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ORIGINAL RESEARCH ARTICLE

Reduced excitatory neurotransmission in the hippocampus after inflammation and sevoflurane anaesthesia

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Abstract

Background: Inflammation and general anaesthesia likely contribute to perioperative neurocognitive disorders, possibly by causing a neuronal imbalance of excitation and inhibition. We showed previously that treatment with lipopolysaccharide (LPS) and sevoflurane causes a sustained increase in a tonic inhibitory conductance in the hippocampus; however, whether excitatory neurotransmission is also altered remains unknown. The goal of this study was to examine excitatory synaptic currents in the hippocampus after treatment with LPS and sevoflurane. Synaptic plasticity in the hippocampus, a cellular correlate of learning and memory, was also studied.

Methods: Mice were injected with vehicle or LPS (1 mg kg^{-1} i.p.), and after 24 h they were then exposed to vehicle or sevoflurane (2.3%; 2 h). Hippocampal slices were prepared 48 h later. Excitatory synaptic currents were recorded from pyramidal neurones. Long-term potentiation (LTP) and long-term depression (LTD) were studied in the Schaffer collateral–cornu ammonis 1 pathway.

Results: The amplitude of miniature excitatory postsynaptic currents (EPSCs) was reduced after LPS+sevoflurane ($P<0.001$), whereas that of spontaneous EPSCs was unaltered, as evidenced by cumulative distribution plots. The frequency, area, and kinetics of both miniature and spontaneous EPSCs were unchanged, as were LTP and LTD.

Conclusions: The reduced amplitude of miniature EPSCs, coupled with the previously reported increase in tonic inhibition, indicates that the combination of LPS and sevoflurane markedly disrupts the balance of excitation and inhibition. Restoring this balance by pharmacologically enhancing excitatory neurotransmission and inhibiting the tonic current may represent an effective therapeutic option for perioperative neurocognitive disorders.

Keywords: excitatory neurotransmission; lipopolysaccharide; mEPSC; perioperative neurocognitive disorders; sevoflurane; synaptic plasticity

Perioperative neurocognitive disorders (PNDs), including postoperative delirium, occur frequently in adult patients.[1,2] Delirium typically occurs within hours to days after surgery, whereas deficits in attention, memory, and executive function can develop and persist for weeks to months. These disorders are associated with poor long-term outcomes, including early retirement, loss of independence, and increased mortality.[3] Unfortunately, no effective pharmacological strategies are currently available to prevent or treat PNDs. Therefore, identifying the molecular basis for PNDs has emerged as an active and high-priority area of anaesthesia research.[3,4]

PNDs are likely multifactorial in origin, with inflammation and general anaesthetic drugs identified as two potential contributing factors.[5,6] To study the multifactorial aetiology of PNDs, rodent models combining these two factors have been recently developed.[7,8] In these aseptic, non-surgical models, inflammation is induced by injection of the endotoxin lipopolysaccharide (LPS); the rodents are then treated with either

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an injectable or an inhaled general anaesthetic drug. We and others have observed cognitive deficits in adult and immature animals treated with the combination of LPS and an anaesthetic drug that were not observed after treatment with either factor alone.[7,8] Identifying the mechanisms that contribute to deficits caused by the combination of inflammation and anaesthetic drugs is important, as it could lead to strategies for mitigating PNDs.

One potential mechanism is an imbalance between excitatory and inhibitory neurotransmission mediated by the major neurotransmitters glutamate and γ-aminobutyric acid (GABA), respectively. We observed previously that treatment with LPS and sevoflurane caused a sustained increase in a tonic inhibitory conductance in pyramidal neurones of the cornu ammonis 1 (CA1) region of the hippocampus.[9] This increase in tonic inhibition is likely because of increased cell-surface expression of extrasynaptic GABA Type A ($GABA_A$) receptors.[10,11] Importantly, increased tonic inhibition may have functional consequences, including reduced neuronal excitability, altered synaptic plasticity, and impaired cognition.[11–13]

The increase in tonic inhibition indicates that the ratio of excitation to inhibition (the so-called E/I balance) is dysregulated after exposure to LPS and sevoflurane. However, it remains unknown whether excitatory neurotransmission is also altered in the injury model. Several lines of evidence support concurrent dysregulation of excitatory neurotransmission. Notably, crosstalk between excitatory and inhibitory neurotransmitter systems is well established in the CNS. As an example, the activity of *N*-methyl-D-aspartic acid (NMDA) receptors alters cell-surface expression of $GABA_A$ receptors, with increased activity of GluN2A subunit-containing NMDA receptors increasing tonic inhibition in neurones.[14,15] Conversely, increasing $GABA_A$ receptor activity by treatment with the positive allosteric modulator midazolam reduces α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor expression in a mouse model of chronic constriction nerve injury.[16] Other researchers have reported that each of LPS and sevoflurane alone alters the expression of AMPA and NMDA receptor subunits in the hippocampus. For example, LPS decreased GluA1 and GluA2 expression when measured 24 h after treatment, whereas sevoflurane increased GluN1 and GluN2B expression.[17–19] Collectively, these results support the hypothesis that the combination of LPS and sevoflurane will alter excitatory neurotransmission, although the exact nature of the disruption remains unclear.

The goal of this study was to examine excitatory synaptic transmission in the hippocampus after treatment with LPS and sevoflurane. In addition, we studied synaptic plasticity in the Schaffer collateral–CA1 pathway, which is thought be a molecular substrate for learning and memory, as memory can be impaired after anaesthesia and surgery.

Methods

Experimental mice

C57BL/6 mice (age 6–10 weeks) were purchased from Charles River (Saint-Constant, QC, Canada). Mice were group-housed in a temperature-controlled facility on a 14 h/10 h light/dark cycle (lights on at 6:00 a.m.), with food and water provided *ad libitum*. All studies were performed in male mice to eliminate the confounding effect of previously reported sex differences in both excitatory and inhibitory neurotransmission between male and female mice.[13,20]

A block randomisation strategy was used to randomly assign mice in each cage to different treatment groups. Mice were treated as per our previous studies.[7,9] Briefly, inflammation was induced by intraperitoneal injection of LPS that was derived from *Escherichia coli* (1 mg kg^{-1}; O111:B4; Sigma-Aldrich, Oakville, ON, Canada). At this moderate dose, LPS induces neuroinflammation that persists for at least 24 h, as indicated by elevated levels of pro-inflammatory cytokines.[7] One day after LPS treatment, mice were anaesthetised in a warmed, airtight acrylic chamber with sevoflurane (2.3%; 2 h) delivered in O_2 30% (1.3 L min^{-1}). This anaesthetic treatment regimen was not associated with substantial changes in blood gas values, including pH, PCO_2, and PO_2 in mixed venous and arterial blood samples that were collected immediately after sevoflurane anaesthesia.[9] The vehicle controls for LPS and sevoflurane were saline (0.9% w/v of NaCl, i.p.) and O_2 30% (20 min), respectively. Weight loss, which is a hallmark of LPS-induced inflammation, was studied in an independent cohort of mice by measuring body weight sequentially for 8 days.

