Anesthesiology 2008; 108:233–42

Attenuation of the 40-Hertz Auditory Steady State Response by Propofol Involves the Cortical and Subcortical Generators

Gilles Plourde, M.D., M.Sc., Alfonso Garcia-Asensi, M.D.,† Steven Backman, M.D., Ph.D.,* Alain Deschamps, M.D., Ph.D.,‡ Daniel Chartrand, M.D., Ph.D.,§ Pierre Fiset, M.D.,§ Terence W. Picton, M.D., Ph.D.‖*

Background: **The 40-Hz auditory steady state response (40-Hz ASSR) provides a reliable marker of anesthetic-induced unconsciousness. Brain electric source analysis indicates that the 40-Hz ASSR arises from cortical and subcortical generators. The authors used source analysis to assess the effect of propofol anesthesia on the cerebral generators of the 40-Hz ASSR. They also examined the effect of propofol on two auditory evoked potentials of cortical origin: the N1 and the sustained potential.**

Methods: **Eleven healthy human volunteers were anesthetized with propofol given in target-concentration mode at the minimal concentration causing unconsciousness. The 40-Hz ASSR was recorded before, during, and after anesthesia. The source model consisted of five concurrently active generator dipoles: two in the contralateral auditory cortex (one tangentially oriented, one radially oriented), two in the ipsilateral auditory cortex (same orientations), and one in the midline brainstem.**

Results: **During anesthesia, the strength of the cortical and brainstem dipoles was reduced to the same extent (to 54% of baseline for the four cortical dipoles pooled *vs.* 53% for the brainstem dipole). Dipole strength during anesthesia was significantly less ($P < 0.01$) than during baseline and recovery for both cortical and brainstem dipoles. The N1 and sustained potential were no longer recordable during anesthesia.**

Conclusions: **The attenuation of the 40-Hz ASSR during propofol anesthesia results from a reduction of similar magnitude of the activity of the cortical and brainstem generators. The N1 and sustained potential are so profoundly attenuated during propofol anesthesia that they are no longer recordable from the scalp.**

AUDITORY evoked potentials have been used extensively to monitor the effects of general anesthesia on the brain and the level of consciousness.[1] By contrast, the mechanisms by which general anesthetics alter auditory evoked potentials have received little attention. As a first step to examine these mechanisms, we evaluated the effects of propofol on the cerebral generators of the 40-Hz auditory steady state response (40-Hz ASSR), a sensory evoked potential that provides a reliable and sensitive measure of the hypnotic effects of general anesthetics.[2–8]

The middle latency auditory evoked response also provides a reliable measure of the effect of general anesthetics (including propofol[9]) on the brain and has been more widely used than the 40-Hz ASSR.[10] We chose the 40-Hz ASSR instead of the middle latency auditory evoked response because the stability of steady state responses over long temporal windows facilitates the localization of the cerebral generators. With transient (as opposed to steady state) responses, such as the middle latency auditory evoked response, the location of the generators changes with the poststimulus latency as brain activation ascends the sensory pathways. Attempts to localize the generators must therefore be done on small temporal windows (5–10 ms), and the recording time needed to obtain adequate signal-to-noise ratio is much longer than for the 40-Hz ASSR.

Brain electric source analysis (BESA) is widely used to characterize the intracerebral generators of sensory evoked potentials recorded noninvasively at the scalp.[11,12] With BESA, the activity of a circumscribed region of the brain is represented by a single equivalent dipole. The adjective "equivalent" is added to stress that a dipole provides a description of the compound activity of a large number of synchronized neuronal elements. A dipole is characterized by its location (three parameters), its orientation (two angles), and its strength (one parameter). Strength is the only parameter that varies over time. BESA allows for multiple, concurrently active dipoles. The activity recorded at the scalp can be predicted as the sum of the contribution of each dipole. The goal of the analysis is to obtain a prediction that closely resembles the observed data. The mismatch between the observed and predicted data is quantified as the residual variance. The analysis can iteratively modify the location or the orientation of the postulated sources to minimize the residual variance.

Brain electric source analysis does not allow complete determination of the cerebral sources generating of known distribution of scalp potentials[11] because an infinite set of intracranial sources may produce the same distribution, *i.e.*, the inverse problem (identifying the generators of a known distribution of scalp potentials) has no unique solution. Constraints must therefore be placed on the number, type, and spatial arrangements of

* Professor, § Associate Professor, Department of Anesthesia, McGill University, Montreal, Quebec, Canada. † Research Assistant, Department of Anesthesia, McGill University, Montreal, Quebec, Canada. Current position: OSR Medical Inc., Montreal, Quebec, Canada. ‡ Assistant Professor, Department of Anesthesia, McGill University, Montreal, Quebec, Canada. Current position: Département d'anesthésie, Institut de Cardiologie de Montréal, Montreal, Quebec, Canada. ‖ Professor, Rotman Research Institute at Baycrest, University of Toronto, Toronto, Ontario, Canada.

Received from the Department of Anesthesia, McGill University, Montreal, Quebec, Canada. Submitted for publication April 20, 2007. Accepted for publication October 31, 2007. Supported by grant No. MOP-49583 from the Canadian Institute of Health Research, Ottawa, Ontario, Canada. Presented in part at the Annual Meeting of Society for Neuroscience, New Orleans, Louisiana, November 8–12, 2003.

Address correspondence to Dr. Plourde: Department of Anesthesia, Montreal Neurological Hospital, 3801 University Street, Montreal, Quebec, Canada, H3A 2B4. gilles.plourde@mcgill.ca.

the postulated sources. The constraints are based on the anatomy and physiology of the sensory pathways.

Dipole strength depends on the magnitude and synchrony of the neuronal field potentials induced by the stimuli as well as on the geometrical arrangements of the activated neurons.[12] Alterations of sensory evoked potentials by general anesthetics[13] likely reflect changes in the strength and temporal pattern of dipole activation because general anesthetics impair synaptic transmission.[14]

Localization of the generators of the human 40-Hz ASSR with BESA has proposed that the response mainly arises from six concurrently active dipoles: D1, a cortical, tangentially oriented dipole in the contralateral supratemporal plane; D2, a cortical, radially oriented dipole in the contralateral supratemporal plane; D3 and D4, duplicating D1 and D2 in the ipsilateral supratemporal plane; D5, a vertically oriented dipole in the midline brainstem; and D6, a laterally oriented dipole in the midline brainstem.[15] The brainstem dipole source may reflect activity in the medial geniculate, reticular thalamus, and inferior colliculus, all of which are very close and cannot be resolved with the spatial resolution of the source analysis technique.

