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General Anesthesia Increases Temporal Precision and Decreases Power of the Brainstem Auditory-evoked Response-related Segments of the Electroencephalogram

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Background: **Brainstem auditory-evoked responses (BAEP) have been reported to be unchanged in the presence of drugs used for induction and maintenance of general anesthesia. The aim of this study was to investigate if the signal segments after the auditory stimulus that are used to average the evoked response change under the influence of general anesthesia.**

Methods: **BAEPs of 156 patients scheduled for elective surgery under general anesthesia were investigated. Anesthetic regimen was randomized as a combination of one of four hypnotic drugs supplemented by one of four opioids. Signal segments after the auditory stimulus were obtained at six different periods of anesthesia. Power and phase properties of wavelet-filtered single-sweep auditory-evoked activity accounting for the waveform of the averaged BAEP wave V and the stability of amplitude and latency of the averaged BAEP wave V over periods were analyzed.**

Results: **Amplitude and latency of wave V change slightly with no significant difference between the periods. During anesthesia, however, the power of single sweeps is significantly reduced, whereas phase-locking properties of the according signal segments are significantly enhanced. This effect is independent of the anesthetic or opioid used.**

Conclusions: **General anesthesia affects phase and power of the segments of the electroencephalogram related to BAEP wave V. This study's results support the idea that temporally precise responses from a large number of neurons in the brainstem might play a crucial role in encoding and passing sensory information to higher subcortical and cortical areas of the brain.**

THE midlatency auditory-evoked potential has been proposed as a possible measure of anesthetic depth because morphologically well defined changes in the evoked signal (amplitude and latency of certain peaks and troughs) could be mapped to clinically evaluated depth of anesthesia.[1] The preceding part of the signal, the brainstem auditory-evoked potential (BAEP), has been reported as being mainly stable over different regimens of anesthesia and different dosing protocols.[2] The BAEP is generated in the brainstem and represents electrophysiological activity starting at the eighth cranial nerve and extending into medulla and pons. Waves III to V reflect central brainstem conduction pathways.[3] Thus the BAEP has served in assessing the structural integrity of the brainstem during certain surgical procedures, *i.e.*, during resection of cerebellopontine tumors (acoustic neurinoma) as well as during decompression of the trigeminal and facial nerves.[4] The BAEP also plays a role in monitoring brainstem function in comatose patients.[5] So far, the features of the underlying signal that lead to morphological stability or changes within the evoked signal have attracted little interest. The averaged potential might be regarded as a reoccurring temporal structure within each signal segment (sweep) after an auditory stimulus. From a time-frequency point of view, this reoccurring temporal structure can be looked at as a precise temporal development of power and phase. The morphology of the BAEP remains stable under periods that range from an awake to a deeply anesthetized patient[2]; therefore, we formulated a hypothesis as follows: general anesthesia does not alter power and phase properties of single-sweep signals used for averaging BAEPs.

We investigated wave V of BAEPs of patients scheduled for elective surgery. We concentrated on evaluating wave V because it satisfies two criteria: Wave V has been shown to be mainly stable across different combinations and dosing protocols of anesthesia (for an overview please see Banoub *et al.*[2]), and it can be interpreted as a preprocessing stage before the neuroelectric signals enter other subcortical and cortical areas.

We will show that, in accordance with the literature, the averaged brainstem evoked potential wave V remains widely unaffected by pharmacologically different forms of general anesthesia at six clinically different levels of anesthesia. However, the power and the precision of the oscillations responsible for the morphology of the averaged wave V are heavily influenced by the same forms of general anesthesia for these different levels.

Materials and Methods

After approval of the study protocol by the local ethics committees (Munich, Heidelberg, Luebeck, and Friedrichs-

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hafen; all in Germany), all patients between 18 and 65 yr of age who were scheduled for elective surgery at one of the University Clinics of Munich, Heidelberg, Luebeck, or the Hospital Friedrichshafen were candidates for inclusion in the study. They were not included if any of the following exclusion criteria were present: (1) American Society of Anesthesiologists physical status classification III or higher, (2) drug abuse, (3) known or suspected neurologic disorder, (4) known or suspected hearing disorder, (5) emergency surgery, (6) obesity (Body Mass Index greater than 25, or (7) indication for rapid sequence induction. All patients underwent a comprehensive medical evaluation and gave their written informed consent the day before surgery. The study was performed in the anesthesia induction rooms, and the corresponding operating theaters of the hospitals participating in this study. An anesthesiologist and a resident performed the study with the aid of varying nursing staff.

This prospective, single-blind study was performed for 209 patients after written informed consent was obtained. Of these 209 data sets, 192 data sets were eligible to be used for evaluation in this study. Criteria for eligibility were: (1) all four channels had to be recorded consistently (*i.e.*, impedance had to be below 5 kOhm in all channels, and no channel experienced a dropout), (2) certain key events had to have been available (key event periods). Of these 192 data sets, 36 data sets were discarded after artifact detection. The results are based on 156 data sets.

Anesthetic regimen was designed in a randomized single-blinded way (please see Appendix, second paragraph, Randomization–sequence generation). Several drug combinations, commonly used in clinical practice, were chosen for induction and maintenance of general anesthesia. Induction of anesthesia was performed with either 2.5 $mg \cdot kg^{-1}$ propofol or 6 $mg \cdot kg^{-1}$ thiopentone. Anesthesia was maintained with a combination of either one of the volatile anesthetics isoflurane, sevoflurane, or desflurane or the intravenous hypnotic agent propofol, supplemented by one of the opioids fentanyl, sufentanil, alfentanil, or remifentanil.

