Supporting Information

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**SI Materials and Methods**

**Animals.** We used a line of transgenic mice—dominant-negative SNARE (dnSNARE) mice—in which there is an astrocyte-specific expression of an inducible dominant-negative form of synaptobrevin II that inhibits the release of chemical transmitters, including ATP. The generation of the dnSNARE mice has been previously reported (1). Bigenic GFAP.tTA and tetO.dnSNARE transgenic animals (i.e., dnSNARE mice) and their WT littermates were used. GFAP.tTA mice and tetO.dnSNARE mice were backcrossed at least 10 generations onto the C57BL/6J background before intercrossing. Mice were bred and housed in a room with controlled photoperiods (12 h light and 12 h darkness) and temperature (21–23 °C) with food and water ad libitum. WT and dnSNARE mice were fed a diet containing doxycycline (40 mg/kg) from conception until weaning (3 wk of age). All procedures were in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Tufts University Institutional Animal Care and Use Committee.

**Pilocarpine-Induced Epilepsy, Video EEG Monitoring, and Seizure Classification.** Adult male dnSNARE and WT mice (2 mo old; weight, >20 g) were used in these experiments. WT and dnSNARE mice were maintained on doxycycline containing food until weaning (postnatal day 21), when they were switched to a regular diet to allow the transgene expression in the dnSNARE animals. Thirty minutes before the injections of pilocarpine, methylscopolamine (a muscarinic antagonist) was administered intraperitoneally (1 mg/kg; Sigma) to reduce adverse, peripheral effects. Then, mice were intraperitoneally injected with repeated low-dose pilocarpine hydrochloride (a muscarinic agonist, 100 mg/kg; Sigma) every 20 min until onset of status epilepticus (SE) characterized by continuous tonic-clonic seizures. Ninety minutes after the onset of SE, diazepam (4 mg/kg; Hospira) was administered s.c. to reduce seizure activity. Mice that did not develop SE after the fourth injection of pilocarpine were used as controls. After SE, all animals were intraperitoneally injected with lactated Ringer solution and fed with soaked rodent food. The seizure severity was scored according to the scale of Racine (2): score 0, no signs of motor seizure activity; score 1, immobility, rigid posture; score 2, repetitive movements, head bobbing; score 3, severe seizures with rearing without falling; score 4, severe seizures with rearing and falling, running and jumping, loss of righting ability; and score 5, SE characterized by continuous tonic-clonic seizures. The electrographic features of spontaneous recurrent seizures (SRSs) were analyzed with a video EEG monitoring system (Pinnacle Technology). Two days after SE, mice were deeply anesthetized with a mixture of ketamine hydrochloride and xylazine hydrochloride. They were perfused sequentially via the left ventricle with 20 mL chilled PBS solution (1×) and 100 mL 4% (wt/vol) paraformaldehyde in PBS solution (1×, pH 7.4). Brains were removed and postfixed in the same fixative for 24 h at 4 °C. After fixation, they were soaked 24 h in a solution of 30% (vol/vol) sucrose and dried with paper towels. Mice that experienced SRS during a trial were excluded from the analysis.

