Linear transformation of the encoding mechanism for light intensity underlies the paradoxical enhancement of cortical visual responses by sevoflurane

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Key points

- The mechanisms of action of anaesthetics on the living brain are still poorly understood. In this respect, the analysis of the differential effects of anaesthetics on spontaneous and sensory-evoked cortical activity might provide important and novel cues.
- Here we show that the anaesthetic sevoflurane strongly silences the brain but potentiates in a dose- and frequency-dependent manner the cortical visual response.
- Such enhancement arises from a linear scaling by sevoflurane of the power-law relation between light intensity and the cortical response.
- The fingerprint of sevoflurane action suggests that circuit silencing can boost linearly synaptic responsiveness presumably by scaling the number of responding units and/or their correlation following a sensory stimulation.

Abstract General anaesthetics, which are expected to silence brain activity, often spare sensory responses. To evaluate differential effects of anaesthetics on spontaneous and sensory-evoked cortical activity, we characterized their modulation by sevoflurane and propofol. Power spectra and the bust-suppression ratio from EEG data were used to evaluate anaesthesia depth. ON and OFF cortical responses were elicited by light pulses of variable intensity, duration and frequency, during light and deep states of anaesthesia. Both anaesthetics reduced spontaneous cortical activity but sevoflurane greatly enhanced while propofol diminished the ON visual response. Interestingly, the large potentiation of the ON visual response by sevoflurane was found to represent a linear scaling of the encoding mechanism for light intensity. To the contrary, the OFF cortical visual response was depressed by both anaesthetics. The selective depression of the OFF component by sevoflurane could be converted into a robust potentiation by the pharmacological blockade of the ON pathway, suggesting that the temporal order of ON and OFF responses leads to a depression of the latter. This hypothesis agrees with the finding that the enhancement of the ON response was converted into a depression by increasing the frequency of light-pulse stimulation from 0.1 to 1 Hz. Overall, our results support the view that inactivity-dependent modulation of cortical circuits produces an increase in their responsiveness. Among the implications of our findings, the silencing of cortical circuits can boost linearly the cortical responsiveness but with negative impact on their frequency transfer and with a loss of the information content of the sensory signal.

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Abbreviations BSR, burst-suppression ratio; CCF, cross-correlation function; EEG, electroencephalography; L-AP4, L-2-amino-4-phosphonobutyric acid; MAP, mean arterial pressure; $mGluR_6$, metabotropic glutamate receptor 6; *N*, number of readily releasable synaptic vesicles; *p*, release probability of synaptic vesicles; P1, first positive peak of the visual evoked potential; PSD, power spectral density; RMS, root mean square; V1, primary visual cortex; VEP, visual evoked potential.

Introduction

In the past, the silencing effect of general anaesthetics has been studied and interpreted as the result of interactions with molecular targets that control neuronal excitability(Patel *et al.* 1999; Ouyang *et al.* 2003; Bieda & MacIver, 2004) or the strength of excitatory pathways. The latterscenario might relate to a direct inhibition of excitatory synaptic transmission (Yamakura *et al.* 1995; Nishikawa & MacIver, 2000; Westphalen & Hemmings, 2003; Wu*et al.* 2004; Solt *et al.* 2006; Ishizeki *et al.* 2008; Haseneder *et al.* 2009) or might follow the enhancement of inhibitory transmission (Mihic *et al.* 1997; de Sousa *et al.* 2000; Asahi*et al.* 2006; MacIver, 2014).

Because of the complexity of cortical circuits, the final*in vivo* outcome of anaesthetic treatment might be counterintuitive, for example resulting in a reduction of the strong synaptic inhibitory tone observed in wakefulness, which generates a more balanced ratio between excitation and inhibition (Haider *et al.* 2013). Furthermore, during general anaesthesia neuronal activity is not simply suppressed, but the brain seems to shift into a distinct electrical and functional mode (Brown *et al.* 2010). In this state, many sensory stimuli can still effectively reachthe cerebral cortex, where a variable degree of cortical activation can be detected (see for review Kumar *et al.* 2000). These sensory responses are not simply altered intheir amplitude but anaesthetics change their shape and duration compared to wakefulness (Imas *et al.* 2005, 2006;Saxena *et al.* 2013). While a depression of the amplitude ofsensory responses would be expected (Saxena *et al.* 2013), because of the well-known depressant effect of anaestheticson thalamo-cortical and cortico-cortical firing rates, their maintenance and/or amplitude enhancement (Imas *et al.* 2005, 2006) is more difficult to reconcile with the currentlyavailable information.

Here, we explored the effects of the volatile anaesthetic sevoflurane on the visual processing of dark-to-light and light-to-dark transitions from rat primary visual cortex (V1) and compared its actions with those of another general anaesthetic molecule, propofol, which belongs to a different class of compounds. Our findings reveal an inactivity-dependent modulation of cortical circuits by sevoflurane underlying a previously unrecognized multiplicative action of this compound on light-intensitycoding mechanisms.

Methods

Ethical approval and animal care procedures

Research and animal care procedures were approvedby our Institutional Animal Care and Use Committee for Good Animal Experimentation in accordance with the Italian MIUR code of practice for the care and use of animals for scientific purposes (IACUC number: 541). Experiments were carried out on adult male Sprague–Dawley rats (350–540 g; *n* 33). As indicated below, every effort was made to minimize the animals' distress, pain and suffering during the entire course of the experimentation. All animals were individually caged with free access to food and water *ad libitum* and were exposed to 12 h light–dark cycles at 23°C constant roomtemperature.

