SUPPLEMENTAL MATERIAL

Expanded Materials & Methods

Animal models

All surgical procedures and protocols were approved by the Animal Care and Use Committee of The University of Texas MD Anderson Cancer Center. Male and female Sprague-Dawley rats (13–15 weeks old) were purchased from Envigo (Indianapolis, IN). Heterozygous (*Cacna2d1*$^{+/-}$, C57BL/6 genetic background) mice were purchased from Medical Research Council (Harwell Didcot, Oxfordshire, UK). *Cacna2d1* knockout (KO, *Cacna2d1*$^{-/-}$) mice and wild-type (WT, *Cacna2d1*$^{+/+}$) littermates were obtained by breeding the heterozygous mice as we described previously.[25] Adult WT and *Cacna2d1* KO mice (8–10 weeks old, age- and sex-matched) were used in the final experiments. Because we did not find any sex difference in response to FK506 treatment, we pooled the data from male and female mice for final data analysis. All the animals were housed at a controlled temperature (25°C) and lighting (12-hour light/dark cycles). The animals had free access to rodent food and tap water. Tacrolimus (FK506; #3631, Tocris Bioscience, Bristol, UK) was dissolved in dimethyl sulfoxide. For induction of CIH, FK506 was injected intraperitoneally at a dose of 3 mg/kg once daily for 14 consecutive days in rats and mice.[5] Animals received a same amount of dimethyl sulfoxide as the vehicle group. Final experiments were conducted 5–7 days after the last FK506 injection. Gabapentin (#10008346, Cayman Chemical, Ann Arbor, MI) was injected intraperitoneally or given through drinking water.

PVN tissue collection and synaptosome preparation

Under anesthesia induced by 3% isoflurane, rats were decapitated, and their brains were quickly removed and placed in ice-cold artificial cerebrospinal fluid (aCSF) containing 124.0 mM NaCl, 3.0 mM KCl, 1.3 mM $MgSO_4$, 2.4 mM $CaCl_2$, 1.4 mM NaH_2PO_4, 10.0 mM glucose, and 26.0 mM $NaHCO_3$, which was continuously gassed with a mixture of 95% O_2 and 5% CO_2. Hypothalamic tissues were sectioned at 1.08–2.12 mm caudal to the bregma, and punched PVN tissues from 2 rats were pooled for each sample. Synaptosomes from the PVN tissues were prepared as we described previously.[13,27] Briefly, PVN tissues were homogenized by using 10 volumes of ice-cold HEPES-buffered sucrose solution (0.32 mM sucrose, 1 mM EGTA, and 4 mM HEPES at pH 7.4) containing a protease inhibitor cocktail (#P8340, Millipore Sigma). The homogenates were centrifuged at 2,000 *g* for 10 minutes at 4°C to remove nuclei and large fragments, and then the supernatant was centrifuged at 20,000 *g* for 30 minutes to obtain crude synaptosomes. The synaptosome pellets were subjected to lysis via hypoosmotic shock in 9 volumes of the ice-cold HEPES buffer containing a protease inhibitor cocktail for 30 minutes. Finally, the lysates were centrifuged at 25,000 *g* for 45 minutes at 4°C to obtain synaptosome fractions.

Coimmunoprecipitation and immunoblotting

Coimmunoprecipitation and immunoblotting were performed as we described previously.[13,27]. The protein concentration in tissue lysates and synaptosomes was quantified by using a protein assay kit (#5000001; Bio-Rad).

The protein samples were incubated with Protein G bead (#16-266; Millipore Sigma) prebound to a rabbit anti-GluN1 antibody (#G8913; dilution 1:100; Sigma-Aldrich) at 4°C overnight. Protein G beads prebound to a rabbit IgG (#A2909, Sigma-Aldrich) were used as controls. Protein G beads were rotated at 4°C overnight and washed 3 times with an immunoprecipitation buffer (#87787; Thermo Fisher Scientific). Protein samples on the beads were isolated using the loading sample buffer and were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis with 4% to 15% gel (#NP0335BOX; Invitrogen) and then transferred to a polyvinylidene difluoride membrane. The membrane was blocked with 5% nonfat dry milk and then incubated with a mouse anti–α2δ-1 antibody (#sc271697; 1:1,000; Santa Cruz Biotechnology), a mouse anti–PSD-95 antibody (#36233S; 1:1,000; Cell Signaling Technology), or a mouse anti-GluN1 antibody (#75-272; 1:1,000; NeuroMab) at 4°C overnight. On the second day, the membranes were incubated with a horseradish peroxidase–conjugated anti-mouse antibody (#7076; 1:5,000; Cell Signaling) for 1 hour at 22°C. The specificity of primary antibodies has been validated in our previous studies.[13,25,28] The protein bands were detected and quantified by using an Odyssey Fc Imager (LI-COR Biosciences).

Arterial blood pressure measurement with telemetry and power spectral analysis

Arterial blood pressure (ABP) in free-moving rats was measured by using a Millar telemetry system (Telemetry Research Ltd.), and the ABP in free-moving mice was measured by using a DSI telemetry system (Harvard Bioscience Inc.). Animals were anesthetized with 2% isoflurane, and a midline laparotomy was performed aseptically. A catheter connected to the telemetry transmitter was inserted into the descending aorta.[29-31] The transmitter was then fixed in the abdominal cavity, and the abdominal wound was closed with interrupted sutures. After surgery, the animals were given buprenorphine (0.05 mg/kg, subcutaneously) every 12 hours for 2 days and enrofloxacin (5 mg/kg, subcutaneously) daily for 3 days. ABP signals were continuously recorded. ABP signals in rats were sampled and analyzed by using LabChart 7 (AD Instruments), and ABP signals in mice were analyzed by using Ponemah software (Harvard Bioscience Inc.). Heart rate (HR) values were derived from ABP pulse signals.