**Immunofluorescence Staining.** After completion of the behavioral study, mice were deeply anesthetized with a mixture of ketamine hydrochloride and xylazine hydrochloride. They were perfused sequentially via the left ventricle with 20 mL chilled PBS solution (1×) and 100 mL 4% (wt/vol) paraformaldehyde in PBS solution (1×, pH 7.4). Brains were removed and postfixed in the same fixative for 24 h at 4 °C. After fixation, they were soaked 24 h in a solution of 30% (vol/vol) sucrose. Brains were cut serially at 200-μm intervals on a freezing microtome (SM2000R; Leica) into coronal sections at a thickness of 40 μm and then stored in an antifreeze solution at −20 °C until immunostaining. Immunostaining was conducted on free-floating sections incubated with 5% (vol/vol) normal goat serum blocking solution for 1 h at room temperature and then with primary antibodies overnight at 4 °C on a shaking platform. The following antibodies were used: mouse anti-NeuN (1:1,000; Chemicon), rabbit anti-GFAP (1:1,000; Sigma), and chicken anti-vimentin (1:1,000; Abcam). Sections were rinsed three times with 1× PBS solution and then incubated with secondary antibodies for 2 h at room temperature. The following secondary antibodies were used: goat anti-mouse Alexa 546 (1:1,000; Invitrogen), goat anti-rabbit Alexa 633 (1:1,000; Invitrogen), and goat anti-chicken Alexa 488 (1:1,000; Invitrogen). For biocytin staining, slices were fixed overnight with 4% (wt/vol) paraformaldehyde in PBS solution (1×, pH 7.4) at 4 °C, then rinsed several times with PBS solution and incubated with 0.4% Triton X-100/PBS solution and streptavidin Alexa 546 (1:250;...
Invitrogen) for 4 h at room temperature. All sections were counterstained with DAPI, mounted on slides, and coverslipped with Vectashield antifade mounting medium. The fluorescent images were acquired with a confocal laser scanning microscope (Nikon A1) and analyzed by using ImageJ and MetaMorph. Areas CA1 and CA3 and the dentate gyrus (DG) of hippocampus were independently outlined, and an intensity threshold was set to identify pixels that were GFAP- and vimentin-positive. The proportion of total pixels containing GFAP and vimentin immunoreactivity was then determined. NeuN-positive cells were counted, and the density of neurons in CA1, CA3, and DG was plotted and analyzed.

**In Vivo Pharmacological Manipulation.** Osmotic minipumps (model 1002; Alzet; flow rate, 0.25 μL/h; 2-wk duration) were filled with the NMDA receptor (NMDAR) antagonist d-(-)-2-amino-5-phosphonopentanoate [d-AP5; 5 mM in artificial cerebrospinal fluid (ACSF); Tocris] or vehicle (ACSF for a group of control mice), secured with the flow moderator, and primed overnight in 0.9% saline solution at 37 °C before to be connected to the brain cannula (Alzet Brain Infusion Kit 3) by flexible catheter tubing. Two days after SE, mice were implanted with EEG as described earlier, and the brain cannula was stereotaxically placed into the right lateral ventricle (anteroposterior, -0.2 mm; mediolateral, -1.0 mm; dorsoventral, -2.2 mm). Animals were allowed to recover for 3 d. Osmotic minipumps were externalized, placed in a sealed conic tube of 1 ml filled with 0.9% saline solution, and maintained at 37 °C with a iBlock Mini Dry Bath (Midsci) placed out of the cage. The catheter tubing was filled with d-AP5 (5 mM in ACSF; Tocris) or vehicle (ACSF for a group of control mice). A small air bubble was inserted into the catheter tubing during its connection with the pump to monitor the flow rate estimated at 0.25 μL/h in our conditions. The flexible catheter tubing was carefully aligned and attached to the EEG cable. The presence of a swivel (Pinnacle Technology) prevented the torsion of tubing and cable.

**Slice Preparation.** Coronal cortical–hippocampal slices (310–400 μm) were obtained from dnSNARE and WT mice at postnatal days 21 to 30. At birth, WT and dnSNARE mice that were to be used for brain slice studies were fed a doxycycline-free diet to allow transgene expression in the dnSNARE mice. Mice were anesthetized with isoflurane, and, after cervical dislocation, the brain was rapidly removed and put in ice-cold artificial cerebrospinal fluid (ACSF). Areas CA1 and 3 and the dentate gyrus of hippocampus (DG) were independently outlined, and an intensity threshold was set to identify pixels that were GFAP- and vimentin-positive. The proportion of total pixels containing GFAP and vimentin immunoreactivity was then determined. NeuN-positive cells were counted, and the density of neurons in CA1, CA3, and DG was plotted and analyzed.

**Patch-Clamp Recordings.** CA1 pyramidal neurons from coronal cortical–hippocampal slices (310 μm) were visually identified with a 16× objective and 2x magnification in an upright Nikon Eclipse FN1 microscope by using IR-differential interference contrast. Whole-cell patch-clamp recordings were performed in current- and voltage-clamp modes by using a Multiclamp 700B amplifier (Axon Instruments). Data were filtered at 1 kHz and sampled at 5 kHz with Digidata 1322A interface and pClamp software (Axon Instruments).