This source model is consistent with functional magnetic resonance imaging that showed increased blood flow in the auditory cortex and upper brainstem during 40-Hz auditory stimulation.[16] The model is also in line with animal data indicating that the 40-Hz ASSR can be recorded in the auditory cortex,[17–20] in the thalamus (medial geniculate),[20] and in the brainstem, particularly the inferior colliculus.[17,19,21,22]

We considered two hypotheses to account for the attenuation of the 40-Hz ASSR by propofol. The first hypothesis proposed that propofol exerts strong inhibition (>90%) of the cortical sources with a mild to moderate (<40% inhibition) effect on the subcortical generator. This proposal is based on the available animal literature comparing the effects of general anesthetics (barbiturates) on the cortical and subcortical generators of the 40-Hz ASSR.[20,22] This hypothesis is also in line with observations suggesting that propofol disrupts sensory processing by acting mainly on the cerebral cortex.[23] The second hypothesis was based on the suggestion that the 40-Hz ASSR critically depends on reciprocal excitation between cortical and subcortical sources.[24] In this case, one expects near equal attenuation of the cortical and subcortical sources. Therefore, the *cortical hypothesis* predicts that during anesthesia, the cortical and subcortical generators will be attenuated by 90% or more and 40% or less, respectively. The *reciprocal excitation hypothesis* predicts that the cortical and subcortical generators will be attenuated to the same extent.

The protocol used for recording the 40-Hz ASSR made it also possible to assess the effects of propofol on two evoked potentials of cortical origin: the N1 and the sustained potential (SP). The N1 evoked potential peaks approximately 100 ms after stimulus onset and is influenced by the subject's level of attention.[25] The N1 gradually decreases in amplitude during sleep onset and disappears during definite stage 2 sleep.[26] The changes in N1 are especially apparent when the subject no longer signals awareness of the external stimulus. The N1 is abolished during general anesthesia with isoflurane or thiopental.[27,28] However, there is controversy about the N1 during propofol anesthesia: Two studies reported its persistence,[29,30] and one study reported its absence.[31] BESA revealed that the N1 arises mainly from bilateral, vertically oriented dipoles on the supratemporal plane located near the primary auditory cortex.[32,33] The SP is a negative deflection that occurs during prolonged stimulus presentation and that lasts as long as the stimulus.[34,35] The SP arises from bilateral, anteriorly oriented dipoles located slightly in front of the N1 dipoles.[33] The effects of general anesthetics on the SP have not been studied.

Materials and Methods

Subjects

After approval of the McGill University Health Center ethics board (Montreal, Quebec, Canada), we recruited 11 American Society of Anesthesiologists physical status I, paid volunteers (5 men and 6 women) aged 20–38 yr (mean, 29 yr). Subjects had no history of neurologic or hearing disorders. They gave written consent, underwent a comprehensive medical evaluation, and were screened for normal hearing with pure tone audiometry.

Anesthesia

The auditory evoked responses were recorded during three periods: baseline (subject awake, lying quietly with eyes closed before any medication); propofol anesthesia (at the lowest concentration of propofol producing unconsciousness); and recovery (20 min after return of consciousness after discontinuation of propofol). Three blocks of 8 min were recorded for each period. Unconsciousness was defined as failure to respond to verbal commands.

Testing took place in the morning after an overnight fast. After placement of the electroencephalographic electrodes, we gave sodium citrate (30 ml orally) and inserted an 18-gauge cannula in a vein of the left forearm. Oxygen was given by facemask. Monitoring included three-lead electrocardiography, pulse oximetry, and inspired and expired concentrations of oxygen and carbon dioxide of gas sampled from nasal prongs. Propofol was administered with a pump (Harvard 22; Harvard Apparatus, Holliston, MA) driven by a personal computer running STANPUMP, version of

May 11, 1996,# using the kinetic variables of Tackley *et al.*[36] The effect site concentration was initially set to 1.5 μg/ml and was increased every 10 min by 0.5-μg/ml steps until the subject became quietly immobile and unresponsive to verbal commands. The target concentration required for unconsciousness was 2.0–4.5 μg/ml (mean, 3.0 μg/ml).

Stimuli and Electrophysiology

The recording protocol is based on that used by Makela and Hari.[37] The stimuli consisted of 1,000-Hz tone bursts (10 ms total duration with 2 ms rise/fall time, 100% amplitude modulation, 75 dB peak equivalent sound pressure level) delivered to right ear at the rate of 40/s in trains of 25 stimuli (625 ms). The interval between train onsets was 2.5 s. Stimuli were created using an ATHLON-XP–based (AMD, Sunnyvale, CA; 1.33 GHz) personal computer (Biostar M7MIA 2.0 RAID; BMA Industrial Inc., City of Industry, CA) using Matlab version 6.0 (MathWorks, Natick, MA) under Microsoft Windows 98 (Microsoft, Redmond, WA). They were delivered *via* the integrated sound card connected to Sennheiser HDA 200 earphones (Wedemark, Germany). The Matlab routine also produced a brief (3-ms) synchronization marker coinciding with the onset of each tone burst using the digital-to-analog function of an AT-MIO-64E-3 board (National Instruments Corp., Austin, TX).

The electroencephalogram was recorded with gold disk cup electrodes from 32 scalp sites of the extended 10-20 system[38] (FP1/2, F9/10, F7/8, F3/4, FC5/6, FC1/2, T9/10, T7/8, C3/4, CP5/6, CP1/2, P9/10, P7/8, P3/4, O1/2, Fz, and Cz) referenced to A2. Ground was Fpz. Impedances were below 5 kΩ. The electroencephalogram was amplified (Isolated Bioelectric Amplifier; SA Instrumentation Corp., San Diego, CA; 0.01- to 300-Hz bandpass), digitized at 1,024 Hz and stored on disk with Harmonie software (version 5.1; Stellate Systems, Montreal, Quebec, Canada) using the above computer and the AT-MIO-64E-3 board for analog-to-digital conversion. An additional channel was used to record stimulus onset synchronization markers.

Averaging and Preprocessing

The electroencephalogram was averaged off-line using Matlab (version 7.3; MathWorks). The epoch for averaging was from −100 to 1,000 ms relative to onset of the first tone burst of each train. Epochs contaminated by artifacts (10% of points outside ±70 μV) were rejected automatically. The average waveform for each subject and period was based on approximately 500 epochs (24 min of recording).

The amplitude at Cz of the N1 was calculated as the mean amplitude over the 75- to 125-ms interval; that of the SP was calculated as the mean amplitude over the 275- to 675-ms interval. To search for residual N1 and SP activity during anesthesia, the mean amplitude at Cz over successive intervals of 50 ms centered at 50, 100, 150, . . . 900 ms was measured. This was done to determine subsequently whether the mean amplitude differed significantly from zero. This technique of using sequential means to look at a slowly changing waveform has long been used in event-related potential research.[39]

The amplitude of the 40-Hz ASSR was obtained with a 512-point fast Fourier transform over a time window from 125 to 625 ms after the start of the stimulation on the Cz waveform of each subject for each period. The measurement was started at 125 ms to remove the effects of the transient γ band response and to allow build-up of the 40-Hz ASSR.[40]

Source Analysis

N1 and SP. Source analysis will not be reported for N1 (and SP) because these responses were completely abolished by propofol. Therefore, no source analysis could be obtained for the anesthesia data.

