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Journal of Neuroscience Methods 129 (2003) 19–31

JOURNAL OF NEUROSCIENCE METHODS

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On the use of isoflurane versus halothane in the study of visual response properties of single cells in the primary visual cortex☆

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Received 20 February 2003; received in revised form 16 June 2003; accepted 17 June 2003

Abstract

Halothane is a widely used anesthetic in research. It produces several alterations in organs, especially in the brain. Recently, isoflurane emerged in neuroscience laboratories. For many reasons it appears to be better than halothane for animal brain research (e.g. isoflurane induces lower intracranial pressure, and is less detrimental on the cardiovascular system). However, no one is in a position to recommend it in electrophysiology research because its effects on specific brain functions are relatively unknown. Given that both anesthetics yield different actions on gross brain activity (EEG, VEP), it is likely that they differentially affect single neuron activity. The goal of this study is to determine whether halothane or isoflurane use is best suited to study the receptive field properties of neurons in the cat's primary visual cortex. Extra-cellular recordings were made for both anesthetics in area 17 of adult cats under different levels of anesthesia. Results indicate that various cell parameters differ under halothane anesthesia when compared with isoflurane. The main difference between the two anesthetics is the greater depression of the cell optimal visual response amplitude induced by isoflurane at equipotent concentration. Due to its stronger depressive effects, isoflurane may not be the ideal anesthetic for single-cell recordings in the primary visual cortex.
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Keywords: Anesthesiology; Cat; Electrophysiology; Pharmacodynamics; Receptive fields; Striate cortex; Vision

1. Introduction

Halothane is one of the most widely used volatile anesthetics in animal research. As other anesthetics, it produces several specific alterations of bodily functions, especially brain functions. For instance, through its action on the cardio-vascular system, halothane causes an increase in cerebral blood flow (Wollman et al., 1964; Theye and Michenfelder, 1968; Cucchiara et al., 1974; Eger, 1985) and impairs cerebral autoregulation, which in turn lead to an increase in intra-cranial pressure (Drummond et al., 1983; Todd and Drummond, 1984). It also produces a dose-related depression of cardiac output (Eger, 1985) and may produce cardiac arrhythmia (Katz and Katz, 1966; Johnston et al., 1976; Bosnjak and Kampine, 1983; Hikasa et al., 1996). In addition to these effects, repeated exposure to halothane may be detrimental to the experimenter's health: this anesthetic produces hepatic toxicity (Allan et al., 1987; Franco, 1989) and may also alter neurobehavioral performance (Lucchini et al., 1996).

In recent years, some neuroscience laboratories have used another volatile anesthetic, isoflurane, because, when compared with halothane, it has little impact on cardio-vascular function and intra-cranial pressure (Drummond et al., 1983; Todd and Drummond, 1984; Frost, 1984). Despite the fact that isoflurane appears to be a better anesthetic than halothane for animal brain research, no one is in a firm position to recommend switching from halothane to isoflurane in electrophysiology because the effects of the latter on specific in-vivo brain functions are virtually unknown. To our knowledge, only a few studies have demonstrated that isoflurane and halothane differentially affect electroen-

☆ This work is attributed to the École d'optométrie of the Université de Montréal, Quebec, Canada.

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0165-0270/03/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0165-0270(03)00198-5

cephalographic recordings (Grundy, 1983; Ogawa et al., 1992; Tsushima et al., 1998) and visual evoked potentials (Domino et al., 1963; Chi and Field, 1986; Sebel et al., 1986; Uhl et al., 1980; Ogawa et al., 1992). Given that both anesthetics yield different actions on gross brain activity, it is likely that they may also differentially affect single neuron activity. This is of fundamental importance considering that many studies investigate the function of brain areas based on the response properties of comprising neurons.

The main goal of this study was to determine, using a widespread anesthesia protocol utilized in neurosciences, whether isoflurane may be used to study the receptive field properties of neurons in the cat's primary visual cortex. More specifically, the effect of halothane and isoflurane at varying concentrations on the orientation, direction, and spatial frequency tuning functions of striate neurons was evaluated. Part of this study was presented in abstract form (Villeneuve et al., 2000).

2. Materials and methods

2.1. Animal preparation

Nine adult cats of either sex weighing 2.5–4.5 kg were used in this study. They were treated in accordance with the guidelines of the Canadian Council for the Protection of Animals. The cats were food-deprived 24 h before the anesthesia. Animals were premedicated with a subcutaneous injection of acepromazine maleate (Atravet™ 10 mg/ml; 0.05 ml/kg; Ayerst Veterinary Laboratories, Ontario, Canada) and atropine (0.4 mg/ml; 0.1 ml/kg; Sabex®, Inc., Québec, Canada). Thirty minutes after the injection, anesthesia was induced by mask inhalation of 5% isoflurane (Forane®, Bimeda-MTC Santé Animale, Inc., Ontario, Canada) mixed with O_2/N_2O (50:50) and gradually lowered and maintained at 2% for the preparatory surgery. The depth of anesthesia was determined by the lack of response to clamping the inter-digital web of the posterior paws. N_2O was added to the mixture to reproduce the anesthetic condition most frequently used in visual neuroscience protocols (Hoffmann and Fischer, 2001; Schröder et al., 2002; Sengpiel and Bonhoeffer, 2002; Suder et al., 2002; Young et al., 2002).

Heart rate and oxygen blood saturation were monitored using an oxygen saturation meter (Model 8500, Nonin Medical, Inc., Minnesota, USA). Lidocaine hydrochloride (Xylocaine® 2%, Astra Pharma, Inc., Ontario, Canada) was infused or applied at all points of incision or pressure. Following cephalic vein cannulation and tracheotomy, the animal was paralyzed with gallamine triethiodide (2%; Sigma Chemical, Missoury, USA) and placed in a stereotaxic frame (D. Kopf). Muscular relaxation provided by the gallamine triethiodide supplies stability of eye position and constant location of the visual receptive field while increasing the stability of electrophysiological recordings. Throughout the experiment, the animal was artificially ventilated using a respiratory pump (Model 665, Harvard Apparatus) with an O_2/N_2O (33/66%) mixture supplemented using agent-specific Tec3-Ohmeda vaporizers of halothane (Fluothane®, Bimeda-MTC Santé Animale, Inc.) or isoflurane (Forane®, Bimeda-MTC Santé Animale, Inc.). This protocol was chosen because it is generally used in the investigation of receptive field properties in long-lasting neurosciences experiments. The use of 66% N_2O allows one to reduce the toxicity of O_2: since the experiment lasted several days, the administration of 100% O_2 over such a long time period would have been very detrimental for the animal physiology (Winter and Smith, 1972; Jenkinson, 1993). End-tidal gas samples were drawn from a non-rebreathing circuit, through a tube positioned at a Y-piece connection at the oral end of the endotracheal tube. End-tidal anesthetic concentration and CO_2 partial pressure was monitored by a capnometer (Normocap® 200, Datex-Ohmeda, Inc.) and the CO_2 partial pressure was maintained between a range of 28 and 34 mmHg by adjusting the rate and stroke volume of the respiratory pump between testing periods, i.e. that the pump settings were not modified during the experimental protocols. The temperature was maintained at 37 ± 0.5 °C by means of a feedback-controlled heating pad linked to a rectal probe. Electroencephalogram (EEG) recordings were made with stainless steel screws of 2 mm in diameter inserted in the frontal bone. Both EEG and electrocardiogram (ECG) were monitored throughout the experiment (Axoscope, Axon Instruments, Inc., USA). The animals were continuously infused with 5% dextrose in lactated Ringer's injection solution (Baxter Corporation, Ontario, Canada) containing gallamine triethiodide (50:50, 20 mg/kg per h). Pupils were dilated with atropine sulfate 1% (Isopto®, Alcon Canada, Inc., Ontario, Canada) and nictitating membranes were retracted with local application of phenylephrine hydrochloride 2.5% (Mydfrin®, Alcon Canada, Inc.). The eyes were also protected using contact lenses of appropriate power.

Following a craniotomy overlying area 17, the dura was incised to be able to visualize the cortex. Once the electrode was positioned, the exposed cortex was covered with warm agar (Difco Laboratories, Michigan, USA), over which melted wax was applied to create a sealed recording chamber.

