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Enhanced isoflurane suppression of excitatory synaptic transmission in the aged rat hippocampus

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1 The effects of the volatile anaesthetic, isoflurane, were investigated on evoked dendritic field excitatory postsynaptic potentials (f.e.p.s.p.) and antidromic and orthodromic population spikes recorded extracellularly in the CA1 cell layer region in the *in vitro* hippocampal slice taken from young mature (2–3 months) and old (24–27 months) Fisher 344 rats.

2 Isoflurane depressed the f.e.p.s.ps and the orthodromically-evoked population spikes in both old and young hippocampi. However, the magnitude of the anaesthetic-induced depression was greater in slices taken from old rats compared to those taken from young rats during the application of different isoflurane concentrations (0.5–5%).

3 In the presence of the $GABA_A$ antagonist, bicuculline methiodide (15 μM), isoflurane suppressed the f.e.p.s.ps to the same extent as was observed in the absence of the $GABA_A$ antagonist.

4 Orthodromically evoked population spikes were suppressed by isoflurane in a manner quantitatively similar to the suppression of the f.e.p.s.ps. However, antidromic population spikes and presynaptic volleys evoked in young and old slices were resistant to anaesthetic action. In addition, paired pulse facilitation ratio of the evoked dendritic f.e.p.s.ps was not affected in both young and old slices during the application of isoflurane.

5 When slices were exposed to low Ca^{2+}/high Mg^{2+} solution, isoflurane (1 and 3%) depressed the f.e.p.s.ps in aged slices to the same extent as in young slices.

6 The augmented anaesthetic depression of f.e.p.s.ps in old compared to young hippocampi in the absence and presence of bicuculline, and the lack of anaesthetic effects on antidromic population spikes and presynaptic volleys in old and young slices, suggest that the increased sensitivity of anaesthetic actions in old hippocampi is due to age-induced attenuation of synaptic excitation rather than potentiation of synaptic inhibition. Furthermore, elimination of the increased sensitivity of old slices to anaesthetic actions when the slices were perfused with low Ca^{2+}/high Mg^{2+} medium, which presumably would decrease intracellular [Ca^{2+}], suggests that the enhanced anaesthetic effects in aged neurones might be related to increased intraneuronal [Ca^{2+}] in the synaptic terminal.

Keywords: Ageing; hippocampal slice; anaesthetic; isoflurane; electrophysiology; synaptic transmission; paired pulse facilitation; Fisher 344 rats

Introduction

Anaesthetic effects on pre- and postsynaptic sites at different levels of the central nervous system of young animals have been studied in detail (Krnjevic, 1992; Pocock & Richards, 1993; Franks & Lieb, 1994). However, the mechanisms of ageing-induced potentiation of anaesthetic actions have not been explored before. Recently, however, preliminary studies showed that ageing enhances the anaesthetic-induced depression of the evoked dendritic field postsynaptic potentials (f.e.p.s.p.) without any effect on the presynaptic volley in hippocampal slices (El-Beheiry *et al*., 1996a). This suggests that age-induced changes in nerve fibre conduction are not involved in the mechanism of increased vulnerability of old synapses to anaesthetic action. Other synaptic sites are probably involved in the age-dependent potentiation of anaesthetic suppression of synaptic transmission. In agreement with this concept is the early investigation in young animals by Larrabee and Posternak (1952). They showed that general anaesthetics greatly interrupted the interneuronal transmission of action potentials and did not alter their conduction along nerve axons in young animals (Richards, 1983).

Anaesthetic interruption of neurotransmission could be due to depression of excitation (Richards & White, 1975; Berg-Johnson & Langmoen, 1987; MacIver & Roth, 1988; Fujiwara *et al*., 1988; Miu & Puil, 1989; El-Beheiry & Puil, 1989b; Kendig *et al*., 1991; Perouansky *et al*., 1995) and/or potentiation of inhibition (Gage & Robertson, 1985; Collins, 1988; Orser *et al*., 1994). Interestingly, such anaesthetic actions on synaptic transmission are similar to the reported ageing-induced changes in central synapses. For example, the depolarization and the number of binding sites of the excitatory amino acid, N-methyl-D-aspartate (NMDA), are reduced in the aged rat hippocampus and neocortex (Baskys *et al*., 1990; Gonzales *et al*., 1991; Magnusson & Cotman 1993). In addition, GABA-evoked currents in aged neurones displayed significantly greater maximal response and lesser degree of use-dependent receptor desensitization compared to young neurones (Griffith & Murchison 1995; Abdulla *et al*., 1995). Therefore, age-induced augmentation of anaesthetic actions could be due to synergism between neuronal ageing and anaesthesia in depressing synaptic excitation and/or enhancing synaptic inhibition.

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The purpose of these experiments was to study the mechanisms of the increased vulnerability of synaptic transmission to anaesthetic depression in old neurones. The results of these studies have been presented in an abstract form (El-Beheiry *et al*., 1996b).

Methods

Slice preparation

Young-mature (2–3 months) and old (24–27 months) Fisher 344 rats were decapitated under halothane anaesthesia. The brain was quickly removed and submerged for two minutes in oxygenated (95% O_2–5% CO_2) iced artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl 125, KCl 2.5, $NaHCO_3$ 26, NaH_2PO_4 1.25, $CaCl_2$ 2, $MgCl_2$ 2 and glucose 10. Whole brain slices (400–500 μm thick) were obtained using a Vibroslicer. Slices were then incubated at 30–31°C into a humidified holding chamber and allowed to recover for at least 1–2 h before recording.

Electrophysiological recording

Slices were transferred to a submerged recording chamber and continuously perfused with oxygenated ACSF. The flow rate of the perfusate was set at $\sim$2 ml min^{-1}. All experiments were performed at 35.5$\pm$0.5°C.

