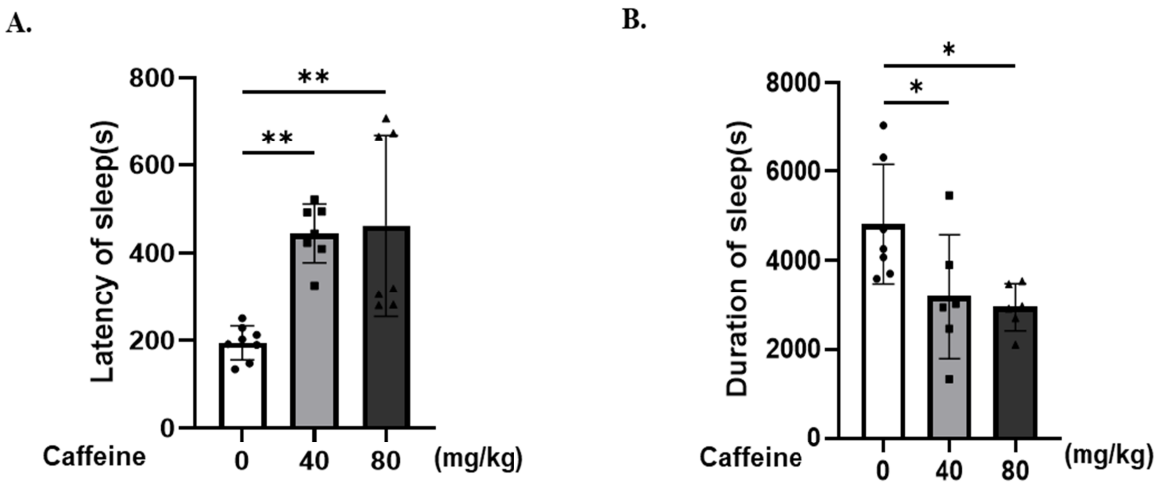
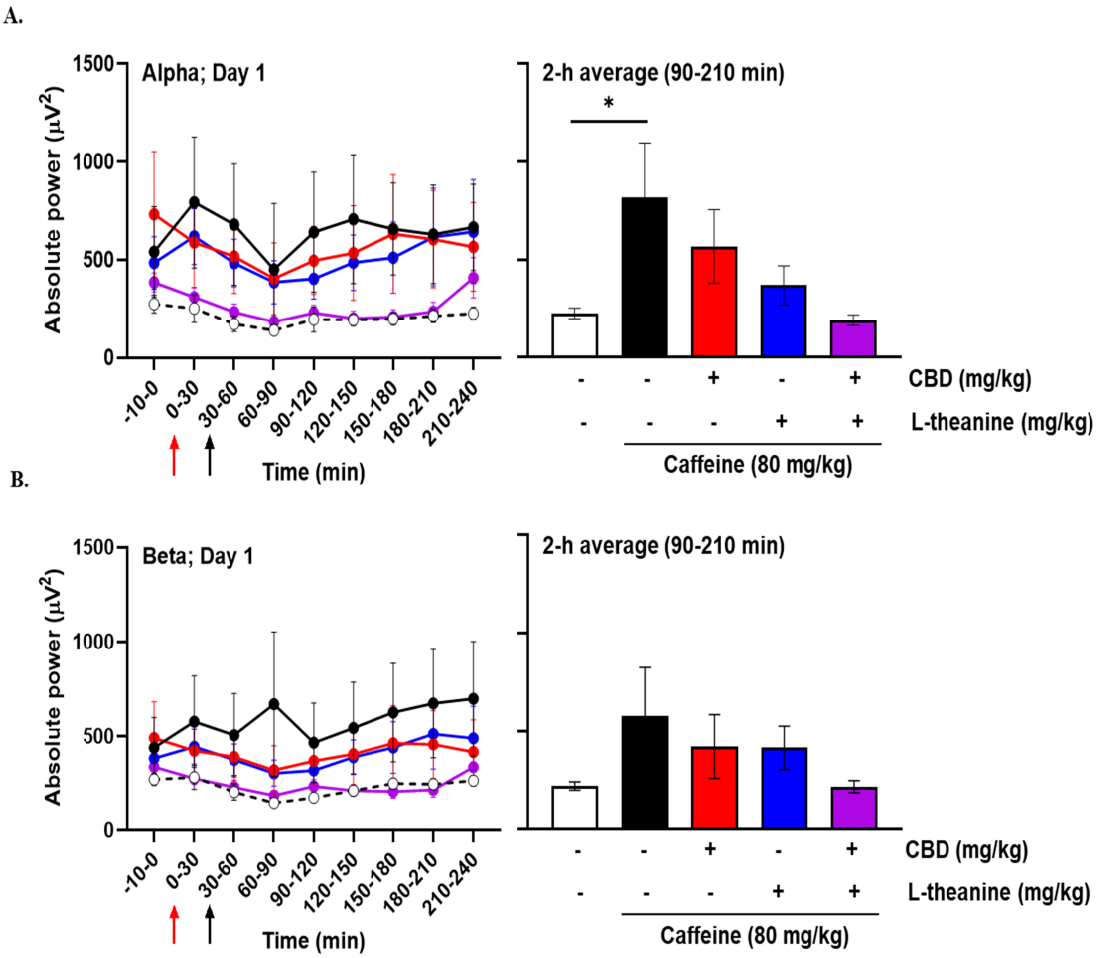
Supplementary Figures and Methods