Patients were premedicated with 7.5 mg of midazolam administered orally 30 min before planned arrival at the operating theater. Standard monitoring of vital parameters (pulse oximetry, electrocardiogram, oscillometric blood pressure monitoring, temperature monitoring orally or rectally) was started, and an 18-gauge intravenous canula was inserted in a vein at one of the upper extremities. After placing the electrodes for the signal collection and starting collection of electroencephalographic signals, the opioid was administered. Hypnosis was induced 2 min later by administering the hypnotic drug. In the case of a planned tracheal intubation, muscle relaxation was achieved after secure mask ventilation by 0.5 $mg \cdot kg^{-1}$ atracuriumbesilate. Whenever a laryngeal mask was used for ventilation of patient lungs, no muscle relaxant agent was given. General anesthesia was maintained by one of the volatile anesthetics isoflurane, sevoflurane, or desflurane, each dosed at 0.9 minimum alveolar concentration[6] (age-adjusted, measured end-tidally) at the beginning, or by intravenous administration of propofol beginning at 5 $mg \cdot kg^{-1} \cdot min^{-1}$, according to the randomization. Anesthesia was deepened or lowered, and further doses of opioid were administered when deemed appropriate, leaving the decision to the expertise of the attending anesthesist.

Electroencephalographic signals were recorded in a four-channel montage (A1/Fp1, A2/Fp2, A1/Cz, A2/Cz, with Fpz as common ground, fig. 1). Preparations for data acquisition were started after completing a first measurement of vital signs and after installing the intravenous canula. Silver/silver-chloride adhesive electrodes (Neuroline 7200 00-S; Ambu/Medicotest, Ballerup, Denmark) were placed after skin preparation with acetone at A1, A2, Fp1, Fp2, Fpz, and Cz according to the international 10/20 system. Interelectrode impedances were kept below 5 kOhm. The signals on the electrodes were amplified within a preamplifier (POD; Siemens Medical, Erlangen, Germany; sensitivity 0.0170 μV, sampling rate 4 kHz, internal bandpass 0.01–1000 Hz, 3 dB). Patients were asked to lie comfortably and advised to close their eyes after the placement of earphones to reduce myogenic artifacts.

After achieving a calm environment, data acquisition was started. Auditory stimuli were delivered binaurally as rarefaction clicks at a stimulus rate of 9.1 Hz, a stimulus duration of 98 μs, and at an intensity of 95 dB. Stimuli were created by a personal computer (Neuroscreen; Toennies/Viasys, Hoechberg, Germany). Neuroelectric signals were stored on a computer as a continuous data stream. An additional channel served for storing a synchronization marker coinciding with the onset of the stimulus. In addition, an .xml file allowed the storage of intraoperative key events coded by hotkeys or free text to be entered by the attending anesthesist. These key events were supposed to allow the identification of clinically interesting time points, *i.e.*, before and after intubation, reduction of anesthetic delivery, *etc*.

The primary goal of the study was to investigate whether general anesthesia affects the segments of the electroencephalogram related to BAEP wave V. Secondary criteria were whether general anesthesia affects the averaged BAEP wave V. In addition, we tested for differences in outcome measures resulting from anesthetic regimen. The main parameters were based on the basic signal properties power and phase at the datapoints 20 to 36 (accounting for the shape of BAEP wave V) of the single-sweep electroencephalogram. Amplitude and latency of wave V of the BAEP were evaluated.