The power spectral analysis of systolic ABP variability was conducted using Welch method (512 points, 50% overlap and Hamming window). For data from rats, the analysis was performed using LabChart 7, and power spectral density was integrated in the low frequency range of 0.20 to 0.75 Hz and high frequency range of 0.75 to 3.00 Hz).[32] For the mouse data, the analysis was performed using the variability analysis module of Ponemah software, and power spectral density was integrated in the low frequency range of 0.15–0.60 Hz and high frequency range of 2.5–5.0 Hz.[33]

Retrograde labeling of spinally projecting PVN neurons

Spinally projecting PVN neurons were retrogradely labeled as described previously.[34,35] In brief, animals were anesthetized using 2% isoflurane, and the spinal cord at T2 to T4 levels was exposed via laminectomy. Red FluoSpheres (50 nL, #F8770; Thermo Fisher Scientific) were bilaterally injected into the intermediolateral region

at T2 to T4 levels through a glass pipette (2–3 separate injections). After surgery, the animals were given buprenorphine (0.05 mg/kg, subcutaneously) every 12 hours for 2 days and enrofloxacin (5 mg/kg, subcutaneously) daily for 3 days. The animals were allowed to recover for 5–7 days to permit the FluoSpheres to be retrogradely transported to the PVN.

Whole-cell voltage-clamp recording of labeled PVN neurons in brain slices

Brain slices at the hypothalamus level were prepared from FluoSphere-injected animals as previously described.[35,36] In brief, animals were anesthetized using 2% isoflurane and decapitated. Their brains were quickly removed and placed in ice-cold artificial cerebrospinal fluid containing 124.0 mM NaCl, 3.0 mM KCl, 1.3 mM $MgSO_4$, 2.4 mM $CaCl_2$, 1.4 mM NaH_2PO_4, 10.0 mM glucose, and 26.0 mM $NaHCO_3$, which was continuously gassed with a mixture of 95% O_2 and 5% CO_2. Coronal hypothalamic slices (300 μm) were sectioned and incubated in artificial cerebrospinal fluid continuously gassed with a mixture of 95% O_2 and 5% CO_2 at 34°C for at least 1 hour before recording.

Brain sections were transferred from an incubation chamber to a recording chamber, which was continuously perfused with the artificial cerebrospinal fluid (saturated with 95% O_2 and 5% CO_2) at a speed of 3 mL/minute at 34°C. Labeled spinally projecting PVN neurons were identified under an upright microscope equipped with epifluorescence illumination and differential interference contrast optics. Recording electrodes were pulled from borosilicate capillaries using a micropipette puller. The electrodes' resistance was 3–6 MΩ when filled with internal solution containing 140.0 mM potassium gluconate, 2.0 mM $MgCl_2$, 0.1 mM $CaCl_2$, 10.0 mM HEPES, 1.1 mM EGTA, 0.3 mM Na_2-GTP, and mM 2.0 Na_2-ATP (adjusted to pH = 7.25 with 1 M solution of KOH, 270–290 mOsm). Whole-cell configuration was performed to record the miniature of excitatory postsynaptic currents (mEPSCs) and puff NMDAR currents in spinally projecting PVN neurons. The mEPSCs in labeled PVN neurons were recorded in the presence of 1 μM tetrodotoxin (TTX) and 20 μM bicuculline at a holding potential of –60 mV. To record postsynaptic NMDAR activity, we puffed NMDA (100 μM) directly to the labeled PVN neuron at a holding potential of –60 mV in the presence of 1 μM TTX.[5,36] The puff pipette (tip diameter around 10 μm) was placed about 150 μm away from the recorded neuron, and NMDA was directly ejected to the recorded neuron using a Toohey Pressure System (4 psi, 50 ms; Toohey Company). Because NMDAR is blocked by Mg^{2+} at a negative holding potential and co-activated by glycine, puff-elicited NMDAR currents were recorded in Mg^{2+}-free artificial cerebrospinal fluid in the presence of 10 μM glycine.[36,37] The electrical signals were processed using a MultiClamp 700B amplifier, filtered at 1–2 kHz, and digitized at 10 kHz using a Digidata 1440 digitizer (Molecular Devices, San Jose, CA). The liquid junction potential, defined as the voltage difference between the recording pipette solution and external solution, was corrected for all the recordings.

All agents were freshly dissolved in artificial cerebrospinal fluid before the recording. TTX (#HB1035), bicuculline (#HB0896), and 2-amino-5-phosphonopentanoic acid (AP5, #HB0252) were purchased from Hello Bio Inc. NMDA (#454575) was obtained from Sigma Millipore. α2δ-1 C terminus peptide

(VSGLNPSLWSIFGLQFILLWLVSGSRHYLW) and scrambled control peptide (FGLGWQPWSLSFYLVWSGLILSVLHLIRSN), both fused to the Tat domain (YGRKKRRQRRR) at the end of α2δ-1 C terminus, were synthesized by Bio Basic Inc. (Markham, ON, Canada) and validated using liquid chromatography and mass spectrometry.