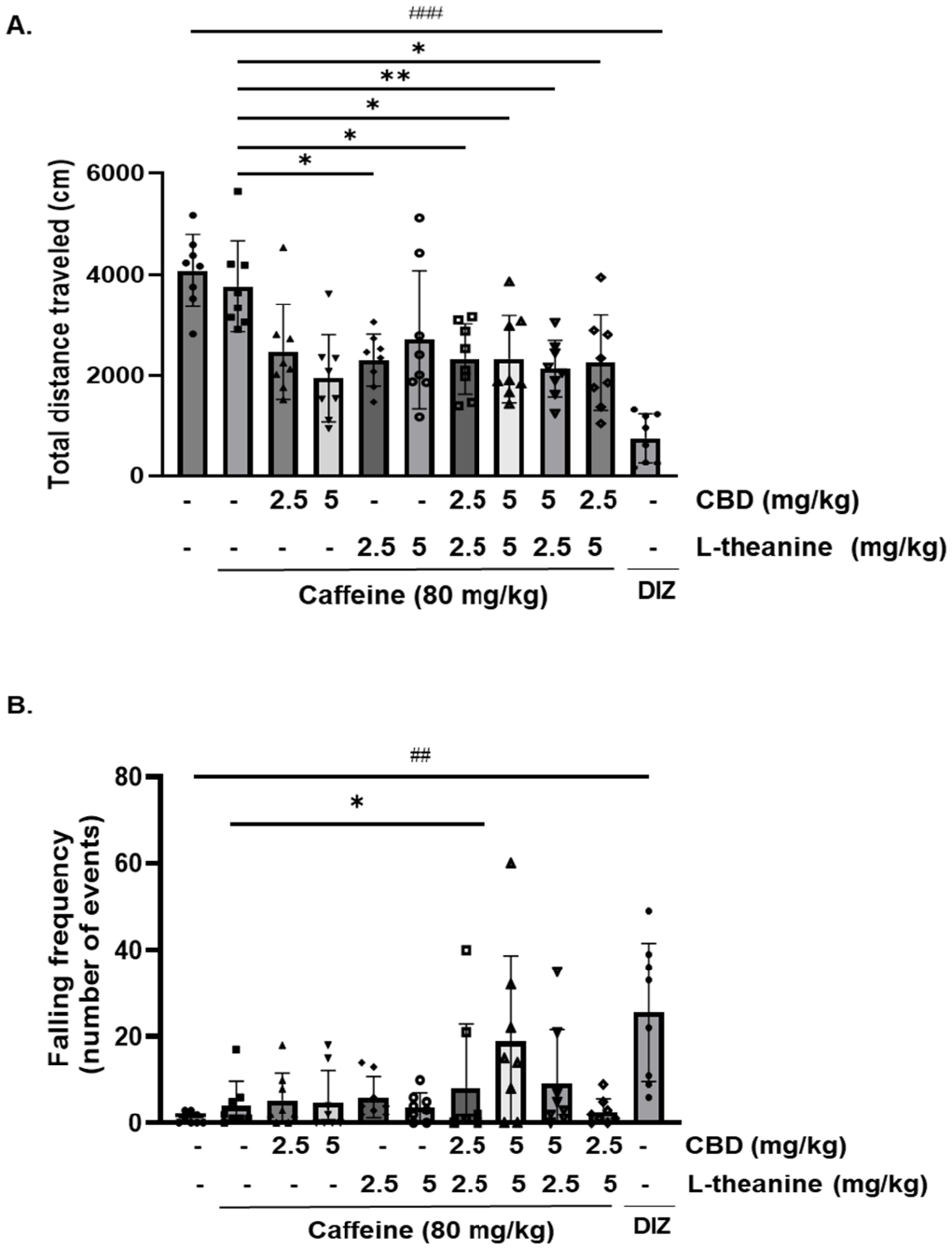
Supplementary Figure 1. Effects of CBD or L-theanine as single treatments on pentobarbital-induced sleep in mice. Mice were orally pretreated with single doses of L-theanine or CBD (1–40 mg/kg). Thirty minutes later, pentobarbital (42 mg/kg i.p.) was administered to induce sleep. Diazepam (DIZ, 2 mg/kg, p.o.) was used as a positive control. Sleep latency (left panel) and sleep duration (right panel) following L-theanine administration were measured (n = 12 per group) (A). Sleep latency (left panel) and sleep duration (right panel) following CBD administration were measured (n = 12 per group) (B). Data are presented as mean ± SD. Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparisons test. $^{**}p < 0.01$, $^{***}p < 0.001$, vs. control. CBD, cannabidiol; DIZ, diazepam.