For the AMPA/NMDA ratio measurements, a glass pipette (3–4 MΩ) was filled with the following internal solution (in mM): 120 Cs-MesO2, 20 CsCl, 0.2 EGTA, Na-phosphocreatine 10, 5 QX-314, 4 Mg-ATP, 0.2 Na3-GTP, and 10 Heps, pH 7.3 with CsOH. Biocytin (2 mg/mL; Sigma) was added to the internal solution in some experiments. Biocytin was allowed to passively diffuse into the cell for at least 10 min. Synaptic AMPA and NMDA currents were evoked by electrostimulation of the Schaffer collateral pathway at 0.033 Hz with a bipolar extracellular electrode (FHC) placed 100 to 200 μm apart from the recorded cell. Picrotoxin (100 μM; Sigma) was always added to the ACSF while measuring AMPA/NMDA ratio. After a stable synaptic current was obtained, AMPA currents were first recorded at ~70 mV, then the AMPA receptor antagonist 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide (10 μM; Tocris) was added to the ACSF, and finally the NMDA current was measured at +40 mV. The NMDAR antagonist d-AP5 (50 μM; Tocris) was added to the perfusion system at the end of the recording to confirm the nature of the evoked synaptic current. During AMPA/NMDA ratio measurements, a high-Mg2+ and -Ca2+ (4 mM of MgCl2 and CaCl2) ACSF was always used to increase membrane stability in the presence of picrotoxin. AMPA and NMDA currents were analyzed with Clampfit 9.2 (Axon Instruments). In some experiments, spontaneous excitatory post-synaptic currents (EPSCs; sEPSCs) were recorded at ~70 mV before recording evoked AMPA and NMDA currents and analyzed using Minianalysis 6.0 software (Synaptosoft).

To measure the AMPA and NMDA components of miniature EPSCs (mEPSCs), cells were recorded at holding potential ~70 mV in ACSF containing 0 Mg2+ and 1 μM TTX (Tocris). Internal solution contained the following (in mM): 145 K-glucanate, 2 MgCl2, 5 EGTA, 2 Na2-ATP, 0.2 Na3-GTP, and 10 Heps, pH 7.2 with KOH. Analysis of mEPSCs was performed by using Minianalysis 6.0 software (Synaptosoft). Events were detected automatically by using a threshold of 15 pA and verified visually. The average mEPSC waveform of each cell was aligned at their half-maximal rise time. The peak of an average mEPSC was taken as the AMPA peak. NMDA current amplitude was measured 20 ms after the AMPA peak. Before recording mixed AMPA and NMDA mEPSCs, cells were recorded in normal ACSF containing 1 mM Mg2+ and a series of constant current pulses (from ~300 to +300 pA for 300 ms with 20-pA increments, at 1-s intervals) was used to characterize their intrinsic membrane properties and excitability. Intrinsic membrane properties of CA1 pyramidal cells were not altered in dnSNARE mice (n = 15 cells from five animals) compared with WT mice (n = 11 cells from three animals). No difference was found in their resting membrane potential (WT, -74.00 ± 0.55 mV, vs. -72.46 ± 1.34 mV in dnSNARE; Student t test, P > 0.05) and membrane input resistance (WT, 146.94 ± 11.50 MΩ, vs. 154.16 ± 8.53 MΩ in dnSNARE; Student t test, P > 0.05). We also observed no difference in their excitability and the amplitude of evoked action potentials measured at rheobase (WT, 88.36 ± 2.28 mV, vs. 83.06 ± 2.80 mV in dnSNARE; Student t test, P > 0.05). Junction potentials were determined to allow correction of membrane potential values.

**Glutamate and o-Serine Biosensor Recordings.** Biosensor micro-electrodes (Sarissa Biomedical) coated with an enzymatic matrix
surrounding a platinum electrode (50-μm diameter) were polarized to +500 mV (vs. Ag/AgCl reference). Electrochemical detection occurred via amperometric measurement of hydrogen peroxide produced by the degradation reaction mediated by the enzymes included in the matrix. The current generated was proportional to the concentration of the analyte of interest. Glutamate release was measured by using a glutamate sensor, comprising glutamate oxidase and using a null sensor (possessing only the polymeric coat without enzymes), as background signals. Signals from the null sensor were subtracted from the signals generated by the glutamate sensor to give a measure of net glutamate concentrations. D-Serine release was measured by using a D-serine sensor, comprising D-amino acid oxidase and using a null sensor, as background. The principles and operation of glutamate and D-serine sensors have been previously described (4–7).