40-Hz ASSR. The montage was first converted to average reference. The grand-average baseline data were used to obtain a source template with the program BESA (version 3.0; MEGIS Software GmbH, Gräfelfing, Germany) using a four-shell head model. The source model was similar to that of Herdman *et al.*[15] with two exceptions. First, only one brainstem dipole (D5, vertically oriented) was used because the laterally oriented brainstem dipole in Herdman *et al.*[15] is small and mostly reflects ear differences. Second, the radial and tangential cortical dipoles were allowed to separate in location (see Scherg *et al.*[32]). The cortical dipoles were constrained to be symmetrical in location and mirror image in orientation. This template of five dipoles was then used to obtain the source waveforms of the 40-Hz ASSR of each subject and recording period. The period selected for this analysis was from 125 to 625 ms after train onset. The data were collapsed to two cycles (50 ms) and filtered 30–50 Hz before source analysis to increase signal-to-noise ratio. This procedure is called "within-epoch" averaging and is equivalent to conventional averaging using the first stimulus of each train and every other stimulus afterward. It was used by Herdman *et al.*[15] and by others.[41] Dipole strength was assessed as the root-mean square of source waveform across the two cycles. The onset phase (*i.e.*, the section of the waveform that occurs at time 0) of the dipole was obtained by fast Fourier transform of the source waveform across the two cycles. This measurement can be converted to phase lag by subtracting from 360°. Phase lag is related to latency, but the measurement is ambiguous because of the unknown number of cycles preceding the measured cycle.

STANPUMP program. Available at: http://anesthesia.stanford.edu/pkpd. Accessed October 15, 2007.

Statistics

Unless indicated otherwise, all results are reported as mean ± SD and were analyzed with analyses of variance for repeated measures with Geisser-Greenhouse adjustment and Tukey honest significant difference for *post hoc* tests.[42] The criterion for significance was 0.05. Procedures were performed with Statistica (version 4.1 for the Macintosh; Statsoft, Tulsa, OK). Power calculations were calculated with the function `sampsizepwr` from the Matlab statistical toolbox (version 5.3, MathWorks).

Additional procedures were performed to address specific needs as described below.

Sequential Interval Testing for N1 and SP. To establish the presence of response, one-sample *t* tests were conducted to determine whether the mean Cz amplitude over successive intervals of 50 ms differed significantly from zero. The two-tailed criterion for significance was 0.0028 after Bonferroni correction (18 comparisons per recording period) for an overall error rate of 0.05 for each component.

40-Hz ASSR Dipoles: Comparison of the Cortical Dipoles as a Group *versus* the Brainstem Dipole. To compare the effect of propofol on cortical *versus* brainstem dipoles during anesthesia, dipole strength during anesthesia was divided by dipole strength during baseline to obtain a relative measure. The relative values for the four cortical dipoles were then combined in a weighted average to obtain a value for the cortical dipoles as a group. This was done for each subject separately. The weights reflected the relative contribution of each cortical dipole to total cortical dipole strength during baseline. This was necessary because Herdman *et al.*[15] found 3.5-fold differences in strength among the cortical dipoles. Therefore, the global impact of the attenuation of a given cortical dipole by propofol depends on the contribution of the dipole to total cortical strength. A similar process was performed for recovery relative to baseline. Paired *t* tests were used to compare relative strength of cortical dipoles as a group *versus* the brainstem dipole during anesthesia and recovery. One-sample *t* tests were used to determine whether the normalized dipole strength during anesthesia or recovery was different from baseline (*i.e.*, from one).

40-Hz ASSR Dipoles: Phase Statistics. Circular statistics[43] were used to compute the mean and SD of the phase obtained by fast Fourier transform. Phase is reported in degrees (0–360°), counterclockwise from horizontal and relative to a cosine. Pairwise comparisons (between dipoles during baseline; between baseline and anesthesia) were obtained with a Hotelling T^2 test[44] implemented in Matlab (version 7.3). To limit the number of tests and the risk of spurious significance when amplitudes were small, tests of phase differences were obtained only for the three main dipoles (D1, D3, and D5).

Results

Effects of Propofol on the Electroencephalogram

Inspection of the raw electroencephalographic tracings revealed increased amplitude of background rhythms, mainly affecting the α and β bands. This pattern is similar to the stage 1 of light anesthesia reported by Reddy *et al.*[45]

N1 and SP

Figure 1 shows the original waveforms recorded at Cz from each subject as well as the grand-average waveforms across all the subjects. The 0- to 20-Hz filtered data reveal clear N1 and SP during baseline and recovery. During anesthesia, there is no clear response. The amplitude of N1 (mean ± SD) was −1.18 ± 0.36, 0.15 ± 1.20, and −0.49 ± 0.38 μV during baseline, anesthesia, and recovery, respectively ($P < 0.01$ for anesthesia *vs.* baseline and recovery). The amplitude of the SP was −1.46 ± 0.63, −0.26 ± 0.98, and −1.46 ± 0.78 during baseline, anesthesia, and recovery, respectively ($P < 0.01$ for anesthesia *vs.* baseline and recovery).

The mean amplitude of the intervals corresponding to the N1 (100 ms) and to the SP (intervals centered at 300–650 ms) differed significantly (one-sample *t* tests; $P < 0.0028$) from zero during baseline and recovery. By contrast, the mean amplitude at Cz over successive 50 ms-intervals (table 1) did not differ significantly from zero during anesthesia, indicating that the N1 and SP were no longer recordable.

40-Hz ASSR

The 40-Hz ASSR is visible as the 30- to 50-Hz filtered traces in figure 1 and in figure 2, which shows the grand-average waveforms (all subjects pooled) used for source analysis after collapsing the period from 125 to 625 ms after train onset to a two-cycle (50 ms). Inspection of figure 2 reveals that propofol reduced the amplitude of the 40-Hz ASSR on all channels. Measures from individual subjects showed that the amplitude of the 40-Hz ASSR at Cz was (μV) 0.17 ± 0.07 during baseline, 0.08 ± 0.03 during propofol, and 0.23 ± 0.09 during recovery ($P < 0.001$ for propofol *vs.* baseline or recovery; $P < 0.01$ for baseline *vs.* recovery). Figure 2 shows that propofol also changed the phase of response on most channels.

Dipole Fit and Dipole Source Waveforms

Residual variance (%) for the dipole fit on the grand-average waveforms was 4, 22, and 3 during baseline, anesthesia, and recovery, respectively. Residual variance (%) for the fit when applied to individual subjects was 12 ± 7, 30 ± 13, and 12 ± 8 during baseline, anesthesia, and recovery, respectively. The specific contribution of each dipole to the reduction of the variance during baseline was measured by removing from the model each source, one at a time. The contributions were 12, 5, 9, 6, and 8%