Preparation of brain slices

Whole-cell voltage clamp recordings were performed 48 h after sevoflurane anaesthesia. To study excitatory neurotransmission, brain slices were prepared using a 'protective' slice preparation technique.[21] Briefly, brains were extracted and placed in an ice-cold carbogenated (O_2 95%/CO_2 5%) slicing solution that was composed of (in mM) 92 *N*-methyl-D-glucamine (NMDG), 25 D-glucose, 30 $NaHCO_3$, 1.25 NaH_2PO_4, 2.5 KCl, 0.5 $CaCl_2$, 10 $MgCl_2$, 12 *N*-acetyl cysteine, 20 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES), 2 thiourea, 5 Na ascorbate, and 3 Na pyruvate (pH 7.3–7.4; 300–310 mOsm). Sagittal brain slices (300 μm thick) containing the hippocampus were sectioned using a Leica VT1200 S vibratome (Deerfield, IL, USA). Slices were allowed to recover in a warmed slicing solution (32°C) for 11–12 min and were then transferred to an NMDG-free holding solution, which was similar in composition to the slicing solution except that it contained (in mM) 0 NMDG, 92 NaCl, 2.5 $CaCl_2$, and 1.3 $MgCl_2$. Slices were maintained at room temperature for at least 1 h before starting the electrophysiological recordings.

To study synaptic plasticity in the hippocampus, brain slices were prepared for extracellular field recordings 48 h after mice were treated with sevoflurane. For studies of long-term depression (LTD), 350-μm-thick coronal slices were prepared using the protective slice preparation methods, as described previously. For studies of long-term potentiation (LTP), slices were prepared and stored in a carbogenated artificial cerebrospinal fluid (aCSF) containing (in mM) 125 NaCl, 10 D-glucose, 25 $NaHCO_3$, 1.25 NaH_2PO_4, 2.5 KCl, 2.5 $CaCl_2$, and 1.3 $MgCl_2$ (pH 7.3–7.4; 300–310 mOsm). This alternative method was used for the LTP studies because preliminary experiments showed that slices that were prepared using protective techniques exhibited weak or no LTP under control conditions, as reported by others.[22]

Whole-cell voltage clamp recordings

Slices were placed in a submersion recording chamber where they were perfused with aCSF at a flow rate of 2–4 ml min^{-1} at room temperature. Recordings were performed from pyramidal neurones in the CA1 under visual control (Olympus BX52WI; Olympus Canada, Toronto, ON, Canada) using a

MultiClamp 700A amplifier (Molecular Devices, Sunnyvale, CA, USA) that was controlled with pCLAMP 9.2 software *via* a Digidata 1322A interface (Molecular Devices). Borosilicate glass pipettes (2.5–5 MΩ; World Precision Instruments, Sarasota, FL, USA) were filled with intracellular solution containing (in mM): 140 KCl, 10 HEPES, 5 ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid, 3 Na_2ATP, 1 $MgCl_2$, and 0.5 $CaCl_2$ (pH adjusted to 7.3; 290–300 mOsm). Cells were recorded at a holding potential of −70 mV, with currents sampled at 10 kHz. Access resistance was monitored before and after recordings, and cells that exhibited a baseline shift greater than 20% were not included in the analyses.

After achieving the whole-cell configuration, spontaneous excitatory postsynaptic currents (sEPSCs) were first recorded from each cell in the presence of the $GABA_A$ receptor inhibitor picrotoxin (100 mM). Subsequently, tetrodotoxin (TTX; 0.5 mM) was perfused to block the generation of action potentials, and miniature EPSCs (mEPSCs) were recorded from the cell.

Analyses of EPSCs

Recordings collected in the presence and absence of TTX were used to analyse mEPSCs and sEPSCs, respectively, using MiniAnalysis software (Synaptosoft Inc., Fort Lee, NJ, USA). Traces were filtered using a 2000 Hz Bessel filter, and synaptic events were automatically detected as negative deflections greater than 5 pA in amplitude. Subsequently, all records underwent a visual inspection to include missing events and remove incorrectly detected events. The inter-event interval and frequency were computed using these captured events, utilising standard formulae in Excel (Microsoft Corporation, Redmond, WA, USA). The recordings included instances of compound synaptic events and events where the decay was distorted because of spontaneous fluctuations in the baseline. Because these events would skew the analyses of amplitude and decay kinetics, we manually selected a subset of mEPSCs and sEPSCs that exhibited a single well-defined peak and then decayed to baseline. These subsets of synaptic currents were used to determine the amplitude, rise time, area, and decay of events using the curve-fitting capabilities of MiniAnalysis. To calculate decay time constants, each trace was fitted with a bi-exponential decay curve, and the weighted decay time was calculated using the following equation: $(\tau_1A_1+\tau_2A_2)/(A_1+A_2)$, where τ_1 and τ_2 are the fast and slow decay components, respectively, and A_1 and A_2 are the corresponding weighting factors.

After deriving event parameter, the average amplitude, frequency, rise time, decay time, and area of mEPSCs and sEPSCs were calculated for each cell. We also performed an additional analysis by plotting the cumulative distributions of the event amplitudes and inter-event intervals from each cell and then calculating the mean cumulative distribution for each treatment group. Both the average parameters and cumulative distributions were then compared between treatment conditions.

Recordings and analyses of synaptic plasticity

To examine synaptic plasticity in the Schaffer collateral–CA1 pathway, field postsynaptic potentials (fPSPs) were recorded by placing an aCSF-filled pipette in the stratum radiatum of the CA1 and stimulating the Schaffer collaterals using a concentric electrode (MicroProbes for Life Science, Gaithersburg, MD, USA). The baseline properties of synaptic transmission were first assessed. Input–output (I–O) relationships were studied by incrementally increasing the stimulation intensity and plotting the amplitude of the fibre volley (input) against the initial slope of the fPSPs (output), until a maximal fPSP response was reached. For each slice, a linear regression line was fitted through these points, and the slope was calculated. Paired-pulse facilitation (PPF) was studied by applying two consecutive stimuli at an intensity that produced a half-maximal response. The inter-stimulus interval was varied in 50 ms increments from 50 to 300 ms, and PPF was quantified by dividing the slope of the second fPSP (P2) by the slope of the first fPSP (P1).