Pairs of sensors (glutamate/null or D-serine/null; 150–200 μm apart) were inserted sequentially into stratum radiatum of area CA1 of cortical–hippocampal slices (400 μm) continuously perfused (2 mL/min) with oxygenated ACSF at 31 °C. Experiments commenced 30 min after the slices recover and the sensor signal asymptotes to a baseline level. Experiments consisted of collecting 20 min of stable baseline, sensor withdrawal, and sensor calibration. Upon sensor withdrawal, the glutamate and D-serine signals decreased below the preceding baseline level recorded in the slices. Subtraction of the pre- and postwithdrawal signals was used as an indirect measure of glutamate or D-serine tone. Calibration was performed after each experiment by washing in 10 μM L-glutamate (Sigma) or D-serine (Sigma). The 0-burst stimulation (TBS) of the Schaffer collaterals was performed with a bipolar extracellular electrode (FHC) positioned in the stratum radiatum 150 to 200 μm from the glutamate sensor. The TBS protocol was conducted 20 min after a stable baseline signal and consisted of one single train of five 0-bursts (75–100 μA) at 100 Hz separated by 200 ms (8). Measurements were taken at the peak amplitude of the response. Potentiostat-based recordings were made by using the ME200+ Duo-Stat (Sycopel International) and sampled at 10 kHz with Digidata 1322A interface and pClamp software (Axon Instruments). Analysis was performed with Clampfit 9.2 (Axon Instruments).

**Statistical Analysis.** The difference between several groups was analyzed by one-way ANOVA. Two-way ANOVA was used when more than two comparisons (with time and genotype) were made. Repeated-measures ANOVAs were used when multiple measurements were made over time in the same groups. ANOVA was followed by a Student–Newman–Keuls post hoc multiple comparisons test and Bonferroni test for all behavior experiments. Comparisons between two groups were conducted with the paired or unpaired Student t test or the nonparametric Mann–Whitney test as appropriate. Proportions were compared as appropriate with the Fisher exact test or χ² test. Cumulative probability plots were calculated, and a Kolmogorov–Smirnov test was used to determine statistical differences. Kaplan–Meier analysis was performed, and the log-rank test was used to determine statistical differences.

Fig. S1. Behavioral and electrographic features of pilocarpine-induced SE in WT and dnSNARE mice. (A) The seizure-related behavior scored every 5 min according to the Racine scale (2) during four successive injections of low-dose pilocarpine (20 min apart) was similar between WT (n = 12) and dnSNARE mice (n = 10; ANOVA, P > 0.05). (B) The latency to onset of SE after the first injection of pilocarpine was comparable in WT mice (n = 12) and dnSNARE mice (n = 10; Student t test, P > 0.05). (C) The percentage of mice that developed and survived SE induced with multiple injections of low-dose pilocarpine was similar in WT (n = 71) and dnSNARE mice (n = 58; χ² test, P > 0.05). (D and E) EEG recordings before and during SE induced with a single high dose of pilocarpine were subjected to fast Fourier transformation, and the power in different frequency bands was analyzed in WT (D) and dnSNARE mice (E). Representative analyses Legend continued on following page
of band power in the β-band are shown. The zero time point corresponds to the time of scopolamine injection 30 min before the pilocarpine injection. Asterisks mark isolated acute seizures before mice enter continuous SE. The horizontal dashed line indicates the death of the animal. (Inset) Traces represent EEG recordings 15 min before pilocarpine injection (1), during the occurrence of an acute seizure (2), and during continuous SE (3) in WT (D) and dnSNARE mice (E). (Scale bars: 400 µA and 10 s.) (F) As a measure for the severity of acute seizures, the percentage change of power during acute seizures in different frequency bands was calculated relative to the power of the basal EEG recorded before pilocarpine injection. No differences were observed between WT (n = 9) and dnSNARE mice (n = 7; ANOVA, P > 0.05). (G) Similarly, the percentage change in EEG power during continuous SE was not different between WT and dnSNARE mice (n = 10 per genotype; ANOVA, P > 0.05). (H) Average latency to onset of acute seizure after the single injection of high-dose pilocarpine in WT (n = 9) and dnSNARE mice (n = 7; Mann–Whitney test, P > 0.05). (I) Average number of acute seizures occurring between the time of the single injection of high-dose pilocarpine and the beginning of SE in WT (n = 9) and dnSNARE mice (n = 7; Mann–Whitney test, P > 0.05). (J) Average duration of acute seizures occurring after the single injection of high-dose pilocarpine in WT (n = 9) and dnSNARE mice (n = 7; Student t test, P > 0.05). (K) Average latency of SE induced with the single injection of high-dose pilocarpine in WT and dnSNARE mice (n = 10 per genotype; Mann–Whitney test, P > 0.05).