Extracellular recording micropipettes (2–5 MΩ tip resistance) pulled from thin walled-borosilicate capillary tubing, were filled with 150 mM NaCl. Population spikes and the dendritic f.e.p.s.ps were recorded from stratum pyramidal and the stratum radiatum, respectively. A bipolar concentric stimulating electrode was placed either along the Schaffer collateral for orthodromic or along the alveus for antidromic stimulation. Field potentials were evoked at 0.05 Hz. Stimulating intensities were adjusted to evoke $\sim$75% of the maximal field potentials obtained with supramaximal currents. Extracellular signals were processed by an Axoclamp 2A amplifier (Axon Instruments, Foster City, California). Data were acquired, stored and analysed using an IBM clone computer and P-Clamp software, version 5.1 and 6.0 (Axon Instruments, Foster City, California). A slice was considered stable when the orthodromic response varied less than 5% over a fifteen minute interval. Additionally, a slice was included in the study if the amplitude of the dendritic field and the population spike were greater than 1.5 and 2 mV in amplitude, respectively. Input/output (I/O) curves were determined by signal averaging of 3–4 consecutive runs of 5 stimulation intensities ranging from those evoking minimal to maximal responses.

Drug preparation and application

The volatile anaesthetic, isoflurane (Anaquest, Pointe Claire, Quebec), was vaporized at 1.5 l min^{-1} flow rate with 95% O_2–5% CO_2 mixture at 23°C ambient temperature using an Ohmeda vaporizer (Ohio Medical Products, Madison, Wisconsin). Isoflurane vapour was then bubbled in ACSF reservoirs (inverted 60 ml syringes) for a minimum of 20 min before bath application at designated volume/volume concentration values. Isoflurane vapour also was introduced into the chamber atmosphere immediately above the submerged slice. The volume/volume concentration values (vol. %) are the concentrations of isoflurane bubbled into the superfusate which are produced by the vaporizer. Previous studies have shown that there is a linear relationship between the anaesthetic concentrations in the bubbled superfusate and the concentrations in the superfusate reaching the slice (Miu & Puil, 1989; El-Beheiry & Puil, 1989a). In the latter investigations isoflurane was measured in the perfusing medium by 19-Flurine nuclear magnetic resonance spectroscopy.

In a separate set of experiments, slices were perfused with the γ-aminobutyric $acid_A$ ($GABA_A$) blocker, bicuculline 15 μM (Sigma, St. Louis), for 20–30 min while continuously monitoring the f.e.p.s.ps or population spikes. GABA-ergic blockade was assumed to be adequate when epileptic discharges were observed using the same stimulus strength pre-bicuculline application.

Data analysis

The peak amplitude of f.e.p.s.p. was considered to be the distance between the isoelectric line and the peak downward deflection. The duration of the f.e.p.s.p. was measured from the onset to the offset of the synaptic transient, respectively. Population spike amplitudes were measured from the onset of the spike to its negative peak. The amplitude of the antidromic population spike was defined as the distance between the most negative potential of the field and the midpoint of a line drawn between the isoelectric line and the positive potential succeeding the negative deflection. Percentage depression of peak amplitudes was calculated as follows: (control response – isoflurane response/control response) $\times$ 100. Results obtained from young and aged slices during the application of isoflurane concentrations were tested with non-parametric two-way ANOVA (Friedman test) for the statistical significance between anaesthetic actions at different concentrations in each group. Comparisons between effects of isoflurane in young *versus* old slices at every anaesthetic concentration were achieved by using non-parametric one-way ANOVA and Mann-Whitney U-test. Statistical significance was always assumed to be accepted at $P<0.05$.

The dose-response curves for f.e.p.s.p. depression by isoflurane in young and old hippocampi were fitted to the logistic function (De Lean *et al*., 1978):

$$Y = [a - d/1 + (X/c)^b] + d \quad (1)$$

where Y is % depression of f.e.p.s.p. at the concentration of isoflurane X; a and d are the response when X = 0 and the response for 'infinite' dose respectively, b is the slope factor and c is isoflurane concentration that produces half-maximal effect (EC_{50}). Non-linear regression analysis with Jandel Scientific software was used to fit the data to the logistic function (equation 1) and to estimate the EC_{50}s and maximum inhibition values for the effect of isoflurane in young and aged slices.

Results

The effects of isoflurane (0.5–5%) applications were observed on 32 young and 29 old slices. In the initial investigations on young and old slices (n = 4 and 3 respectively), 2% isoflurane was applied continuously for about 18 min. The maximal effects were evident at 6–8 min with a perfusion rate of $\sim$2 ml min^{-1}. To ensure reproducibility of isoflurane effects, different vapour concentrations were applied 2–3 times to the same slice in about 50% of experiments. The consistent reproducibility of the different isoflurane effects indicates that the anaesthetic concentrations did not fluctuate at the site of action within the hippocampal slice. Steady-state isoflurane concentrations in the slice were ensured by using relatively fast