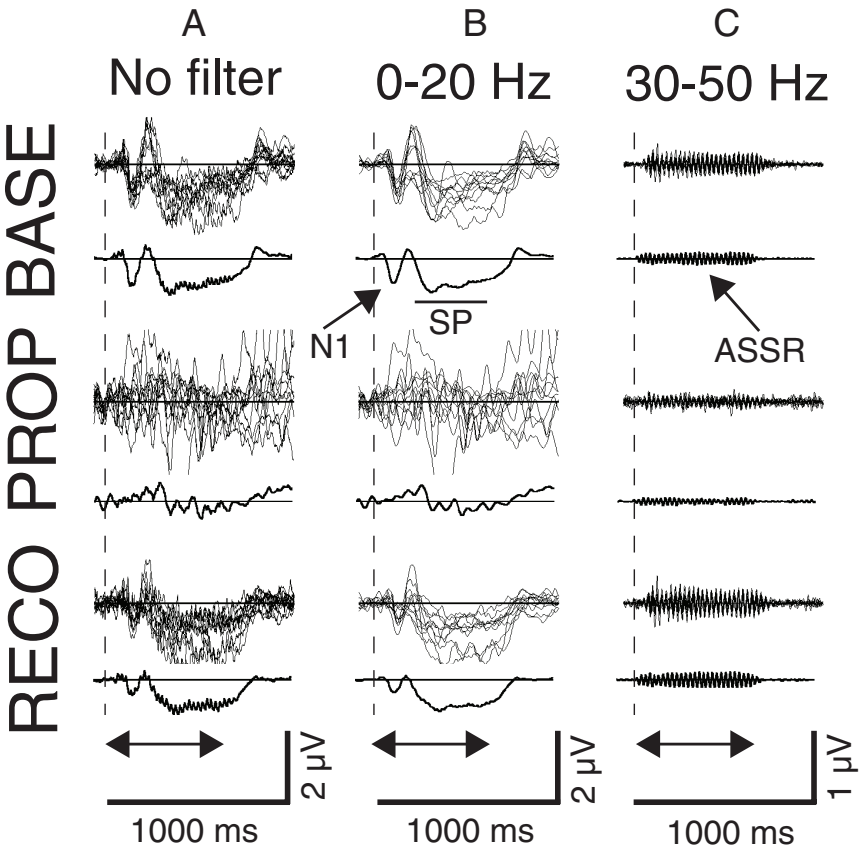

Fig. 1. For each period, the *upper row* shows the traces obtained at Cz (vertex) from each subject. Positivity is upward. The *lower row* shows the grand-averaged traces. Column *A*, labeled "no filter," shows the raw traces. Column *B*, labeled "0–20 Hz," shows waveforms after application of a 0- to 20-Hz digital filter to reveal the N1 and sustained potential (SP). Column *C*, labeled "30–50 Hz," shows waveforms after application of a 30- to 50-Hz digital filter to reveal the 40-Hz auditory steady state response (40-Hz ASSR). The *horizontal arrows* indicate the timing of the stimulus train. Base = baseline; prop = propofol; reco = recovery.

for dipoles 1–5, respectively. (The sum of these values is less than the total variance explained [96%] because, when one source is removed, the increase in residual variance is absorbed in part by the remaining sources.)

Table 1. Range Amplitude at Cz for N1 (100 ms) and SP (300–700 ms)

Time, ms	Baseline	Anesthesia	Recovery
50	0.21 (0.27)	0.02 (0.63)	0.12 (0.21)
100	−1.18 (0.36)*	0.15 (1.20)	−0.49 (0.38)*
150	−0.48 (0.62)	0.30 (1.05)	−0.28 (0.35)
200	0.47 (1.20)	0.58 (1.26)	0.06 (0.85)
250	−0.69 (0.88)	0.70 (0.86)	−0.85 (0.75)
300	−1.76 (0.88)*	0.16 (1.41)	−1.32 (0.76)
350	−1.82 (0.66)*	−0.26 (1.70)	−1.73 (0.90)*
400	−1.52 (0.74)*	−0.42 (1.53)	−1.68 (0.80)*
450	−1.64 (0.71)*	0.01 (1.00)	−1.53 (0.80)*
500	−1.59 (0.78)*	−0.65 (0.93)	−1.54 (0.78)*
550	−1.35 (0.77)*	−0.50 (0.71)	−1.46 (0.85)*
600	−1.31 (0.74)*	−0.19 (0.75)	−1.51 (0.88)*
650	−1.19 (0.63)*	−0.36 (0.74)	−1.32 (0.69)*
700	−0.93 (0.57)*	−0.10 (0.96)	−1.04 (0.73)*
750	−0.46 (0.47)*	−0.09 (1.38)	−0.40 (0.50)
800	0.48 (0.58)	0.31 (1.55)	0.08 (0.38)
850	0.41 (0.51)	0.41 (1.39)	0.06 (0.44)
900	0.20 (0.33)	0.61 (2.01)	0.00 (0.41)

Data are mean (SD), in microvolts. Time designates the center of interval (*i.e.*, 50 refers to the 25- to 75-ms range).

* Significant at $P < 0.0028$.

Cz = scalp location of the 10–20 system and corresponding to the vertex; SP = sustained potential.

Figure 3A shows the dipole diagrams and figure 3B shows the activity of the source waveforms for each period. Inspection of this figure reveals the following. During baseline, the strength of the brainstem dipole (D5) is largest, followed by that of the two tangential cortical dipoles (D1 and D3). The two radial dipoles (D2 and D4) have the lowest strength. During baseline, the phase of the source waveforms are such that the latency of the first positive peak is shortest for the brainstem dipole (D5, arrow), followed by that of the two tangential cortical dipoles (D1 and D3) and, last, by that of the two radial dipoles (D2 and D4). This pattern is consistent with ascending activation in the auditory pathways. The figure also reveals that propofol reduced the strength of all dipoles and changed the phase of the cortical dipoles (D1–D4). The source strength during recovery appears slightly larger than during baseline. Propofol did not alter the phase of the brainstem dipole (D5).

Individual Measures of Dipole Strength

Measures from individual subjects were subjected to statistical analysis to validate the observations described above. There were significant differences in dipole magnitude (fig. 4) during baseline: D1 (tangential left cortical) was larger than D2 (radial left cortical) ($P < 0.05$) and D4 (radial right cortical) ($P < 0.01$); D3 (tangential right cortical) was larger than D2 (radial left cortical) ($P < 0.001$) and D4 (radial right cortical) ($P < 0.01$); D5 (brainstem)