2.2. Electrophysiological recordings

Varnished tungsten microelectrodes (2–4 MΩ; A-M Systems, Inc®., Carlborg, WA, USA) were utilized to record single-unit activity in area 17. Craniotomies were

performed over area 17 on both hemispheres, at Horsley-Clark coordinates AP −1 to −7, and ML 1.5–5. An angle of descent of $16 \pm 1^\circ$ in the coronal and sagittal plane with respect to the vertical was used, so the electrode was advanced roughly perpendicular to the surface of the cortex. The signals were amplified, displayed on an oscilloscope and played through an audio monitor. Neuronal activity of the recorded units was isolated from the overall signal using a window discriminator (WPI, Inc., Florida, USA) and fed to an acquisition program (spike2 v3.x, CED Cambridge, UK) via an analogue digital interface (1401, CED). Responses were recorded as post-stimulus time histograms (PSTH) of 10 ms bin width.

2.3. Visual stimulation

Receptive fields were first mapped and characterized with manually controlled stimuli (bars and circles) projected onto the tangent screen facing the animal, using a hand-held projector and an ophthalmoscope. Each unit was then quantitatively tested with sinusoidal gratings (60% contrast) drifting in the frontoparallel plane generated by the PIXX software (v. 2.03; Sentinel Medical Research Corp., Québec, Canada) driven by a Mcintosh G3 computer. The stimuli were back projected by a LCD projector (InFocus Systems) onto a translucent screen placed 57 cm in front of the animal and covering $70 \times 85^\circ$ of visual angle. The screen (Da-Lite) is made of a precise optical coating applied to an acrylic substrate (Da-Plex) allowing for a display of high optical quality and uniform light diffusion. The image had a resolution of 6.8 pixels/degree and the refresh rate was 67 Hz. Full-screen stimuli were presented for all cells, except for those showing inhibitory surrounds (end-stop cells) where only the receptive field was stimulated.

Grating orientation was varied over 360° in 12 or 24 steps of 30 and 15°, respectively. An orientation range of 360° also specifies the direction of motion. For example, 90 and 270° are both horizontal and denote opposite directions of motion. Cell responses at optimal orientation were also studied as a function of spatial and temporal frequencies. During each test, the activity for a blank screen of equal mean luminance was recorded (spontaneous activity level). Each stimulus presentation and spontaneous activity recording lasted 4 s and was repeated four times. Presentations were randomly interleaved and in most cases only the dominant eye was stimulated.

2.4. Anesthesia parameters

To be able to compare different anesthetics, here halothane and isoflurane, Merkel and Eger (1963) described an "index of comparison" between anesthetics which uses the term minimum alveolar concentration (MAC). They defined 1.0 MAC as the "minimal anesthetic concentration in the alveoli required to keep an animal from responding by gross purposeful movement to a painful stimulus". With this index, anesthetic doses could be transformed into multiples of MAC and compared on a single scale. Halothane and isoflurane have a 1.0 MAC value of 1.14% (Steffey et al., 1974) and 1.63% (Steffey and Howland, 1977), respectively. However, N_2O, whose MAC is at 250% for the cat, has an additive effect on MAC values of the primary agent (Steffey et al., 1974; Hikasa et al., 1996) and therefore, modifies those values. Knowing the effect of the addition of 66% N_2O on the primary agent potency from work by Hikasa et al. (1996), it was possible to calculate the true 1.0 MAC values for this protocol: 0.72% for halothane + 66% N_2O (halo-N_2O) and 1.19% for isoflurane + 66% N_2O (iso-N_2O).

There is a possibility that the premedicated agents have influenced MAC values. However, the recording period occurred several hours after the injection of acepromazine maleate and atropine, hence this possible influence is not considered here.

2.5. Experimental protocol

Following the isolation of a single cell, the characterization of its visual properties (orientation, spatial and temporal frequency tuning functions) was done and the neuron was then tested under different anesthetic conditions. The waveform of the action potential as well as the optimal orientation of the cell was continuously monitored to insure that the same unit was recorded throughout the anesthetic protocol. All tests were set at the cell's optimal parameters to reduce the variability of the neuronal response. Three interleaved orientation (varied over 360°) and spatial frequency (varied in relation to the bandwidth of each cell) tests were performed over a 30 min period (one test starting every 5 min) at a control anesthetic condition. The control condition was set at a low clinical level of anesthesia, usually 0.42 MAC of halo-N_2O, to increase the probability of isolating visually driven cells. This concentration corresponded to 0.5% of halothane on readings from a calibrated vaporizer, and 0.3% in the expired end-tidal (E-T) concentration reading on the capnometer which is a generally used concentration in electrophysiological studies (Hoffmann and Fischer, 2001; Schröder et al., 2002; Sengpiel and Bonhoeffer, 2002; Suder et al., 2002; Young et al., 2002). The depth of anesthesia was verified four times a day by a lack of heart rate change to clamping the digits of the anterior paws. Once control tests were performed, a pseudo-random change in anesthetic and/or concentration was made. Response properties were then recorded when either the concentration of the anesthetic was modified

or the agent itself was changed. After the change in anesthetics and/or concentration, no recording were made for the next 30 min. Following this period, it is generally agreed that there is no longer a mix of anesthetic and/or concentration neither in the lungs, the blood or the brain that could affect cell response (Ikeda and Wright, 1974; Ogawa et al., 1992; Tsushima et al., 1998)[1]. Orientation and spatial frequency tests were then performed to evaluate the effect of the new anesthesia condition (change in agent and/or concentration). This procedure was repeated for each new condition of anesthesia. Finally, at the end of the testing session, a single recovery condition consisting of a return to a low concentration condition was tested. The purpose of this condition was to assess whether the cell was still responsive even after the extended testing period and whether its response profile was maintained. Eight experimental conditions were generally used (two agents, and in most cases, four concentrations each) for a maximal recording time for each cell of 9–10 h (including characterization of the optimal parameters). During each recording, the heart rate, pCO_2, EEG and temperature were also quantitatively assessed. Each experiment lasted several days and we did not notice any deterioration of the physiological functions of each animal throughout the experiments. It is our experience that any decline in the animal health will result in aberrant recordings in the primary visual cortex (e.g. bursty pattern, increased spontaneous activity). This was never observed for the cell sample presented here.

2.6. Statistical analysis

The direction selectivity of each cell was measured by computing a direction index:

$$\mathrm{DI} = 1 - \frac{\text{response in the nonpreferred direction} - \text{spontaneous activity}}{\text{response in the preferred direction} - \text{spontaneous activity}}$$

An index value of >0.5 indicates that the cell was selective for the direction of motion of the stimulus (Minville and Casanova, 1998). Cells with $\mathrm{DI} < 0.5$ were considered as non direction selective.

The bandwidth of the orientation curve, which represents a measure of the sharpness of the selectivity of a cell, was calculated as the half-width of the tuning curve at half-height. Bandwidth of the spatial frequency tuning function was calculated as the full width at half-height. The organization of the sub-regions of a cell's receptive field was also examined by measuring a modulation index (MI; Skottun et al., 1991) from the cells' responses to drifting gratings:

$$\mathrm{MI} = \frac{\text{first harmonic of the optimal response (AC component)}}{\text{mean optimal response (DC component)} - \text{spontaneous activity}}$$

The MI can be used to differentiate between simple and complex cells. A value >1 indicates that a cell responded with a high degree of modulation in its discharge. This is the case of simple cell whose receptive fields are characterized by adjacent ON and OFF sub-regions. Neurons exhibiting a $\mathrm{MI} < 1$ did not show a strong modulation of their responses as do complex cells in the primary visual cortex because their receptive field is composed of superimposed ON and OFF sub-regions.

All response properties were normalized as the standard deviation (S.D.) of the mean of the control condition for every cell. With this procedure, all cells could be compared on a single level.

$$z\text{-score} = \frac{x - \mu}{\sigma}$$

Linear regressions were then performed on the cellular parameters for both agents under all the concentrations.

$$Y = A + B^{*}X$$

All cell parameters were best described with a linear fit. Analyses of covariance were then conducted with PRISM 3.0 (GraphPad Software, Inc.) to compare the linear regression slopes and y-intercepts. Slopes provided information on the influence of agent concentration on the measured property. If the responses presented the same slopes, y-intercepts were compared and provided information on the effect of the agent.

2.7. Histology

Electrolytic lesions were made along recording tracks. At the end of each experiment, the animal was killed by an intravenous overdose of pentobarbital sodium (euthanyl, 240 mg/ml, 2 cm^3/4.5 kg, Bimeda-MTC Santé Animale, Inc.). The brain was removed from the skull and immersed in a solution of buffered formalin (10%). Coronal serial sections of 40 μm were cut using a Microtome Cryostat HM500 OM (Microm International GmbH, Walldorf, Deutschland) and stained with Cresyl Violet. The laminar position of the recorded cells was then assessed.