Fig. S2. Normalized EEG spectral power in SE mice during an SRS (Left) and control no-SE mice (Right; 3-min recording). Power spectra were similar in WT and dnSNARE mice during the two different conditions (n = 5 per genotype; ANOVA, P > 0.05).
Fig. S3. Seizure and interictal spike development after pilocarpine-induced SE in WT and dnSNARE mice. (A) Average number of seizures per day in seven WT (Upper) and eight dnSNARE mice (Lower) after SE. Horizontal dashed lines represent the maximum number of seizures reached at day 101 and day 126 after SE in WT mice. Note the progressive increase in seizure frequency over time in WT mice (Upper) but not in dnSNARE mice (Lower; ANOVA: genotype, $P = 0.002$; time, $P = 0.089$; genotype $\times$ time interaction, $P = 0.248$; post hoc test, *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$). (B) Individual average number of seizures per day during the fifth month after SE plotted as a function of average number of seizures per day during the first month after SE. Linear regressions indicate a progressive increase in seizure frequency over time in WT mice (dashed line, $n = 5$) but not in dnSNARE mice (solid line, $n = 8$). (C) Bar graph illustrating the
average duration of seizures for each 1-mo block after SE in WT ($n = 4–6$) and dnSNARE mice ($n = 6–8$). Note that no difference was found in the seizure duration over time between genotypes (ANOVA, $P > 0.05$). (D) Average number of interictal spikes per day in seven WT (Upper) and eight dnSNARE mice (Lower) after SE. Horizontal dashed lines represent the maximum number of interictal spikes reached at day 144 after SE in WT mice. Note the reduced interictal spikes frequency in dnSNARE mice (Lower) compared with WT mice (Upper; ANOVA: genotype, $P = 0.02$; time, $P < 0.001$; genotype × time interaction, $P = 0.960$; post hoc test, *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$).

Fig. S4. Analysis of behavioral parameters of eight WT no-SE mice, seven WT SE mice, seven dnSNARE no-SE mice, and eight dnSNARE SE mice in the open field over a 30-min test period. (A) Average cumulative distance traveled over the 30-min test period (ANOVA, $P > 0.05$). (B) Line graph showing the distance traveled in 1-min bins over the 30-min test period.
Fig. S5. NeuN and GFAP staining in WT and dnSNARE mice after pilocarpine treatment. (A and B) Representative hippocampal sections from control no-SE and SE-experienced WT and dnSNARE mice stained with an antibody directed against the neuron-specific epitope NeuN 8 mo after pilocarpine treatment. A pronounced neuronal cell loss in areas CA1 (A, arrowheads) and CA3 (B, arrowheads) was detected in WT and dnSNARE mice that developed SE. Average neuronal density in CA1 (A) and CA3 (B) are presented under each corresponding section (WT no-SE, n = 3 animals; WT SE, n = 7 animals; dnSNARE no-SE, n = 5 animals; dnSNARE SE, n = 4 animals; ANOVA followed by post hoc test, *P < 0.05, **P < 0.01, and ***P < 0.001; ns, nonsignificant). (C and D) Same hippocampal sections as in A and B but stained with an antibody directed against the astrocyte-specific epitope GFAP. GFAP expression was increased in areas CA1 (C) and CA3 (D) in WT mice that entered SE but not in dnSNARE mice. Average GFAP area in CA1 (C) and CA3 (D) are presented under each corresponding section (ANOVA followed by post hoc test, *P < 0.05 and **P < 0.01; ns, nonsignificant). (Scale bars: 100 μm.)
Fig. S6. Vimentin staining in WT and dnSNARE mice after pilocarpine treatment. (A–C) Representative hippocampal sections from WT and dnSNARE mice that entered or did not enter SE stained with an antibody directed against the astrocyte-specific epitope vimentin 8 mo after pilocarpine treatment. Note the increased expression of vimentin in areas CA1 (A) and CA3 (B) and in the hilus of the DG (C) in WT mice that entered SE but not in dnSNARE mice. Average vimentin area in CA1 (A), CA3 (B), and hilus of the DG (C) are presented under each corresponding section (WT no-SE, n = 11 animals; WT SE, n = 11 animals; dnSNARE no-SE, n = 9 animals; dnSNARE SE, n = 7 animals; ANOVA followed by post hoc test, *P < 0.05, **P < 0.01, and ***P < 0.001; ns, nonsignificant). (Scale bars: 100 μm.)