Surgical and electrophysiological protocols

Cortical activity was recorded by three metal electro- des (stainless steel screws, 1.4 mm diameter) chronically implanted on the skull, positioned at the level of left–right V1 and right motor cortex. The coordinates for implantation were as follows: for V1, mediolateral ± 4.6 mm from the sagittal axis and rostrocaudal 7.00 mm below bregma; for the motor cortex, mediolateral –1.82 mm and rostrocaudal 2.4 mm above bregma. For electrode implantation, rats were placed in an anaesthetic induction chamber (sevoflurane 5%; Abbvie, North Chicago, IL, USA) for a few minutes until unconscious. Rats were then removed from the chamber,the loss of the righting reflex was tested, and then they were quickly connected through a nose cone to a mechanical ventilator (sevoflurane 3.75%; SERVO900C, Siemens, München, Germany; gas vaporizer, Vapor 2000, Drägerwerk, Lübeck, Germany). Rats then received intraperitoneal gentamicin (1.5 mg kg^{-1}, Italfarmaco,Milano, Italy), subcutaneous carprofen (5 mg kg^{-1}, Pfizer, Latina, Italy) and dexamethasone (0.2 mg kg^{-1}, Hospira, Napoli, Italy). Body temperature during the procedure was maintained at 36–37°C using a heating pad (ATC1000, WPI, Sarasota, FL, USA). Electrodes were secured by methylmethacrylate cement (Salmoiraghi Produzione Dentaria, Mulazzano, Italy). For the following 3 days, animals received intraperitoneal gentamicin (1.5 mg kg^{-1}) twice a day.

After 1 week of recovery from electrode implantation, rats underwent two successive recording trials run under full anaesthesia, interleaved with a resting period of at least 3 days (3–6 days), during which their recovery and health conditions were carefully monitored. As described below, during these two experimental trials, animals were neither physically restrained nor exposed to any furthersurgical procedure or noxious stimuli. At the end of the second trial, before regaining consciousness, animals werekilled by a lethal dose of thiopental (100 mg kg^{-1}; I.V.), which induced a rapid flattening of the EEG (see below).Animals were disposed of only after death was confirmed by the clear onset of *rigor mortis*.

For the two recording trials, rats were anaesthetized as described above, quickly intubated (endotracheal polyethylene 16 GA cannula; the cannula was moistened using a 2.5% lidocaine chlorhydrate gel; Luan, Molteni, Italy) and the endotracheal cannula connected to the mechanical ventilator (sevoflurane 2.5%). Volume-controlled air ventilation (tidal volume6 ml; respiratory rate 70–80 breaths min^{-1}) was set and the end-expiratory sevoflurane and CO_2 concentration continuously monitored by a gas analyser (Vamos, Drägerwerk, Lübeck, Germany). Rats were placed on a custom-built apparatus for electrophysiological recordings, where body temperature was maintainedat 36–37°C using a heating pad. The tail vein was then cannulated (26 GA cannula) and from this point on, the anaesthesia was maintained either with sevoflurane (2.5–5%) or with propofol (intravenous 1–2 mg kg^{-1} min^{-1}; AstraZeneca, London, UK), using as starting dosage for both compounds the lowest one (sevoflurane 2.5%; propofol 1 mg kg^{-1} min^{-1}). For propofol anaesthesia, the intravenous injectionof an initial bolus (10 mg kg^{-1}) was followed by continuous intravenous infusion of the anaesthetic at maintenance dosage (Pump11-elite, Harvard Apparatus, Holliston, MA, USA). Following the induction of anaesthesia, electrodes were connected to the recording system. During the initial preparation phase (~20 min),
the stability of anaesthesia was evaluated by on-line monitoring of the EEG and its spectrum as well as by evaluating the loss of the righting reflex. The lowest anaesthetic dosages used in these experiments werefound to abolish the righting reflex in 100% of cases (sevoflurane 2.5%; propofol 1 mg kg^{-1} min^{-1}). Following this preparation phase, atracurium (GlaxoSmithKline, Brentford, UK) was administered as an intravenous bolus (5 mg kg^{-1} every 18 min) in order to suppress involuntary eye bulb movements, which are preserved during anaesthesia (Nair *et al.* 2011). This treatment was used to maximize visual responses reproducibility. EEG monitoring was maintained throughout the experiment to evaluate the stability and depth of anaesthesia. Regarding the anaesthetic concentrations,

in all experiments, the sequence used always went from the lowest to the highest concentration (one anaesthetic compound, three sequential anaesthetic concentrations for each trial). For intravitreal injection of the metabotropic glutamate receptor 6 ($mGluR_6$) agonist L-2-amino-4-phosphonobutyric acid (L-AP4; Tocris Bioscience, Bristol, UK), during the initial pre-paration phase (sevoflurane 2.5%), 5 μl of a standard Tyrode solution containing 20 mM L-AP4 was injected into the eye bulb (estimated final concentration of L-AP4 2 mM). In some experiments the mean arterial pressure (MAP) was recorded by a catheter (26 GA) inserted into the femoral artery and connected to a pressure trans- ducer. Electrical activity was recorded by a custom-built amplifier featuring a total gain of 5000 and band-pass filtering of 0.1–3000 Hz. The signal was digitalized at20 kHz (16 bit) using the ITC-18 data acquisition interface(HEKA Elektronik, Lambrecht, Germany) controlled by a custom software developed in the LabVIEW environment (National Instruments, Austin, TX, USA). A groundelectrode was positioned on the ear. The inter-hemispheric occipito-occipital derivation was used to record visual evoked potentials (VEPs) and the inter-hemispheric occipito-frontal derivation was used for spontaneousactivity.