[1] It is essential to point out here that the animal was continuously under anesthesia. Switching from one volatile agent to the other consisted in turning off one agent and concomitantly turning on the other one. Therefore, during a brief epoch, both agents were present in the animal, and the 30 min period was aimed at going back to stable anesthetic conditions. There was never a moment during which isoflurane or halothane was not administered.

3. Results

3.1. Physiological measures

3.1.1. Heart rate

The heart rate of the animal was affected by general anesthesia under both halo-N_2O and iso-N_2O. Table 1 shows the mean heart rate of all cats under every conditions. It illustrates the close relationship between an increase in anesthetic concentrations of both agents and the slowing of the heart rate of the animal (halo-N_2O: $F_{1,96} = 81.75$, $P < 0.0001$; iso-N_2O: $F_{1,45} = 59.19$, $P < 0.0001$). This suppressive action was identical for both agents ($F_{1,141} = 2.97497$, $P = 0.08675$), i.e. an increase in halo-N_2O concentration produces the same diminution of heart rate as an equivalent increase in iso-N_2O concentration. Due to this correlation (halo-N_2O: $r = -0.67817$, $P < 0.0001$; iso-N_2O: $r = -0.75372$, $P < 0.0001$) and taking into consideration that both agents are similar at a given MAC value ($F_{1,142} = 3.06986$, $P = 0.08191$), the heart rate is visibly a good indicator of the anesthetic depth. Taken as a whole, halo-N_2O and iso-N_2O clearly alter, in a similar manner, the heart rate of the animal in an inverse concentration–response relationship.

3.1.2. Lungs pCO_2

The partial pressure of CO_2 (pCO_2) in the lungs was reduced when halo-N_2O or iso-N_2O concentrations were increased (halo-$N_2O = F_{1,66} = 21.70$, $P < 0.0001$; iso-$N_2O = F_{1,30} = 5.474$, $P = 0.0262$) as shown in the Table 1. This relationship (halo-N_2O: $r = -0.49743$, $P < 0.0001$; iso-N_2O: $r = -0.39282$, $P = 0.02615$) between an increase of concentration and the diminution of pCO_2 is identical for both anesthetics ($F_{1,96} = 0.0069$, $P = 0.934$). However, a difference emerges between the two anesthetics when compared at a single concentration. For a fixed MAC value, pCO_2 was more reduced under halo-N_2O than iso-N_2O ($F_{1,97} = 16.9136$, $P < 0.0001$).

3.2. Cerebral functions

3.2.1. Electroencephalogram

EEG recordings, which measure the general activity of the brain, were greatly altered by a change in the administered concentration of either halo-N_2O or iso-N_2O. The recordings presented in Fig. 1 show representative recordings of cat's EEG. At lower concentrations, there were no differences between halo-N_2O and iso-N_2O anesthesia. However, as the concentration is increased to 1.0 MAC (i.e. 0.72 and 1.19% E-T for halo and iso, respectively), the optimal frequency decreased for both agents as revealed by the power spectrum analysis. Despite these similar changes, a specificity of iso-N_2O anesthesia emerged at 1.38 MAC (i.e. 1.0 and 1.64% E-T for halo and iso, respectively). At this concentration of iso-N_2O, burst–suppression patterns appeared which reflect a deep suppression of brain activity. This activity was not present in any of the recordings made under halo-N_2O anesthesia. Burst–suppression patterns gradually became dominant with

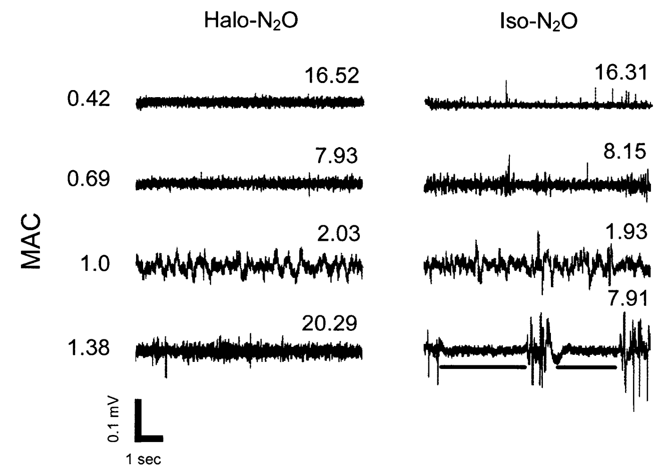

Fig. 1. EEG recordings under different levels of anesthesia (MAC) for halothane and isoflurane mixed with 66% N_2O and 33% O_2. The numbers in the above right corner of traces are the optimum frequencies (Hz) revealed by FFT analysis. The presence of burst–suppression patterns observed under 1.38 MAC of iso-N_2O are underlined. MAC values of 0.42, 0.69, 1.0 and 1.38 correspond to 0.30, 0.5, 0.72 and 1.0% E-T for halo and 0.5, 0.82, 1.19 and 1.64% E-T for iso, respectively.

Table 1
Physiological measurements

	Halothane				Isoflurane			
MAC values	0.42	0.69	0.97	1.38	0.25	0.69	1.0	1.38
Heart rate (h.b./min)	221 ± 18	210 ± 23	210 ± 14	174 ± 13	223 ± 12	216 ± 2.5	213 ± 20	161 ± 14
pCO_2 (mmHg)	31.92 ± 1.5	32.45 ± 2.3	28.25 ± 1.7	28.4 ± 1.9	32.2 ± 2.3	33.9 ± 2.5	32.5 ± 2.3	28.8 ± 2.5

Summary of physiological measurements. Mean ± S.D. Some MAC values do not closely correspond between agents because we either study their influence on body function at similar concentration or MAC values. MAC values of 0.42, 0.69, 0.97 and 1.38 correspond to 0.30, 0.5, 0.70, and 1.0% E-T for halo. For iso, MAC values of 0.25, 0.69, 1.0 and 1.38 correspond to 0.30, 0.82, 1.19, and 1.64% E-T, respectively.

time to the point where the brain became almost electrically silent, suggesting that it was not responsive. This is not the case, since EEG bursts at 1.38 MAC (i.e. 1.64% E-T) of iso-N_2O could be generated by non-specific peripheral stimulation (hand clapping, touch or visual stimulation). This suggests that even if EEG recordings show a deep state of anesthesia, known as isoelectric, the brain is not necessarily silenced or unresponsive; it retains the ability to react to basic stimulation. At 1.38 MAC of halo-N_2O (i.e. 1.0% E-T), a return to a high frequency mode was observed, without the presence of burst–suppression patterns. These findings indicate that the suppression of general brain activity, as measured by EEG, is similar for both agents at low level of anesthesia. However, for higher concentrations, it seems that the two agents produce different modifications of the brain activity.

3.2.2. Single cell response amplitude

A total of 85 neurons were recorded in the primary visual cortex. Twenty-two units were kept for analysis, and consisted in 15 simple and seven complex cells. The remaining units could either not be tested with confidence (high level of variability) or were lost before the completion of the long-lasting protocol.

Optimal responses to orientation and spatial frequency were strongly affected by the anesthetic: iso-N_2O anesthesia reduced the optimal amplitude of cell's visual responses to a larger extent than halo-N_2O ($F_{1,219} = 4.98845$, $P = 0.0265$). Fig. 2A shows the PSTHs of a representative area 17 cell recorded under different anesthetic conditions, and Fig. 2B illustrates the corresponding optimal cellular response amplitude. It can be seen that for both agents, responsiveness varied as a function of concentration (halo-N_2O: $F_{1,139} = 4.574$, $P = 0.0342$; iso-N_2O: $F_{1,79} = 4.484$, $P = 0.0374$). As the MAC value of halo-N_2O and iso-N_2O increases, a decrease in the optimal amplitude of the cell's visual response is observed. This inverse relationship (halo-N_2O: $r = -0.17848$, $P = 0.03422$; iso-N_2O: $r = -0.23176$, $P = 0.03736$) is similar for both agents ($F_{1,218} = 0.00256$, $P = 0.9597$), i.e. an identical increase in halo-N_2O or iso-N_2O concentration produces a similar diminution of the optimal response. More interestingly is the fact that the amplitude of the optimal response is much more strongly reduced in presence of iso-N_2O. At 0.42 and 0.69 MAC (i.e. 0.42 MAC = 0.30 and 0.5% E-T for halo and iso, respectively; 0.69 MAC = 0.5 and 0.82% E-T for halo and iso, respectively), the mean discharge of the neuron was greater by a factor of 1.99 and 1.61 with halo-N_2O when compared with iso-N_2O, respectively (see panel B). No reliable responses could be recorded when the cell was studied under iso-N_2O 1.38 MAC (i.e. 1.64% E-T) anesthesia. The more depressant effect of isoflurane is illustrated in panel C of Fig. 2 that presents, for all neurons, the relationship between the normalized optimal discharge amplitudes and the agents' concentrations. Clearly, even at low concentrations, iso-N_2O had a more profound effect on the optimal neuronal responses than halo-N_2O. The strength of the response is an important key for a successful experiment and halo-N_2O anesthesia appears to provide better recording conditions than iso-N_2O.