To study synaptic plasticity, half-maximal fPSPs were recorded at a frequency of 0.05 Hz until a stable baseline was reached for at least 10 min. LTD was induced by applying 600 stimuli at 10 Hz. LTP was induced using a theta burst stimulation (TBS) protocol that consisted of 10 stimulus trains at 5 Hz, with each train composed of four pulses delivered at 100 Hz. fPSPs were recorded at 0.05 Hz for 60 min after either 10 Hz stimulation or TBS, and the average fPSP slope in the last 5 min of recording was used to quantify LTD and LTP, respectively. The average fPSP slope in the first minute after stimulation was also quantified as a measure of post-tetanic potentiation. In addition, the fPSP slopes from the initial 20 min after TBS were fitted with a mono-exponential decay curve using GraphPad Prism 9 (GraphPad Software Inc., San Diego, CA, USA). The decay constant (τ) for the curve was used as a measure of short-term potentiation.

Statistical analyses

Data are expressed as means [standard deviation]. All analyses were performed using GraphPad Prism 9 (GraphPad Software Inc., San Diego, CA, USA). No data were excluded from analyses. Normality of data and residuals was assessed using the D'Agostino–Pearson test in all cases, except for the data presented in Fig 4, where the Shapiro–Wilk test was used because of the small sample sizes. Two-tailed hypothesis tests were used with $P<0.05$ being considered statistically significant. Multiplicity-adjusted *P*-values are reported, where appropriate. Sample sizes were selected based on previous experience with the assays.[7,9,10,12]

Body weights were compared using the two-way repeated measures analysis of variance (ANOVA) followed by Bonferroni's multiple comparisons test. An unpaired Student's *t*-test or Mann–Whitney *U*-test was used to compare the average characteristics of mEPSCs and sEPSCs. To compare cumulative distribution plots, all recorded events were pooled for each treatment group, and the distribution of amplitudes and inter-event intervals were compared using the Kolmogorov–Smirnov test. An unpaired Student's *t*-test was used to compare I–O slopes, and two-way ANOVA followed by Bonferroni's multiple comparisons test was used to compare PPF. The unpaired Student's *t*-test was used to analyse data for LTD, LTP, and the first minute after stimulation. The decay time courses for short-term potentiation were compared by determining whether there was overlap between the 95% confidence intervals (CIs).

Results

Weight loss after LPS+sevoflurane

Male adult mice were treated with LPS then the following day were anaesthetised with sevoflurane. The body weight of the