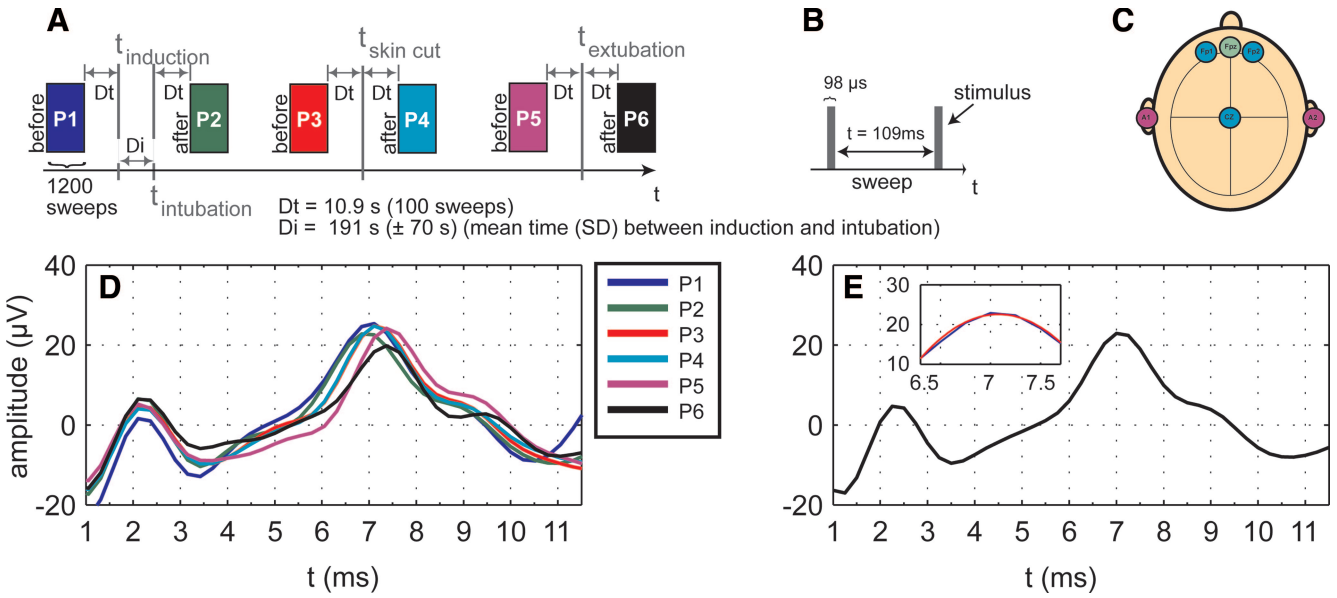

Fig. 1. Visualization of data collection, period selection, grand mean, and overall grand mean (*A*) Periods for data extraction were chosen such that possible effects of general anesthesia were expected to be maximal. (*B*) Stimuli consisted of brief binaural clicks at 80 dB; sweeps were defined as following signal segments with a length of 111 ms, collected as a differential signal at A1/Fp1, A2/Fp2, A1/Cz, A2/Cz, with Fpz as common ground, according to the 10/20-system (*C*). (*D*) The normalized grand means for the brainstem auditory-evoked potential, pooled over patients, channels, and anesthetic drug combinations, for the periods P1-P6 (before induction [*P1*], after intubation [*P2*], before skin cut [*P3*], after skin cut [*P4*], before extubation [*P5*], and after extubation [*P6*]) mainly resemble. (*E*) Fit of a polynom second order for the overall grand mean, which consists of the data in addition pooled over periods, for the brainstem auditory-evoked potential wave V. This polynom second order was used to automatically evaluate normalized amplitude and latency of the peak of brainstem auditory-evoked potential wave V.

Data Analysis

A total of 192 datasets were analyzed offline on a Linux cluster (8 knots, 2 GB RAM per knot, 2 × 3.2 GHz processors at each knot; Max-Planck Institute for Brain research, Frankfurt, Germany) by using Matlab version 6.5 (MathWorks, Natick, MA) and Matlab's Wavelet Toolbox with the programmers being blinded to the drug combination used to achieve general anesthesia.

After filtering procedures and artifact rejection, calculations resulted in three parameters: (1) an averaged BAEP with an estimation of amplitude and latency of peak V for each patient, channel, and period; (2) the mean angular deviation, an estimate for the stimulus locking of oscillations, for each time point for six bandpass filtered signals for each patient, channel, and period; (3) an estimation of the power for each time point for six bandpass filtered signals for each patient, channel, and period. For a detailed description of the data flow, please see "Schematic illustration of the data flow" in the Appendix.

We included all four channels of each patient to increase the database. Data from all four channels were filtered by applying two bandpass filters, 2–450 Hz combined with a Notch filter and 450–1000 Hz to the complete datastream. At or around the key events, data representing 1,200 sweeps were extracted as a whole.

We used the time period Dt (length of 100 sweeps = 10.9 s; fig. 1A) as a spacer from the so called events (event markers stored along with the electroencephalogram) to extract a data period representing 1,200 sweeps as a whole. This means that for the events denoted "before," the 1,200 sweeps ended 100 sweeps (equivalent to 10.9 s, denoted as Dt) before the event. For the events marked as "after," the extraction of the 1,200 sweeps started 100 sweeps after the event marker. The event markers were as follows: P1, induction (before); P2, intubation (after); P3, skin incision (before); P4, skin incision (after); P5, extubation (before); P6, extubation (after). The time period between "end of P3"/"beginning of P4" and "end of P5"/"beginning of P6" is around 20 s. The time difference between the "end of P1" and "beginning of P2," however, is different for individual patients and averages to 213 s. From a clinical point of view, patients at time periods P1 and P6 are definitively awake patients, since wakefulness was a criterion for extubation (P6, post extubation). P2 to P5 might be assigned to levels of deep general anesthesia; however, a situation of insufficient anesthesia cannot be excluded at these time points, *i.e.*, at P4 (after skin incision) or P5 (before extubation). We must emphasize, however, that clinical signs of insufficient anesthesia or arousal (sweating, tearing, motor reactions, and coughing) were stored as additional event markers along with the electroencephalographic data in the case of occurrence. For the patients included in the analysis, no such event was stored by the attending anesthesist for the time points P2 to P5. We would, therefore, expect effects to be maximal between the time points P1 and P2

and P5 and P6 and possibly minimal between the time points P2 to P5.

An artifact detection programmed by the authors was used. For the signal filtered at 2–450 Hz, sweeps contaminated by artifacts (any point within the sweep outside ± 2,500 μV) and sweeps with an amplitude overshooting four times the SD of the amplitude of all 1,200 sweeps were marked. In addition, for the signal that had been filtered with the bandpass 450–1000 Hz, sweeps inheriting a frequency component exceeding 800 Hz (outside ± 400 μV or overshooting four times the SD of the amplitude of all 1,200 sweeps) were marked. Data were filtered into two frequency bands to detect single sweeps distorted by external "nonbiological noise," such as cauterizing, line noise, *etc*. For the very high-frequency band 450–1,000 Hz, we aimed at mostly external electrical noise such as cauterizing (high-frequency, high-amplitude frequencies starting at 300 Hz, main frequency representation 800–2,000 Hz for the electrocautery devices in use in this study) or a high transient peak, which might pollute all frequency bands due to the frequency representation. High-frequency oscillations of the brain are expected to be lower in amplitude than lower-frequency oscillations; therefore, we used two different cutoff values for the maximum allowed amplitude for the two frequency bands (2,500/400 μV).

At all the defined periods around the key events for each condition and each channel, data quality must have been such that at least 600 of 1,200 single sweeps were left over after artifact detection; in other words, at least 600 artifact-free sweeps with a length of 111 ms had to be available within a period of 130 s for every channel and condition. We discarded datasets of 36 patients that did not meet these criteria. In the end, data from 156 patients were considered for further evaluation.