Experimental design and visual stimuli

To reduce animal-to-animal variability, the same animals were exposed to both anaesthetics in sequential experimental sessions. In these two separate recording trials, the administration sequence for the two anaesthetic compounds was randomly assigned toanimals. Spontaneous activity recordings were performed in complete darkness without light stimulation. For light stimulation protocols, rats were maintained in complete darkness with their left eye covered with a black tape. The light source was a white, high-brightness light-emitting diode placed 2 cm away from the right eye. Light stimuliwere applied at a fixed rate (0.1 Hz). The irradiance and the pulse duration were randomly varied by the software during the course of experiments (irradiance range, 1–330 μW cm^{-2}; pulse duration range, 20–800 ms). In some experiments the rate of light stimuli was also varied (frequency range, 0.1–1 Hz) with fixed light pulse intensity and duration (150 μW cm^{-2} and 300 ms, respectively).

EEG and VEP analysis

Power spectral density (PSD) of raw electro-encephalographic (EEG) signal in the resting condition was computed as follows: in each condition 110 s of spontaneous brain activity was subdivided into 10 s sweeps, band-pass filtered (0.5–90 Hz) and band-block

filtered (49–51 Hz) for removal of line noise. Each sweep was Hamming-windowed followed by fast Fourier trans- form (FFT). For normalization the square magnitude of FFT was divided by the number of samples *N*and by the sampling frequency F_s. A set of successive power spectra (*n* 11) were averaged. For the delta (0.5–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), sigmaor spindle (12–15 Hz), beta (15–25 Hz) and gamma (25–80 Hz) bands, the average PSD and overall sumfor all densities was calculated. For burst detection, 10 s sweeps were down-sampled to 5 kHz, band-pass filtered (12–15 Hz) and rectified; burst events were thendetected based on their amplitude (mean voltage and its SD) and duration (minimal duration ? 500 ms;
when inter-event interval was ≤ 300 ms, the events were considered as parts of the same burst. Burst peak-amplitude (rectified signal), duration and PSD (same procedure as above) were then extracted for analysis. Short-time Fourier transform (STFT) was performed on raw signals with 120 s Hamming windows (99% overlapping). The state of burst suppression was quantified by the burst-suppression ratio (BSR), which specifies the fractional time spent in suppression (100%, all suppression; 0%, no suppression; Vijn & Sneyd, 1998).

Quantitative estimates of VEPs were obtained from ensemble averages as well as from single traces. VEP latency was expressed as the latency of 50% of the first positive peak from the beginning of the light pulse (50% P1 latency). VEP amplitude was obtained as the root mean square (RMS) of the first 150 ms on the ensemble average startingfrom stimulus onset:

$$\mathrm{RMS}_{\mathrm{VEP}} = \sqrt{\frac{1}{n}\left(x^2_1 + x^2_2 + \cdots + x^2_n\right)}$$

Where *x* is the voltage sample and n is the sample number. For the OFF response, the end of the stimulus was used as reference starting point.

The weighted least-square method was used to fit linear functions. A non-linear fitting process was performed using the Levenberg–Marquardt algorithm. Error bars were used as weights in fitting procedures. Light-intensity and pulse-rate response curves were fitted with thefollowing power-function:

$$y = ax^b$$

where *y* is the RMS amplitude, *b* is the power exponent,*a* is the multiplicative coefficient and *x* either the light intensity or stimulation frequency. Power exponents *b* were computed by linear fitting of log–log plots and then averaged for every condition. For the effects of light-pulse stimulation frequency on VEP amplitude, the amplitudes of single VEPs that were uncorrupted by spontaneous cortical noise (selection threshold equal to the mean RMS + 1 SD of the first 20 ms from stimulus onset) were measured, averaged and fitted by the following exponentialdecay function:

$$y = (y_0 - y_\infty)e^{-x/T} + y_\infty$$

where *y* is the RMS amplitude, *x* is the stimulus number,*T* is the time constant, *y* is the plateau value, and y_0 is setto the amplitude of the first VEP in the series.

Cross-correlation functions (CCFs) were computed using a 150 ms window starting from stimulus onset (same temporal window used for RMS analysis). Thelag of the first positive peak of the cross-correlationfunction (between pairs of VEP traces) was detectedby searching for the first local maximum of the CCFusing a moving search window of 3 neighbouring samples.