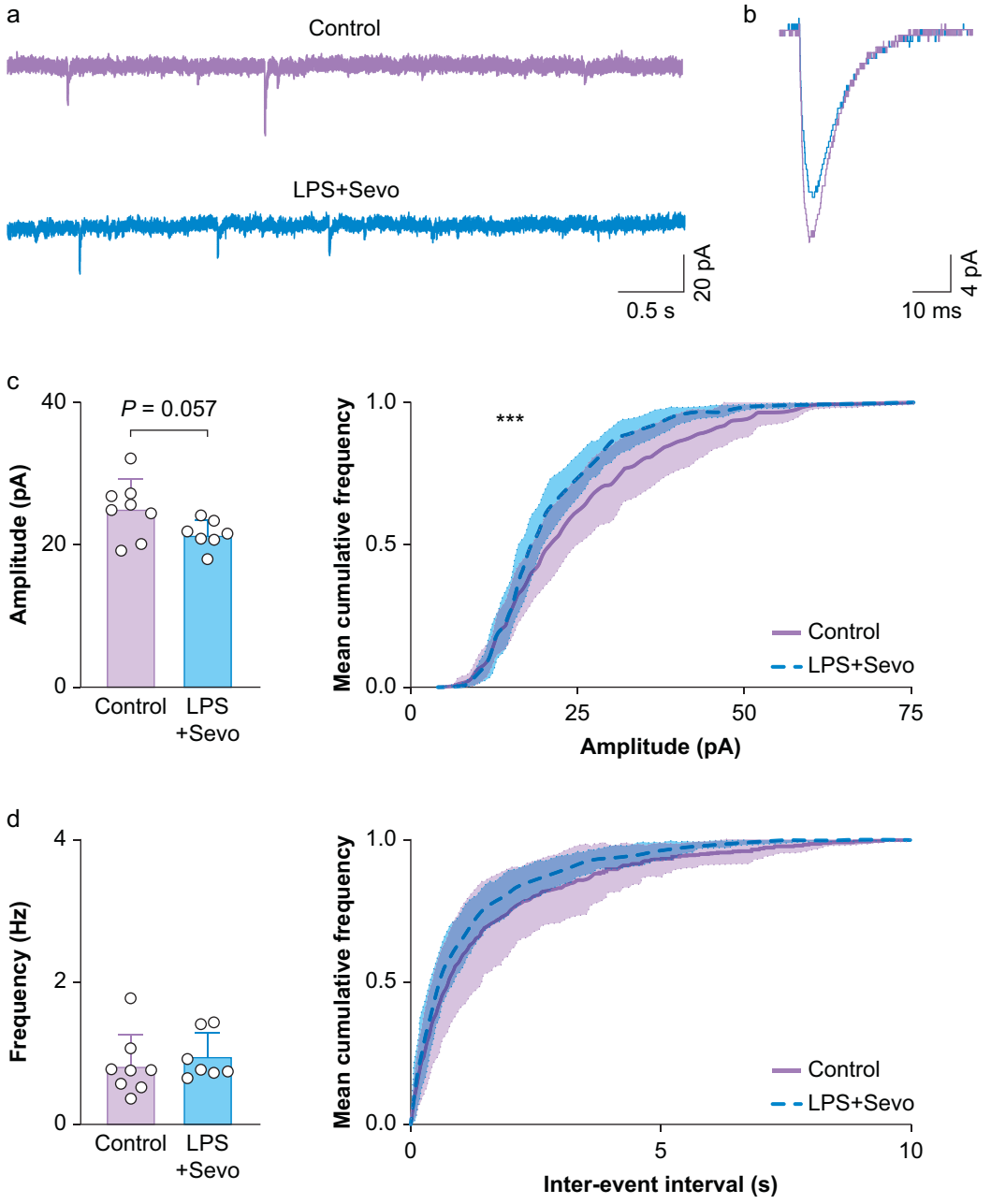

Fig 1. Amplitude, but not frequency, of mEPSCs was reduced after LPS+sevoflurane. (a) Representative traces for mEPSC recordings. (b) Averaged mEPSC traces representing 52 (control) and 69 (LPS+sevoflurane) individual events. (c) Left: summarised data show a trend towards reduced amplitude of mEPSCs after LPS+sevoflurane. n=8; 7 cells (control *vs* LPS+sevoflurane). Unpaired Student's t-test. Right: there was a leftward shift in the cumulative distribution of event amplitudes after LPS+sevoflurane, indicating a reduction in mEPSC amplitude. n=525; 546 events (control *vs* LPS+sevoflurane). ***P<0.001, Kolmogorov–Smirnov test. (d) Left: summarised data show that the frequency of mEPSCs was not changed after LPS+sevoflurane. P=0.482, Mann–Whitney U-test. Right: the cumulative distribution of inter-event intervals was also not different between treatment groups. n=797; 834 events (control *vs* LPS+sevoflurane); P=0.513. Data are presented as means [standard deviation]. LPS, lipopolysaccharide; mEPSCs, miniature excitatory postsynaptic currents; Sevo, sevoflurane.

mice decreased significantly, reaching a nadir 2 days after LPS (86.3 [2.7]% of baseline; P<0.001, n=9) then gradually recovered close to baseline (Supplementary Fig 1). In contrast, the body weight of control mice increased by 2.1 [1.2]% over a similar 8 day period (P=0.0496; Day 8 compared with Day 1; n=8). The weight loss after LPS+sevoflurane suggests the induction of an acute inflammatory state and is consistent with previous reports.[7,23]

Table 1 mEPSC variables after LPS+sevoflurane. $P>0.05$ for all variables, with $P=0.057$ for amplitude and $P=0.070$ for area. Amplitude, rise time, decay time, and area were compared using unpaired Student's *t*-tests, and the Mann–Whitney *U*-test was used to compare frequency. Data are presented as means [standard deviation]. LPS, lipopolysaccharide; mEPSCs, miniature excitatory postsynaptic currents; Sevo, sevoflurane.

Treatment	Amplitude (pA)	Frequency (Hz)	Rise time (ms)	Decay time (ms)	Area (pA ms^{-1})
Control, $n=8$	25.1 [4.1]	0.82 [0.4]	2.5 [0.4]	8.0 [0.9]	371.8 [54.2]
LPS+Sevo, $n=7$	21.6 [2.0]	0.95 [0.3]	2.6 [0.1]	8.4 [0.5]	326.1 [29.7]

LPS+sevoflurane reduced excitatory synaptic transmission

Two days after sevoflurane treatment (3 days after LPS), *ex vivo* hippocampal slices were prepared, and excitatory neurotransmission was studied in CA1 pyramidal neurones using whole-cell voltage clamp recordings. First, mEPSCs, which are transient synaptic events triggered by the spontaneous release of glutamate from presynaptic terminals, were studied (Fig 1a and b). The amplitude of mEPSCs recorded from LPS+sevoflurane neurones was 21.6 [2.0] pA ($n=7$) compared with 25.1 [4.1] pA ($n=8$) for controls (95% CI of the difference: −0.14 to 7.14; $P=0.057$; Fig 1c, left). To further analyse the amplitude of mEPSCs, all recorded events were pooled, and cumulative distribution plots were constructed. The distribution of mEPSC amplitudes recorded in neurones from LPS+sevoflurane mice was shifted to the left compared with controls ($P<0.001$; Fig 1c, right). Collectively, these data indicate that the amplitude of mEPSCs in pyramidal neurones was reduced in the LPS+sevoflurane treatment group.

The frequency, rise time, decay time, and area of mEPSCs were also compared. The frequency of events, measured as the average frequency of mEPSCs per cell, was similar between treatment groups (control: 0.8 [0.4] Hz; LPS+sevoflurane: 0.9 [0.3] Hz; $P=0.482$; Fig 1d). Consistent with these findings, the inter-event intervals were unchanged ($P=0.513$). Similarly, the rise time, decay time, and area were not significantly different between treatment groups, although a trend towards reduced area was observed after LPS+sevoflurane ($P=0.070$; Table 1).

Next, sEPSCs, which are excitatory postsynaptic events in response to both spontaneous and action potential-dependent release of glutamate, were examined (Fig 2a and b). Interestingly, both the average amplitude of sEPSCs (control: 21.2 [3.6] pA, $n=8$; LPS+sevoflurane: 20.6 [4.6] pA, $n=7$; $P=0.800$) and their cumulative distribution ($P=0.078$) were similar between the LPS+sevoflurane and control groups (Fig 2c). Also, the frequencies of sEPSCs in the two groups were similar (control: 1.6 [0.8] Hz; LPS+sevoflurane: 1.7 [0.7] Hz; $P=0.740$), as were the distributions of inter-event intervals ($P=0.075$; Fig 2d). The additional parameters of sEPSCs were not significantly different between control and LPS+sevoflurane groups (Table 2).

Collectively, these results indicate a reduction in the amplitude but not frequency or time course of mEPSCs in slices from LPS+sevoflurane mice. This reduction was specific to mEPSCs, as the characteristics of sEPSCs were unchanged.

LPS+sevoflurane did not impair synaptic plasticity

The observed reduction in mEPSCs, together with our previously reported increase in tonic inhibitory current, suggests that the E/I balance was disrupted after LPS+sevoflurane.[9] Given that changes in E/I balance are well known to impair synaptic plasticity in the hippocampus, we postulated that synaptic plasticity would be impaired after LPS+sevoflurane. To test this hypothesis, we recorded fPSPs in the Schaffer collateral–CA1 pathway and examined responses to stimulation paradigms that either weaken or strengthen synaptic responses.

To begin, synaptic weakening was examined by studying LTD. For these recordings, slices were prepared utilising the same protective solutions and techniques that were used for whole-cell recordings described earlier. First, the baseline properties of the field responses were evaluated. The I–O relationship was studied by stimulating incoming Schaffer collaterals with progressively stronger pulses (input) and recording the field response (output). The slope of the I–O relationship was then calculated for each slice. No differences were observed in slopes between the treatment groups, indicating that baseline excitability was unaltered by LPS+sevoflurane (control: 2.7 [1.0] ms^{-1}, $n=12$; LPS+sevoflurane: 2.9 [0.7] ms^{-1}, $n=10$; $P=0.612$; Fig 3a). Next, to assess presynaptic transmitter release, PPF was examined by delivering pairs of stimuli in rapid succession. The response to the second stimulus was consistently greater than the first in all recordings. There was no difference in PPF between treatment groups (effect of treatment: $F_{(1,120)}=0.27$; $P=0.604$; Fig 3b).