A total of 600 unmarked sweeps with a length of 445 datapoints equivalent to a signal duration of 111 ms at the following key events were used for evaluation: pre (P1) and post intubation (P2), pre and post skin incision (P3, P4), and pre and post extubation (P5, P6). We chose these time periods for inclusion in the analysis because we expect the highest variability of anesthesia, ranging from awake to clinical very deep anesthesia, possibly producing a maximum effect on the variability of the signals and their derivatives. The BAEP was averaged from these 600 sweeps for a time range from 0 ms to 11.2 ms (equivalent to 45 datapoints), each of them being offset-corrected, at each period for each patient and channel. The BAEPs were used in calculating a grand average. For each BAEP for each patient, channel, and period, we fitted a mathematical function (polynomial second order) to the data section 20–36 datapoints corresponding to the signal fragment 5–9 ms post stimulus. We estimated amplitude and latency of the wave V peak. We chose a polynomial second order to make sure that there is only one maximum in the fit to estimate peak amplitude and peak latency. The fit of such a polynom is illustrated in figure 1E.

To avoid boundary effects for the wavelet-filtering procedures, each of the unmarked sweeps was flanked by the preceding and by the following sweep. On these data pieces, the discrete wavelet transform as introduced by Mallat[7] was used as an effective bandpass filter,[7,8] separating the original signals in six bandpass-filtered signals for the following frequency bands: 7–14 Hz, 14–28 Hz, 28–57 Hz, 57–114 Hz, 114–228 Hz, and 228–457 Hz.[9,10] The signals were filtered with the Daubechies 4 wavelet.[11] The squared wavelet coefficients of the frequency bands served to estimate the power of the oscillations equivalent to the datapoints 20–36 of the middle sweep.[12,13] We used the Hilbert transform to obtain the instantaneous phase of the bandpass-filtered signal for each frequency band.[14] As an estimator of variability of the phase across sweeps, we calculated the mean angular deviation at each datapoint 20 to 36 of the middle sweep.[15] We used the mean angular deviation to describe the intertrial phase-locking (ITPL), which can vary from 0 to 1. A value close to zero indicates a purely random distribution of phases across sweeps, and a value of 1 is in accordance with the interpretation that all phase values resemble at the according datapoint. To cover the period of wave V, we pooled ITPL values by computing the average ITPL for samples 20 to 36 of the middle sweep.

The calculations resulted in (averaged) brainstem evoked responses with corresponding latencies and amplitudes for peak V over patients, channels, and periods as well as a pooled grand average, including all the patients, channels, and periods. Figure 1A shows the periods and the relevant sweeps used for evaluation. A total of 600 sweeps at the periods before induction (P1), after intubation (P2), before skin cut (P3), after skin cut (P4), before extubation (P5), and after extubation (P6) were used to assess the variability of basic signal properties. Fig. 1B visualizes stimulus length and length of the sweeps. Figure 1C illustrates part of the 10/20 system. Figure 1E illustrates the fit of the polynomial second order to the great grand mean, which results when the data are also pooled over periods. This fit of the polynomial second order to wave V of the BAEPs served in automatically assessing the amplitude and latency of respective wave complex for all patients and all periods.

In addition, figure 2 shows 100 wavelet-filtered single sweeps of a representative patient for the frequency bands 57–114 Hz, 114–228 Hz, and 228–457 Hz within the time range 0–25 ms after stimulus at each of the periods P1 to P6. The average of these wavelet-filtered single sweeps is plotted in gray for each frequency band.

Statistical Methods

Normalized amplitude and latency of BAEP peak of wave V were analyzed with the Kruskal-Wallis test. Esti-

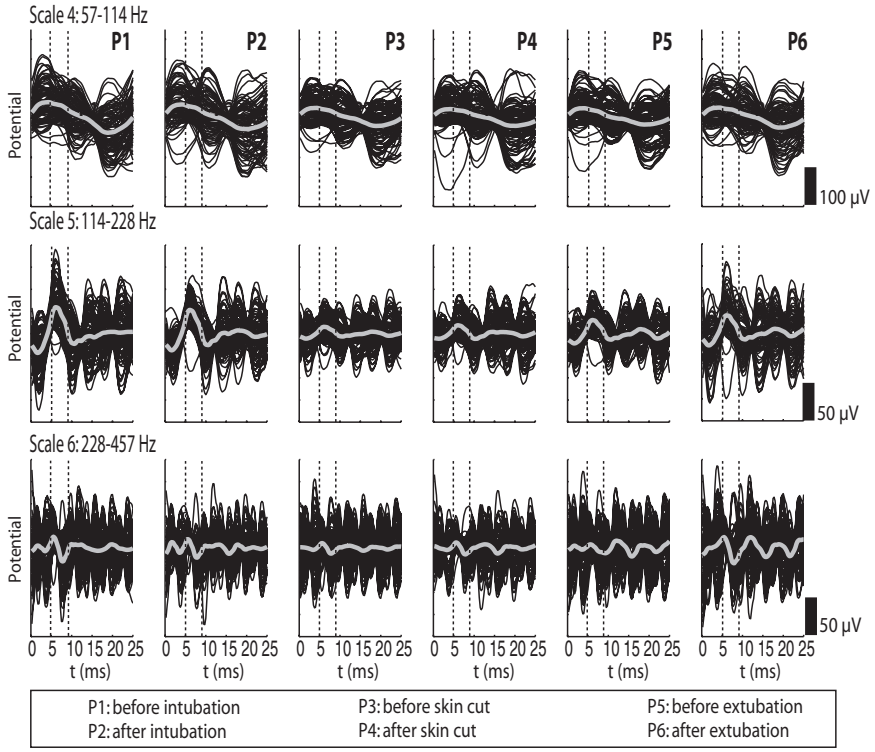

Fig. 2. Wavelet-filtered single-sweep auditory-evoked responses (here 100 sweeps) of a representative patient illustrate the applicability of the wavelet transform for the separation of stimulus-triggered responses for three frequency bands. Subplots show the wavelet-filtered signals for the frequency bands 57–114 Hz, 114–228 Hz, and 228–457 Hz in rows and for the time periods before induction (*P1*), after intubation (*P2*), before skin cut (*P3*), after skin cut (*P4*), before extubation (*P5*) and after extubation (*P6*) in columns on a time scale from 0 to 25 ms after auditory stimulus. The period between the *dotted lines* represents the analyzed period.