Statistics

Results are expressed as the mean ± standard errorof the mean. The normality of distribution of 50% P1 latency and of VEP RMS of ON and OFF responses in the experimental population was assessed by applying the Shapiro–Wilk test. In a repeatedmeasures design, principal and interaction effects were tested with one-way or two-way repeated measuresANOVA (rANOVA). Greenhouse–Geisser correction was applied when sphericity could not be assumed. Group comparisons in repeated measures design were tested withStudent's paired-samples *t* test and the Bonferroni–Holm correction was applied to multiple hypothesis testing. The two-samples *t* test was chosen for non-repeated measures design and the Welch correction for unequal variance
was applied. To evaluate the goodness of fit, adjusted R^2 was computed and the *F* test was performed to establish a *P* value. For the analysis of the effects of light-pulse stimulation frequency on VEP amplitude, the Spearman correlation coefficient (ρ) was calculated and the *t* test used to compute a *P* value. Non-parametric statistics were applied to test comparisons between ratio estimates and standard deviations. In the repeated measures design,principal effects were tested with Friedman's test, while group comparisons in repeated measures design were tested with Wilcoxon's signed-rank test. The criterion for significance was set at $P < 0.05$. All statistical tests were two-tailed. Symbols used to indicate *P*-values are as follows: $P > 0.05$, ns; *, $P < 0.05$; **, $P < 0.01$; ***,$P < 0.001$.

Results

Initial evaluation of anaesthesia depth

To evaluate the depth of anaesthesia we recorded spontaneous cortical activity and tested the effects

of increasing dosages of sevoflurane and propofol. Concentrations used in these experiments ranged for sevoflurane from 2.5 to 5% and for propofol from 1 to 2 mg kg^{-1} min^{-1}. At the lowest dosages, spontaneous cortical activity showed the characteristic high-voltage low-frequency oscillations consistent with non-REM sleep and anaesthesia (Fig. 1*A*; Brown *et al.* 2010). When the concentrations of these agents were increased (sevoflurane, 3.75 and 5%; propofol, 1.5 and 2 mg kg^{-1} min^{-1}), a clear burst-suppression pattern was induced (Fig. 1*A* and *B*), reflecting a strong brain inactivation which is characteristic of deep general anaesthesia and coma (Kroeger & Amzica, 2007; Ching *et al.* 2012). The burst-suppression ratio (BSR; Vijn & Sneyd, 1998) confirmed that at low dosages this behaviour remains close to zero for both anaesthetics (Fig. 1*B*; *n* = 7 rats; BSR: sevoflurane 2.5%, 3.44 ± 2.01%; propofol 1 mg kg^{-1} min^{-1}, 1.39 ± 0.34%; $P > 0.05$, Wilcoxon's signed-rank test) while a large increase of BSR was found when drug concentration was increased (Fig. 1*B*; *n* = 7 rats; $P < 0.001$ for both sevoflurane and propofol, Friedman's test). At the highest dosages, no significant differences in BSR were found between the two anaesthetics, suggesting a similar state of cortical inactivation (Fig. 1*B*; *n* = 7 rats; BSR: sevoflurane 5%, 95.35 ± 0.78%; propofol 2 mg kg^{-1} min^{-1}, 94.53 ± 0.81%; $P > 0.05$, Wilcoxon's signed-rank test). This burstingbehaviour emerged as a dose-dependent reduction of EEG power spectral density (Fig. 1*C–F*; *n* = 7 rats, 0.5–80 Hz window; $P_{sevoflurane} < 0.01$; $P_{propofol} < 0.001$; one-way rANOVA) even at the level of individual frequency bandswith the sole exception of the gamma band (25–80 Hz) under sevoflurane anaesthesia (Fig. 1*E* and *F*; gamma band $P_{sevoflurane} > 0.05$; for all other bands and conditions in all cases $P < 0.05$; one-way rANOVA).

The analysis of individual bursts also showed that while burst occurrence and duration were significantly decreased at high dosages with anaesthetics, burst amplitude and spectral power were differentially affected (Fig. 2).

Selective potentiation of visual cortical responsesby sevoflurane

We began by studying the action of sevoflurane on evoked cortical responses induced by light stimuli (VEPs) and compared its effects with those of propofol. These sensory responses displayed the standard set of positiveand negative deflections (Creel *et al.* 1974) and remained stable both in amplitude and waveform up to the endof recordings for both pharmacological agents (pulse rate 0.1 Hz; stable up to 2 h from the beginning of anaesthesia).As depicted in Fig. 3, a dose-dependent potentiation of VEP amplitude by sevoflurane was found (light-pulseduration 20 ms; pulse irradiance 25 $\mu W\ cm^{-2}$; pulse rate

0.1 Hz) while this was not seen with propofol, which produced the opposite effect (Fig. 3*A* and *B*; *n* 6 rats; RMS amplitude estimated from the average VEP waveform; principal effect of anaesthetic concentration, $P < 0.05$ for both sevoflurane and propofol, one-way rANOVA; significant multiple comparisons, $P_{sevoflurane}$ (2.5 *vs*. 3.75%) < 0.05, $P_{sevoflurane}$ (2.5 *vs*. 5%) < 0.05, $P_{propofol}$ (1 *vs*. 2 mg kg^{-1} min^{-1}) < 0.05, paired-samples *t* test). In these experiments a clear dose-dependent incremental delay of the onset of the P1 wave was foundwith both anaesthetics (Fig. 3*C*; 50% P1 latency; $P_{sevoflurane} < 0.01$; $P_{propofol} < 0.05$; principal effect of anaesthetic concentration, one-way rANOVA). Since sevoflurane and propofol are known to reduce arterial blood pressure (Claeys *et al.* 1988; Ebert *et al.* 1995), we wondered if the differential effects of these two molecules on visual responses could be explained by an uneven action on the cardiovascular system. We found that stepwise increments in sevoflurane and propofol concentrations produced comparable drops in the mean arterial pressure (Fig. 4), suggesting that their specific mode of action could not be attributed to differential effects on the