After the assessment of baseline properties, LTD was induced using a 10 Hz stimulation protocol. As expected, in control slices, the initial slope of fPSPs was reduced to 86.5 [7.8]% of baseline when measured 1 h after stimulation, indicating LTD (Fig 3c and d). Surprisingly, slices from mice treated with LPS+sevoflurane exhibited a similar level of LTD (89.0 [13.2]%) as controls ($P=0.591$). However, potentiation was observed immediately after stimulation (within 1 min) in slices from mice treated with LPS+sevoflurane, but this did not reach statistical significance (control: 100.1 [12.0]%; LPS+sevoflurane: 117.6 [27.6]%; $P=0.061$).

Next, LTP was studied to examine synaptic strengthening using a TBS protocol. Preliminary experiments showed that slices prepared using the protective solutions exhibited only weak LTP ($n=5$, three dissections; unpublished observations), as reported by others.[22] Therefore, the studies of LTP were performed with hippocampal slices that were prepared using different slicing methods and holding solutions (see Methods). Interestingly, under these experimental conditions, the I–O slope differed between control and LPS+sevoflurane groups, suggesting an increase in overall network excitability (control: 2.4 [0.8] ms^{-1}, $n=6$; LPS+sevoflurane: 3.7 [1.1] ms^{-1}, $n=4$; $P=0.0497$; Fig 4a). In contrast, PPF was not significantly different between control and LPS+sevoflurane groups (effect of treatment: $F_{(1,48)}=3.716$; $P=0.060$; Fig 4b).

Long-term potentiation was observed in slices from control mice, as evidenced by the increase in the initial slope of fPSPs

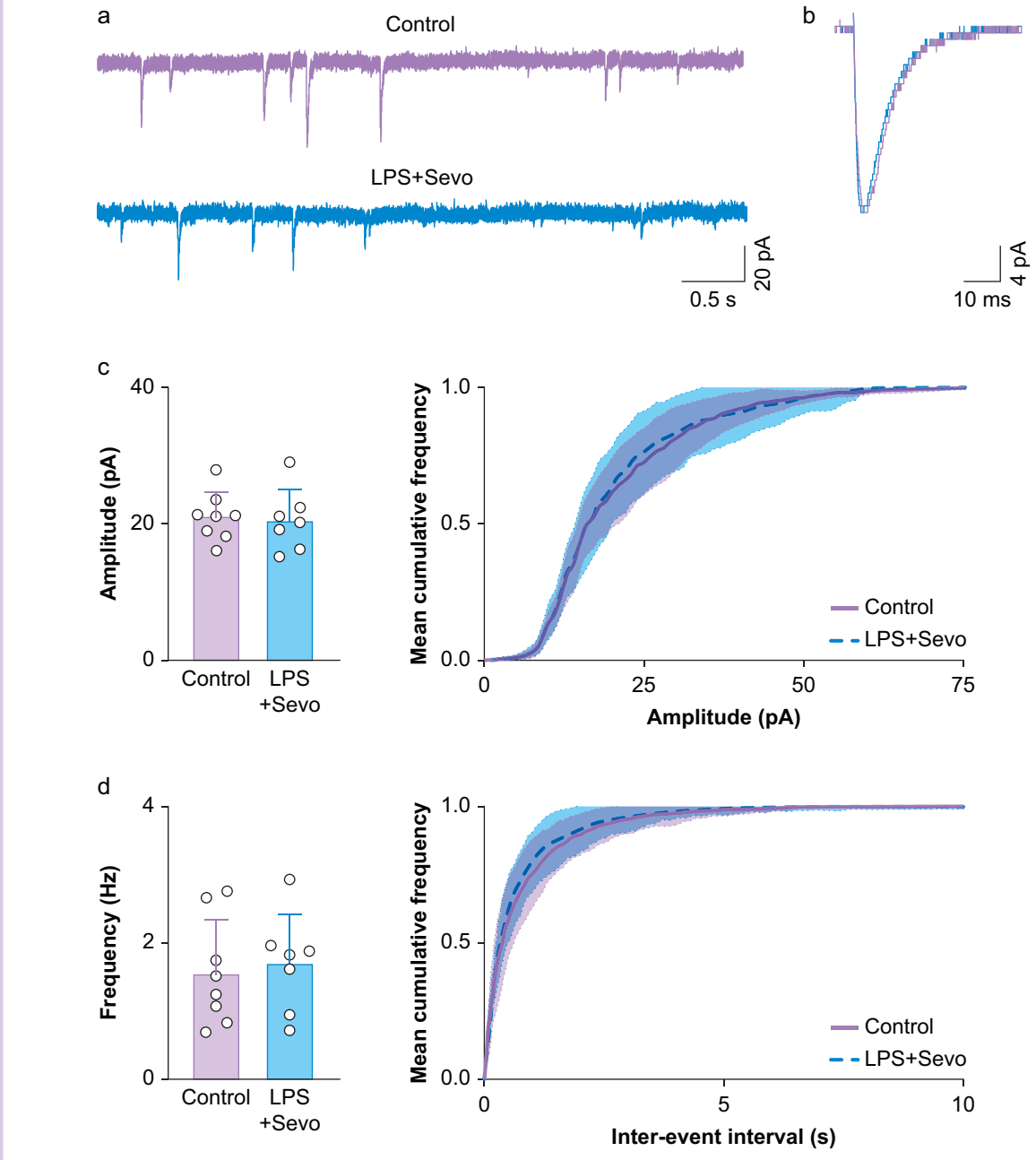

Fig 2. Amplitude and frequency of sEPSCs were unchanged after LPS+sevoflurane. (a) Representative traces for sEPSC recordings. (b) Averaged sEPSC traces representing 120 (control) and 90 (LPS+sevoflurane) individual events. (c) Left: summarised data show that the amplitude of sEPSCs was not changed after LPS+sevoflurane. n=8; 7 cells (control *vs* LPS+sevoflurane). P=0.800, unpaired Student's t-test. Right: the cumulative distribution of amplitudes was not different between treatment groups. n=1056; 941 events (control *vs* LPS+sevoflurane). P=0.078, Kolmogorov–Smirnov test. (d) Left: summarised data show that the frequency of sEPSCs was not changed after LPS+sevoflurane. P=0.740, unpaired Student's t-test. Right: the cumulative distribution of inter-event intervals was also not different between treatment groups. n=1612; 1530 events (control *vs* LPS+sevoflurane); P=0.075. Data are presented as means [standard deviation]. LPS, lipopolysaccharide; sEPSCs, spontaneous excitatory postsynaptic currents; Sevo, sevoflurane.