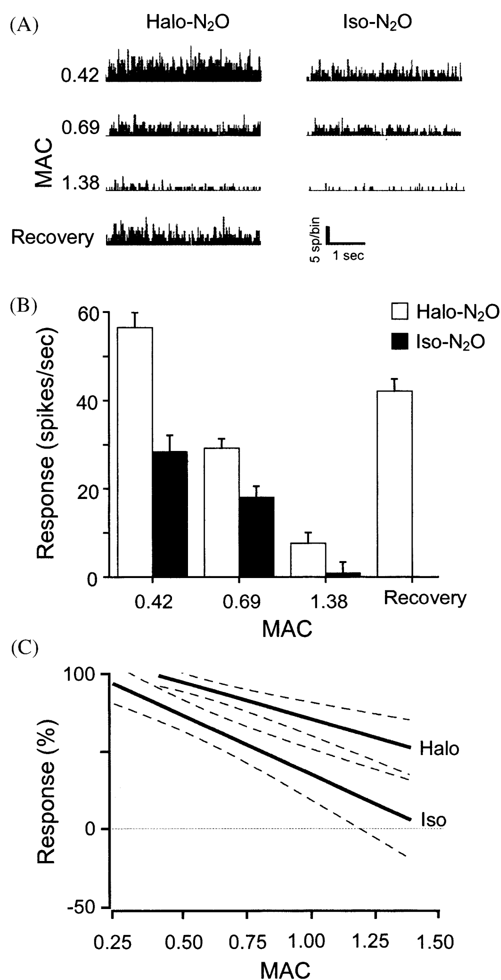

Fig. 2. A: Response shows PSTHs of a complex cell (MI = 0.48) in area 17 to a drifting grating presented at optimal parameters (Orientation: 240°, Spatial frequency: 0.3 c/degree, Temporal frequency: 4 Hz) as a function of anesthesia level and type. Panel B shows the corresponding amplitude of the responses (mean and S.E.M.). Note that the neuronal discharges remain unmodulated (complex cell) throughout the testing period (unaltered response profile) despite the strong reduction in visual responsiveness. Panel C illustrates the effect of anesthetic agent and concentration on the normalized optimal cellular response of the whole population tested. The dotted lines represent the confidence intervals. MAC values of 0.42, 0.69 and 1.38 correspond to 0.30, 0.5, and 1.0% E-T for halo and 0.5, 0.82 and 1.64% E-T for iso, respectively. sp/bin: spikes per bin.

3.2.3. Optimal spatial frequency and tuning function

Spatial frequency was also measured as a function of the concentration of halo-N_2O and iso-N_2O. Fig. 3 illustrates the spatial frequency tuning functions of area

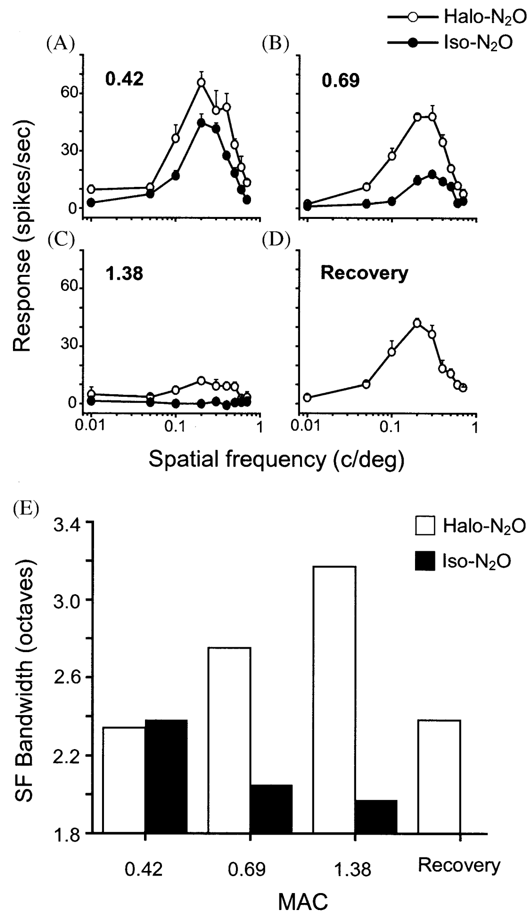

Fig. 3. Panel A–C present spatial frequency tuning functions (mean response and S.E.M.) of a complex cell (MI = 0.48) tested at 0.42, 0.69, 1.38 MAC, respectively, for halo-N_2O (empty symbols) and iso-N_2O (filled symbols). Panel D shows the spatial frequency tuning function for the recovery condition that corresponds to 0.42 MAC of halo-N_2O. Panel E presents the spatial frequency bandwidths of another complex cell (MI = 0.27). For this cell the recovery condition was also set at halo-N_2O 0.42 MAC. MAC values of 0.42, 0.69 and 1.38 correspond to 0.30, 0.5, and 1.0% E-T for halo and 0.5, 0.82 and 1.64% E-T for iso, respectively.

17 cells in different conditions of anesthesia. Panels A–C present the spatial frequency tuning functions of a complex cell at 0.42, 0.69 and 1.38 MAC under halo-N_2O and iso-N_2O anesthesia (i.e. MAC values of 0.42, 0.69 and 1.38 correspond to 0.30, 0.5 and 1.0% E-T for halo and 0.5, 0.82 and 1.64% E-T for iso, respectively). Fig. 3D illustrates the recovery condition that was set at 0.42 MAC halo-N_2O (0.30% E-T) and illustrates the fact that the cell exhibited the same response profile (optimal spatial frequency and tuning functions) at the end of the recording session. As for the cell shown in Fig. 2A–B, iso-N_2O has a much more profound effect on responses amplitude than halo-N_2O. In addition, Fig. 3A–C show that the preferred spatial frequency was not modified by changing the anesthetic agent ($F_{1,91} = 0.416293$, $P = 0.5204$) or the concentration (halo-N_2O: $F_{1,61} = 0.1571$, $P = 0.6936$; iso-N_2O: $F_{1,29} = 3.156$, $P = 0.0862$). The level of anesthesia did not interfere with the preferred spatial frequency (halo-N_2O: $r = 0.05069$, $P = 0.6936$; iso-N_2O: $r = -0.31327$, $P = 0.08616$). This indicates that under all tested conditions of anesthesia, the optimal spatial frequency remains constant in area 17.

A different picture emerged when considering spatial frequency selectivity. Fig. 3E shows the bandwidths of the spatial frequency tuning curves of another area 17 cell. The cell's behavior is representative of the units tested and can be summarized as follows: (1) an increase in halo-N_2O concentration reduces the spatial selectivity of the cell by increasing its spatial frequency bandwidth ($F_{1,34} = 11.17$, $P = 0.002$); (2) iso-N_2O enhances spatial frequency selectivity with increasing concentration ($F_{1,20} = 4.41$, $P = 0.0486$). The differing effects of halo-N_2O and iso-N_2O on spatial frequency selectivity are emphasized by the different relation between an increase of halo-N_2O and iso-N_2O on spatial selectivity (halo-N_2O: $r = 0.49726$, $P = 0.00203$; iso-N_2O: $r = -0.42505$, $P = 0.04861$). This indicates that an increase in the concentration of one agent produces divergent results compared with an increase in concentration of the other ($F_{1,54} = 14.164$, $P = 0.0004$).