mations of the instantaneous phase and power of the wavelet bandpass-filtered signals at each timepoint (more precise datapoint) were analyzed by means of variance (ANOVA) to unmask significant differences between the periods. In a subgroup analysis, we plotted power and phase values for the different anesthesia regimens subdivided into a group of opioids and a group of anesthetics. Where appropriate, Bonferroni correction ($P < 0.01$) was applied for multiple comparisons. To characterize the effect size for the same ANOVA analysis, we used η^2, which is calculated as the ratio of the effect variance (SS_{effect}) to the total variance (SS_{total}).[16] The value of η^2 describes the degree of association between the effect and the dependent variable. Two-way ANOVA was performed including the factors "period" and "anesthetic drug," with the interaction term between period and anesthetic drug pointing towards a possibly combined influence of period and anesthetic drug. In the case of multiple measurements on a single subject as *i.e.*, four channels per subject and six periods per surgery repeated measurement ANOVA may be more appropriate than the standard ANOVA. Therefore, we also applied the repeated-measurement ANOVA in all cases for which we used the standard one-way ANOVA. In figures, data are presented by median, upper, and lower quartile, 1.5 times interquartile range, and outliers. In tables, values are presented as mean ± SD.

Results

A total of 209 patients were enrolled in the study. One patient withdrew his written informed consent 2 days after the surgical procedure without presenting a reason. We analyzed data of 192 patients in a preliminary step. For the final analysis, we used data of 156 patients, and data of 36 patients were excluded from the final step because of artifact rejection.

Outcomes and Estimation

This investigation shows that amplitude and latency of the averaged BAEP are widely unaffected by general anesthesia. The underlying signals though are significantly affected by general anesthesia as far as power and stimulus-locking are concerned.

For all results, the number of patients is n = 156; the number of used channels is 4. There was no significant difference in the demographic data of the patients included in the final evaluation (split in groups for opioids and for anesthetics in table 1).

Table 2 shows the distribution of drug combinations used to maintain general anesthesia.

Figure 1D shows the grand mean (pooled over subjects and channels) of the BAEP for the six different periods. The population statistics across patients reveals that neither peak amplitude nor peak latency of the BAEP wave V changes (figs. 3A and 3B, Kruskal-Wallis, both with $P > 0.01$). Boxplots represent the mean, the upper and lower quartile of the data (box), and 1.5 times the interquartile range as whiskers. Crosses represent outliers.

In a two-way ANOVA, the subgroup analysis reveals that there are no significant differences ($P = 0.44$) for the amplitudes and the peak latencies among the anesthetics isoflurane, sevoflurane, desflurane, and propofol (table 3).

Table 1. Demographic Data of the Patients Included in the Final Evaluation

	Opioids				Anesthetics			
	Fentanyl	Sufentanil	Alfentanil	Remifentanil	Isoflurane	Sevoflurane	Desflurane	Propofol
Patients, n	48	47	34	27	33	49	28	46
Gender, M/F	13/35	16/31	8/26	10/17	9/24	14/35	13/15	11/35
Age, yr	44 ± 14.0	46 ± 10.6	40 ± 12.3	39 ± 11.8	40.8 ± 13.7	42.9 ± 11.5	42.6 ± 14.2	45.3 ± 11.6
Height, cm	168.5 ± 8.0	167.4 ± 12.0	167.3 ± 9.2	169.2 ± 11.4	168.0 ± 8.1	168.4 ± 9.9	167.4 ± 14.6	167.9 ± 8.5
Weight, kg	76.0 ± 15.2	77.6 ± 22.2	73.4 ± 14.8	75.7 ± 21.6	72.2 ± 13.6	74.2 ± 15.6	80.6 ± 27.2	77.5 ± 17.8

There are no significant differences in age, height, and weight for the patients in the different groups of anesthetics or opioids. Group sizes are different for the groups of opioids and the groups of anesthetics. More female patients were included in the final evaluation. Data are shown as mean ± standard deviation.

Since BAEP wave V amplitude and latency are not changed by either anesthetic, we hypothesized that the stimulus-induced information in the underlying stimulus-associated sweeps might not be influenced either. Figure 4 row 1 (A1 to A6) depicts the population statistics for the power contained in the BAEP single sweeps after bandpass filtering with the Daubechies 4 wavelet for datapoints 20–36, equivalent to 5–9 ms after the auditory stimulation.