Recording of renal sympathetic nerve activity and PVN microinjection

The ABP and renal sympathetic nerve activity (RSNA) in rats were recorded as we descried previously.[5,27] Rats were anesthetized with intraperitoneal injection of a mixture of α-chloralose (60–75 mg/kg) and urethane (800 mg/kg). The rats were subjected to mechanical ventilation with a rodent ventilator (CWE, Ardmore, PA). A cannula was inserted into the left femoral artery for ABP measurement, and HR was calculated from the peak interval of the blood pressure wave. RSNA was recorded from a branch of left renal nerve with the distal end cut. The RSNA signal was amplified (gain, 20,000–30,000) and band-pass filtered (100-3000 Hz) by using an alternating current amplifier (model P511; Grass Instrument, West Warwick, RI). ABP and RSNA were recorded by using a 1401-PLUS analog-to-digital and Spike2 system (Cambridge Electronic Design, Cambridge, UK). The background noise was determined after rats were euthanized with intraperitoneal injection of an overdose of sodium pentobarbital (200 mg/kg) at the end of each experiment.[5,27] The background noise was subtracted from integrated RSNA values.

PVN microinjections were performed as we reported previously.[12,29] Briefly, a small hole was drilled on the surface of the skull, and a glass pipette (tip diameter, 20–30 μm) was advanced into the PVN at the following stereotactic coordinates: 1.6 to 2.0 mm caudal to the bregma, 0.5 mm lateral to the midline, and 7.0 to 7.5 mm ventral to the dura. The drug solution was pressure-ejected via a calibrated microinjection system under an operating microscope. The location of the pipette tip in the PVN was examined and confirmed histologically after each experiment by including 5% rhodamine-labeled fluorescent microspheres (0.04 μm) in the drug solution.[13,29] At the end of the experiment, the rat brain was removed, fixed, and sectioned to examine injection sites according to the Paxinos and Watson brain atlas.[38] In total, 3 rats were excluded from analysis because the pipette tips were misplaced outside the PVN.

Study design and data analysis

All data are expressed as mean ± SEM. The sample sizes used in the study were estimated using an α value of 0.05 and statistical power of 85% and were based on similar studies we published previously.[5,27,35] The minimum number of samples required per group for the primary endpoint ABP, protein levels, and mEPSCs was 6, 6, and 10, respectively. The investigators performing the biochemical assays and electrophysiological recording were blinded to the mouse genotypes and experimental treatments. The animals were assigned to the control and treatment groups in a 1:1 ratio as they became available. Clinical studies indicate that CIH occurs similarly in male and female patients.[39-41] Because we did not find any sex differences in responses of ABP and HR to prolonged FK506 treatment between male and female rats, we mainly used male rats for this study. No test for outliers in the data was conducted.

For brain slice recordings, at least four animals were used in each recording protocol, and only one neuron was recorded from each slice. Cell capacitance was compensated, and the recording was discontinued if series resistance changed more than 15%. The amplitude and frequency of mEPSCs was calculated using MiniAnalysis software program (Synaptosoft). The cumulative probability of amplitudes and inter-event intervals of mEPSCs were compared using Kolmogorov–Smirnov test. The amplitude of puff NMDAR currents was determined using pCLAMP 10 (Molecular Devices). The repeated measures models were fitted to evaluate the treatment/genetic KO effects on mEPSCs and NMDAR currents. The AR(1) within-subject correlation structure was used, and statistical analyses were performed using SAS 9.4 (SAS, Cary, NC) and S-Plus 8.2 (TIBCO Software Inc., Palo Alto, CA).

The target protein band was initially normalized to that of loading controls or input proteins on the same gel and then normalized to the protein level in vehicle-treated animals. The protein level in the vehicle group was considered 1. RSNA signals were integrated over a 1-second period from the original recording traces, and the integrated background noise was subtracted using Spike2 software. The resulting integrated RSNA value was then normalized to its corresponding baseline value. To perform statistical analysis, the percentage change of integrated RSNA following each PVN microinjection was calculated. The baseline values of mean ABP, HR, and RSNA were determined as averages over a period of 3 min immediately before each treatment. Integrated RSNA values were normalized to the respective baseline, which was set as 100%. Response values after each intervention were averaged over 60 seconds when maximal responses occurred. ABP and HR were averaged during light and dark cycles, and mean ABP was calculated with a standard formula (diastolic ABP + 1/3 [systolic ABP – diastolic ABP]). Data normality was assessed using Shapiro-Wilk test before selecting a statistical test. Student *t* test was used to determine difference between 2 groups, and one-way or two-way ANOVA followed by Bonferroni's *post hoc* test was used to determine differences among 3 or more groups. Repeated measures ANOVA followed by Dunnett's *post hoc* test was performed to compare values at different time points within a group. Statistical analyses were performed using Prism (version 8, GraphPad Software Inc., San Diego, CA). $P < 0.05$ was considered statistically significant.