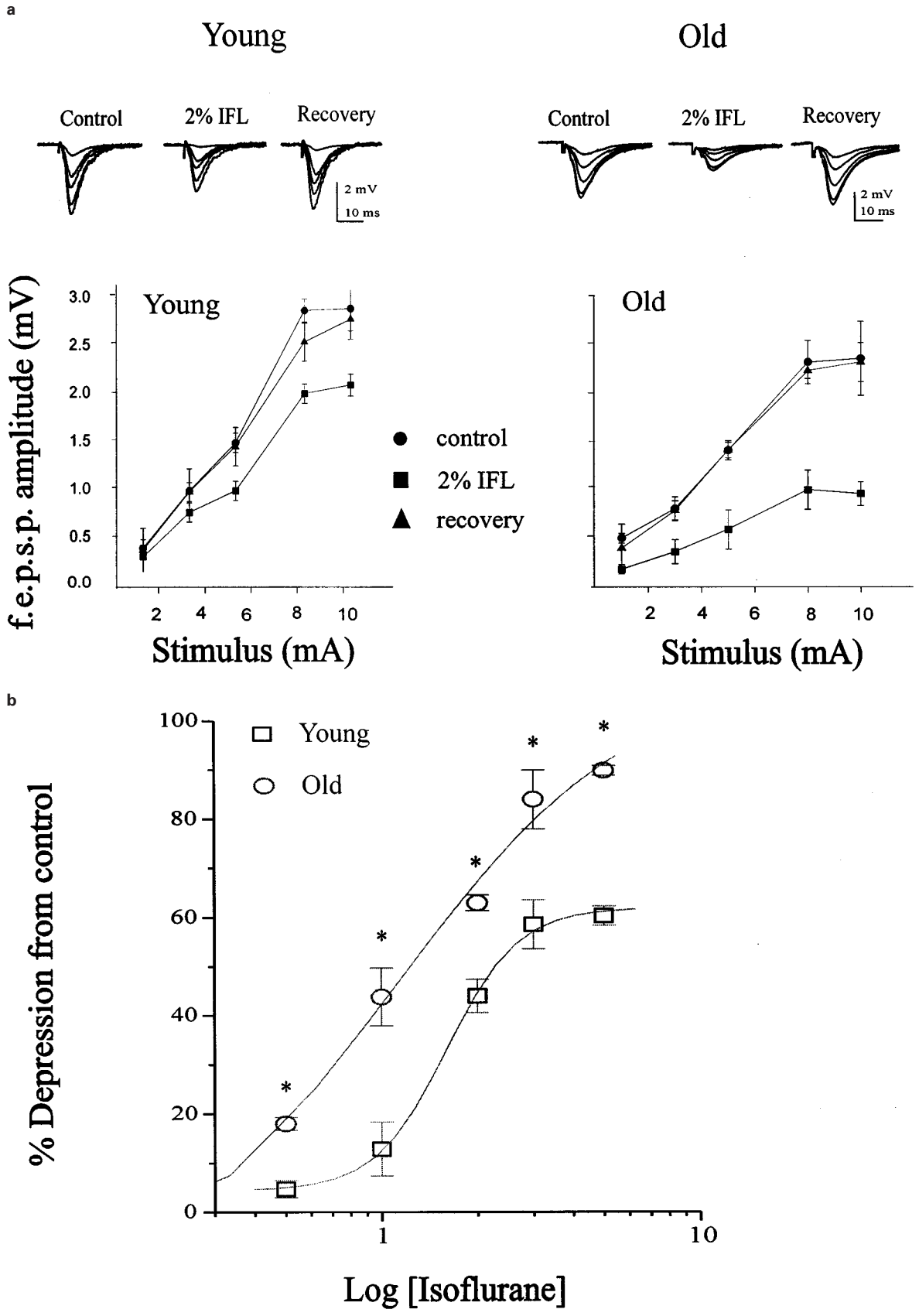

Figure 1 Isoflurane actions in young and old hippocampal formation. (a) Isoflurane (IFL) reversibly reduced the f.e.p.s.p. amplitude at different stimulation intensities. Figure (a) shows records obtained from an old and a young slice. Input-output curves for f.e.p.s.p, amplitude *versus* the stimulation intensity were generated by averaging the f.e.p.s.p. amplitude at the different stimulation intensities from four slices taken from young and old animals. (b) Concentration-dependent effects of IFL in young and old slices. Data represent mean% depression of f.e.p.s.p. ($n \geqslant 5$ slices); vertical lines show s.e.mean. *Indicates statistical significance ($P < 0.05$) between young and old at corresponding concentrations. The line curves shown in figure represent the best fit for the dose-response relationships using a logistic function (see text).

perfusion rates (~2 ml min^{-1}), bubbling the perfusate with isoflurane for at least 20 min before application and introducing the anaesthetic vapour into the chamber atmosphere immediately above the slice.

Effects on orthodromic and antidromic evoked responses

Isoflurane application depressed the orthodromically evoked f.e.p.s.ps produced by using submaximal stimulation (~75%) in slices taken from young and old animals. In order to eliminate the possibility that a particular subset of excitatory synapses was sensitive to the anaesthetic at submaximal stimulation, the effect of isoflurane 2% on different stimulation intensities was examined by generating an I/O relationship (Figure 1a). The effect, i.e. reduction of the field amplitude, was observed at all stimulation intensities suggesting that this phenomenon is not peculiar to a small number of synapses. A striking feature in the I/O relationship was the increased vulnerability of old slices to isoflurane action. Such increased sensitivity to anaesthetic action in the old slices was consistently observed with the administration of different isoflurane concentrations (0.5–5 vol %). The concentration-response relationships for isoflurane depression of f.e.p.s.ps in slices taken from young and old animals is shown in Figure 1b. The relationships could be best fitted to a rectangular hyperbolic logistic function with a correlation coefficient, $r^2 = 0.99$ and 0.98 and with statistical significance, $P < 0.05$ and < 0.005 for data obtained from young and old slices, respectively (cf. Figure 1b). The EC_{50}s for isoflurane depression of f.e.p.s.ps in the young and old slices are 1.6 and 1.1 vol %, respectively. The relative potency of isoflurane actions in young and old slices ($EC_{50,\ young}/EC_{50,\ old}$) was 1.4. In addition, the maximum inhibition was apparently greater in old slices compared to young slices (Figure 1b).

The effects of isoflurane on the population spike amplitudes recorded from the CA1 pyramidal cell layer were dependent on the stimulating mode. Population spikes evoked by antidromic stimulation in young and old slices were resistant to anaesthetic action at high concentrations (Figure 2a and Table 1). In contrast, orthodromically evoked population spikes were suppressed by isoflurane in a manner similar to the suppression of the f.e.p.s.ps; i.e. the anaesthetic-induced attenuation was age- and dose-dependent (Figure 2b and Table 1). The presynaptic volleys seen during orthodromic stimulation were unaffected by the application of multiple anaesthetic concentrations (Figure 2b).