Statistical evaluation shows significant differences in induced power for six different frequency bands, except for the frequency band 28–57 Hz for the anesthetic Isoflurane (table 4). The pattern exhibited by the population statistics for induced power over periods is different for the frequency bands. The induced power in the frequency band 7–14 Hz appears inconsistent with the tendency to drop over periods. For the frequency band 14–28 Hz, there is a significant increase in the induced power for the periods when patients are anesthetized. Induced power in the 28–57 Hz band is not statistically different over periods. For the three higher frequency bands, 57–114 Hz, 114–228 Hz, and 228–457 Hz, population statistics are alike. Most prominently, we see a significant ($P < 0.001$) reduction in power for the frequency bands 57–114 Hz, 114–228 Hz, and 228–457 Hz for the periods P2, P3, P4, P5 (fig. 4, A4–A6). To characterize the effect size, we calculated η^2 (division of the sum of squares between groups by the sum of squares total) as a way to measure the proportion of variance explained. η^2 can reach numbers between 1 (if there is no error variance) and 0 (if all the group means are equal). In the former case, 100% of the variance is explained by the treatment; in the latter case, 0% of the variance is explained. Numbers of 0.2 are usually determined as small, 0.5 as medium and 0.8 as large effect sizes. Effect sizes for the power can be described as between small and large, depending on the frequency band and the hypnotic used. Effect sizes are smaller for propofol (between 0.095 and 0.399, with the higher-frequency bands exhibiting a stronger effect) than for the volatile anesthetics (between 0.195 and 0.635, with the strongest effects in the high-frequency bands) (table 4).

Table 2. Drug Combinations (n) Used for Maintaining General Anesthesia

	Opioids			
	Fentanyl	Sufentanil	Alfentanil	Remifentanil
Anesthetics				
Isoflurane	6	11	10	6
Sevoflurane	20	17	8	4
Desflurane	4	11	9	4
Propofol	18	8	7	13

All of these combinations of drugs used for maintenance of general anesthesia are commonly in use in hospitals in Germany. For this set of data, general anesthesia was maintained most often with a combination of sevoflurane and fentanyl, followed by a combination of propofol and fentanyl. Sevoflurane/sufentanil and propofol/remifentanil anesthesia ranked at places three and four, respectively, for our dataset.

This reduction in power is accompanied by a significant increase of up to almost factor 2 in the intertrial phase-locking for the frequency bands 114–228 Hz and 228–457 Hz (fig. 4, B5 and B6; table 5). The intertrial phase-locking serves as a measure of how tightly the signal is locked to the stimulus. The lower-frequency bands do not show statistically significant differences among the periods (fig. 4, B1–B4).

Figure 5 presents the population statistics of the calculated power for the different anesthetic regimens (in rows: isoflurane, sevoflurane, desflurane, propofol) for the three highest frequency bands (in columns: 57–114 Hz, 114–228 Hz, and 228–457 Hz) and for the four different opioids (fentanyl, sufentanil, alfentanil and remifentanil) used accordingly.

The subplots look very similar for all these three high-frequency bands and for all four kinds of anesthetics and opioids. For each of the anesthetics isoflurane, sevoflurane, desflurane, and propofol, there is a decrease of induced power for the periods P2, P3, and P4, which correspond to the states in which patients are anesthetized. Shortly before extubation (P5) and after extubation (P6) induced power in these frequency bands rises again for the time segment correlating to BAEP wave V. Basically the same pattern is seen in a subgroup analysis for the opioids fentanyl, sufentanil, alfentanil, and remifentanil. For both panels, the variability decreases for the states of patients being anesthetized. Two-way ANOVA shows that the factor "period" is the influencing

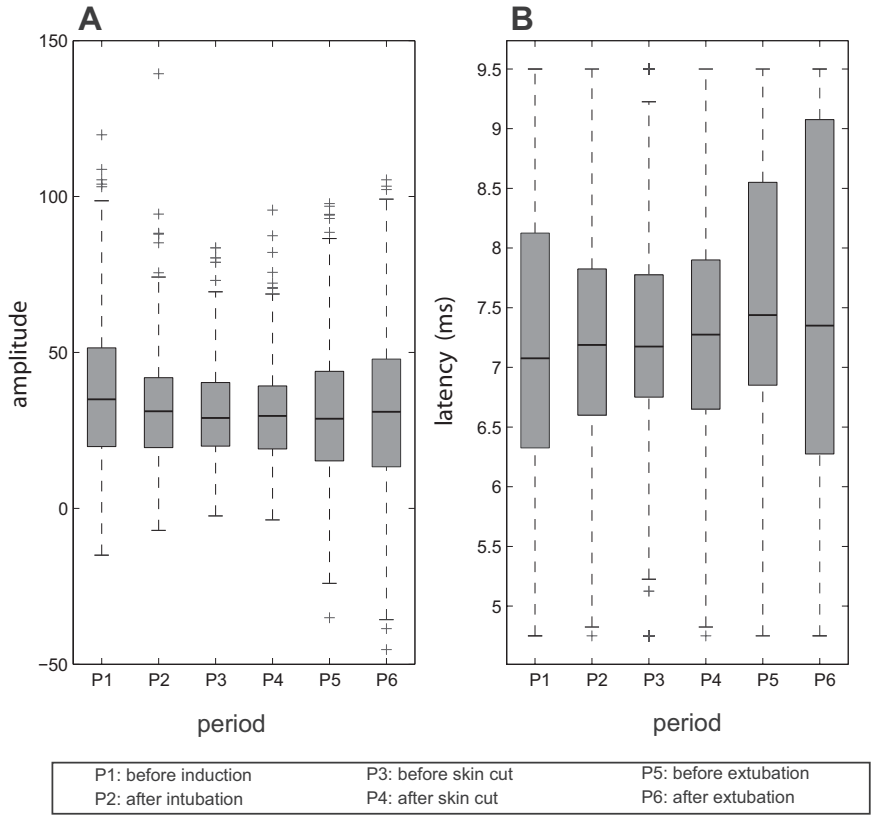

Fig. 3. Population statistics for the normalized amplitude and latency of the brainstem auditory-evoked potential peak V. The distribution of the normalized amplitude (*A*) and latency (*B*) of brainstem auditory-evoked potential (BAEP) peak V does not show significant differences over periods. The latency shows a tendency to lengthen for the periods of general anesthesia, and interindividual variability seems to be less for the periods of anesthesia as well for both amplitude and latency (n = 156). Boxplots show the median, upper and lower quartile as *box*, 1.5 interquartile range as *dotted line*, and outliers as *crosses*.

factor for all frequency bands (all $P < 0.0001$), whereas the factor anesthetic drug does not show a significant impact (all $P > 0.9$).