Online Figures

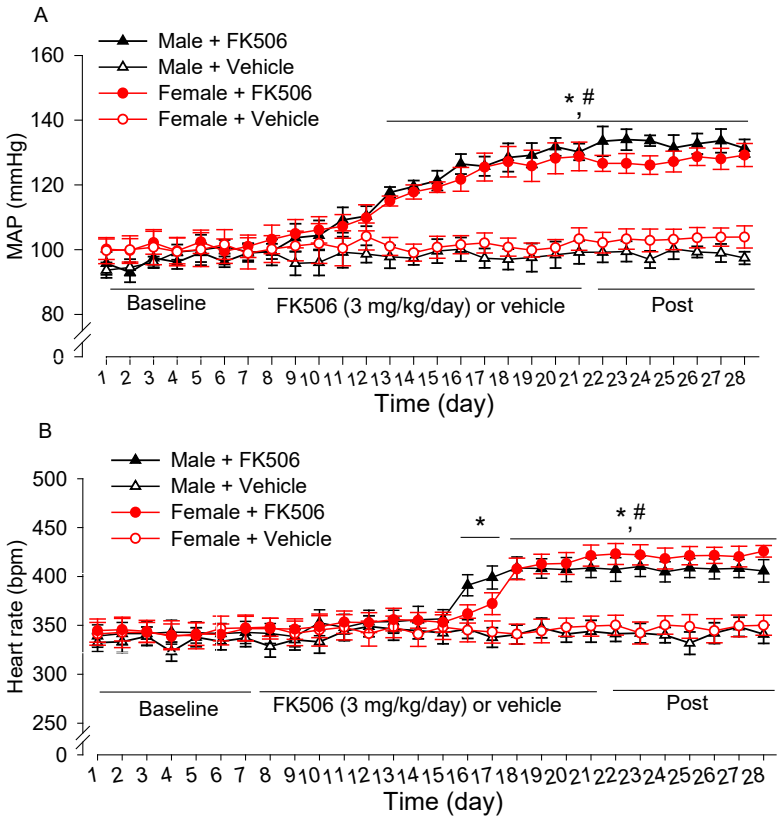

Figure S1. Prolonged treatment with FK506 similarly increases arterial blood pressure and heart rate in male and female rats.

A and **B,** Radiotelemetry recording data show the time course of changes in mean arterial blood pressure (MAP, **A**) and heart rate (HR, **B**) in male and female rats treated with vehicle or FK506 (n = 6 rats per group). Rats were treated with vehicle or FK506 (3 mg/kg per day, via intraperitoneal injection) for consecutive 14 days. Data in male rats were from our recent study[5] and were plotted here for comparisons with data from female rats. Data are expressed as mean ± SEM. **P* < 0.05, compared with respective baseline values within the group (repeated measures ANOVA with Dunnett's *post hoc* test). $^{\#}P < 0.05$, compared with respective values in vehicle-treated rats with the same sex at the same time point (two-way ANOVA with Bonferroni's *post hoc* test). Exact *P* values are shown in Tables S7 and S8.

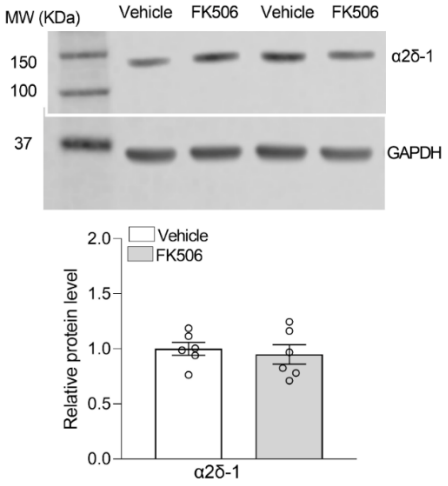

Figure S2. Systemic treatment with FK506 has no effect on the total α2δ-1 protein level in the PVN.
Representative blot images and summary data show total protein levels of α2δ-1 in the PVN lysate from FK506-treated and vehicle-treated rats (n = 6 male rats per group). MW, molecular weight. Data are expressed as mean ± SEM.

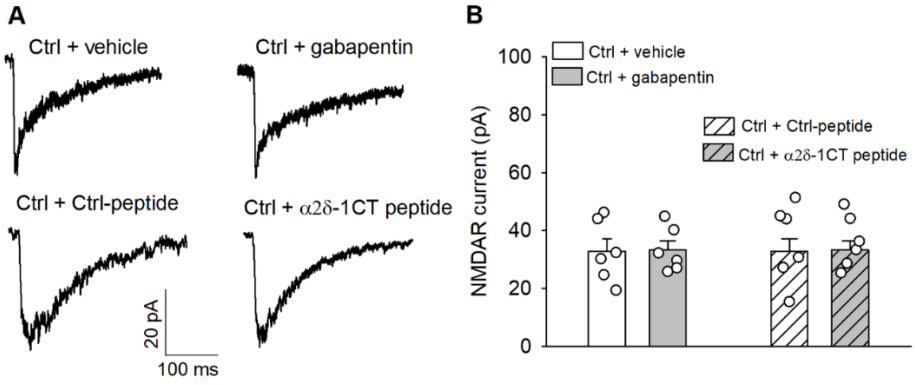

Figure S3. Inhibiting α2δ-1 or disrupting the α2δ-1-NMDAR interaction does not affect NMDAR activity of PVN presympathetic neurons in control rats.
A and **B**, Original recording traces (**A**) and summary data (**B**) show puff-elicited NMDAR currents in labeled PVN neurons in brain slices pretreated with 100 μM gabapentin, 1 μM control peptide (Ctrl-peptide), or 1 μM α2δ-1 C terminus peptide (α2δ-1CT peptide) in vehicle-treated control rats (n = 6 neurons from 3 male rats per group).