3.2.4. *Orientation and direction selectivity functions*

Orientation selectivity was not affected by the condition of anesthesia. A representative example is shown in Fig. 4. Changing the agent ($F_{1,159} = 0.210216$, $P = 0.6472$) or concentration (halo-N_2O: $F_{1,106} = 0.8668$, $P = 0.3540$; iso-N_2O: $F_{1,49} = 2.762$, $P = 0.1029$) has no significant influence on the cell's orientation selectivity, as measured by bandwidth. A simple change of anesthetic, for a fixed MAC value, does not modify the orientation selectivity, i.e. both agents have the same action on orientation selectivity ($F_{1,159} = 0.2102$, $P = 0.6472$). The absence of a relationship between concentration and orientation selectivity (halo-N_2O: $r =$

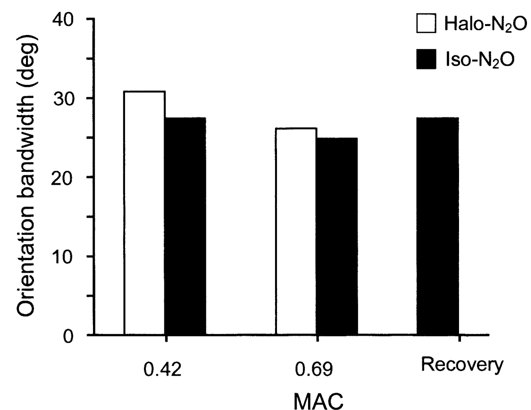

Fig. 4. Orientation tuning bandwidths of a complex cell (MI = 0.30) under different concentrations of halo-N_2O and iso-N_2O. The recovery condition consisted of halo-N_2O 0.42 MAC. MAC values of 0.42 and 0.69 correspond to 0.30 and 0.5% E-T for halo and 0.5 and 0.82% E-T for iso, respectively.

0.09006, $P = 0.35397$; iso-N_2O: $r = -0.231$, $P = 0.10291$) indicates that this parameter is independent of the anesthetic level.

Direction selectivity was also assessed under different conditions of anesthesia. As shown by the cells presented in Fig. 5A and B, direction selectivity was not affected by the agent of anesthesia ($F_{1,159} = 0.210216$, $P = 0.6472$) nor by concentration (halo-N_2O: $F_{1,103} = 0.1678$, $P = 0.6829$; iso-N_2O: $F_{1,55} = 0.4478$, $P = 0.5062$). This was observed for direction selective (panel A) and non-direction selective cells (panel B). Here again, for a fixed MAC value, both agents are identical ($F_{1,158} = 0.210216$, $P = 0.6472$) when the direction selectivity is considered. The absence of a relationship between concentration and direction selectivity (halo-N_2O: $r = -0.04013$, $P = 0.68438$; iso-N_2O: $r = -0.089870$, $P = 0.50618$) indicates that the level of anesthesia did not affect this parameter.

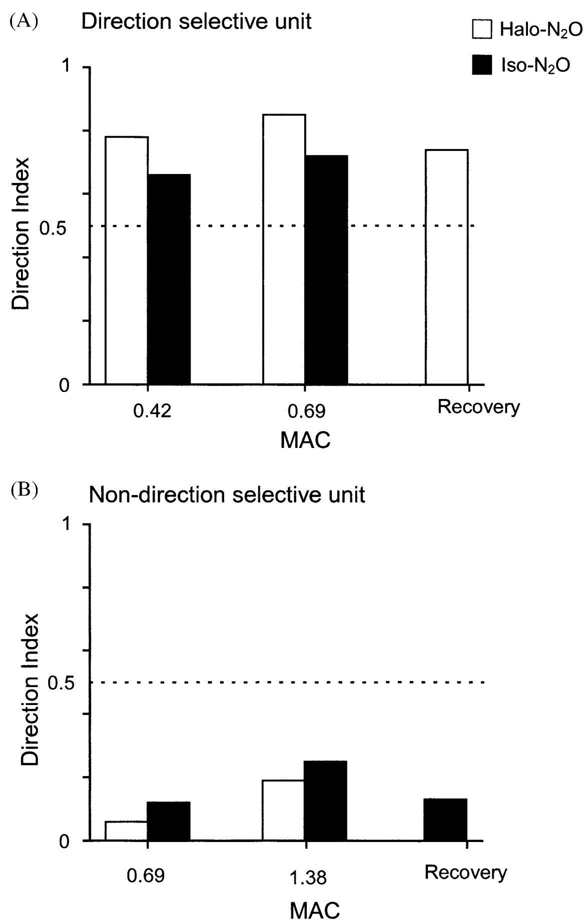

Fig. 5. Direction indices of two cells under different levels of halo-N_2O and iso-N_2O anesthesia. Panel A presents the data for a direction selective complex cell (MI = 0.47) and panel B, for a non-direction selective simple cell (MI = 1.81). The recovery conditions was 0.42 MAC of halo-N_2O for cell A and 0.69 MAC of iso-N_2O for cell B. The dotted line represents the limit at which direction selective (> 0.5) and non-direction selective cells (< 0.5) are distinguished. MAC values of 0.42, 0.69 and 1.38 correspond to 0.30, 0.5, and 1.0% E-T for halo and 0.5, 0.82 and 1.64% E-T for iso, respectively.

3.2.5. Response profile

The response profile to drifting gratings provides information on the structural organization of the subregions of the receptive field (Skottun et al., 1991). Fig. 6A presents the response profile of a simple cell to a drifting grating, and panel B shows the corresponding computed MIs. Panel C and D depict the response profiles and MIs of a complex cell. These data illustrate that the level of modulation of the neuronal discharge was not affected by the anesthetic ($F_{1,163} = 0.212827$, $P = 0.6452$) nor the concentration (halo-N_2O: $F_{1,102} = 0.1760$, $P = 0.6757$; iso-N_2O: $F_{1,60} = 0.4229$, $P = 0.5180$) despite the fact that the strength of the responses of the two neurons was strongly reduced, particularly in the iso-N_2O condition. Both cells' response profiles remained identical throughout the testing period, suggesting that the organization of the ON–OFF regions was kept constant. In other words, simple and complex cells remained as such under any condition of anesthesia (agent/concentration). No level of anesthesia influenced the structural composition of the sub-regions of the receptive field (halo-N_2O: $r = -0.04151$, $P = 0.6757$, iso-N_2O: $r = -0.08366$, $P = 0.51796$), indicating the absence of a relationship between anesthetic concentration and modulation index. When compared on a single

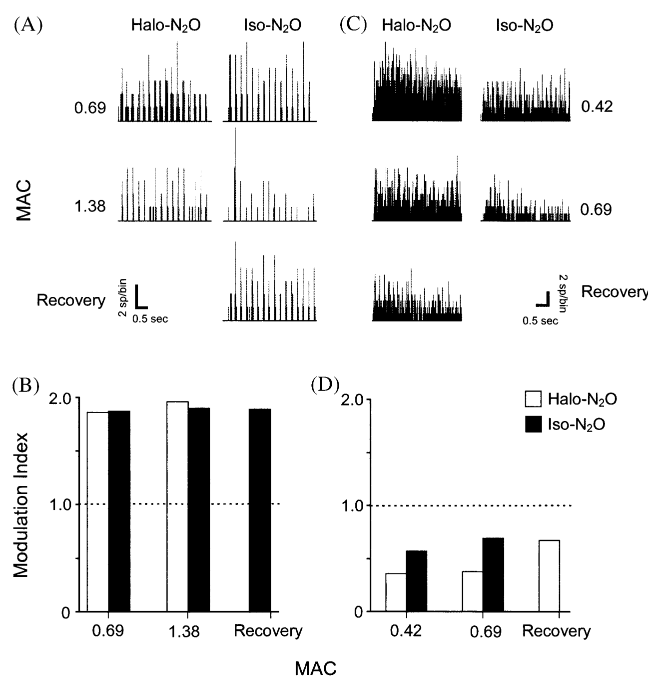

Fig. 6. Modulation indices of two cells under different conditions of anesthesia. Panel A shows the responses (PSTHs) of a simple cell to a drifting grating in different anesthetic conditions and panel B, the corresponding MIs. Panels C and D depict the response profile and MIs of complex cell, respectively. The recovery conditions were set at 0.69 MAC of iso-N_2O for cell A and 0.42 MAC of halo-N_2O for cell C. The dotted lines represent the value at which simple (< 1) and complex cells (> 1) are separated. MAC values of 0.42, 0.69 and 1.38 correspond to 0.30, 0.5, and 1.0% E-T for halo and 0.5, 0.82 and 1.64% E-T for iso, respectively. sp/bin: spikes per bin.