Figure 6 shows the population statistics for the ITPL for the frequency bands 57–114 Hz, 114–228 Hz, and 228–457 Hz and follows the same apportionment as figure 5.

Whereas the ITPL is not significant over periods for the frequency band 57–114 Hz except for the anesthetic sevoflurane, the population statistics of the ITPL of the higher frequency bands 114–228 Hz and 228–457 Hz results in a pattern contrary to the pattern exhibited for the induced power in figure 5. ITPL as an estimator of the stability of phase values almost doubles its value for the periods of deep anesthesia. Within these frequency bands, variability of the distribution is higher for the periods in which patients are anesthetized. The effect size is different for the anesthetics and the frequency bands (varying from 0.078 for the volatile anesthetic sevoflurane for the frequency band 57–114 Hz to 0.497 for the volatile anesthetic desflurane for the frequency band 114–228 Hz) and ranges between the classification small and medium for the ITPL (table 5). Two-way ANOVA again shows the influence of the factor "period" (highest $P = 0.003$) for all frequency bands and statistically no influence of the factor "anesthetic drug" (lowest $P = 0.23$).

Discussion

Key Findings

We show with electroencephalographic data collected in 156 patients during routine surgery that the BAEP,

Table 3. Mean Latencies of the Brainstem Auditory-evoked Potential (BAEP) Wave V for the Different Anesthetics at Various Conditions

		Period					
Latencies Peak Wave V	N	P1	P2	P3	P4	P5	P6
Isoflurane	33	6.92 ± 1.50	7.16 ± 1.12	7.26 ± 1.02	7.24 ± 1.50	7.75 ± 1.56	7.92 ± 1.62
Sevoflurane	49	7.32 ± 1.48	7.13 ± 1.33	7.17 ± 1.15	7.32 ± 1.11	7.58 ± 1.31	7.17 ± 1.71
Desflurane	28	7.19 ± 1.46	7.46 ± 1.18	7.28 ± 1.12	7.50 ± 1.25	7.61 ± 1.34	7.26 ± 1.73
Propofol	46	7.18 ± 1.57	7.27 ± 1.33	7.35 ± 1.16	7.12 ± 1.17	7.44 ± 1.35	7.23 ± 1.59
Average		7.18 ± 1.51	7.23 ± 1.27	7.26 ± 1.12	7.27 ± 1.13	7.57 ± 1.38	7.35 ± 1.68

There are nonsignificant differences in the mean latency of the BAEP wave V for the subgroup anesthetics with no clear tendency. Whereas the mean latency prolongs from period 1 to period 6 for the volatile anesthetic isoflurane throughout, the picture for the anesthetics sevoflurane, desflurane and propofol is inconsistent. We infer that the differences are the result of a statistical variability of the signals. Data are shown as mean ± standard deviation.