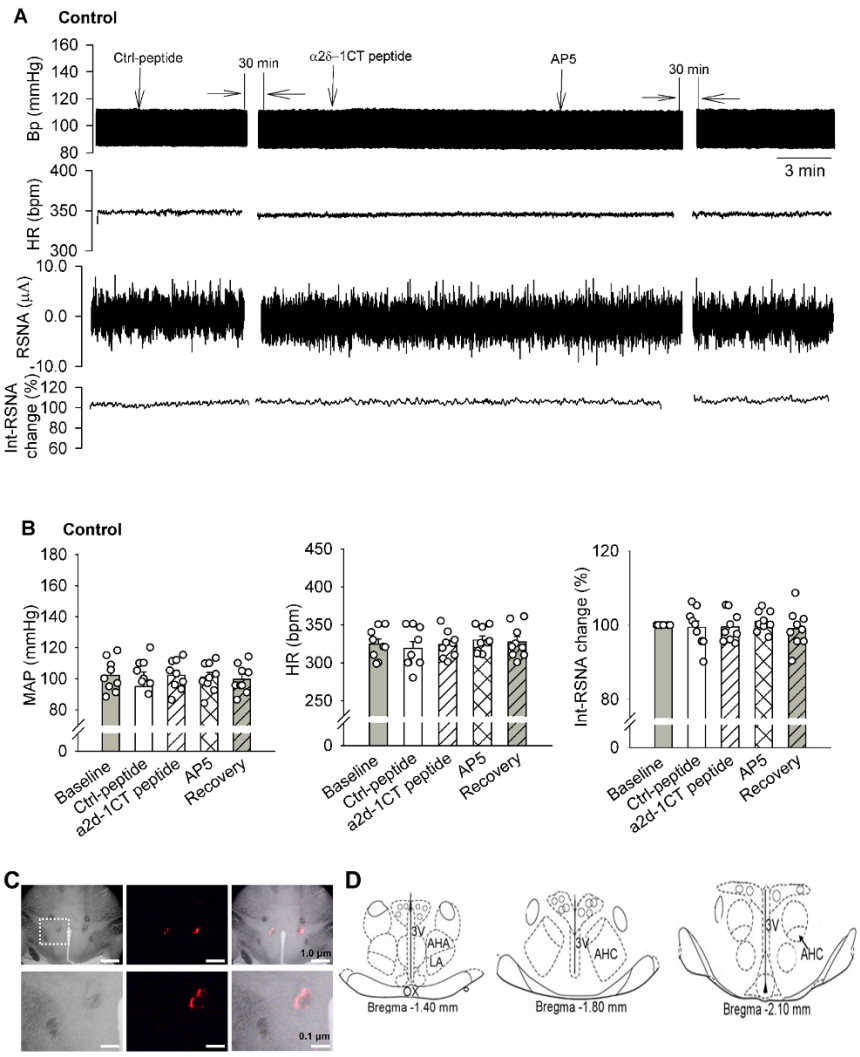

Figure S4. α2δ-1–bound NMDARs in the PVN do not control sympathetic vasomotor activity in normotensive control rats.

A and **B**, Original recording traces (**A**) and summary data (**B**) show that bilateral microinjection of control (Ctrl) peptide (50 pmol in 50 nL), α2δ-1CT peptide (50 pmol in 50 nL), or AP5 (1.0 nmol in 50 nL) into the PVN had no significant effect on mean arterial blood pressure (MAP), heart rate (HR), or integrated renal sympathetic activity (Int-RSNA) in vehicle-treated control rats (n = 9 male rats). **C** and **D**, Representative low- and high-magnification brightfield and fluorescence images (**C**) and schematic drawings (**D**) show microinjection sites in the PVN in control rats. ○ represents microinjection sites. 3V, third ventricle; AHA, anterior hypothalamic area; AHC, central division of the anterior hypothalamus; LA, latero-anterior hypothalamus; OX, optic chiasm. Data are expressed as mean ± SEM.

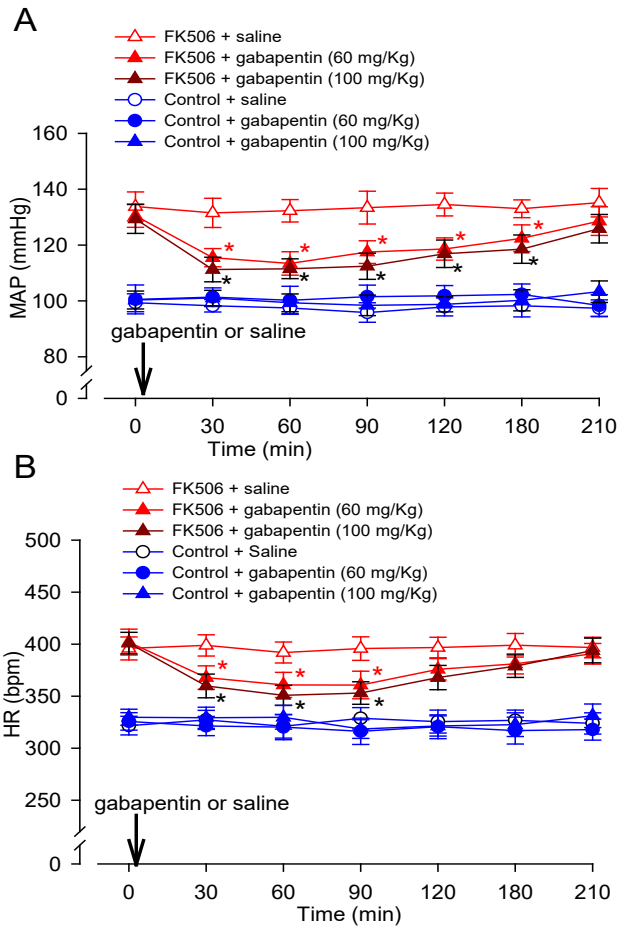

Figure S5. Gabapentin effectively reduces arterial blood pressure and heart rate elevated by FK506 treatment in female rats.

A and **B,** Radiotelemetry recording data show the effect of a single intraperitoneal injection of gabapentin (60 mg/kg or 100 mg/kg) or the same volume of saline on mean arterial blood pressure (MAP, **A**) and heart rate (HR, **B**) in female rats after treatment with FK506 or vehicle control for 14 days (n = 6 female rats per group). Data are expressed as mean ± SEM. **P* < 0.05, compared with respective baseline values within the group (repeated measures ANOVA with Dunnett's *post hoc* test). Exact *P* values are shown in Tables S9 and S10.