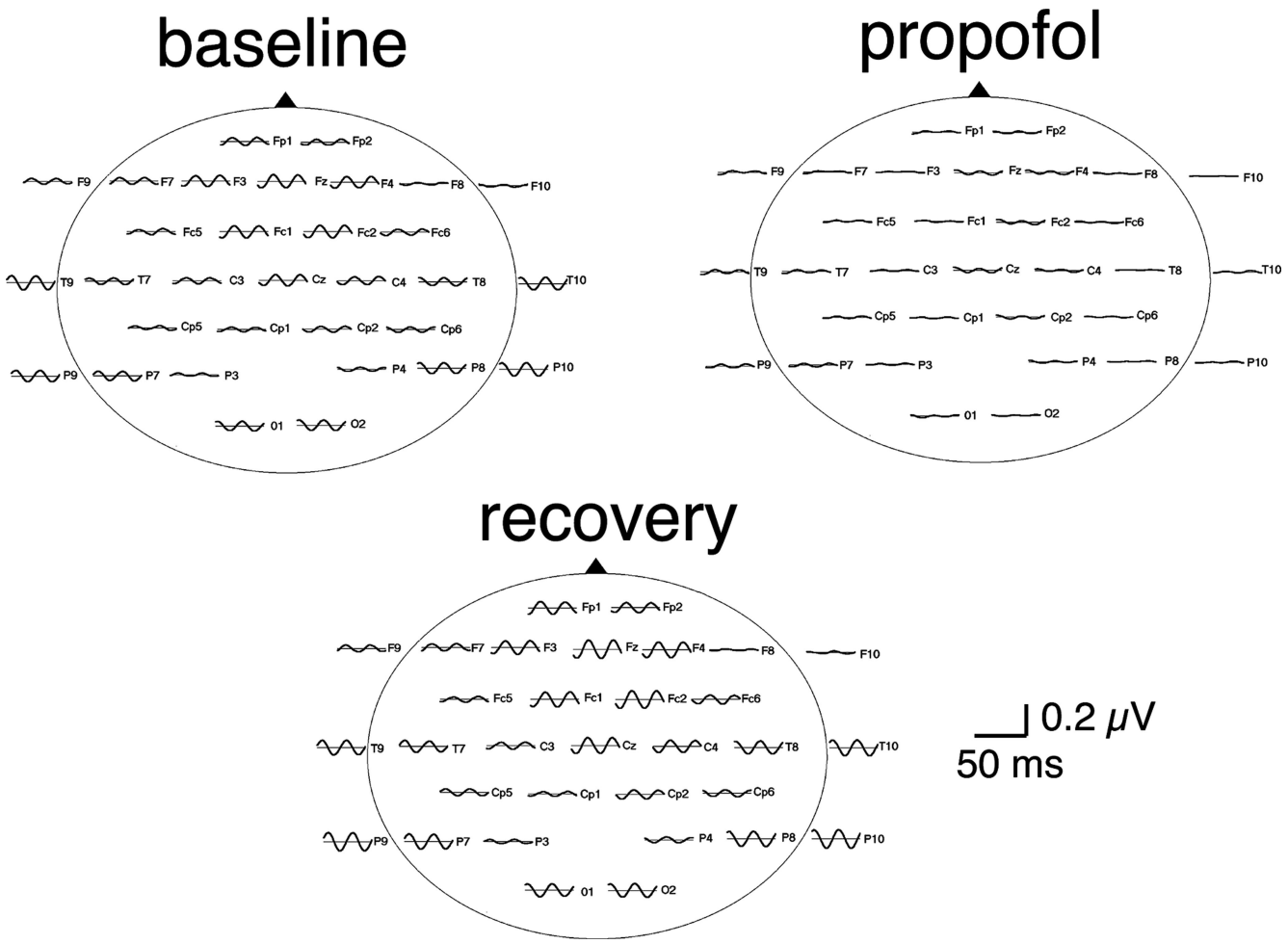

Fig. 2. Grand-averaged waveforms used for source analysis. These waveforms were obtained after collapsing the period 125–625 ms after train stimulus onset to further increase signal-to-noise ratio. They are shown on a diagram of the head seen from above, with the nose as a triangle. The labels of the extended 10–20 system are indicated to the right of each trace. Positivity is upward.

was larger than all other dipoles ($P < 0.001$). The magnitude of all dipoles was significantly less during anesthesia than during baseline and recovery except for D2 (left cortex radial) compared with baseline (table 2 and fig. 4). The magnitude of all dipoles during recovery was higher than during baseline (fig. 4; see normalized measures below for statistical comparisons).

The relative strength of the cortical dipoles during anesthesia (baseline = 1) was 0.41 ± 0.26 for D1, 0.68 ± 0.58 for D2, 0.61 ± 0.36 for D3, and 0.51 ± 0.40 for D4. There were no significant differences between these values, which were all significantly less than baseline ($P < 0.01$; one-sample t test). The pooled relative strength during anesthesia of the four cortical dipoles as a group was 0.54 ± 0.24, and it did not differ significantly from the relative strength of the brainstem dipole (D5), 0.53 ± 0.40 (paired t test; $t = 0.12$, $P = 0.91$; fig. 5). These two values were significantly smaller than baseline ($P < 0.01$; one-sample t test). Therefore, the cortical dipoles as a group and the brainstem dipole were attenuated to the same extent during anesthesia. The difference between the relative strength of the pooled cortical dipoles and of the brainstem dipole that could have been detected with a power of 0.8 at $P < 0.05$ (one-tailed) was 0.37 (*i.e.*, cortical attenuation exceeding brainstem attenuation by 0.37).

During recovery, the relative value for the pooled cortical dipoles and the brainstem dipole was 1.3 ± 0.3 and 1.2 ± 0.0.8, respectively (fig. 5). The cortical value was significantly higher than baseline ($P < 0.01$; one-sample t test). The difference between the two values was not significant (paired t test; $t = -0.71$, $P = 0.50$).

Individual Measures of Dipole Phase

Phase comparisons were limited to the three dipoles with the largest magnitude: the two tangential cortical dipoles (D1 and D3) and the brainstem dipole (D5). During baseline, the onset phase of D5 (195 ± 18, in degrees) was significantly ($P < 0.05$) greater than that of the two tangential dipoles: D1 (131 ± 25) and D3 (81 ± 29), which were also significantly ($P < 0.001$) different from each other. In terms of phase lag, the D1 and D3 responses occurred later than the brainstem responses.

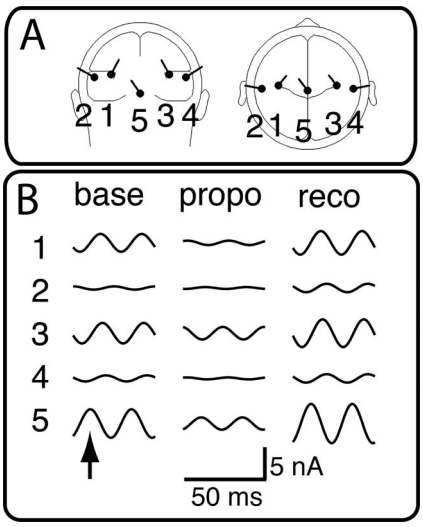

Fig. 3. (*A*) Posterior and top view of the dipoles. The dipoles are not scaled to magnitude. (*B*) Current strength time course for each dipole in nanoamperes. The *arrow* points to the first positive peak of dipole 5 during baseline. Base = baseline; propo = propofol; reco = recovery.

There were significant phase differences between baseline and anesthesia for the two tangential dipoles (D1: 131 ± 25 during baseline *vs.* 325 ± 45 during anesthesia; D3: 81 ± 29 *vs.* 34 ± 29). Both of these can be interpreted as increased phase lag. The phase of the brainstem dipole did not change significantly (195 ± 18 *vs.* 218 ± 40).

Discussion

40-Hz ASSR

The main finding of this study is that the cortical and subcortical generators of the 40-Hz ASSR are affected to the same extent during propofol anesthesia This observation clearly supports the *reciprocal excitation hypothesis*, whereby the 40-Hz ASSR involves recurrent excitation between cortical and brainstem sources[24] and appears at first glance at variance with the prevalent view that impairment of (nonnociceptive) sensory processing by propofol involves mostly cortical neurons, with only minimal, if any, alterations at the level of the brainstem. It must be noted, however, that these observations[23] supporting the *cortical hypothesis* concern the somesthetic system.