presented in Fig. 3*A*). Individual responses obtained at all sevofluraneconcentrations seemed highly reproducible despite aclear dose-dependent reduction in pre-stimulus and late cortical background activity. Similarly to estimates from average responses, a significant sevoflurane concentration-dependent effect was found for both VEPamplitude (Fig. 5*B*; $P < 0.05$; compare with Fig. 3*B*) and background cortical activity (Fig. 5*C*; $P < 0.001$; compare with Fig. 1*E*) when using individual responses (*n* 6 experiments; principal effect by one-way rANOVA). The reliability of individual visual responses was confirmed by a cross-correlation analysis (Fig. 5*D*–*F*). At all sevofluraneconcentrations, single trials showed a similar degree of lag 0 cross-correlation with the corresponding VEP ensemble averages (*n* 6; $P > 0.05$, Friedman's test), suggesting a similar representativeness of the latter descriptors. Furthermore when the cross-correlation function (CCF) was computed in the same experiments among all pairs of individual VEPs, the distributions of the lag of the first CCF positive peak were found to bevery similar at all sevoflurane concentrations (Fig. 5*F*), without any significant difference in their variability (Fig. 5*E*; *n* 6; $P > 0.05$, Friedman's test). These results indicate that the potentiation of VEP responses by sevoflurane does not arise from an increased trial-to-trial synchronicity but represents a real modification of VEP amplitude.

Effects of sevoflurane and propofol on light-intensityresponse curves

As for other types of sensory signalling, the V1 response toa light pulse is sensitive to its strength across a wide range of stimulus intensities (Stevens, 1970). The cortical visual response grew as a power function of irradiance (pulse irradiance 1–330 μW cm^{-2}; pulse duration 20 ms; pulse rate 0.1 Hz). This was found to be true for both sevoflurane and propofol Fig. 6*B*; at the lowest dosages the power exponent was ~0.1). Interestingly, when the dose of thetwo anaesthetics was varied, a clear discrepancy between these two agents emerged (Fig. 6*C*–*E*). With increasing concentration of sevoflurane, the entire light-intensity response curve was found to be shifted upward in log–log coordinates (Fig. 6*C*; n 6 rats; $P < 0.01$; principal effect of sevoflurane concentration, two-way rANOVA; $P_{\text{sevoflurane}}$ (2.5 *vs*. 3.75%) < 0.001, $P_{\text{sevoflurane}}$ (2.5 *vs*. 5%) < 0.001, $P_{\text{sevoflurane}}$ (3.75 *vs*. 5%) < 0.01; multiple comparisons, paired-samples t test) and an interaction between the concentration of sevoflurane and the irradiance could be excluded (P > 0.05, two-way rANOVA). These results contrasted with the effects of propofol. On the one hand, increasing propofol concentrations produced a dose-dependent decrement of the VEP amplitude with significantly different light-intensity response curves (Fig. 6*D*; n 6 rats; $P < 0.001$; principal effect of propofol concentration, two-way rANOVA; P_{propofol} (1 *vs.* 1.5 mg kg^{-1} min^{-1}) < 0.05, P_{propofol} (1 *vs.* 2 mg kg^{-1} min^{-1}) < 0.001, P_{propofol} (1.5 *vs.* 2 mg kg^{-1} min^{-1}) < 0.001;

multiple comparisons, paired-samples *t* test). On the other hand at high stimulus intensities, these curves converged to a comparable response amplitude, causing a significant interaction between light-intensity and propofol dosage ($P < 0.01$, two-way rANOVA). This suggests some form of relief of propofol-induced cortical inhibition at high stimulation intensities, which was not seen with sevoflurane.

Differential effects of sevoflurane and propofol on ON and OFF visual responses

Because of the composite ON and OFF nature of the visual cortical response (Reid & Alonso, 1995; Martinez *et al.* 2005; Lien & Scanziani, 2013), we evaluated the effects of sevoflurane and propofol on the individual ON and OFF components (VEP_{On} and VEP_{Off}, respectively). With low concentration of both anaesthetics, the prolongation of the light pulse produced two clear and well separated ON and OFF transients, seen at the beginning and at the end of the stimulus, respectively (Fig. 7*A*; sevoflurane, 2.5%; light-pulse duration, 300–800 ms; pulse irradiance, 150 μW cm^{-2}; pulse rate, 0.1 Hz). When the light-pulse duration was incremented, the OFF response remained time-locked to the end of the stimulus, indeed suggesting that it represents a *bona fide* OFF response (Fig. 7*A*). Similarly to the ON response, the VEP_{Off} was sensitive to the light intensity, but it was smaller in amplitude than the VEP_{On} (Fig. 7*B*; sevoflurane, 2.5%; light-pulse irradiance, 6.5–290 μW cm^{-2}; pulse duration, 300 ms; pulse rate, 0.1 Hz). Therefore both VEP_{On} and VEP_{Off} amplitudes grew as a power function of irradiance with a similar exponent (Fig. 7*B*; n = 7 rats; power exponent of VEP_{On}, 0.24 ± 0.04; power exponent of VEP_{Off}, 0.19 ± 0.04; $P > 0.05$, paired-samples *t* test), but the entire light-intensity response curve of the VEP_{Off} was scaled toward smaller amplitude values compared with ON responses (Fig. 7*B*; n = 7 rats; $P < 0.001$, paired-samples *t* test).