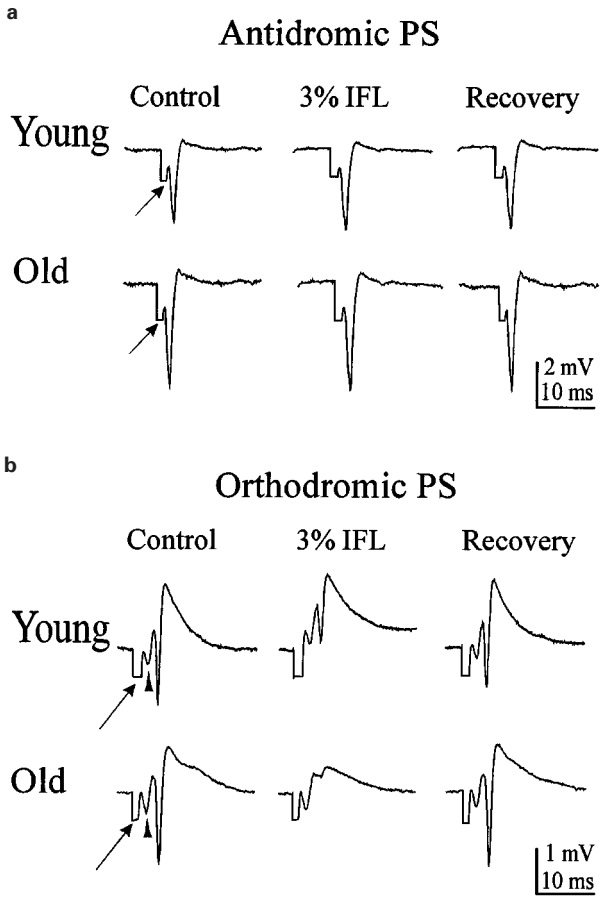

Figure 2 Effects of isoflurane (IFL) on the somatic population spike (PS) amplitude recorded antidromically and orthodromically from young and aged hippocampal brain slices. (a) PSs were evoked antidromically. After 20 min of control recording the slices were perfused with ACSF containing 3% IFL. Note the lack of effect of IFL on the antidromic PS amplitudes. Similar results were obtained from eight other slices taken from young and old animals. (b) Population spikes were evoked orthodromically. The same paradigm was used as in (a). IFL suppressed the PS amplitude in a manner approximately similar to the depression of dendritic f.e.p.s.p. (see also Table 1). (→) Indicates stimulus artefact and (➤) indicates presynaptic volley.

Anaesthetic evoked changes in f.e.p.s.ps during GABAergic-blockade by bicuculline

Bicuculline (15 μM) was used in the present investigations to block the inhibitory synapses in the hippocampal slice for more accurate determination of anaesthetic effects on 'pharmacologically isolated' f.e.p.s.ps. During bicuculline application, the stimuli which were used to evoke the control f.e.p.s.ps initiated epileptiform bursts that were completely suppressed with isoflurane application in young and old slices ($n = 6$ and 5, respectively; Figure 3a,b). In order to obtain monosynaptic f.e.p.s.ps during bicuculline perfusion, the stimulus intensities were reduced to evoke just f.e.p.s.ps without multiple discharges. The application of isoflurane 2% to young and old slices depressed these f.e.p.s.ps; these effects were similar to those observed in the absence of bicuculline (Figures 1 and 3a,b, third panels).

Table 1 The effects of isoflurane (IFL) on the dendritic field excitatory postsynaptic potentials (f.e.p.s.ps.) and the somatic population spikes (PS) recorded from the hippocampal CA1 region

		% depression		
		Dentritic	*Somatic population spike*	
	Isoflurane	*f.e.p.s.p.*	*Orthodromic*	*Antidromic*
Young	1%	14±7	23±2.9	1.5±0.7
($n = 5$)	3%	58.7±6.1	47±3.1	1.7±0.3
Old	1%	43±5.8*	52±8.7*	1.3±0.1
($n = 5$)	3%	84±5.6*	91±7.3*	1.9±0.3

Values are mean±s.e.mean. *Indicates statistical significance ($P < 0.05$) between young and old at corresponding concentration.

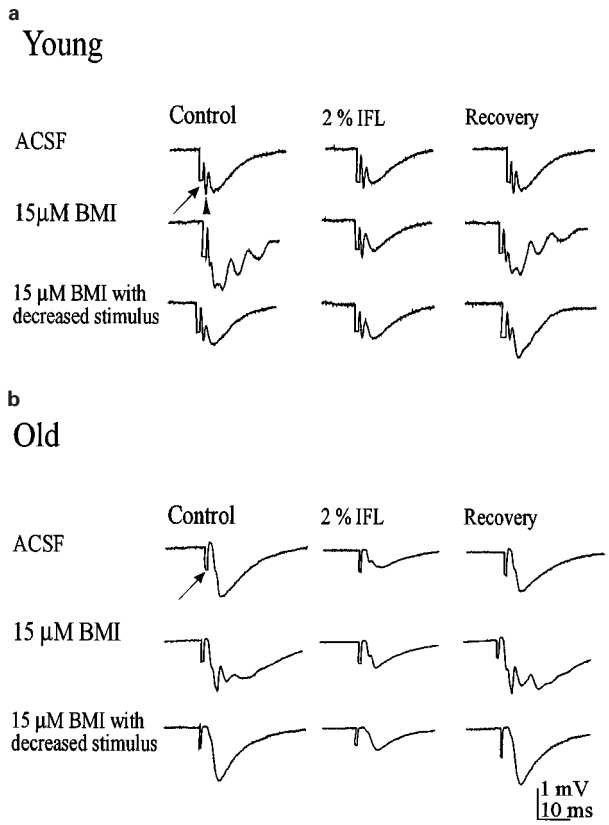

Figure 3 The anaesthetic isoflurane (IFL) attenuates dendritic f.e.p.s.p. amplitude in the presence of bicuculline methiodide (BMI). (a) Records from a young slice showing the effects of IFL during perfusion with normal ACSF and the $GABA_A$ antagonist, bicuculline. Epileptic discharges induced by bicuculline were suppressed by IFL. The stimulation intensity was then reduced to study the effect of IFL on the 'pharmacologically isolated' f.e.p.s.p. (b) Records of f.e.p.s.ps from an old hippocampal slice. Note that IFL was more potent in depressing the evoked f.e.p.s.p. (after stimulus reduction in panel 3) obtained during bicuculline application than in (a) (panel 3). (→) Indicates stimulus artefact and (➤) indicates presynaptic volley.