mEPSCs in TTX, 0 Mg

WT

dTTP

1 s

dnSNARE

Fig. S7. Representative whole-cell recordings of CA1 pyramidal cells depicting mEPSCs in the presence of 1 μM TTX and 0 Mg²⁺ in WT (Upper) and dnSNARE mice (Lower).

Fig. S8. (A) D-AP5 (1 μM) reduced the amplitude of evoked synaptic NMDA currents by 32% in CA1 pyramidal neurons from WT slices (n = 4 slices from four animals). (B) Average of 15 consecutive synaptic NMDA currents before (control) and during application of 1 μM D-AP5.
Fig. S9. Glutamate and D-serine microelectrode biosensor measurements in hippocampal slices from WT and dnSNARE mice. (A) Response of the glutamate sensor (Glu, Upper) to different concentrations of glutamate (10, 5, 1, 0.5, and 0.2 μM) applied through the perfusion of the recording chamber. (Bottom) Trace (Glu – Null) obtained by subtracting the signal from the null sensor (Middle) from the glutamate trace (Glu, Upper). (B) Plot depicting the calibration of the glutamate sensor performed in A (linear regression: y = 0.1041x – 0.0009, r² = 0.9990). (C) Picture showing the typical placement of microelectrodes at the surface of hippocampal slices. Glutamate and null sensors were placed closed together into the stratum radiatum (s.r.). (Scale bar: 200 μm.) (D) Example of traces illustrating basal tone measurements of glutamate in WT (Left) and dnSNARE slices (Right). Subtracted signals (Glu – Null) are presented. Glutamate and D-serine measurements continued on following page.
null sensors were removed from the slices after a baseline recording of 20 min. Subtraction of the pre- and postremoval signals was used to determine the basal tone of glutamate that was calculated by using a calibration of 10 μM glutamate at the end of the experiment. Dashed lines indicate the baseline signal before sensors removal. (E) Average glutamate tone in WT (n = 11 slices from six animals) and dnSNARE slices (n = 10 slices from five animals; Mann–Whitney test, P > 0.05). (F) Representative traces showing the response of the glutamate sensor to TBS (arrow, one train of five bursts at 100 Hz separated by 200 ms) in WT (Upper) and dnSNARE slices (Lower). Subtracted signals (Glu – Null) are presented. The dashed line indicates the baseline signal before TBS. (G) Average concentration of glutamate detected in response to TBS in WT (n = 9 slices from five animals) and dnSNARE slices (n = 9 slices from six animals; Mann–Whitney test, P > 0.05). (H) Response of the α-serine sensor (α-ser; Upper) to different concentrations of α-serine (10, 5, 1, 0.5, and 0.2 μM). (I) Plot depicting the calibration of the α-serine sensor performed in H (linear regression: y = 0.1090x – 0.0082, r² = 0.9994). (J) Example of traces illustrating basal tone measurements of α-serine in the stratum radiatum of WT (Left) and dnSNARE hippocampal slices (Right). Subtracted signals (D-ser – Null) are presented. The α-serine signal was calibrated with 10 μM α-serine at the end of the experiment. (K) Average α-serine tone in WT (n = 9 slices from six animals) and dnSNARE slices (n = 10 slices from five animals; Mann–Whitney test, P > 0.05).