When rats were exposed to prolonged light pulses at all anaesthetic dosages (Fig. 7*C–E*; sevoflurane, 2.5, 3.75 and 5%; propofol, 1, 1.5 and 2 mg kg^{-1} min^{-1}; light-pulse duration, 300 ms; pulse irradiance, 150 μW cm^{-2}; pulse rate, 0.1 Hz), the simultaneous analysis of the ON and OFF responses revealed that the potentiation of the amplitude of the VEP_{On} by sevoflurane contrasted with the dose-dependent reduction of the amplitude of the VEP_{Off}. (Fig. 7*C*). In contrast to the effects on the VEP_{On}, the reduction of the VEP_{Off} was seen with both anaesthetic compounds (Fig. 7*C–E*;

effect of sevoflurane was conserved, we varied the concentration of this anaesthetic agent immediately after the administration L-AP4 (Fig. 8*C* and *D*; L-AP4, 2–8 mM; sevoflurane, 2.5, 3.75 and 5%; light-pulse duration, 300 ms; pulse irradiance, 150 μW cm^{-2}; pulse rate, 0.1 Hz). Interestingly, when the ON response was absentbecause of the L-AP4 administration, sevoflurane induceda paradoxical dose-dependent potentiation on the VEP_{Off} amplitude, thus reverting the inhibitory action into a potentiation (Fig. 8*C* and *D*; n 5 rats; $P < 0.05$; one-way rANOVA; compare with Fig. 7*D*). Strikingly, the dose-dependent augmentation of the ON response by sevoflurane in the absence of L-AP4 matched almost perfectly the potentiation of the OFF response seen in the presence of L-AP4 (Fig. 8*E*; $P > 0.05$, two-sample t test for each sevoflurane concentration). The visualization of

individual VEP traces (Fig. 8*F*) highlights that as for ON responses, OFF responses occurred with precise timing, with a consistent potentiation across trials.

An increase in the frequency of stimulation abolishes the sevoflurane-induced potentiation and unmasks a depression

Since the ON and OFF pathways are known to converge onto single cortical neurons in layer IV (Reid & Alonso, 1995; Martinez *et al.* 2005; Lien & Scanziani, 2013), we tested for the induction of a use-dependent depressive state triggered by the initial ON response. To evaluate the pre- sence of an activity-dependent change, rats were exposed to trains of brief light stimuli at different frequencies (0.1–1 Hz) in the presence of increasing dosages of sevoflurane (sevoflurane, 2.5, 3.75 and 5%; light-pulse duration, 20 ms; pulse irradiance, 150 μW cm^{-2}; pulse rate range, 1–0.1 Hz; n 4 rats). At low frequencyof light stimulation (0.1 Hz), no significant change in VEP_{On} amplitude could be detected along the sequence of stimuli for all sevoflurane concentrations (Fig. 9*A*; n = 4

rats; sevoflurane 2.5%: $\rho = -0.01$, $P > 0.05$; sevoflurane 5%: $\rho = 0.12$, $P > 0.05$; Spearman correlation, *t* test). Conversely, when rats were stimulated by trains of light pulses at higher frequency (1 Hz), a quick decrement of VEP amplitude could be observed, which was much more evident at the higher anaesthetic dosages (Fig. 9*B*; *n* 4rats; sevoflurane 2.5%: $\rho = -0.47$, $P < 0.05$; sevoflurane 5%: $\rho = -0.62$, $P < 0.01$; Spearman correlation, *t* test). In the latter condition, the amplitude time course could be well fitted by an exponential decay function, whose steady state value unmasked a depression (Fig. 9*B*). The level of this activity-dependent depression increased as a function of the anaesthetic dosage (Fig. 9*D*; *n* 4rats; $P < 0.05$; one-way rANOVA). When these steady state values were plotted as a function of stimulation frequency, the slope of the resulting curves in log–log plotsdecreased in a dose-dependent fashion (Fig. 9*G*; *n* 4 rats; $P < 0.001$, one-way rANOVA). These results confirm a clear interaction between the concentration of sevofluraneand the frequency of stimulation (Fig. 9*F*; $P < 0.05$, two-way rANOVA) and indicate that by increasing the frequency of sequential stimulation, a gradual conversion of the dose-dependent potentiation into a dose-dependent depression could be elicited.

Discussion

Silencing induced potentiation

Here we report that two general anaesthetic compounds, sevoflurane and propofol, display opposite actions on the sensory visual response recorded on the V1 cortex (Fig. 3). The attenuation by propofol, which agrees with a previous report (Saxena *et al.* 2013), is clearly consistent with the general reduction in neuronal excitability and firing (see for review Franks, 2008), here transpiring as a decrease in spectral power of spontaneous activity (Fig. 1) and increase in VEP latencies (Fig. 3). To the contrary, the large amplitude enhancement of cortical visual responses by sevoflurane, which is consistent with previous reports on the effects of other volatile anaesthetics (Imas *et al.* 2005, 2006), is more difficult to understand. One possible explanation is that cortical silencing and the enhancement of visual sensory responses are not independent phenomena. Indeed, in wakefulness, the primary visual cortex processes a large amount of spontaneous circuital activity, whichis induced and sustained to a large extent by two-way thalamo-cortical interactions (Reinhold *et al.* 2015). These cortical dynamics are likely to induce various forms of activity-dependent short-term plasticity whose balance contributes to the input–output properties of the cortex (Markram *et al.* 1997; Zucker & Regehr, 2002). This might result in a persistent activity-dependent synaptic depression in the awake state (Castro-Alamancos & Oldford, 2002; Swadlow *et al.* 2002; Reinhold *et al.* 2015), more evident at those terminals which are prone to depressas the thalamo-cortical synapses (Gil *et al.* 1997).