MAC value level, both agents are indistinguishable ($F_{1,159} = 0.210216$, $P = 0.6472$).

3.2.6. *Spontaneous activity*

Spontaneous activity reflects the non-specific activity of a neuron. As shown by a representative cell in Fig. 7, iso-N_2O significantly reduces the level of spontaneous activity of the cell when compared with equipotent concentration of halo-N_2O ($F_{1,176} = 12.3902$, $P = 0.0005495$). At all MAC values, there is less spontaneous activity under iso-N_2O anesthesia than under halo-N_2O. It seems that an increase in anesthetic concentration diminishes the level of spontaneous activity, though this effect does not reach significance for either agent (halo-N_2O: $F_{1,109} = 0.4188$, $P = 0.5189$; iso-N_2O: $F_{1,66} = 0.2953$, $P = 0.5886$). The absence of a link between the concentration of an agent and spontaneous activity (halo-N_2O: $r = -0.06187$, $P = 0.51891$; iso-N_2O: $r = 0.06675$, $P = 0.58864$) indicates that spontaneous activity was less dependent on the level of anesthesia than were evoked responses.

There was no clear relationship between the cells signal-to-noise ratio and the nature or concentration of the agent used. Statistical analysis revealed that this ratio did not significantly differ between halo-N_2O and iso-N_2O conditions ($F_{1,200} = 0.0385$, $P = 0.8446$), and did not vary as a function of the concentration of a given anesthetic (halo-N_2O: $F_{1,131} = 3.64$, $P = 0.0584$; iso-N_2O: $F_{1,70} = 2.021$, $P = 0.1596$).

4. Discussion

The main result of this study is that iso-N_2O anesthesia lowers the optimal response amplitude of cortical neurons when compared with equivalent levels of halo-N_2O. However, specific response properties such as orientation and direction selectivity, optimal spatial frequency, and response profile were not significantly altered by the condition of anesthesia. The latter observation does not stands for spatial frequency selectivity that appeared to be agent and concentration dependent.

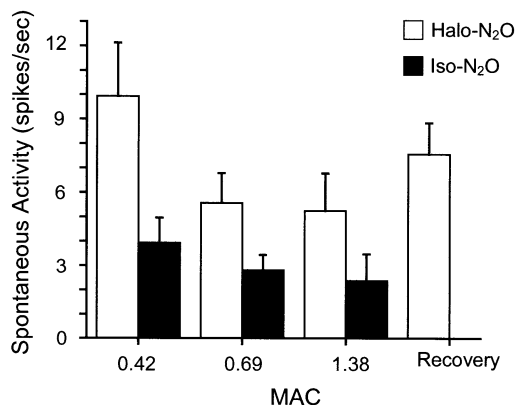

Fig. 7. Spontaneous activity levels (mean and S.E.M.) of a complex cell (MI = 0.47) under different conditions of anesthesia. The recovery condition consisted of 0.42 MAC of halo-N_2O. MAC values of 0.42, 0.69 and 1.38 correspond to 0.30, 0.5, and 1.0% E-T for halo and 0.5, 0.82 and 1.64% E-T for iso, respectively.

The animal's heart rate and pCO_2 was also very sensitive to the level of anesthesia. When the concentration of both agents was increased, both physiology parameters decreased significantly. In addition, agent specific modifications occurred. For a given MAC value, halo-N_2O maintained lungs pCO_2 at lower levels than an equipotent concentration of iso-N_2O.

4.1. *Cardiopulmonary functions*

Heart rate is often used to determine the level of anesthesia (McKelvey and Hollingshead, 2000). In our protocol, there was a close relationship between the concentration of both anesthetics and the heart rate of the animal. Increasing the MAC of both agents yielded a decrease in heart rate. This was observed for both agents in a similar fashion: halo-N_2O and iso-N_2O appear to have a comparable action on cardiac frequency, regardless of the concentration used. The inverse concentration–heart rate relationship reported here is in agreement with previous findings (Ikeda and Wright, 1974; Hartsfield and Sawyer, 1976; Ingwersen et al., 1988; Hikasa et al., 1997; Hodgson et al., 1998). This suggests that monitoring heart rate would help the experimenter in determining and controlling the level of anesthesia, even though it remains a crude indicator. A few studies, however, do not support this statement. Grandy et al. (1989) reported that halothane does not produce heart rate alterations in cats when the concentration is increased from 1.3 to 2.0 MAC. In addition, Steffey and Howland (1977) described that isoflurane had virtually no effect on the cat heart frequency when the anesthetic dose was increased from 1 to 2.4 MAC. These two studies suggest that heart frequency is not a reliable indicator of anesthetic depth. However, the range of concentrations used in their protocol differs greatly from ours. The main explanation for these conflicting results may be that there is a non-linear relationship between anesthesia level and heart frequency. It is possible that the slope of the relationship between concentration and heart rate changes around 1.3 MAC (floor effect). One may also propose that the divergent results may arise from differences between experimental protocols (spontaneous vs. controlled ventilation; N_2O-rich gas mixture vs. N_2O free gas). These factors, however, are not believed to significantly alter heart rate (Grandy et al., 1989; Hikasa et al., 1996; Hodgson et al., 1998). However, the neuromuscular blocking drug that was used in the present research, gallamine triethiodide, has a strong vagolytic effect

(Hughes and Chapple, 1976) and may have altered the present findings. Even if the dosage was the same in every condition, the interaction with the anesthetic agents could be different. The results presented here reflect the impact of different anesthetic agents on heart rate in commonly used neuroscience experimental protocols. The interpretation of the heart rate results is somehow difficult because other drugs must be administered to increase the success of the experiment (gallamine triethiodide, N_2O). A specific study of anesthetic impact on cardiac parameters would provide more insight in this matter.

pCO_2 values are commonly used to evaluate the metabolism of the animal and to adjust the respiration parameters in artificially ventilated animals. In our experimental conditions, halo-N_2O tended to maintain pCO_2 readings to values lower than those observed during an equipotent iso-N_2O anesthesia. For both anesthetics, there is a dose–dependent relationship such that increasing the agent concentration produces a decrease in pCO_2. This finding is at odds with those previously described. Steffey and Howland (1977), Grandy et al. (1989) and Hodgson et al. (1998) reported that an increase in the concentration of halothane or isoflurane produces an increase in pCO_2. This discrepancy may result from important differences between their protocols and ours. Again, the concentrations they used were very different from ours. In addition, the time course of the experiments differed largely (several hours vs. several days); this is an important factor given that pCO_2 is influenced by the time course of surgery and consequently of the anesthesia (Hikasa et al., 1998). Furthermore, the ventilation modes were dissimilar (spontaneous vs. artificially controlled) and may have contributed to the discrepancy, as controlled ventilation appears to reduce the pCO_2 when compared with spontaneous breathing (Grandy et al., 1989; Hodgson et al., 1998). Nevertheless, it is important to note that, even if halo-N_2O reduces lungs pCO_2 to lower values than equipotent concentrations of iso-N_2O, the CO_2 partial pressure of the expired volume always remains within a normal range, i.e. 28–34 mmHg.

4.2. Brain recordings

In animal neuroscience research, gross brain activity is almost always monitored to determine the state of activity (synchronized vs. desynchronized) and by extension the level of anesthesia. In agreement with Ogawa et al. (1992) and Tsushima et al. (1998), the present study demonstrates the presence of burst–suppression patterns in the EEG of isoflurane anesthetized animals but not in halothane preparations. This indicates that isoflurane has a different effect on gross brain activity than halothane. This affirmation is confirmed by the fact that multi-unit activity in the reticular formation (source of unspecific projections to the cortex, involved in the state of vigilance and EEG (Mori et al., 1971)) is also more reduced with isoflurane (Ogawa et al., 1992; Tsushima et al., 1998). More interestingly perhaps, are the several reports (Domino et al., 1963; Chi and Field, 1986; Sebel et al., 1986; Uhl et al., 1980) that indicate the gross visual activity of primary visual cortex, as measured with visually evoked potentials (VEPs), is considerably more affected by isoflurane than halothane anesthesia. Isoflurane reduced VEPs amplitude and enhanced the peak implicit time, whereas halothane only influenced the latter (also by enhancing latency). Altogether, these data suggest that the recording and characterization of single neurons would be more difficult under isoflurane. Our main findings support this suggestion. To our knowledge, this study is the first to clearly demonstrate that, when compared with equipotent halo-N_2O dosages, iso-N_2O has a more profound depressing effect on the spontaneous activity and optimal visual responses of neurons in the cat primary visual cortex. The dose–response relationship (reduced optimal discharges with increasing concentration) was observed for both agents but differed quantitatively for each. The iso-N_2O-induced reduction was so robust at higher MAC values that reliable responses could not be elicited, whereas equipotent concentrations of halo-N_2O allowed clear responsiveness. One may suggest that the greater effect of iso-N_2O as compared with halo-N_2O on response amplitude might be due to the fact that isoflurane has a more profound effect on blood pressure and on the level of cerebral perfusion (Drummond et al., 1983; Todd and Drummond, 1984; Frost, 1984). However, most of our data do not support such an assumption, mainly due to the fact that there is a maintained level of spontaneous activity while increasing isoflurane concentrations.