when measured 1 h after TBS (Fig 4c and d). Specifically, the slope increased to 164.7 [23.5]% of baseline. The magnitude of LTP was similar after treatment with LPS+sevoflurane (163.5 [11.0]%; P=0.933). Also, post-TBS potentiation, which was measured during the first minute after TBS, was similar between treatment groups (control: 213.3 [41.8]%; LPS+sevoflurane: 230.3 [24.2]%; P=0.488). Visual inspection of the responses after TBS revealed an apparent increase in the slope

Table 2 sEPSC variables after LPS+sevoflurane. $P>0.05$ for all variables; amplitude, frequency, rise time, and decay time were compared using unpaired Student's *t*-tests, and the Mann–Whitney *U*-test was used to compare area. Data are presented as means [standard deviation]. LPS, lipopolysaccharide; sEPSCs, spontaneous excitatory postsynaptic currents; Sevo, sevoflurane.

Treatment	Amplitude (pA)	Frequency (Hz)	Rise time (ms)	Decay time (ms)	Area (pA ms^{-1})
Control, n=8	21.2 [3.6]	1.6 (0.8)	2.6 (0.4)	9.5 (1.0)	333.6 (58.8)
LPS+Sevo, n=7	20.6 [4.6]	1.7 [0.7]	2.4 [0.3]	8.7 [1.5]	309.2 [34.7]

of fPSPs in the early post-TBS phase termed short-term potentiation. Thus, a secondary analysis was performed. To examine short-term potentiation, the mean slope values in the first 20 min after TBS were fitted with a single-exponential decay curve. The decay constant τ for the fitted curve was not significantly different between controls (2.28 [CI 1.45 to 3.70] min) and LPS+sevoflurane (2.69 [CI 1.95 to 3.83] min), indicating no significant difference in short-term potentiation.

In summary, these data indicate that LTP and LTD were not altered in slices from mice treated with LPS+sevoflurane.

Discussion

The goal of this study was to examine whether excitatory synaptic transmission and synaptic plasticity were altered in mice that were treated with LPS and sevoflurane. The results showed that at 2 days after sevoflurane treatment, the amplitude of mEPSCs was reduced, whereas the amplitude of sEPSCs was unaltered. Additional variables, including the frequency, area, and kinetics of both mEPSCs and sEPSCs, remained unchanged. These results indicate a reduction in glutamate-mediated neurotransmission at the quantal level, whereas LTP and LTD in the Schaffer collateral–CA1 pathway were unaltered.

The decrease in amplitude of mEPSCs implies a reduction in the size of quantal events, which refers to postsynaptic responses to the spontaneous release of a neurotransmitter from a single vesicle. At the synaptic level, this decrease may result from a reduction in the amount of glutamate released from presynaptic vesicles or a reduction in the function or number of postsynaptic AMPA receptors. Both LPS and sevoflurane have been shown to alter the expression of postsynaptic AMPA receptors; however, few studies have examined the effects of either LPS or sevoflurane on presynaptic vesicle filling or vesicle volume.[18,19,24] A decrease in the expression of AMPA receptor subunits GluA1 and GluA2 in the hippocampus and cortex has been reported 24 h after LPS.[18,19] In addition, repeated exposure of neonatal P7 mice to sevoflurane (3%; 2 h daily for 3 days) decreased the surface expression of GluA1, GluA2, and GluA3 in the hippocampus when measured in adulthood.[24] Similarly, exposing cultured hippocampal neurones to sevoflurane (4%) reduced surface expression of GluA1.[24] Thus, altered expression levels of postsynaptic receptors may decrease quantal size, as we observed here.

Interestingly, the amplitude of sEPSCs was not altered. As reported by others, alterations in quantal size are not always reflected in the amplitude of sEPSCs, which include postsynaptic responses to action potential-evoked glutamate release.[25,26] For example, Gerkin and colleagues[27] reported that an increase in quantal size, as indicated by increased amplitude of mEPSCs, was not associated with changes in the amplitude of action potential-evoked EPSCs. Such evoked transmitter release leads to saturating concentrations of glutamate in the synaptic cleft, which may mask subtle changes in quantal size. Alternatively, homeostatic changes in the probability of vesicle release may compensate for altered quantal size.[28] Similar mechanisms may explain the discrepancy observed in our study between mEPSCs and sEPSCs. Nevertheless, altered quantal size on its own is associated with impaired function at the network level.[25]

The finding that both LTP and LTD in the Schaffer collateral–CA1 pathway were unaltered after exposure to LPS+sevoflurane was unexpected, given that a reduction in mEPSCs and an increase in tonic inhibition can both independently impair synaptic plasticity.[11,13,20,25] Parallel or compensatory changes in the Schaffer collateral–CA1 or additional pathways may have counteracted the effect of reduced mEPSCs and increased tonic inhibitory current on synaptic plasticity. Such changes may include global homeostatic alterations in E/I balance at the circuit level that maintain network stability.[29,30] Additionally, the threshold for induction of LTP and LTD can shift in response to E/I imbalance, a process known as metaplasticity.[31] Evidence hinting at such parallel changes in our results includes the observed increase in I–O slopes in a subset of slices after LPS and sevoflurane, and a trend towards early potentiation in the LTD experiments.