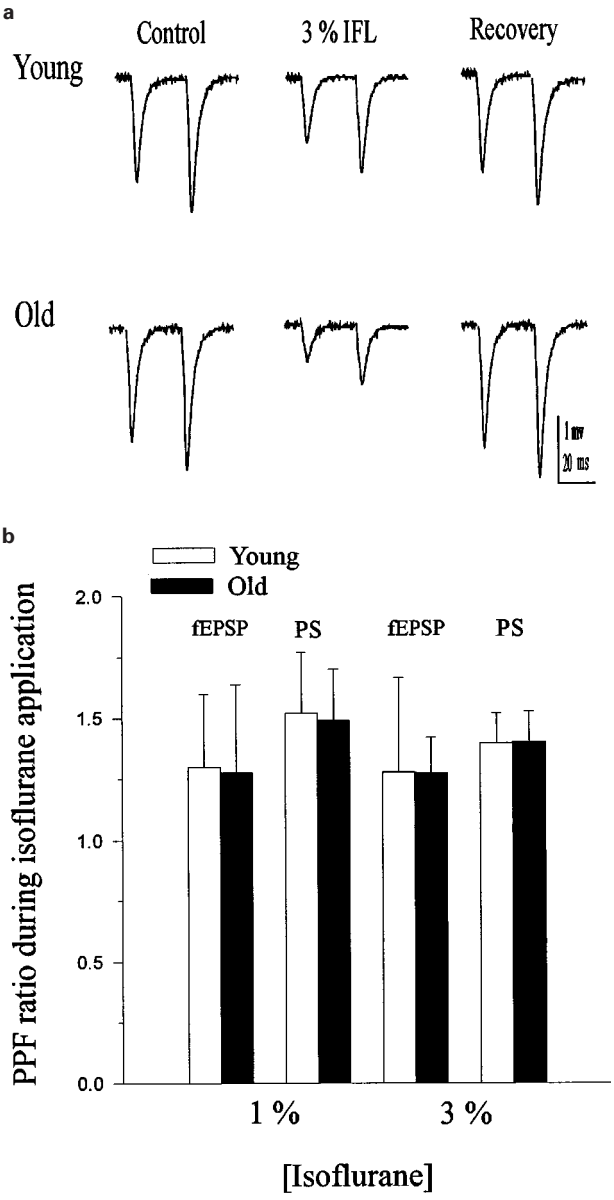

Figure 4 Isoflurane (IFL) did not affect the paired pulse facilitation (PPF) ratio in young or old slices. (a) Sample tracings of f.e.p.s.p. during control recording and during IFL application. Note the attenuation of f.e.p.s.ps without a change in the PPF ratio. Similar results were seen in five other slices. (b) Summary figure showing the effects of different concentrations of IFL on the PPF ratio during recording from the soma (PS) and dendrites (f.e.p.s.p.) of CA1 hippocampal neurones. Columns indicate mean ± s.e.mean, $n = 5$ for each column.

Anaesthetic action on paired-pulse facilitation (PPF)

Because the PPF phenomenon in the hippocampus is attributed to presynaptic activation of $GABA_B$ receptors following the first stimulus (Leung & Fu, 1994), the effects of isoflurane were studied on PPF in both young and old slices. This might answer the question whether the observed sensitivity to anaesthetic actions in old neurones is related to $GABA_B$ actions in the synaptic cleft. In all the slices double-pulse orthodromic stimulation of the Schaffer collateral with an interpulse interval of 50 ms produced a larger second f.e.p.s.p. or populations spike response (Figure 4a). This paired-pulse facilitation phenomenon (PPF) was abolished at an interpulse interval of 10–20 ms as described by other investigators (Creager *et al.*, 1980; Leung & Fu, 1994). To analyse qualitatively the PPF, the second/first response ratio was calculated for every evoked pair of f.e.p.s.ps and population spikes. There was no statistically significant difference between the PPF ratios calculated for the responses obtained in young and old slices. The PPF phenomenon also was entirely preserved during the administration of isoflurane (Figure 4a). As can be seen from Figure 4b, the mean control f.e.p.s.p. and population spike PPF ratios were not statistically different from those calculated during isoflurane 1 and 3% administration to young and old slices ($n = 5$ for each anaesthetic application).

Effects of isoflurane on young and old slices perfused with low Ca^{2+}/high Mg^{2+} ACSF

Previous electrophysiological studies indirectly indicated that intracellular Ca^{2+} ($[Ca^{2+}]_i$) is increased in ageing neurones (cf. Hartmann *et al.*, 1994). We hypothesized that a presumed increase in neuronal calcium in the presynaptic terminal may lead to the observed enhanced vulnerability of hippocampal neurones to isoflurane. To investigate this assumption, we lowered the extracellular calcium concentration to 1 mM (from

2 mM), and increased Mg^{2+} to 3 mM (from 2 mM). This manoeuvre has been shown to reduce cytoplasmic calcium by Mg^{2+}-induced blockade of Ca^{2+} channels and a consequent decrease in Ca^{2+} influx (Rahamimoff, 1968; Martin, 1977). Application of the above medium caused a reduction in the f.e.p.s.ps in both young and old slices. In six young slices the low Ca^{2+}/high Mg^{2+} ACSF caused a $33 \pm 5\%$ reduction of the f.e.p.s.p. and in four old slices the same medium caused a reduction of $30 \pm 7\%$. These effects were completely reversed upon re-perfusion of the slices with normal ACSF. Isoflurane application (1 and 3%) during perfusion of the slices with low Ca^{2+}/high Mg^{2+} ACSF depressed the f.e.p.s.p. in young slices to the same extent as in aged slices (Figure 5a). This is in contrast to the observations recorded under normal conditions where f.e.p.s.ps recorded from old slices were more vulnerable to anaesthetic depression compared to those recorded from young slices (Figures 1b, 4a and 5a,b).