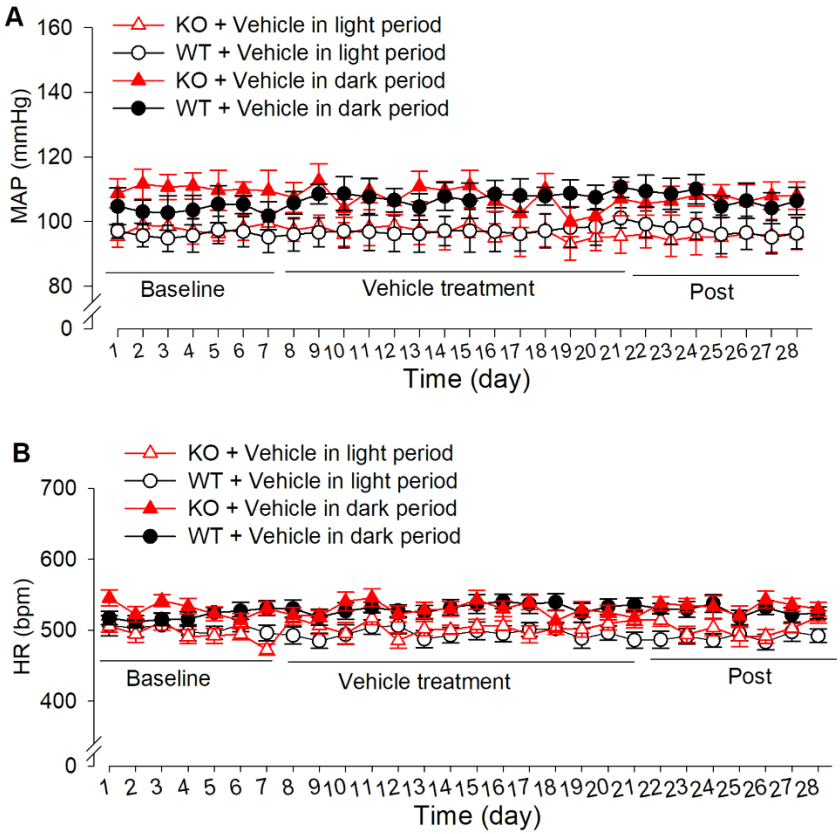

Figure S6. Treatment with vehicle has no effect on baseline arterial blood pressure or heart rate in wild-type and *Cacna2d1* knockout mice.

A and **B**, Radiotelemetry recording data show the time course of changes in mean arterial blood pressure (MAP, **A**) and heart rate (HR, **B**) in wild-type (WT) and *Cacna2d1* knockout (KO) mice treated with vehicle during light and dark cycles (n = 3 male and 3 female mice per group). Data are expressed as mean ± SEM.

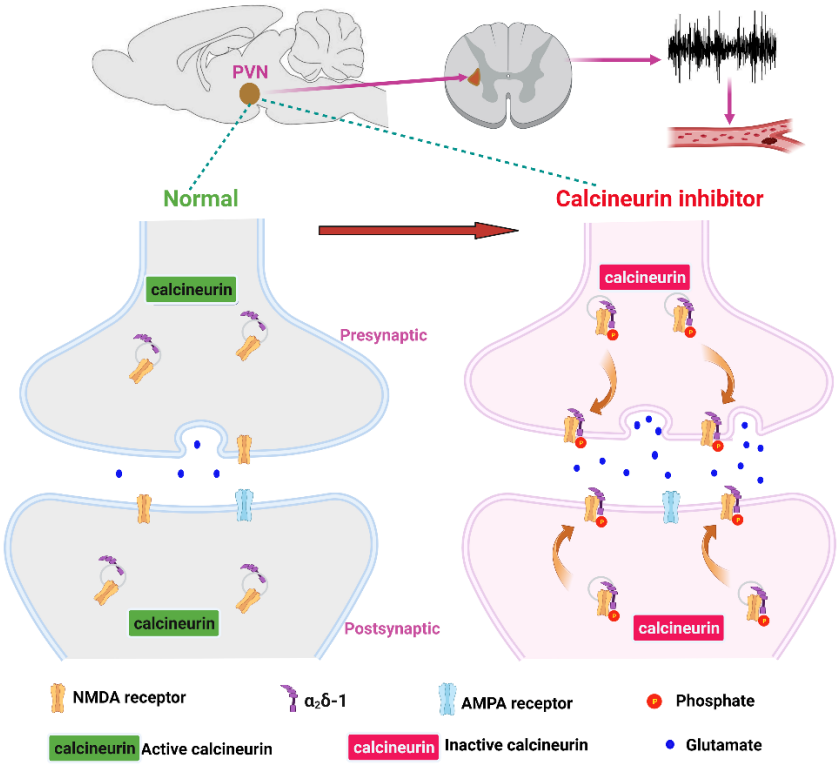

Figure S7. Schematic depicting the role of α2δ-1 in calcineurin inhibitor-induced synaptic NMDAR hyperactivity in the PVN and sympathetic vasomotor activity.

Under normal conditions, because basal activity of calcineurin keeps NMDARs dephosphorylated, NMDARs do not interact with α2δ-1 proteins and are little expressed at presynaptic and postsynaptic sites in the PVN. Systemically administered calcineurin inhibitors can access to the PVN to diminish calcineurin activity, causing increased phosphorylation of NMDARs and subsequent physical interactions with α2δ-1. As a result, the α2δ-1-NMDAR complexes traffic to the synaptic membranes to augment the activity of presynaptic and postsynaptic NMDARs. This increased excitatory glutamatergic input to PVN presympathetic neurons potentiates sympathetic outflow and sympathetic nerve discharges, consequently leading to hypertension.

Table S1. Adjusted *P* values for MAP comparisons in Figure 6A.