It is noteworthy that the increase in I–O slopes after LPS+sevoflurane, which indicates increased excitability, was observed only in slices prepared in aCSF and not those prepared using protective techniques. The cause of this incongruity remains unknown, but it may be attributable to differences in the protective slicing solutions, which reduce oxidative stress, increase neuronal glutathione synthesis, and improve survival of inhibitory interneurones.[21,32] All of these factors can affect network function and synaptic plasticity in the hippocampus.[29,33–35]

This study had several limitations. First, we studied the effects of LPS and sevoflurane only in combination. However, the effects of each factor alone may differ from those produced by the combination, as reported previously for changes in executive function.[7] Second, we studied the effects of the anaesthetic sevoflurane, which is administered by inhalation, but the incidence of PNDs and the underlying mechanisms associated with injectable anaesthetics, including propofol, may be different.[36] Third, from our investigations, it remains unknown whether the reduced amplitude of mEPSCs was associated with behavioural deficits. Despite intact synaptic plasticity, reduced mEPSCs may lead to behavioural impairments, and this possibility is worthy of further investigation. Fourth, for this study, we selected the LPS model because it allowed us to investigate the effects of inflammation and general anaesthesia on their

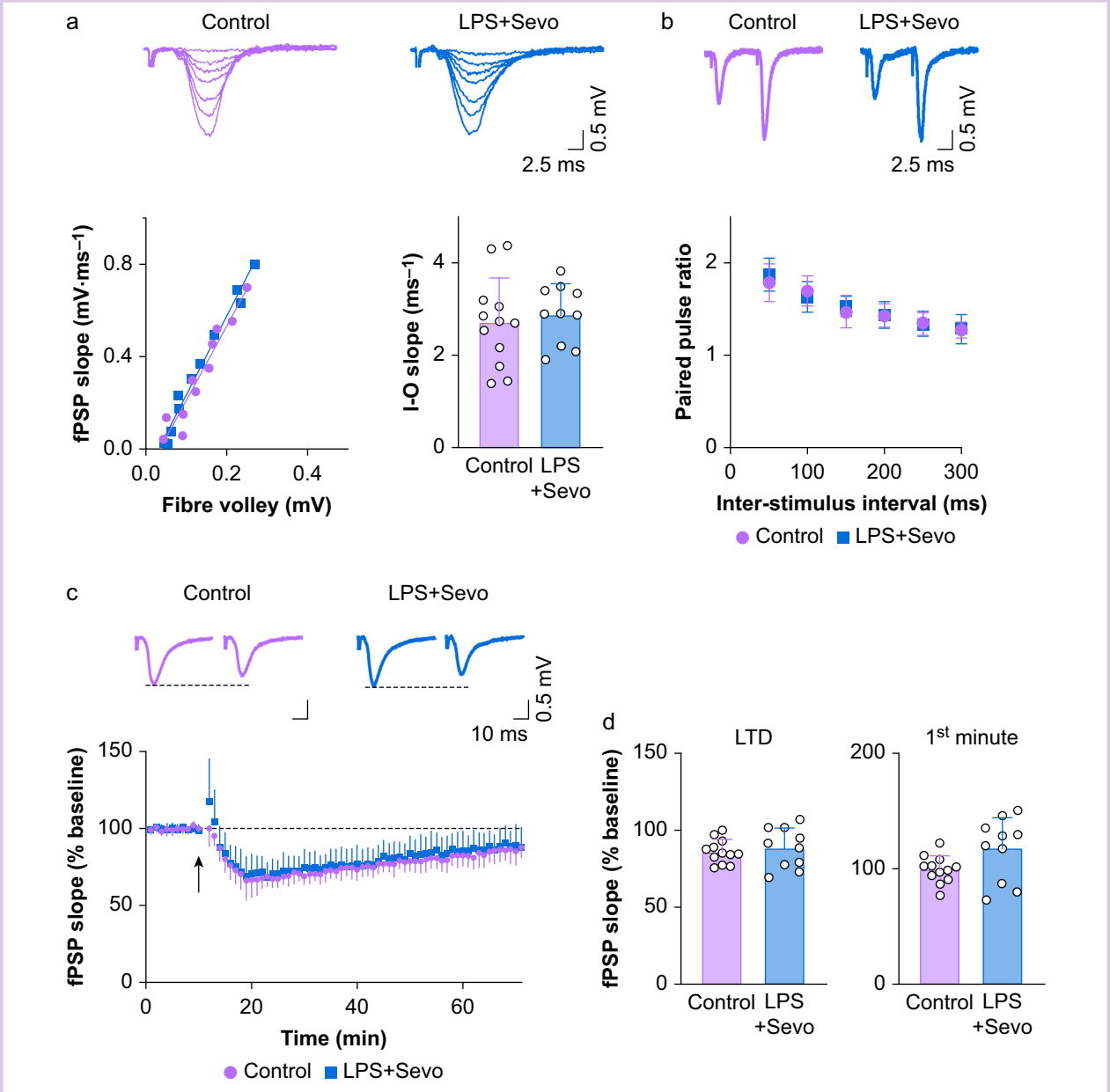

Fig 3. Long-term depression (LTD) was unchanged after LPS+sevoflurane. (a) Top panel: representative traces show the response to progressively stronger stimuli from slices in the control and LPS+sevoflurane groups. Bottom panel, left: I–O responses from the representative slices shown previously were fit with a linear regression line. The slopes of the two linear regression lines were not significantly different. $F_{(1,18)}$=0.37; P=0.553; ANCOVA. Bottom panel, right: summarised I–O slopes from all slices show no change after LPS+sevoflurane. P=0.612, unpaired Student's t-test. (b) Top panel: representative traces show the facilitation of the response to the second stimulus. Bottom panel: summarised data show that paired-pulse facilitation is not different between control and LPS+sevoflurane groups. Effect of LPS+sevoflurane: $F_{(1,120)}$=0.27, P=0.604; effect of inter-stimulus interval: $F_{(5,120)}$=40.85, P<0.001; effect of interaction: $F_{(5,120)}$=0.67, P=0.646; two-way ANOVA. (c) Top panel: representative traces for LTD. Bottom panel: normalised slopes of fPSPs after 10 Hz stimulation, indicated by the arrow. (d) Summarised data show the average slope in the last 5 min of recording (LTD, left; P=0.591) and the first minute (right, P=0.061) after 10 Hz stimulation. n=12; 10 slices (control *vs* LPS+sevoflurane). Unpaired Student's t-test. Data are presented as means [standard deviation]. ANCOVA, analysis of covariance; ANOVA, analysis of variance; fPSP, field postsynaptic potential; I–O, input–output; LPS, lipopolysaccharide; Sevo, sevoflurane.

own, in the absence of surgical pain or tissue trauma. The model of LPS-induced inflammation has been shown previously, by us and others, to induce a robust neuroinflammatory response.[7,37,38] However, the mechanisms, time course of onset and resolution, and behavioural consequences of LPS- and surgery-induced