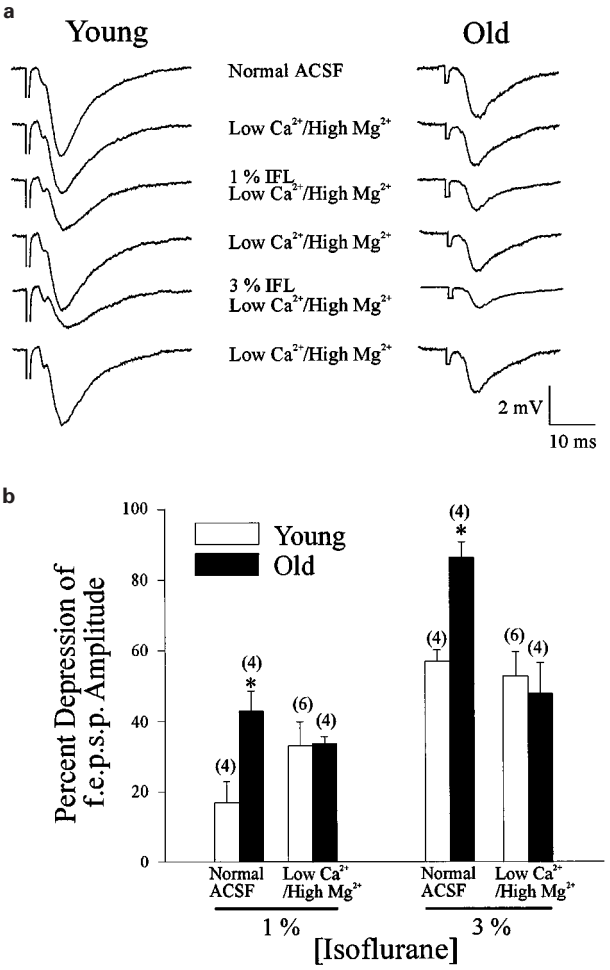

Figure 5 Effects of isoflurane (IFL) on the f.e.p.s.p. amplitude in young and aged slices perfused with low Ca^{2+}/high Mg^{2+} medium. (a) Sample tracings obtained from a young and an old slice. Slices were perfused initially with normal artificial cerebrospinal fluid (ACSF) then with ACSF containing 1 mM Ca^{2+} and 3 mM Mg^{2+}. The f.e.p.s.ps evoked in old slices did not show an increased vulnerability to isoflurane during perfusion with low Ca^{2+}/high Mg^{2+}. (b) Isoflurane induced % depression (mean $\pm$ s.e.mean) of the f.e.p.s.p. amplitude evoked in both young and old slices during perfusion with normal and low Ca^{2+}/high Mg^{2+} ACSF. The f.e.p.s.ps evoked in old slices did not show an increased vulnerability to isoflurane during perfusion with low Ca^{2+}/high Mg^{2+}. *Indicates significant difference, $P<0.05$. Numbers in parentheses represent the number of slices used in each group.

Discussion

These investigations verified the hypothesis that the negative correlation between age and volatile anaesthetic requirements is due to increased vulnerability of an ageing cellular site to anaesthetic agents. The study also suggests that the synergism between ageing and anaesthesia is due to increased sensitivity of synaptic excitation to anaesthetic depression in old neurones. Nonetheless, other mechanisms could have been involved in the age-dependent potentiation of isoflurane actions in hippocampal slices, i.e. decreased cholinergic modulation of excitatory transmission in old animals (Pintor *et al*., 1994). Such possible mechanisms could be explored in future investigations.

Ageing potentiation of anaesthetic actions

The present data confirm previous extracellular and intracellular observations that volatile anaesthetics depress equally excitatory synaptic transients and population spikes evoked in hippocampal slices taken from young animals (Berg-Johnson & Langmoen, 1987; MacIver & Roth, 1988; Miu & Puil, 1989; Perouansky *et al*., 1995). However, this study quantified and compared anaesthetic actions in young and old brain slices. The EC_{50}s for the actions of isoflurane in young and old hippocampi were 1.6 and 1.1 vol %, respectively.

The different potencies of isoflurane in the present investigations might be due to an increased anaesthetic lipophilicity in the old slices. However, isoflurane and other volatile anaesthetics lipophilicities (i.e. brain/gas and brain/blood partition coefficients) do not change with the advancement of age after sexual maturity in man and experimental animals (Cook *et al*., 1981; Webb & Weaver 1981; Lerman *et al*., 1985; 1986). There is also no evidence, to date, that ageing induces alteration in grey matter brain lipid content. Therefore, the enhanced potency of isoflurane in the ageing hippocampus is likely to be due to an increased vulnerability of a specific site of action to the anaesthetic agent.

In young and old slices, the f.e.p.s.ps and the orthodromic population spikes were depressed approximately to the same extent, suggesting that isoflurane decreases the spike discharges of the CA1 neurones by predominantly synaptic actions. Hence, isoflurane-enhanced effects in old slices are probably not due to major changes in membrane electrotonic properties, i.e. decrease in input resistance and increase in discharge thresholds (cf. El-Beheiry 1989a; Ries & Puil 1993; Puil *et al*., 1994). In addition, presynaptic volleys and antidromic population spikes were resistant to isoflurane application in slices taken from young and old animals. This indicates that the increased vulnerability of excitatory synapses to anaesthetic actions in old hippocampi is probably not due to age-induced changes in nerve fibre conduction properties or age-induced alterations in the intrinsic spike generation processes along the dendrites of the CA1 pyramidal neurones.

Anaesthetic actions during GABA-ergic blockade

The f.e.p.s.ps evoked by stimulation of the Schaffer collateral could be contaminated with GABA-ergic mediated field i.p.s.ps due to recurrent and direct feed-forward activation of interneurones (Jones & Heinemann, 1987). Therefore, the observed isoflurane suppression of f.e.p.s.ps may be explained by anaesthetic potentiation of inhibitory transmission. To exclude this possibility, the $GABA_A$ antagonist bicuculline was applied to block selectively a possible shunting effect of the i.p.s.ps on the f.e.p.s.ps (El-Beheiry & Puil, 1989b; Puil *et al*.,

1991). Such pharmacologically isolated f.e.p.s.ps evoked in young and old slices were attenuated by isoflurane administration. The magnitude of f.e.p.s.p. suppression by isoflurane was similar in the presence or absence of bicuculline. These observations suggest that the increased sensitivity of aged synapses to anaesthetic blockade is due to an increased vulnerability of excitatory synaptic neurotransmission.

Isoflurane effects on paired pulse facilitation

Previous studies suggested that the paired pulse facilitation phenomenon in the hippocampus is due to presynaptic actions of the first stimulus. This pre-conditioning impulse will activate presynaptic $GABA_B$ autoreceptors which can attenuate GABA release during the second stimulus and/or increase nerve ending calcium concentration with a consequent increment in excitatory neurotransmitter release (Leung & Fu, 1994). Isoflurane depressed the field responses evoked by a paired pulse stimulation paradigm, but did not change the amplitude ratio between the first and second f.e.p.s.p. or population spike in young and old slices. Hence, isoflurane does not act on presynaptic $GABA_B$ receptors. We can conclude that the enhanced vulnerability of old neurones to anaesthetic depression is most probably not due to interference with presynaptic GABA-ergic activity.