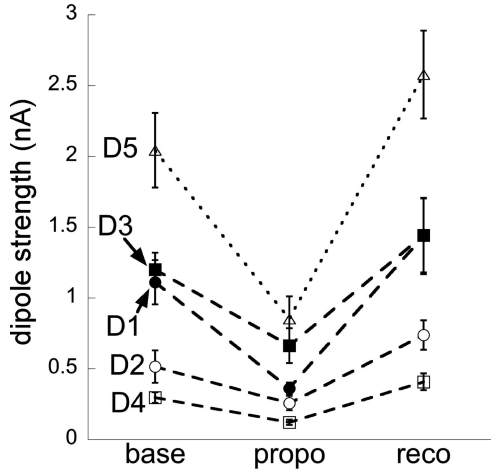

Fig. 4. Mean and SE for dipole strength for each period. n = 11. Base = baseline; propo = propofol; reco = recovery.

Table 2. Results of the ANOVA *Post Hoc* Comparisons

Dipole	Baseline–Anesthesia	Anesthesia–Recovery	Baseline–Recovery
1 left cortex tangential	$P < 0.01$	$P < 0.001$	NS
2 left cortex radial	NS	$P < 0.01$	NS
3 right cortex tangential	$P < 0.01$	$P < 0.01$	NS
4 right cortex radial	$P < 0.01$	$P < 0.001$	NS
5 brainstem	$P < 0.01$	$P < 0.001$	NS

ANOVA = analysis of variance; NS = not significant, $P > 0.10$.

It is likely that the attenuation of the cortical generator results from a direct effect of propofol given the prominent inhibitory effect of this drug on the cerebral cortex.[23] The reduced activity of the brainstem generator of the 40-Hz ASSR may result from a direct or indirect effect of propofol. Brainstem neurons, most notably in the inferior colliculus, which is a probable generator of the 40-Hz ASSR based on animal studies,[17,19,21,22] are clearly affected by general anesthetics.[46,47] There is, however, no data for propofol. Thus, there is partial support from literature for proposing a direct anesthetic effect at the brainstem level. An indirect effect on the brainstem is also possible. The reduction of cortical activity by propofol could indirectly attenuate the activity of brainstem source by decreasing the excitatory cortical input and thus attenuating the recurrent excitation between the brainstem and cortex.[24] Proper evaluation of the relative contributions of the direct and indirect actions of propofol will require animal experimentation.

An important issue to consider is the reliability of the BESA modeling applied to the current data. Reliability can be assessed by the comparing residual variance and the pattern of source activity with published work. The level of residual variance (a measure of the signal-to-noise

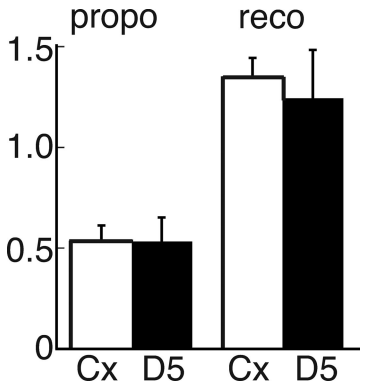

Fig. 5. Mean and SE of dipole strength normalized as function of baseline (set to 1) for the cortical dipoles pooled (Cx) and for the brainstem dipole (D5) during propofol (propo) and recovery (reco). n = 11.

ratio, *i.e.*, quality of the recordings) during baseline and recovery is almost identical to that reported by Herdman *et al.*[15] in waking subjects, and this applies to both grand-average and individual data. As expected, the residual variance during anesthesia was higher than during baseline and recovery. The most likely explanation is a reduction of signal-to-noise ratio because propofol reduces the amplitude of the evoked response[10] and increases the amplitude of background electroencephalogram.[45]

The pattern of 40-Hz ASSR source activity obtained during baseline (greater magnitude of the brainstem source compared with cortical sources; greater magnitude of the tangential cortical sources compared with the radial cortical sources) is similar to that described by Herdman *et al.*[15] The similarity between our findings during wakefulness and those of Herdman *et al.*[15] despite different recording environments provides a strong assurance of reliability.

The higher strength of the midbrain source is unusual when the organization of the assumed generator areas are considered. Evoked potentials probably originate from postsynaptic potentials in parallel oriented neural structures, which are sparse in the midbrain–brainstem and thalamus but abundant in the cortex. Herdman *et al.*[48] also reported higher brainstem source strength. Responses generated earlier in the pathway may be more synchronous, and this may compensate for the geometric advantage of the cortical sources.

A possible explanation for the strong midbrain source is that this source may have captured some activity from the cortical sources. The extent of misallocation can be assessed by how much the removal of the brainstem source increases the source strength of the cortical activations. We accordingly obtained a revised source analysis after excluding the brainstem source (D5) from the model which we applied to the baseline grand-averaged waveforms. Source strength was unchanged for D1 and was increased by 19, 9, and 5% for D2, D3, and D4, respectively. The mean increase for the four dipoles was 8%. This modest increase indicates that the capture of cortical activity by the brainstem dipole (D5) had little impact on its strength. Furthermore, the cortical and brainstem sources were relatively independent in their topographies (data not shown). This provides additional evidence against possible misallocation of cortical activity to the brainstem source (D5).

The significant differences in the phase of the dipole source waveforms provides additional support for the validity of the model. The phase difference between the brainstem dipole and the two main (tangential) cortical dipoles during baseline likely reflects the earlier activation of the brainstem source. Likewise, the phase difference between the two tangential cortical dipoles likely reflects the earlier activation of the contralateral cortex. Finally, the change in phase between baseline and anesthesia noted with the two main tangential cortical dipoles but not with the brainstem dipole is analogous to the observation that general anesthetics affect the latency of cortical evoked potentials much more than that of the brainstem responses.[10]

Another concern is the validity of electrophysiologic source modeling in general. As noted introduction, there is no known method to unequivocally identify the cerebral generators of sensory evoked potentials recorded at the scalp. Despite this limitation, it is reasonable to accept the solution obtained by source modeling if one can demonstrate that the source model (1) explains the observed data as well as can be expected from the level of residual noise, (2) is reproducible (*e.g.*, baseline *vs.* recovery), and (3) makes sense physiologically (*e.g.*, see the previous paragraph on phase changes).[12]

The right tangential cortical dipole (D3) seems to have been less affected by propofol than the other cortical dipoles. Although there was no significant difference between cortical dipoles in the extent of attenuation, the seemingly lesser impact of propofol on D3 remains puzzling. We have no clear explanation, but we think that this finding may be related to the asymmetry of stimulus input (right ear).