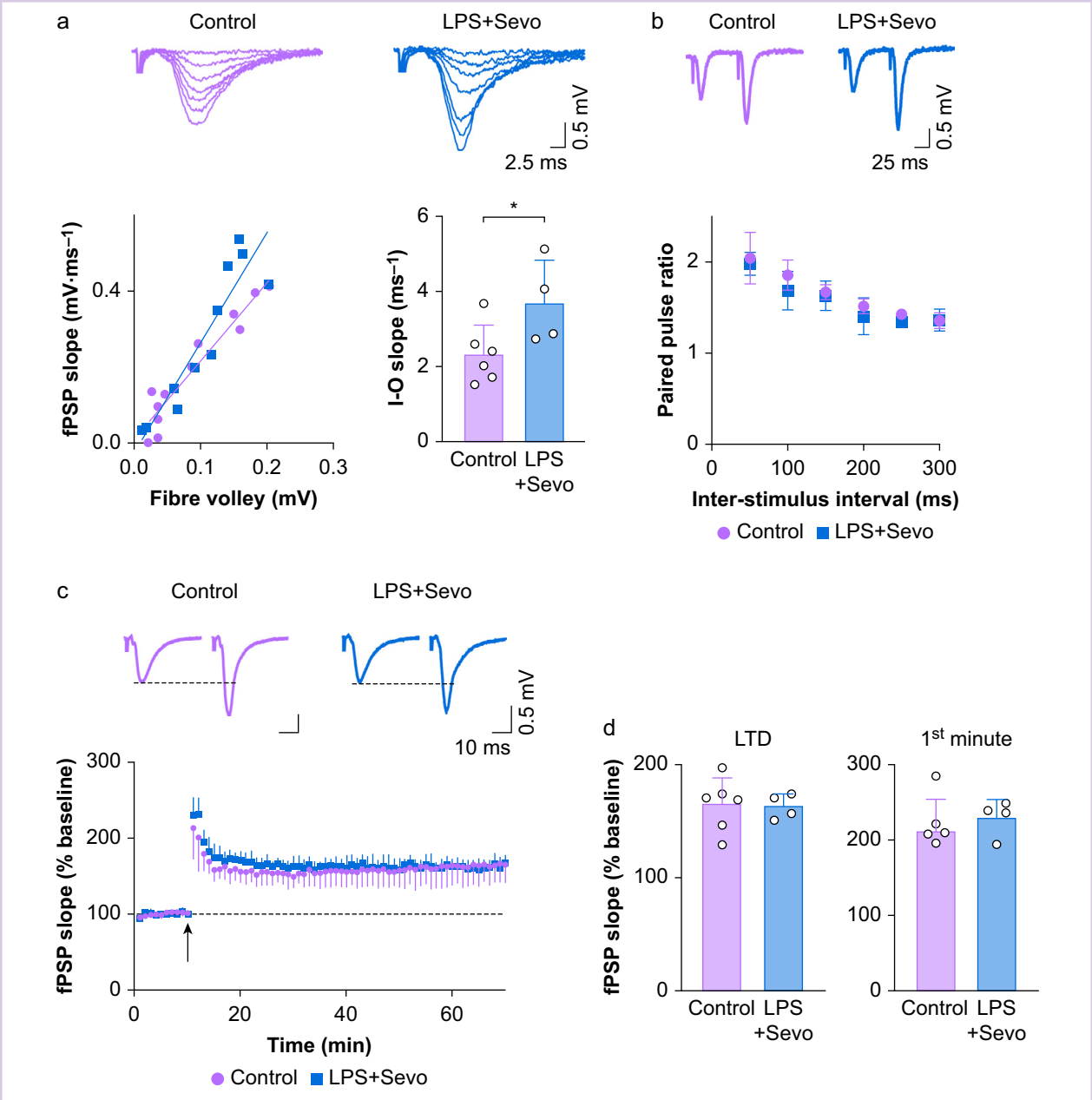

Fig 4. Long-term potentiation (LTP) was unchanged after LPS+sevoflurane. (a) Top panel: representative traces show the response to progressively stronger stimuli from slices in the control and LPS+sevoflurane groups. Bottom panel, left: I–O responses from the representative slices shown previously were fit with a linear regression line. The slopes of the two regression lines were not significantly different: $F_{(1,20)}$=4.12; P=0.056; ANCOVA. Bottom panel, right: summarised I–O slopes from all slices indicate an increase after LPS+sevoflurane. *P<0.05, unpaired Student's t-test. (b) Top panel: representative traces show the facilitation of the response to the second stimulus. Bottom panel: summarised data show that paired-pulse facilitation is not different between control and LPS+sevoflurane groups. Effect of LPS+sevoflurane: $F_{(1,48)}$=3.72, P=0.060; effect of inter-stimulus interval: $F_{(5,48)}$=26.99, P<0.001; effect of interaction: $F_{(5,48)}$=0.40, P=0.848; two-way ANOVA. (c) Top panel: representative traces for LTP. Bottom panel: normalised slopes of fPSPs after TBS, indicated by the arrow. (d) Summarised data show the average slope in the last 5 min of recording (LTP, left; P=0.933) and the first minute (right; P=0.488) after TBS. n=6; 4 slices (control *vs* LPS+sevoflurane). Unpaired Student's t-test. Data are presented as means [standard deviation]. ANCOVA, analysis of covariance; ANOVA, analysis of variance; fPSP, field postsynaptic potential; I–O, input–output; LPS, lipopolysaccharide; Sevo, sevoflurane.

neuroinflammation likely differ.[39–42] As such, future studies examining the role of excitatory neurotransmission in PNDs should be extended to incorporate a surgical procedure.

In conclusion, the results of this study, in combination with our previous work,[9] indicate that the combination of LPS and sevoflurane disrupts the E/I balance in CA1 pyramidal

neurones. Concurrent treatment with a drug that increases AMPA receptor function and a negative allosteric modulator that selectively reduces tonic inhibition may prove to be an effective treatment strategy to restore E/I balance. This study, which identifies a new potential drug target, aligns with several of the research priorities proposed for future PND studies.[4] Overall, our results suggest that reduced excitatory neurotransmission adds to a growing list of changes that occur in the brain during the early postoperative period.

Authors' contributions

Experimental design/development: all authors.
Experimentation: SK.
Data analysis: SK.
Preparation of paper: all authors.
Approval of final paper: all authors.

Declarations of interest

BAO serves on the Board of Trustees of the International Anesthesia Research Society (San Francisco, CA, USA) and is co-director of the Perioperative Brain Health Centre (Toronto, ON, Canada; http://www.perioperativebrainhealth.com). She is a named inventor on a Canadian patent (2,852,978) and two US patents (9,517,265 and 10, 981, 954). The patents, which are held by the University of Toronto, are for new methods to prevent and treat delirium and persistent neurocognitive deficits after anaesthesia and surgery, and to treat mood disorders. BAO also collaborates on clinical studies that are supported by in-kind software. SK and D-SW declare no conflicts of interest.

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Appendix A. Supplementary data

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