Only two studies have previously investigated the effects of halothane and isoflurane on the activity of visual cells in the primary visual cortex, but neither compared both agents (Ikeda and Wright, 1974; Tigwell and Sauter, 1992). Ikeda and Wright (1974) reported findings similar to ours: halo-N_2O decreased the amplitude of responses to all orientations and spatial frequencies, as concentration was increased. In contrast to the present work, they reported that this amplitude reduction was accompanied by a loss of both orientation and spatial frequency selectivity, where we only found the latter. Unfortunately, they did not compared halothane observations with other agents.

Tigwell and Sauter (1992) presented some evidences that reliable visual responses in the monkey primary visual cortex could be elicited under isoflurane. While this study supplied valuable information, it did not provide any comparisons with other concentrations or agents. Their statement that responses could be recorded in an isoflurane preparation was not surprising given the

low concentrations they used (0.5–0.9%; MAC values not given); concentrations at which visual responses in the cat were recorded in the present study. In addition, the authors did not mention whether the concentration used represents vaporizer readings or actual end-tidal volume values, and no 'peripheral' recordings were made. These factors make it virtually impossible to determine the level of anesthesia in their animals and to compare it with ours.

As stated above, both anesthetics yield, in general, an overall decrease in visual responses. Specific properties such as orientation selectivity, direction selectivity and the organization of receptive field sub-regions (revealed by the modulation index) were not affected by the condition of anesthesia, at any MAC values. Interestingly, the mechanisms implicated in shaping orientation selectivity, direction selectivity and receptive field organization are believed to be mediated by γ-aminobutyric acid (GABA; Sillito, 1977; Tsumoto et al., 1979). It is thus surprising that they were not sensitive to halo-N_2O or iso-N_2O given that the activity of $GABA_A$ receptors is enhanced in the presence of clinically-relevant concentrations of volatile anesthetics in vitro (Nakahiro et al., 1989; Jones et al., 1992; Lin et al., 1992; Franks and Lieb, 1994; Mihic et al., 1997; Jenkins et al., 1999; de Sousa et al., 2000). One possibility is that mechanisms that are not GABA-mediated are also involved in shaping receptive field properties in the visual cortex (Alonso et al., 1996). Another possibility would be that the effect of volatile anesthetics is more complex in vivo and/or that they do not act solely as GABA agonists. Both possibilities are not mutually exclusive and may explain the apparent contradictory data obtained when studying spatial frequency selectivity. In contrast to orientation, the spatial frequency tuning width of cortical cells was affected by the anesthesia protocols: while increasing halo-N_2O concentration reduced the spatial frequency selectivity by increasing the bandwidth of the tuning curve (similarly reported by Ikeda and Wright, 1974), iso-N_2O had the opposite effect (Fig. 3). The divergent action of the two anesthetics on spatial frequency selectivity may further suggest that they use a different molecular basis of action. Halo-N_2O tended to broaden the spatial tuning function of the cells, mimicking the effect of $GABA_A$ receptor antagonist (Vidyasagar and Mueller, 1994), while isoflurane seems to act as a $GABA_A$ receptor agonist by increasing the spatial frequency selectivity.

In conclusion, this study provides the first evidence that iso-N_2O has a larger depressing effect on the extracellular single-cell activity of neurons in the primary visual cortex. Given its low toxicity, its weak effect on intracranial pressure and its low blood/gas partition, we propose that, for single cells recording in area 17, iso-N_2O should be used for anesthesia induction, neurosurgery, electrode placement, and in case of prolonged arrhythmia. We recommend switching to halo-N_2O during recording sessions as this anesthetic has proven to be less detrimental to neural discharges, allowing for robust and reliable response recordings.

It is well known that light levels of anesthesia increase the likeliness of finding cells and recording robust visual responses, the latter allowing for a thorough characterization of the cells' receptive fields. For this reason, in a majority of in vivo neuroscience studies, extracellular recordings are made under light anesthesia; the volatile agent concentration on the vaporizer ranging between 0.2 and 0.7% (Dragoi et al., 2000; Hoffmann and Fischer, 2001; Sengpiel and Bonhoeffer, 2002; Suder et al., 2002), which is a very light anesthesia if one considers the E-T concentrations. Based on our study, halo-N_2O anesthesia offers a better environment for single-cell recordings in the primary visual cortex for both the animal and procedural reasons. Halo-N_2O concentration can be set to higher concentrations that insure a better level of anesthesia while permitting the recording of vigorous visual responses. One would not have such latitude in using iso-N_2O considering the more depressant effect of this agent. For example, to obtain robust responses in the iso-N_2O condition comparable to those recorded with halo-N_2O at 0.8 MAC (0.58% E-T), one would have to set isoflurane to values as low as 0.4 MAC (0.45% E-T). On the other hand, increasing the iso-N_2O MAC value to 0.8 (0.95% E-T) will reduce the response by 50% which would not only decrease the success rate in finding cells but also the possibility to adequately drive these neurons with visual stimuli such as drifting gratings, as well as more complex ones.

Acknowledgements

This work was supported by a grant from NSERC (Canada) to C.C. Part of the salary of C.C. was provided by FRSQ. The Réseau FRSQ de recherche en santé de la vision provided a student fellowship to M.V. We thank B. Ouellette and F. Huppé-Gourgues for their help during the experiments and subsequent data analysis, and D. Boire for statistical analysis. We are also grateful to S. Cuvelliez for her helpful comments on a draft of the manuscript.

References

Allan LG, Hussey AJ, Howie J, Beckett GJ, Smith AF, Hayes JD, Drummond GB. Hepatic glutathione *s*-transferase release after halothane anaesthesia: open randomised comparison with isoflurane. Lancet 1987;1:771–4.

Alonso JM, Usrey WM, Reid RC. Precisely correlated firing in cells of the lateral geniculate nucleus. Nature 1996;383:815–9.

Bosnjak ZJ, Kampine JP. Effects of halothane, enflurane, and isoflurane on the SA node. Anesthesiology 1983;58(4):314–21.

Chi OZ, Field C. Effects of isoflurane on visual evoked potential in humans. Anesthesiology 1986;65:328–30.

Cucchiara RF, Theye RA, Michenfelder JD. The effects of isoflurane on canine cerebral metabolism and blood flow. Anesthesiology 1974;40:571–4.

de Sousa SLM, Dickinson R, Lieb WR, Franks NP. Contrasting actions of the inhalational general anesthetics isoflurane and xenon. Anesthesiology 2000;92:1055–66.

Domino EF, Corssen G, Sweet RB. Effects of various general anesthetics on visually evoked response in man. Anesth Analg 1963;42:735–47.

Dragoi V, Sharma J, Sur M. Adaptation-induced plasticity of orientation tuning in adult visual cortex. Neuron 2000;28:287–98.

Drummond JC, Todd MM, Toutant SM, Shapiro HM. Brain surface protrusion during enflurane, halothane and isoflurane anesthesia in cats. Anesthesiology 1983;59:288–93.

Eger EI, II. Isoflurane (Forane): A Compendium and Reference, 2nd ed. Madison, WI: Ohio Medical Products, 1985.

Franco G. Occupationnal exposure to anaesthetics: liver injury, microsomal enzyme induction and preventive aspects. G Ital Med Lav 1989;11:205–28.

Franks NP, Lieb WR. Molecular and cellular mechanisms of general anaesthesia. Nature 1994;367:607–14.

Frost EAM. Inhalation anaesthetic agents in neurosurgery. Br J Anaesth 1984;56(Suppl. 1):47S–56S.