Low extracellular [Ca^{2+}] opposes anaesthetic actions

The 'calcium hypothesis of ageing' (for reviews see: Disterhoft *et al*., 1993; Landfield, 1994; Khachaturian, 1994) suggests that basal intracellular Ca^{2+} is increased in aged animals, and as a result, Ca^{2+}-dependent processes such as neurotransmitter release and postsynaptic receptor responsiveness might be altered. Elevation of basal Ca^{2+} could decrease presynaptic neurotransmitter release by inactivation of presynaptic Ca^{2+} channels (cf. Reynolds & Carlen, 1989), or decrease postsynaptic receptor effects (Baskys *et al*., 1990). In the present study, we demonstrated that by decreasing pre- and postsynaptic intracellular [Ca^{2+}] using a low Ca^{2+}/high Mg^{2+} medium, isoflurane actions in slices taken from old animals were attenuated and resembled responses of slices prepared from young animals. These observations suggest that the enhanced anaesthetic effects in aged neurones may be related to increased intraneuronal calcium concentration.

Conclusion

In conclusion, the enhanced anaesthetic suppression of excitatory synaptic transmission in aged hippocampal formation is due to age-induced attenuation of synaptic excitation rather than potentiation of synaptic inhibition. Ageing-induced changes in nerve fibre conduction and in intrinsic neuronal spike-genesis properties do not play a major role in the observed increased vulnerability of old neurones to anaesthetic actions. Furthermore, the increased sensitivity to anaesthetic actions on excitatory synapses in old neurones might be due to chronic rise of intraneuronal calcium concentration associated with ageing.

Significance

The elderly are known to exhibit an increased incidence of postoperative confusional states including delirium due to ageing enhanced sensitivity to anaesthetic agents (Gustafson *et al*., 1991; William-Russo *et al*., 1992; Parikh & Chung, 1996). The present investigations confirmed the assumption that ageing-induced vulnerability to anaesthesia is due to increased susceptibility of excitatory synaptic neurotransmission to anaesthetic actions. This proposed mechanism might be the basis for developing therapeutic strategies aimed at reversing ageing-potentiation of anaesthetic actions. Consequently, the incidence of perioperative adverse events and clinically significant complications could be decreased in the elderly population.

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References

ABDULLA, F.A., ABU-BAKRA, M.A., CALAMINICI, M.R., STEPHENSON, J.D. & SINDEN, J.D. (1995). Importance of forebrain cholinergic and GABAergic systems to the age-related deficits in water maze performance of rats. *Neurobiol. Aging,* **16,** 41 – 52.

BASKYS, A., REYNOLDS, J.N. & CARLEN, P.L. (1990). NMDA depolarisation and long-term potentiation are reduced in the aged rat neocortex. *Brain Res.,* **530,** 142 – 146.

BERG-JOHNSON, N. & LANGMOEN, I.E. (1987). Isoflurane hyperpolarizes neurones in rat and human cerebral cortex. *Acta. Physiol. Scand.,* **130,** 679 – 685.

COOK, D.R., BRANDOM, B.W., SHIU, G. & WOLFSON, B. (1981). The inspired median effective dose, brain concentration at anaesthesia, and cardiovascular index for halothane in young rats. *Anesth. Analg.,* **60,** 182 – 185.

COLLINS, G.G.S. (1988). Effects of the anaesthetic 2,6-diisopropylphenol on synaptic transmission in the rat olfactory cortex slice. *Br. J. Pharmacol.,* **95,** 939 – 949.

CREAGER, R., DUNWIDDIE, T. & LYNCH, G. (1980). Paired-pulse and frequency facilitation in the CA1 region of the in vitro rat hippocampus. *J. Physiol.,* **299,** 409 – 424.

DE LEAN, A., MUNSON, P.J. & RODBRAD, D. (1978). Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay, and physiological dose-response curves. *Am. J. Physiol.,* **235,** E97 – E102.

DISTERHOFT, J.F., MOYER, J.R., THOMPSON, L.T. & KOWASLKA, M. (1993). Functional aspects of calcium channel modulation. *Clin. Neuropharmacol.* **16,** S12 – S24.

EL-BEHEIRY, H., OUANOUNOU, A. & CARLEN, P.L. (1996a). Ageing potentiates anaesthetic-induced synaptic depression in hippocampal slices. *NeuroReport,* **7,** 502 – 504

EL-BEHEIRY, H., OUANOUNOU, A. & CARLEN, P.L. (1996b). Ageing enhances isoflurane-induced suppression of excitatory synaptic transmission in hippocampal slices. *FASEB J.,* **10,** 920.

EL-BEHEIRY, H. & PUIL, E. (1989a). Postsynaptic depression induced by isoflurane and althesin in neocortical neurones. *Exp. Brain Res.,* **75,** 361 – 368.

EL-BEHEIRY, H. & PUIL, E. (1989b). Anaesthetic depression of excitatory synaptic transmission in neocortex. *Exp. Brain Res.,* **77,** 87 – 93.

FRANKS, N.P. & LIEB, W.R. (1994). Molecular and cellular mechanisms of general anaesthesia. *Nature,* **367,** 607 – 614.

FUJIWARA, N., HIGASHI, H., SHIMOJI, K., SUGITA, S. & YOSHIMURA, M. (1988). Changes in spontaneous firing patterns of rat hippocampal neurones induced by volatile anaesthetics. *J. Physiol.,* **402,** 155 – 175.

GAGE, P.W. & ROBERTSON, B. (1985). Prolongation of inhibitory postsynaptic currents by pentobarbitone, halothane and ketamine in CA1 pyramidal cells in rat hippocampus. *Br. J. Pharmacol.,* **85,** 675–681.

GONZALES, R.A., BROWN, L.M., JONES, T.W., TRENT, R.D., WESTBROOK, S.L. & LESLIE, S.W. (1991). N-Methyl-D-aspartate mediated responses decrease with age in Fisher 344 rat brain. *Neurobiol. Aging,* **12,** 219–225.

GRIFFITH, W.H. & MURCHISON, D.A. (1995). Enhancement of GABA-activated membrane currents in aged Fisher 344 rat basal forebrain neurones. *J. Neurosci.,* **15,** 2407–2416.