Supplementary Figure 2. Establishment of a caffeine-induced sleep disturbance model using the pentobarbital-induced sleep test in mice. Mice were intraperitoneally pretreated with caffeine (40 or 80 mg/kg), followed by administration of pentobarbital (42 mg/kg, i.p.). Sleep latency (A) and sleep duration (B) were measured (n = 8 per group). Data are presented as mean ± SD and were analyzed using one-way ANOVA followed by Dunnett's multiple comparisons test. *$p < 0.05$, **$p < 0.01$ vs. control.



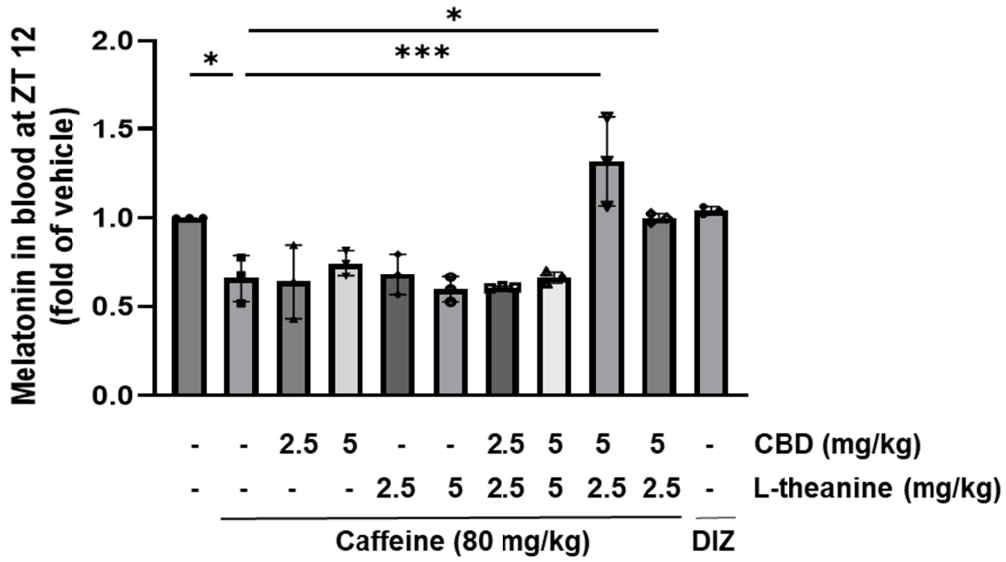
Supplementary Figure 3. Effects of caffeine, CBD and L-theanine on EEG absolute power. Absolute power in the (A) alpha (8.4-14 Hz) and (B) beta (13-30 Hz) frequency bands was analyzed following a single administration of vehicle, caffeine (80 mg/kg), caffeine combined with CBD 5 mg/kg, caffeine combined with L-theanine 2 mg/kg or caffeine combined with CBD and L-theanine at a 2:1 ratio in mice. The red arrow indicates the time of vehicle or caffeine administration, while the black arrow indicates the time of CBD, L-theanine or CBD and L-theanine co-administration. Data are presented as mean ± SEM. (n=4-6 animals per group). Statistical analysis was performed using one-way ANOVA followed by Dunnett's post hoc test. *$p < 0.05$ vs. vehicle.