The expectation is that agents that reduce the thalamo-cortical firing rate, such as anaesthetics, would counteract this synaptic depression. Indeed, our results show a clear inverse correlation between the dose-dependent reduction in cortical spontaneous activity by sevoflurane (Figs 1–3) and the dose-dependent potentiation of visual evoked responses (Fig. 3). Anotherprediction is that a cortex silenced by anaesthetics, when challenged repeatedly with externally controlled stimuli, should re-enter the depressed state. Our results also confirmed this second prediction. After silencing of cortical activity, a series of visual stimuli presented at frequencies above 0.1 Hz were found to produce a decremental cortical response (Fig. 9). Interestingly, the increase in activation frequency not only abolished the dose-dependent facilitation of the visual responses but revealed an underlying dose-dependent depression by sevoflurane (Fig. 9). The reduced ability of brain tissue anaesthetized by sevoflurane to follow a series of close visual stimuli agrees with the recent findings by Reinhold *et al.* (2015), where another volatile anaesthetic, isoflurane, was found to disrupt high-frequency thalamo-cortical transmission.

Our interpretation is reinforced by the here describednegative interaction between ON and OFF responses (Figs 7 and 8), whose elicitations were separated by a short interval (300 ms). Indeed, ON and OFF information are known to converge onto subfields of individual simple cells in the primary visual cortex (Reid & Alonso, 1995; Martinez *et al.* 2005; Lien & Scanziani, 2013).When the dosage of sevoflurane was increased, the clearenhancement of the ON response was accompanied by

a progressive reduction in the OFF response (Fig. 7). As depicted in Fig. 8, the abolition of the ON response by L-AP4 was found to convert the depression ofthe OFF component into a sevoflurane dose-dependent potentiation, with a trend analogous to the potentiationof the ON response seen in control conditions. This resultsuggests that both ON and OFF modalities are shaped by the same use-dependent synaptic phenomena.

A mechanistic synaptic hypothesis for the sevoflurane-induced potentiation of visual responses

Short-term use-dependent changes in synaptic efficacy are in most cases expressed at the level of the presynaptic compartment (Zucker & Regehr, 2002). Central synapses, which are characterized by a low release probability *p*, display a small number of readily releasable synaptic vesicles *N* (Abenavoli *et al.* 2002; Lamanna *et al.* 2015), a figure which is the final outcome of a series of docking, priming and super-priming steps (Lee *et al.* 2013; Jackman *et al.* 2016). These features, on the one hand render their output very unreliable, especially *in vivo* (Borst, 2010), and on the other hand allow large degrees of modulation. If sevoflurane were to scale either *p* or *N*, this change would translate into a large enhancement of visual responses, as the one seen here. The incrementin *p* is unlikely, because of the many *in vitro* studies showing that release probability is reduced by volatile anaesthetics (Westphalen & Hemmings, 2003; Wu *et al.* 2004; Hemmings *et al.* 2005; Xie *et al.* 2013; Baumgart *et al.* 2015). For example, Baumgart *et al.* (2015) have recently reported that in hippocampal cultures, the volatileanaesthetic isoflurane produces a clear decrement of synaptic vesicle fusion probability. An alternative and more likely hypothesis relates to an increase in *N* by sevoflurane. This vesicular pool is not stable but under- goes continuous activity-dependent changes (Zucker & Regehr, 2002). The partial depression of cortical synaptic circuits found in the awake state (Castro-Alamancos & Oldford, 2002; Swadlow *et al.* 2002; Reinhold *et al.* 2015)might then simply represent the use-dependent depletion of the vesicular pool *N* by exuberant spontaneous activity. Conversely, neural inactivity by sevoflurane would counteract the awake low-*N* state generating in some conditions a higher *Np* binomial product, resulting in a larger visual evoked response.

One important caveat of this hypothesis relates tothe fact that the compound propofol, found to silence cortical circuits to a similar extent (Figs 1–3), does not potentiate visual evoked responses (Figs 3 and 6). One possible interpretation is that propofol might antagonize the inactivity-dependent replenishment of the vesicular pool *N*. In support of this interpretation, propofol has beenrecently found to increase spontaneous vesicle exocytosis at the neuromuscular junction (Leite *et al.* 2011). An increase in spontaneous vesicle exocytosis would clearlycompete with the increment of the vesicular pool *N*induced by inactivity postulated here.