Time / *P* values	MAP comparisons with the baseline at 0 min in the FK506 + gabapentin (60 mg/kg) group	MAP comparisons with the baseline at 0 min in the FK506 + gabapentin (100 mg/kg) group
30 min	2.70E-03	7.00E-04
60 min	3.50E-03	1.70E-03
90 min	3.30E-03	6.30E-03
120 min	2.20E-03	1.20E-03
180 min	3.78E-01	1.06E-02
210 min	4.04E-01	1.81E-01

Table S2. Adjusted *P* values for HR comparisons in Figure 6B.

Time / *P* values	HR comparisons with the baseline at 0 min in the FK506 + gabapentin (60 mg/kg) group	HR comparisons with the baseline at 0 min in the FK506 + gabapentin (100 mg/kg) group
30 min	1.30E-02	1.79E-02
60 min	2.00E-04	2.20E-03
90 min	4.49E-02	1.39E-02
120 min	2.86E-01	1.76E-01
180 min	1.15E-01	3.34E-01
210 min	7.55E-01	4.24E-01

Table S3. Adjusted *P* values for MAP comparisons in Figure 6C.

Time / *P* values	MAP comparisons between FK506 + vehicle group and FK506 + gabapentin group in light cycle	MAP comparisons between FK506 + vehicle group and FK506 + gabapentin group in dark cycle	MAP vs. the baseline in the FK506 + vehicle group in light cycle	MAP vs. the baseline in the FK506 + vehicle group in dark cycle
Day 13	1.17E-01	6.93E-02	8.40E-03	6.00E-04
Day 14	3.14E-02	2.58E-02	2.19E-02	2.30E-03
Day 15	1.03E-02	4.27E-02	1.50E-03	1.20E-05
Day 16	5.50E-03	7.00E-03	4.40E-03	3.90E-03
Day 17	5.00E-04	2.10E-03	1.20E-03	1.70E-05
Day 18	1.00E-04	6.00E-04	1.80E-03	3.60E-05
Day 19	2.00E-05	6.00E-04	1.00E-05	8.30E-05
Day 20	5.10E-05	3.00E-04	6.20E-05	7.10E-05
Day 21	3.00E-05	1.00E-03	1.00E-04	1.00E-05
Day 22	2.00E-05	1.20E-03	3.00E-04	2.00E-04
Day 23	2.10E-05	8.00E-04	3.00E-04	6.00E-04

Day 24	3.00E-05	8.00E-04	6.00E-04	5.50E-05
Day 25	3.20E-05	5.20E-05	1.00E-04	1.00E-05
Day 26	3.70E-05	2.00E-04	3.80E-05	4.00E-04
Day 27	2.50E-05	3.00E-04	9.00E-04	1.00E-04
Day 28	2.30E-05	4.00E-04	2.00E-04	6.70E-05

Table S4. Adjusted *P* values for HR comparisons in Figure 6D.

Time / *P* values	HR comparisons between FK506 + vehicle group and FK506 + gabapentin group in light cycle	HR comparisons between FK506 + vehicle group and FK506 + gabapentin group in dark cycle	HR vs. the baseline in the FK506 + vehicle group in light cycle	HR vs. the baseline in the FK506 + vehicle group in dark cycle
Day 16	1.00E+00	1.00E+00	2.80E-03	3.08E-02
Day 17	8.19E-02	1.00E+00	1.00E-05	1.68E-02
Day 18	2.44E-02	1.75E-02	2.00E-04	7.00E-04
Day 19	9.80E-03	2.08E-02	1.40E-03	1.30E-03
Day 20	2.14E-02	3.13E-02	2.00E-04	1.00E-05
Day 21	2.40E-03	2.54E-02	1.00E-04	1.20E-03
Day 22	4.00E-04	5.60E-03	1.00E-05	1.30E-03
Day 23	1.30E-03	1.41E-02	2.00E-04	1.10E-05
Day 24	2.00E-04	3.00E-03	1.80E-05	2.60E-05
Day 25	1.31E-02	1.10E-03	1.00E-05	1.00E-04
Day 26	2.10E-03	2.80E-03	4.30E-05	1.00E-05
Day 27	2.30E-03	1.20E-03	2.90E-05	3.00E-05
Day 28	6.80E-03	1.00E-03	1.00E-05	2.00E-04

Table S5. Adjusted *P* values for MAP comparisons in Figure 7A.

Time / *P* values	MAP comparisons between WT + FK506 group and KO + FK506 group in light cycle	MAP comparisons between WT + FK506 group and KO + FK506 group in dark cycle	MAP vs. the baseline in the WT + FK506 group in light cycle	MAP vs. the baseline in the WT + FK506 group in dark cycle
Day 12	2.25E-01	8.81E-01	1.02E-02	2.90E-03
Day 13	1.59E-01	5.34E-02	1.12E-02	2.80E-03
Day 14	1.09E-02	2.50E-02	6.50E-03	1.40E-03
Day 15	2.20E-03	6.80E-03	3.00E-04	8.00E-04
Day 16	5.50E-03	1.11E-02	1.00E-05	1.00E-04
Day 17	1.89E-02	1.86E-02	3.00E-04	3.00E-04
Day 18	1.82E-02	7.00E-03	1.00E-05	2.00E-04
Day 19	1.26E-02	1.10E-03	1.00E-03	6.00E-04
Day 20	1.17E-02	3.84E-02	6.00E-04	1.20E-03
Day 21	1.98E-02	1.89E-02	6.90E-03	4.00E-04
Day 22	2.69E-02	1.35E-02	3.60E-03	2.10E-03
Day 23	3.19E-02	1.35E-02	1.60E-03	5.00E-04
Day 24	1.93E-02	1.30E-02	6.00E-04	1.00E-05
Day 25	5.90E-03	4.90E-03	1.00E-04	2.50E-03
Day 26	3.33E-02	3.59E-02	2.80E-03	1.30E-02
Day 27	1.33E-02	1.39E-02	1.30E-03	3.00E-04
Day 28	1.06E-02	4.65E-02	1.00E-03	9.00E-04

Table S6. Adjusted *P* values for HR comparisons in Figure 7B.