GUSTAFSON, Y., BRANSTROM, B., BERGGREN, D., RAGNARSSON, J.I., SIGAARD, J., BUCHT, G., REIZ, S., NORBERG, A. & WINBLAD, B. (1991). A geriatric anaesthesiology program to reduce acute confusional states in elderly patients treated from femoral neck fractures. *J. Am. Geriatr. Soc.,* **39,** 655–662.

HARTMANN, H., ECKERT, A. & MULLER, W.E. (1994). Disturbances of the neuronal calcium homeostasis in the aging nervous system. *Life Sciences,* **55,** 2011–2018.

JONES, S.G. & HEINEMANN, U. (1987). Abolition of the orthodromically evoked IPSP of CA1 pyramidal cells before the EPSP during washout of calcium from hippocampal slices. *Exp. Brain Res.,* **65,** 676–680.

KENDIG, J.J., MACIVER, M.B. & ROTH, S.H. (1991). Anaesthetic actions in hippocampal formation. *Ann. New York Acad. Sci.,* **625,** 37–53.

KHACHATURIAN, Z.C. (1994). Calcium hypothesis of Alzheimer's disease and brain aging. *Ann. New York Acad. Sci.,* **747,** 1–11.

KRNJEVIC, K. (1992). Cellular and synaptic actions of general anaesthetics. *Gen. Pharmacol.,* **23,** 965–975.

LANDFIELD, P.W. (1994). Increased hippocampal Ca^{2+} channel activity in brain aging and dementia. *Ann. New York Acad. Sci.,* **747,** 351–364.

LARRABEE, M.G. & POSTERNAK, J.M. (1952). Selective action of anaesthetics on synapses and axons in mammalian sympathetic ganglia. *J. Neurophysiol.,* **15,** 91–114.

LERMAN, J., GREGORY, G.A., WILLIS, M.M., SCHMITT, B.I. & EGER, E.I. II. (1985). Age and solubility of volatile anesthetics in ovine tissue; *Anesth. Analg.,* **64,** 1097–1100

LERMAN, J., SCHMITT-BANTEL, B.I., GREGORY, G.A., WILLIS, M.M. & EGER, E.I. II. (1986). Effect of age on the solubility of volatile anesthetics in human tissue. *Anesthesiology,* **65,** 307–311.

LEUNG, L.S. & FU, X.W. (1994). Factors affecting paired-pulse facilitation in hippocampal CA1 neurones in vitro. *Brain Res.,* **650,** 75–84.

MACIVER, M.B. & ROTH, S.H. (1988). Inhalation anaesthetics exhibit pathway-specific and differential actions on hippocampal synaptic responses in vitro. *Br. J. Anaesth.,* **60,** 680–691.

MAGNUSSON, K.R. & COTMAN, C.W. (1993). Effects of ageing on NMDA and MK801 binding sites in mice. *Brain Res.,* **604,** 334–337.

MARTIN, A.R. (1977). Junctional transmission II. Presynaptic mechanisms. In *Handbook of Physiology: The Nervous System.* ed. Am. Physiol. Soc. 329–355. Bethsda, MD.

MIU, P. & PUIL, E. (1989). Isoflurane-induced impairment of synaptic transmission in hippocampal neurones. *Exp. Brain Res.,* **75,** 354–360.

ORSER, B.A., WANG, L-Y., PENNEFATHER, P.S. & MACDONALD, J.F. (1994). Propofol modulates activation and desensitisation of $GABA_A$ receptors in cultured murine hippocampal neurones. *J. Neurosci.,* **14,** 7747–7760.

PARIKH, S. & CHUNG, F. (1996). Postoperative delirium in the elderly. *Anesth. Analg.,* **80,** 1223–1232.

PEROUANSKY, M., BARANOV, D., SALMAN, M. & YAARI, Y. (1995). Effects of halothane on glutamate receptor-mediated excitatory postsynaptic currents: A patch-clamp study in adult mouse hippocampal slices. *Anesthesiology,* **83,** 109–119.

PINTOR, A., FORTUNA, S. & MICHALEK, H. (1994). Carbachol-induced accumulation of inositol phosphate and its modulation by excitatory amino acids in cortical slices of young and aged rats with down-regulation of mascarinic M-1 receptors. *Neurochem. Res.,* **19,** 1311–1317.

POCOCK, G. & RICHARDS, C.D. (1993). Excitatory and inhibitory synaptic transmission, mechanisms in anaesthesia. *Br. J. Anaesth.,* **71,** 134–147.

PUIL, E., EL-BEHEIRY, H. & BAIMBRIDGE, K.G. (1991). Calcium Involvement in anesthetic blockade of synaptic transmission. *Ann. New York Acad. Sci.,* **625,** 82–90.

PUIL, E., HUTCHEON, B. & REINER, P.B. (1994). Isoflurane inhibits calcium currents in neocortical neurones. *Neurosci. Lett.,* **176,** 63–66.

RAHAMIMOFF, R.A. (1968). A dual effect of calcium ion on neuromuscular facilitation. *J. Physiol.,* **195,** 471–480.

REYNOLDS, J.N. & CARLEN, P.L. (1989). Diminished calcium currents in aged hippocampal dentate gyrus granule neurones. *Brain Res.,* **479,** 384–390.

RICHARDS, C.D. (1983). Actions of general anaesthetics on synaptic transmission in the CNS. *Br. J. Anaesth.,* **55,** 201–206.

RICHARDS, C.D. & WHITE, A.E. (1975). The actions of volatile anaesthetics on synaptic transmission in the dentate gyrus. *J. Physiol.,* **252,** 241–257.

RIES, C.R. & PUIL, E. (1993). Isoflurane prevents transitions to tonic and burst firing modes in thalamic neurones. *Neurosci. Lett.,* **159,** 91–94.

WEBB, E.I. & WEAVER, B.M.Q. (1981). Solubility of halothane in equine tissues at 37°C. *Br. J. Anaesth.* **53,** 479–486.

WILLIAMS-RUSSO, P., URQUHART, B.L., SHARROCK, N.E. & CHARLSON, M.E. (1992). Post-operative delirium: predictors and prognosis in elderly orthopedic patients. *J. Am. Geriatr. Soc.,* **40,** 759–767.

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