Grandy JL, Hogdson DS, Dunlop CI, Curtis CR, Heath B. Cardiopulmonary effects of halothane anesthesia in cats. Am J Vet Res 1989;50:1729–32.

Grundy BL. Electrophysiology monitoring: electroencephalography and evoked potentials. In: Newfield and Cottrell, editor. Handbook of Neuroanesthesia: Clinical and Physiologic Essentials. Boston, Toronto: Little, Brown and Company, 1983. p. 29–59.

Hartsfield SM, Sawyer DC. Cardiopulmonary effects of rebreathing and non-rebreathing systems during halothane anesthesia in the cat. Am J Vet Res 1976;37:1461–6.

Hikasa Y, Kawanabe H, Takase K, Ogasawara S. Comparison of sevoflurane, isoflurane and halothane anesthesia in spontaneously breathing cats. Vet Surg 1996;25:234–43.

Hikasa Y, Ohe N, Takase K, Ogasawara S. Cardiopulmonary effects of sevoflurane in cats: comparison with isoflurane, halothane, and enflurane. Res Vet Sci 1997;63:205–10.

Hikasa Y, Yamashita M, Takase K, Ogasawara S. Prolonged sevoflurane, isoflurane and halothane anaesthesia in oxygen using rebreathing or non-rebreathing system in cats. J Vet Med A 1998;45:559–75.

Hodgson DS, Dunlop CI, Chapman PL, Grandy JL. Cardiopulmonary effects of anesthesia induced and maintained with isoflurane in cats. Am J Vet Res 1998;59:182–5.

Hoffmann KP, Fischer WH. Directional effect of inactivation of the nucleus of the optic tract on the optokinetic nystagmus in the cat. Vis Res 2001;41:3389–98.

Hughes R, Chapple D. Effects of non-depolarizing neuromuscular blocking agent on autonomic mechanisms in cats. Br J Anaesth 1976;48:59–68.

Ikeda H, Wright MJ. Sensitivity of neurones in visual cortex (area 17) under different levels of anaesthesia. Exp Brain Res 1974;20:471–84.

Ingwersen W, Allen DG, Dyson DH, Pascoe PJ, O'Grady MR. Cardiopulmonary effects of halothane/oxygen combination in healthy cats. Can J Vet Res 1988;52:386–91.

Jenkins A, Franks NP, Lieb WR. Effects of temperature and volatile anesthetics on GABAA receptors. Anesthesiology 1999;90:484–91.

Jenkinson SG. Oxygen toxicity. New Horiz 1993;1:504–11.

Johnston RR, Eger EI, II, Wilson C. A comparative interaction of epinephrine with enflurane, isoflurane, and halothane in man. Anesth Analg 1976;55:709–12.

Jones MV, Brooks PA, Harrison NL. Enhancement of γ-aminobutyric acid-activated Cl-currents in cultured rat hippocampal neurones by three volatile anaesthetics. J Physiol 1992;449:279–93.

Katz RL, Katz GJ. Surgical infiltration of pressor drugs and their interaction with volatiles anaesthetics. Br J Anaesth 1966;38:712–8.

Lin LH, Chen LL, Zirrolli JA, Harris RA. General anesthetics potentiate GABA actions on $GABA_A$ receptors expressed by *Xenopus* oocytes: lack of involvement of intracellular calcium. J Pharmacol Exp Ther 1992;263:569–78.

Lucchini R, Placidi D, Toffoletto F, Alessio L. Neurotoxicity in operating room personnel working with gaseous and non-gaseous anesthesia. Int Arch Occup Environ Health 1996;68:188–92.

McKelvey D, Hollingshead JW. Small Animal Anesthesia and Analgesia, 2nd ed. Toronto: Mosby, 2000.

Merkel G, Eger EI, II. A comparative study of halothane and halopropane anesthesia. Including method for determining equipotency. Anesthesiology 1963;24:346–57.

Mihic SJ, Ye Q, Wick MJ, Koltchine VV, Krasowski MD, Finn SE, Mascia MP, Valenzuela CF, Hanson K, Greenblatt EP, Harris RA, Harrison NL. Sites of alcohol and volatile anaesthetic action on GABAA and glycine receptors. Nature 1997;389:385–9.

Minville K, Casanova C. Spatial frequency processing in the posteromedial lateral suprasylvian cortex does not depend on the projections from the striate-recipient zone of the cat's lateral posterior pulvinar complexe. Neuroscience 1998;84:699–711.

Mori K, Kawamata M, Mitani H, Yamazaki Y, Fujita M. A neurophysiologic study of ketamine anesthesia in the cat. Anesthesiology 1971;35:373–83.

Nakahiro M, Yeh JZ, Brunner E, Narahasi T. General anesthetics modulate GABA receptor channel complex in rat dorsal root ganglion neurons. FASEB J 1989;3:1850–4.

Ogawa T, Shingu K, Shibata M, Osawa M, Mori K. The divergent actions of volatile anaesthetics on background neuronal activity and reactive capability in the central nervous system in cats. Can J Anaesth 1992;39:862–72.

Schröder JH, Fries P, Roelfsema PR, Singer W, Engel AK. Ocular dominance in extrastriate cortex of strabismic cats. Vis Res 2002;42:29–39.

Sebel PS, Ingram DA, Flynn PJ. Evoked potentials during isoflurane anaesthesia. Br J Anaesth 1986;58:580–5.

Sengpiel F, Bonhoeffer T. Orientation specificity of contrast adaptation in visual cortical pinwheel centers and iso-orientation domains. Eur J Neurosci 2002;15:876–86.

Sillito AM. Inhibitory processes underlying the directional specificity of simple, complex and hypercomplex cells in the cat's visual cortex. J Physiol 1977;271:699–720.

Skottun BC, De Valois RL, Grosof DH, Movshon JA, Albrecht DG, Bonds AB. Classifying simple and complex cells on the basis of response modulation. Vis Res 1991;31:1079–86.

Steffey EP, Howland D, Jr. Isoflurane potency in the dog and cat. Am J Vet Res 1977;38:1833–6.

Steffey EP, Gillespie JR, Berry JD, Eger EI, II, Munson ES. Anesthetic potency (MAC) of nitrous oxide in the dog, cat, and stump-tail monkey. J Appl Physiol 1974;36:530–2.

Suder K, Funke K, Zhao Y, Kerscher N, Wennekers T, Wörgötter F. Spatial dynamics of receptive fields in cat primary visual cortex related to the temporal structure of thalamocortical feedfoward activity. Exp Brain Res 2002;144:430–44.

Theye RA, Michenfelder JD. The effects of halothane on canine cerebral metabolism. Anesthesiology 1968;29:1113–8.

Tigwell DA, Sauter J. On the use of isoflurane as an anesthetic for visual neurophysiology. Exp Brain Res 1992;88:224–8.

Todd MM, Drummond JC. A comparison of the cerebrovascular and metabolic effects of halothane and isoflurane in the cat. Anesthesiology 1984;60:276–82.

Tsumoto T, Eckart W, Creutzfeldt OD. Modification of orientation sensitivity of cat visual cortex neurons by removal of GABA-mediated inhibition. Exp Brain Res 1979;34:351–63.

Tsushima K, Shingu K, Ikeda S, Kimura H, Yamada K, Murao K. Suppression actions of volatile anaesthetics on response capability in cats. Can J Anaesth 1998;45:240–5.

Uhl RR, Squires KC, Bruce DL, Starr A. Effect of halothane anesthesia on the human cortical visual evoked response. Anesthesiology 1980;53:273–6.

Vidyasagar TR, Mueller A. Function of GABAA inhibition in specifying spatial frequency and orientation selectivities in cat striate cortex. Exp Brain Res 1994;98(1):31–8.

Villeneuve MY, Ouellette BG, Huppé-Gourgues F, Casanova C. On the use of isoflurane versus halothane for single cell recording in the primary visual cortex. SFN Abs 2000;26:140.

Winter PM, Smith G. The toxicity of oxygen. Anesthesiology 1972;37:210–41.

Wollman H, Alexander SC, Chen PJ, Smith TC, Chase PE, Melman E, Behar MG. Cerebral circulation of the man during halothane anesthesia. Anesthesiology 1964;25:180–4.

Young JM, Waleszczyk WJ, Burke W, Calford MB, Dreher B. Topographic reorganization in area 18 of adult cats following circumscribed monocular retinal lesions in adolescence. J Physiol 2002;541:601–12.