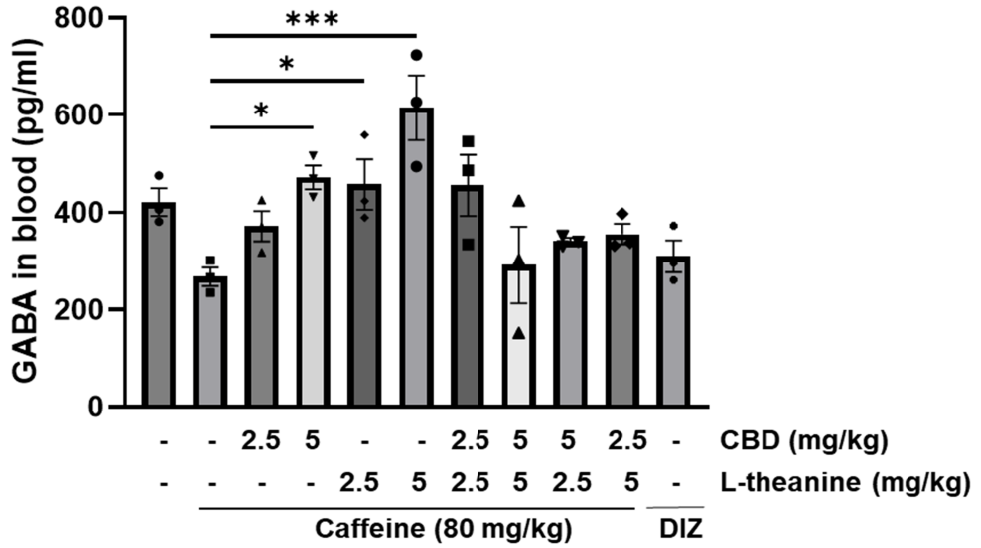


Supplementary Figure 4. Effects of CBD, L-theanine, or their combination on locomotor activity and sleep in caffeine-treated mice. Mice were administered caffeine (80 mg/kg), followed by CBD, L-theanine, or their combination at the indicated doses (n = 8 per group). Thirty minutes after drug administration, locomotor activity was assessed by measuring total distance traveled during a 12-min open field test (OFT) (A), and motor coordination was evaluated by recording the falling frequency during a 10-min rotarod test (B). Diazepam (DIZ)

was used as a positive control. Data are presented as mean ± SD. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparisons test. *$p < 0.05$, **$p < 0.01$, vs. the caffeine-treated group; ##$p < 0.01$, ###$p < 0.001$ vs. the control group. CBD, cannabidiol; DIZ, diazepam.

A.





B.

Supplementary Figure 5. Effects of CBD, L-theanine, or their combination on plasma melatonin and GABA content in caffeine-treated mice. Mice were administered caffeine (80 mg/kg, i.p.), and 30 minutes later received CBD, L-theanine,

or combinations at ratios of 1:1, 1:2, or 2:1. Diazepam (2 mg/kg, p.o.) was used as a positive control. (A) Plasma melatonin levels at zeitgeber time 12 (ZT12) after treatment. Plasma samples were collected at ZT12, and melatonin concentrations were measured using a GABA ELISA kit. (B)Plasma GABA levels after treatment. GABA concentrations were measured using a GABA ELISA kit. Data were analyzed using one-way ANOVA followed by Dunnett's multiple comparisons test. Data are presented as mean ± SD.*$p < 0.05$, ***$p < 0.001$ vs. caffeine-treated group. CBD, cannabidiol; DIZ, diazepam.

Supplementary methods.

Combination Index (CI) Analysis

The interaction between cannabidiol (CBD) and L-theanine on sleep duration was evaluated using a Combination Index (CI) approach conceptually derived from the Chou–Talalay additivity principle. Given the behavioral nature of the endpoint and the use of predefined dose combinations, CI analysis was applied as a single-dose-based, exploratory assessment, rather than a classical median-effect analysis based on full dose-response curves. Sleep duration (seconds) was used as the effect variable. To normalize caffeine-induced sleep suppression, the fractional effect (Fa) was calculated as:

$$F_a = \frac{E_{treatment} - E_{caffeine}}{E_{vehicle} - E_{caffeine}}$$

where $E_{treatment}$ represents the mean sleep duration of the single-compound or combination-treated group, $E_{caffeine}$ represents the caffeine-treated control group, and $E_{vehicle}$ represents the vehicle-treated group. Thus, Fa reflects the relative recovery of sleep duration compared to the caffeine-induced deficit. CI values for each CBD–L-theanine combination were calculated using the simplified Chou-Talalay equation:

$$\mathbf{CI} = \frac{D_1}{D_{x1}} + \frac{D_2}{D_{x2}}$$

where D_1 and D_2 are the doses of CBD and L-theanine used in the combination, and D_{x1} and D_{x2} are the doses of each compound alone producing comparable fractional effects under the same experimentally observed effects at the tested single doses rather than interpolated from fitted median-effect plots. CI values were interpreted descriptively according to conventional thresholds: CI < 1 indicates a synergistic tendency, CI = 1

indicates an additive interaction, and CI > 1 indicates an antagonistic tendency. Given the methodological constraints, CI values are presented as supportive, hypothesis-generating indicators of interaction rather than definitive evidence of pharmacological synergism.