Time / *P* values	HR comparisons between WT + FK506 group and KO + FK506 group in light cycle	HR comparisons between WT + FK506 group and KO + FK506 group in dark cycle	HR vs. the baseline in the WT + FK506 group in light cycle	HR vs. the baseline in the WT + FK506 group in dark cycle
Day 19	9.00E-02	1.00E+00	1.08E-02	6.10E-03
Day 20	1.00E+00	1.00E+00	1.03E-02	6.00E-04
Day 21	3.91E-01	6.82E-01	1.84E-02	3.10E-03
Day 22	3.22E-02	1.47E-02	2.21E-02	2.70E-03
Day 23	2.58E-02	2.73E-02	2.25E-02	1.62E-02
Day 24	4.26E-02	1.85E-02	4.10E-03	2.40E-03
Day 25	2.81E-02	3.94E-02	9.00E-04	1.90E-03
Day 26	3.60E-02	3.16E-02	3.00E-03	5.00E-04
Day 27	1.29E-02	2.66E-02	1.96E-02	1.00E-03

Day 28	3.24E-02	3.22E-02	9.80E-03	4.00E-04

Table S7. Adjusted *P* values for MAP comparisons in Figure S1A.

Time / *P* values	MAP comparisons between Male + FK506 group and Male + vehicle group	MAP comparisons between Female + FK506 group and Female + vehicle group	MAP vs. the baseline in the Male + FK506 group	MAP vs. the baseline in the Female + FK506 group
Day 13	3.75E-02	4.63E-02	1.20E-03	2.89E-02
Day 14	4.00E-04	8.60E-03	5.50E-03	2.90E-03
Day 15	3.34E-02	4.48E-02	6.20E-03	4.20E-03
Day 16	7.90E-03	4.61E-02	3.70E-03	1.00E-05
Day 17	1.50E-03	4.41E-02	6.20E-03	1.00E-04
Day 18	6.80E-03	3.41E-02	2.70E-03	5.00E-04
Day 19	7.90E-03	4.98E-02	1.60E-03	1.60E-03
Day 20	2.50E-03	4.76E-02	1.30E-03	2.00E-04
Day 21	1.50E-03	3.39E-02	6.00E-04	1.00E-05
Day 22	5.00E-03	4.20E-03	6.50E-03	1.00E-05
Day 23	5.00E-04	9.50E-03	5.00E-04	2.00E-05
Day 24	1.00E-05	1.06E-02	4.00E-04	1.10E-05
Day 25	3.70E-03	9.50E-03	2.00E-03	2.30E-05
Day 26	5.00E-04	3.90E-03	3.30E-03	2.20E-05
Day 27	8.00E-04	6.70E-03	2.00E-03	1.00E-05
Day 28	3.20E-05	1.24E-02	8.00E-04	1.00E-05

Table S8. Adjusted *P* values for HR comparisons in Figure S1B.

Time / *P* values	HR comparisons between Male + FK506 group and Male + vehicle group	HR comparisons between Female + FK506 group and Female + vehicle group	HR vs. the baseline in the Male + FK506 group	HR vs. the baseline in the Female + FK506 group
Day 16	3.23E-01	1.00E+00	5.00E-04	4.60E-03
Day 17	7.85E-02	1.00E+00	1.10E-03	2.20E-03
Day 18	3.01E-02	3.59E-02	1.00E-05	1.01E-05
Day 19	4.41E-02	4.28E-02	1.00E-05	1.00E-05
Day 20	4.89E-02	3.62E-02	1.00E-04	5.00E-05
Day 21	4.31E-02	1.88E-02	6.00E-04	3.00E-05
Day 22	4.28E-02	1.67E-02	3.00E-03	7.00E-05
Day 23	2.23E-02	1.10E-02	2.00E-04	1.00E-05
Day 24	1.95E-02	2.14E-02	1.00E-05	2.00E-04
Day 25	1.38E-02	2.59E-02	1.00E-05	2.20E-05
Day 26	3.51E-02	1.76E-02	3.00E-04	1.00E-05
Day 27	3.76E-02	2.58E-02	3.00E-04	1.00E-05
Day 28	4.27E-02	5.70E-03	3.00E-04	1.00E-04

Table S9. Adjusted *P* values for MAP comparisons in Figure S5A.

Time / *P* values	MAP comparisons with the baseline at 0 min in the FK506 + gabapentin (60 mg/kg) group	MAP comparisons with the baseline at 0 min in the FK506 + gabapentin (100 mg/kg) group
30 min	4.60E-03	2.30E-03
60 min	4.05E-02	1.00E-03
90 min	2.28E-02	2.77E-02
120 min	1.94E-02	2.82E-02
180 min	2.57E-02	3.80E-02
210 min	9.67E-01	3.59E-01

Table S10. Adjusted *P* values for HR comparisons in Figure S5B.

Time / *P* values	HR comparisons with the baseline at 0 min in the FK506 + gabapentin (60 mg/kg) group	HR comparisons with the baseline at 0 min in the FK506 + gabapentin (100 mg/kg) group
30 min	2.32E-02	1.19E-02
60 min	2.49E-02	4.70E-02
90 min	4.37E-02	1.15E-02
120 min	1.58E-01	6.97E-02
180 min	4.72E-01	9.97E-02
210 min	1.67E-01	1.50E-01