Previous studies of the effect of general anesthetics on the generators of the 40-Hz ASSR used pentobarbital or isoflurane. Sem-Jacobsen *et al.*[49] recorded the response from the temporal cortex of patients with implanted electrodes to a train of clicks presented at rates near 40/s (based on close examination of fig. 6 after magnification). The authors reported that the cortical response decreased as the depth of thiopental anesthesia increased. Under extremely deep levels of anesthesia, the response could not be elicited. These studies were published in 1956, well before recognition of the 40-Hz ASSR as a separate entity.[50] Makela *et al.*[20] reported that pentobarbital anesthesia (35–40 mg/kg intraperitoneal) in cats causes a near complete suppression of the cortical 40-Hz ASSR recorded from the auditory cortex and approximately a 35% attenuation of the 40-Hz ASSR recorded from the medial geniculate nucleus. Szalda *et al.*[22] showed that pentobarbital anesthesia (40 mg/kg intramuscular) predominantly affects the cortical generator of the ASSR** in the chinchilla, with minimal effect on the inferior colliculus. Kuwada *et al.*[24] suggested, based on the effect of pentobarbital (12–25 mg intravenous) on the amplitude modulation rate transfer, that the drug reduces the ASSR in both the auditory cortex and inferior colliculus but that the cortical response was slightly more attenuated. A higher effective concentration of pentobarbital in the study of Kuwada *et al.*[24] may

** The stimulation frequency yielding the largest or most reliable ASSR depends on the species. The optimal frequency is 40 Hz in humans, 70 Hz in the chinchilla,[22] and 60 Hz in the rabbit.[24] Most studies examine different frequencies to obtain information about the amplitude modulation rate transfer (*i.e.*, plots of the ASSR amplitude as a function of stimulation frequency). We will omit the label "40 Hz" when the findings to which we refer include frequencies other than 40 Hz.

explain why they observed an effect on the inferior colliculus whereas Szalda *et al.*[22] did not. Santarelli *et al.*,[51] using subdural electrodes overlying the auditory cortex of the rat, showed that the amplitude of the 40-Hz ASSR was decreased by half by 0.4–1.1% isoflurane.

N1 and SP

This is the first study to examine the effect of a general anesthetic on the SP, which was no longer recordable during anesthesia. This suggests that the SP is a sensitive marker of anesthetic effect. The SP has features that could be advantageous for clinical monitoring the level of consciousness in clinical anesthesia. Its plateau shape facilitates its extraction from background noise, and its duration depends on the length of the stimulus train, which could be varied to facilitate its recognition. Before further consideration, however, the effect of sedative concentrations of propofol on the SP must be assessed.

The current study shows that the N1 is abolished during propofol anesthesia, in accord with the observations of Simpson *et al.*[31] and in contrast to those of Ypparila *et al.*[29] and van Hooff *et al.*,[30] who reported the persistence of the N1. This discrepancy may be explained by the influence of surgery and the mode of administration of propofol. The study of van Hooff *et al.*[30] was conducted during cardiac surgery, and that of Ypparila *et al.*[29] was conducted immediately after cardiac surgery. By contrast, Simpson *et al.*[31] collected their data before surgery, and our subjects did not undergo surgery. In the two studies involving surgery, surgical pain may have increased arousal, and the patients may have had difficulty signaling their consciousness because of the other drugs used (opiates, muscle relaxants). Furthermore, propofol was not infused in a target-controlled mode in these two studies, in contrast to the study of Simpson *et al.*[31] and the current study.

Why are the N1 and SP more affected than the cortical generator of the 40-Hz ASSR? The most parsimonious explanation is that the cortical ASSR generator mainly arises from a cortical response (the middle latency [15- to 35-ms] auditory response), which precedes the N1 and SP response and is more directly connected to the brainstem input than later responses. The N1 and SP involve more synapses[52] and depend more on cortico-cortical activation, which is highly susceptible to propofol-induced interference.[23]

We conclude that (1) the decrease of the amplitude of the 40-Hz ASSR during propofol anesthesia results from an attenuation of similar magnitude of the activity of the cortical and brainstem generators, and (2) the N1 and SP are so profoundly attenuated during propofol anesthesia that they are no longer recordable from the scalp.

The authors thank Chantale Porlier (Electrophysiology Technician, Department of Anesthesia, Royal Victoria Hospital, Montreal, Quebec, Canada) and Louise Ullyatt, R.N. (Department of Anesthesia, Royal Victoria Hospital), for their help; Jean Gotman, Ph.D. (President and CEO, Stellate Systems, Montreal, Quebec, Canada), for allowing the authors to use the Harmonie software for electroencephalogram recording; the staff of the Recovery Room of the Royal Victoria Hospital for their cooperation; Janet Macdonald (Secretary, Department of Anesthesia, Montreal Neurological Hospital, Montreal, Quebec, Canada) for editorial assistance; and the anonymous reviewers for many useful suggestions.

References

1. Plourde G: Auditory evoked potentials and 40-Hz oscillations. ANESTHESIOLOGY 1999; 91:1187–9
2. Plourde G, Picton TW: Human auditory steady-state response during general anesthesia. Anesth Analg 1990; 71:460–8
3. Plourde G, Villemure C: Comparison of the effects of enflurane/N_2O on the 40-Hz auditory steady-state response *versus* the auditory middle-latency response. Anesth Analg 1996; 82:75–83
4. Plourde G, Villemure C, Fiset P, Bonhomme V, Backman SB: Effect of isoflurane on the auditory steady-state response and on consciousness in human volunteers. ANESTHESIOLOGY 1998; 89:844–51
5. Meuret P, Backman SB, Bonhomme V, Plourde G, Fiset P: Physostigmine reverses propofol-induced unconsciousness and attenuation of the auditory steady state response and bispectral index in human volunteers. ANESTHESIOLOGY 2000; 93:708–17
6. Bonhomme V, Plourde G, Meuret P, Fiset P, Backman SB: Auditory steady-state response and bispectral index for assessing level of consciousness during propofol sedation and hypnosis. Anesth Analg 2000; 91:1398–403
7. Yli-Hankala HL, Edmonds HL, Heine MF, Strickland T, Tsueda K: Auditory steady-state response, upper facial EMG, EEG and heart rate as predictors of movement during isoflurane-nitrous oxide anaesthesia. Br J Anaesth 1994; 73: 174–9
8. Picton TW, John MS, Purcell DW, Plourde G: Human auditory steady-state responses: The effects of recording technique and state of arousal. Anesth Analg 2003; 97:1396–402
9. Tooley MA, Greenslade GL, Prys-Roberts C: Concentration-related effects of propofol on the auditory evoked response. Br J Anaesth 1996; 77:720–6
10. Plourde G: Auditory evoked potentials. Best Pract Res Clin Anaesthesiol 2006; 20:129–39
11. Scherg M: Fundamentals of dipole source potential analysis, Auditory Evoked Magnetic Fields and Electric Potentials. Edited by Grandori F, Hoke M, Romani GL. Basel, Karger, 1990, pp 40–69
12. Picton TW, Lins O, Scherg M: The recording and analysis of event-related potentials, Handbook of Neuropsychology, 2nd edition. Edited by Boller F, Grafman J. Amsterdam, Elsevier, 1995, pp 3–73
13. Thornton C, Sharpe RM: Evoked responses in anaesthesia. Br J Anaesth 1998; 81:771–81
14. Angel A: Central neuronal pathways and the process of anaesthesia. Br J Anaesth 1993; 71:148–63
15. Herdman AT, Lins O, Van Roon P, Stapells DR, Scherg M, Picton TW: Intracerebral sources of human auditory steady-state responses. Brain Topogr 2002; 15:69–86
16. Purdon P, Purdon A, Jaaskalainen I, Iwaki S, Angelone NL, Belliveau J, Brown E, Bonnmassar G: Concurrent recording of 40-Hz auditory steady state response and functional MRI (abstract), Human Brain Mapping 2004. Budapest, Hungary, 2004, p 2
17. Ottaviani F, Paludetti G, Grassi S, Draicchio F, Santarelli RM, Serafini G, Pettorossi VE: Auditory steady-state responses in the rabbit. Audiology 1990; 29:212–8
18. Conti G, Santarelli R, Grassi C, Ottaviani F, Azzena GB: Auditory steady-state responses to click trains from the rat temporal cortex. Clin Neurophysiol 1999; 110:62–70
19. Hori A, Yasuhara A, Naito H, Yasuhara M: Steady-state auditory evoked potentials (SSAEPs) in the rabbit: Contribution of the inferior colliculus. Electroencephalogr Clin Neurophysiol 1993; 88:229–36
20. Mäkelä JP, Karmos G, Molnar M, Csepe V, Winkler I: Steady-state responses from the cat auditory cortex. Hear Res 1990; 45:41–50
21. Tsuzuku T: 40-Hz steady state response in awake cats after bilateral chronic lesions in auditory cortices or inferior colliculi. Auris Nasus Larynx 1993; 20:263–74
22. Szalda K, Burkard R: The effects of Nembutal anesthesia on the auditory steady-state response (ASSR) from the inferior colliculus and auditory cortex of the chinchilla. Hear Res 2005; 203:32–44
23. Angel A, LeBeau F: A comparison of the effects of propofol with other anaesthetic agents on the centripetal transmission of sensory information. Gen Pharmacol 1992; 23:945–63
24. Kuwada S, Anderson JS, Batra R, Fitzpatrick DC, Teissier N, D'Angelo WR: Sources of the scalp-recorded amplitude-modulation following response. J Am Acad Audiol 2002; 13:188–204
25. Näätänen R, Picton T: The N1 wave of the human electric and magnetic response to sound: A review and an analysis of the component structure. Psychophysiology 1987; 24:375–425
26. Campbell KB, Colrain IM: Event-related potential measures of the inhibition of information processing: II. The sleep onset period. Int J Psychophysiol 2002; 46:197–214