The linear scaling of stimulus-response curveby sevoflurane

The visual response requires the activation of several axonal fibres, neurons and synapses whose activity in the end generates the ensemble field potentials seen at the cortical surface. The amplitude of these evoked responses essentially relates to (i) the number and average firing of thalamo-cortical axons; (ii) the number and average strength of thalamo-cortical synapses; (iii) the balance between excitatory and inhibitory cortical circuits (Murphy & Miller, 2003) and the contribution of feed- back mechanisms such as the cortico-thalamic one (Lien & Scanziani, 2013); and (iv) the synchronicity of theresponding neurons as well as the fine spatial distribution of cortical activation (Buzsáki *et al.* 2012). As for other sensory systems, the natural expectation is that an increase in the intensity of light stimulation should produce an increase in cortical response by an increase in the firing rate of the sensory fibres (Maguire & Baizer, 1982; Derrington & Lennie, 1984). Across a large range of stimulation intensities the standard psychophysics power function should then be detected (Stevens, 1970). Inour experiments, the cortical response grew as a powerfunction of light intensity (Fig. 6). The response curve matched closely a straight line in log–log coordinates with a slope close to 0.1, in conditions where the stimulus

intensity values covered a range of ~2.5 log units. When the concentration of sevoflurane was increased, this log–log relation was shifted to higher response values across the entire range of intensities, while its slope wasleft unchanged. This pure linear effect contrasts with the action of propofol which seemed more complex,
including linear and non-linear changes in the response curve (Fig. 6).

Is our mechanistic interpretation for the effect of sevoflurane compatible with this linear scaling of light-intensity response curves? At least in theory, somefactors among those listed above (i–iv) could produce a linear change of the stimulus–response curve, for example the balance between excitation and inhibition (Murphy & Miller, 2003; Ayaz & Chance, 2009; Atallah*et al.* 2012), feedback circuits (Olsen *et al.* 2012) and modulation by background synaptic noise (Chance *et al.* 2002; Murphy & Miller, 2003; Ayaz & Chance,2009). Despite this, it is difficult to reconcile the above mechanisms with the frequency-dependent abolition of the sevoflurane-induced potentiation (Fig. 9), which suggests, also in light of its temporal dynamics, an activity-dependent change in synaptic strength (Zucker & Regehr, 2002). The advantage of our mechanistic hypothesis is that it would explain both the linear scalingof the stimulus–response curve and the use-dependency of potentiation. Indeed an increase in the number of releasable vesicles *N* by the sevoflurane-induced circuit silencing would render the activated synapses more reliable, with an increased average output. Since this change would be independent from the intensity of sensory activation, a linear scaling of stimulus–response curves would be expected. In addition, a series of closely repeated stimuli would deplete again the vesicular pool *N*, restoring the depressed synaptic state (Castro-Alamancos & Oldford, 2002; Swadlow *et al.* 2002; Borst, 2010; Reinhold *et al.* 2015). It is conceivable that with a higher synaptic reliability, postsynaptic targets would become more prone to respond synchronously, an idea which is consistent with earlier suggestions about the effects of some other anaesthetic compounds (Greenberg *et al.* 2008; Hudetz *et al.* 2009).

Conclusions

A formal testing of the here proposed model would require an independent quantification of the two *N* and *p* quantal parameters at *in vivo* brain synapses. In this respect,the analysis of synaptic vesicle dynamics would provide the most definitive answer. This information would be valuable not only to understand the effects of these drugs, but more importantly to make realistic models for sub- tractive and divisive gain modulation of cortical circuits for both tuning and intensity curves (Salinas & Thier, 2000; Murphy & Miller, 2003; Ayaz & Chance, 2009) that

should also integrate presynaptic dynamics. Unfortunately no technique is today available to approach quantitatively vesicle dynamics inthe living brain. Despite this limitation, some experimentally testable predictions can still be made. For example it would be very valuable to combine VEPs and single intracellular recordings from the V1 cortex during sevoflurane anaesthesia. Besides providing a more detailed analysis of the relationships between evoked and spontaneous activity, this combined approach mightrepresent an indirect testing of our presynaptic depressionhypothesis. If the stimulation of the thalamo-corticalpath could be restricted to a single action potential, for example by using modern optogenetic tools (Fenno *et al.*2011), it should be possible to evaluate the interactions between anaesthetics and some forms of short-term plasticity known to be expressed presynaptically (Smith & Augustine, 1988; Zucker & Regehr, 2002; Jackman *et al.* 2016). Conversely, by using the same optogenetic approach under deep anaesthesia, the generation of variable degrees and dynamics of visual stimulus-uncorrelated synaptic activity should restore the depressed level seen at low sevoflurane concentration or in the awake state, where a maximal level of depression should be reached. To conclude, our results might be used in the future to strengthen our understanding of the synaptic mechanisms underlying gain modulation in the cortex (Olsen *et al.* 2012). This would be especially important when it is relevant to address the contribution of background noise or stimulus-uncorrelated activity (Chance *et al.* 2002; Montesano *et al.* 2015) to the modulation of visual and other sensory modalities perception by attention and wakefulness.

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Additional information

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that couldbe construed as a potential conflict of interest.

Author contributions

The experiments were performed at the Scientific Institute San Raffaele, Division of Neuroscience, Neurobiology of Learning Unit, Via Olgettina 58, 20132 Milan, Italy. A.A., J.L. and
A.M. were responsible for study design; A.A. performed the experiments; A.A. and J.L. analysed the electrophysiological data;A.A., J.L., A.M., M.G., M.R., G.R., V.Z., A.D.V. and L.B. helped in data acquisition and interpretation of results. A.A., J.L. and
A.M. wrote the manuscript draft. All authors were then involved in revising the manuscript. All authors have approved the finalversion of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify forauthorship, and all those who qualify for authorship are listed.

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