27. Plourde G, Picton TW: Long-latency auditory evoked potentials during general anesthesia: N1 and P3 components. Anesth Analg 1991; 72:342–50

28. Howard MA, Volkov IO, Mirsky R, Garell PC, Noh MD, Granner M, Damasio H, Steinschneider M, Reale RA, Hind JE, Brugge JF: Auditory cortex on the human posterior superior temporal gyrus. J Comp Neurol 2000; 416:79–92

29. Ypparila H, Karhu J, Westeren-Punnonen S, Musialowicz T, Partanen J: Evidence of auditory processing during postoperative propofol sedation. Clin Neurophysiol 2002; 113:1357–64

30. van Hooff JC, de Beer NA, Brunia CH, Cluitmans PJ, Korsten HH: Event-related potential measures of information processing during general anesthesia. Electroencephalogr Clin Neurophysiol 1997; 103:268–81

31. Simpson TP, Manara AR, Kane NM, Barton RL, Rowlands CA, Butler SR: Effect of propofol anaesthesia on the event-related potential mismatch negativity and the auditory-evoked potential N1. Br J Anaesth 2002; 89:382–8

32. Scherg M, Vajsar J, Picton TW: A source analysis of the late human auditory evoked potentials. J Cogn Neurosci 1989; 1:336–55

33. Picton TW, Alain C, Woods DL, John MS, Scherg M, Valdes-Sosa P, Bosch-Bayard J, Trujillo NJ: Intracerebral sources of human auditory-evoked potentials. Audiol Neurootol 1999; 4:64–79

34. Picton TW, Woods DL, Proulx GB: Human auditory sustained potentials: I. The nature of the response. Electroencephalogr Clin Neurophysiol 1978; 45: 186–97

35. Picton TW, Woods DL, Proulx GB: Human auditory sustained potentials: II. Stimulus relationships. Electroencephalogr Clin Neurophysiol 1978; 45:198–219

36. Tackley RM, Lewis GTR, Prys-Roberts C, Boaden RW, Dixon J, Harvey JT: Computer controlled infusion of propofol. Br J Anaesth 1989; 62:46–53

37. Mäkelä JP, Hari R: Evidence for cortical origin of the 40 Hz auditory evoked response in man. Electroencephalogr Clin Neurophysiol 1987; 66:539–46

38. Committee EPN: Guideline thirteen: Guidelines for standard electrode position nomenclature. J Clin Neurophysiol 1994; 11:111–3

39. Guthrie D, Buchwald JS: Significance testing of difference potentials. Psychophysiology 1991; 240–4

40. Ross B, Picton TW, Pantev C: Temporal integration in the human auditory cortex as represented by the development of the steady-state magnetic field. Hear Res 2002; 165:68–84

41. Ross B, Borgmann C, Draganova R, Roberts LE, Pantev C: A high-precision magnetoencephalographic study of human auditory steady-state responses to amplitude-modulated tones. J Acoust Soc Am 2000; 108:679–91

42. Kirk RE: Experimental Design: Procedures for the Behavioral Sciences, 2nd edition. Belmont, California, Brooks/Cole Publishing, 1982, pp 237–98

43. Fisher NI: Statistical Analysis of Circular Data. Cambridge, Cambridge University Press, 1993, pp 105–33

44. Picton TW, John MS, Dimitrijevic A, Purcell DW: Human auditory steady-state responses. Int J Audiol 2003; 42:177–219

45. Reddy RV, Moorthy SS, Mattice T, Dierdorf SF, Deitch RD Jr: An electroencephalographic comparison of effects of propofol and methohexital. Electroencephalogr Clin Neurophysiol 1992; 83:162–8

46. Kuwada S, Batra R, Stanford TR: Monaural and binaural response properties of neurons in the inferior colliculus of the rabbit: Effects of sodium pentobarbital. J Neurophysiol 1989; 61:269–82

47. Torterolo P, Falconi A, Morales-Cobas G, Velluti RA: Inferior colliculus unitary activity in wakefulness, sleep and under barbiturates. Brain Res 2002; 935:9–15

48. Herrmann CS, Mecklinger A, Pfeifer E: Gamma responses and ERPs in a visual classification task. Clin Neurophysiol 1999; 110:636–42

49. Sem-Jacobsen CW, Petersen MC, Dodge HWJ, Lazarte JA, Holman CB: Electroencephalographic rhythms from the depths of the parietal, occipital and temporal lobes in man. Electroencephalogr Clin Neurophysiol 1956; 8:263–78

50. Galambos R, Makeig S, Talmachoff PJ: A 40-Hz auditory potential recorded from the human scalp. Proc Natl Acad Sci U S A 1981; 78:2643–7

51. Santarelli R, Carraro L, Conti G, Capello M, Plourde G, Arslan E: Effects of isoflurane on auditory middle latency (MLRs) and steady-state (SSRs) responses recorded from the temporal cortex of the rat. Brain Res 2003; 973:240–51

52. Eggermont JJ, Ponton CW: The neurophysiology of auditory perception: From single units to evoked potentials. Audiol Neurootol 2002; 7:71–99