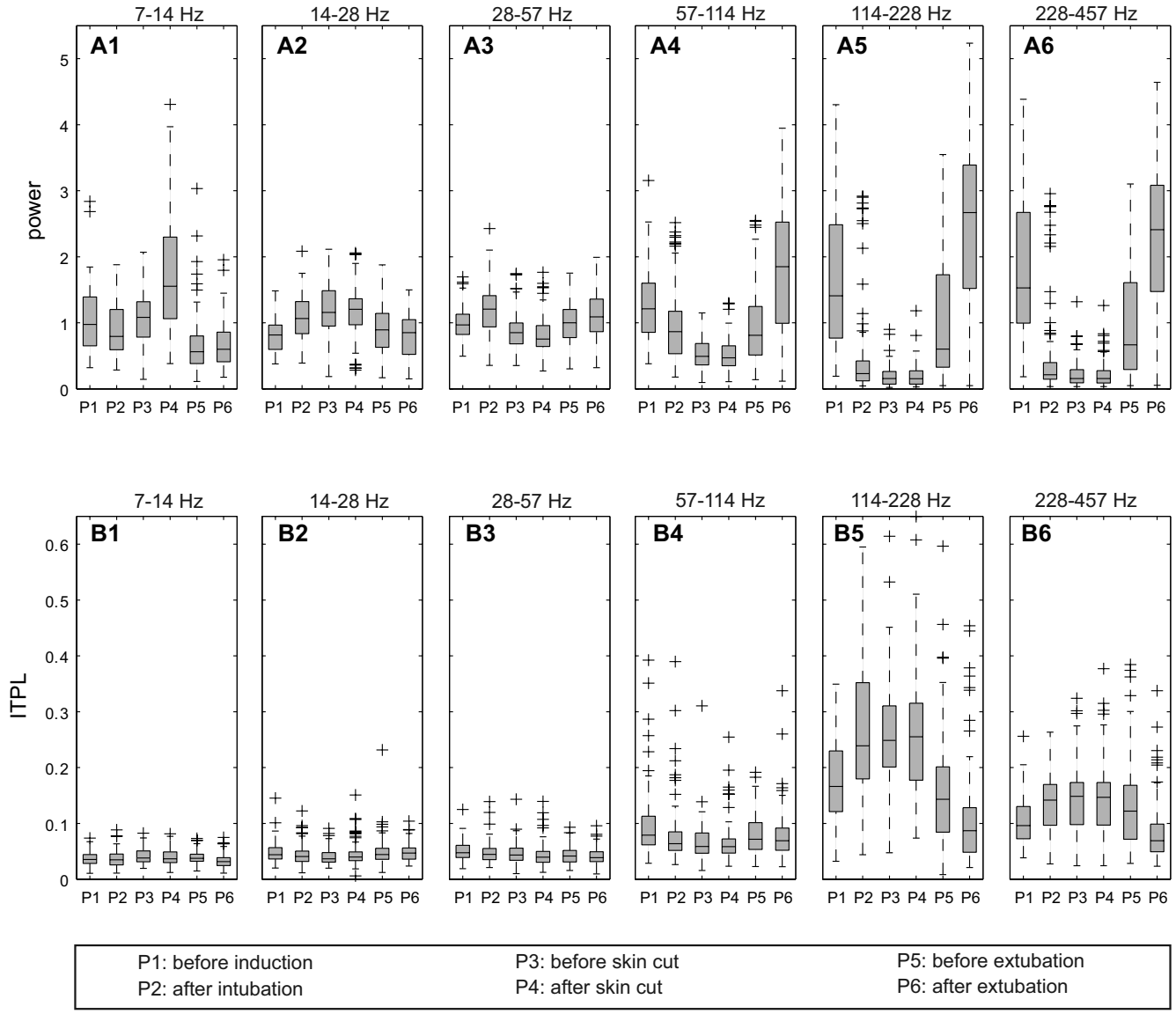

Fig. 4. Population statistics for the power and the intertrial phase-locking (ITPL) accounting for the brainstem auditory-evoked potential (BAEP) wave V for different frequency bands pooled over all drug combinations. Whereas there is no significant difference in induced power for the frequency band 28–57 Hz (*A3*), significant differences exist for the frequency bands 7–14 Hz (*A1*), 14–28 Hz (*A2*), 57–114 Hz (*A4*), 114–228 Hz (*A5*), and 228–457 Hz (*A6*) over periods. The data for the frequency band 7–14 Hz are spread inconsistently; for the frequency band 14–28 Hz, there is a significant rise for the periods when patients are anesthetized. For the three highest frequency bands accounting for the frequencies 58–457 Hz, the pattern resembles and shows a dramatic drop for the periods when patients are anesthetized. This drop is accompanied by a significant increase in the intertrial phase-locking for the frequency bands 114–228 Hz (*B5*) and 228–457 Hz (*B6*). The intertrial phase-locking calculated in the lower frequency bands appears unimpaired (*B1 to B4*). *Boxplots* show the median, upper and lower quartile as *box*, 1.5 interquartile range as *dotted line*, and outliers as *crosses*.

especially wave V, is seemingly unaffected by general anesthesia at varying depths of anesthesia (before induction, after intubation, before skin cut, after skin cut, before extubation, and after extubation). The peak of BAEPs wave V occurs at a latency of 7.31 ms ± 1.35 ms on average (min 7.18 ms, max 7.57 ms). Normalized amplitudes of BAEP wave V show the tendency to decrease for the states in which patients are anesthetized. Neither amplitude nor latency change statistically for the different periods ($P > 0.01$ for both). These findings are consistent with the existing literature.[2] A closer examination of the properties of the underlying stimulus locked sweep and its population statistics, in particular the variability of the locking of the phase of the neuroelectric signal to the stimulus and its corresponding power reveals a big influence of general anesthesia, although the averaged potential seems to be unchanged or only slightly changed. Whereas the power within the stimulus-locked signals is reduced, the precision of their corresponding locking to the auditory stimulus appears enhanced. Two-way ANOVA reveals that this effect is independent of the drug combination used for maintain-

Table 4. Results of the Analysis of Variance (ANOVA) and Effect Size for the Induced Power of Single-sweep Signals Accounting for the Brainstem Auditory-evoked Potential (BAEP) Wave V for Frequency Bands and Anesthetics Over Periods

	7–14 Hz	14–28 Hz	28–57 Hz	57–114 Hz	114–228 Hz	228–457 Hz
P value power						
Isoflurane	0.00E^{+00}*	1.72E^{-12}*	2.23E^{-02}	0*	0*	0*
Sevoflurane	0.00E^{+00}*	3.29E^{-12}*	0*	0*	0*	0*
Desflurane	4.37E^{-13}*	1.30E^{-08}*	5.94E^{-09}*	0*	0*	0*
Propofol	1.00E^{-15}*	7.99E^{-12}*	5.65E^{-05}*	0*	0*	0*
H^2 power						
Isoflurane	0.4142	0.2821	0.0655	0.4199	0.6165	0.6096
Sevoflurane	0.4076	0.1949	0.2658	0.4661	0.5868	0.5541
Desflurane	0.3362	0.2418	0.2495	0.4925	0.5943	0.6346
Propofol	0.2530	0.2009	0.0948	0.3195	0.3924	0.3987

In contrast to band 28–57Hz, all other frequency bands show significant differences for all anesthetics between periods. Test level was 0.01 (*) throughout; Bonferroni correction was applied for multiple comparisons for six frequency bands and four anesthetic drugs. To characterize the effect size for the same analysis of variance, we use η^2, which is calculated as the ratio of the effect variance (SS_{effect}) to the total variance (SS_{total}). The value of η^2 describes the degree of association between the effect and the dependent variable.

* Significant after Bonferroni correction for six frequency bands and four anesthetic drugs (test level 1%).

ing general anesthesia. Restricting to only statistically significant differences the effect sizes can be classified as medium to large for the power and small to medium for ITPL. This demonstrates not only that results are significant on the average across the population, but also that the observed effects are medium to strong for individual patients. Up to 63% of the effect is introduced by general anesthesia for the measure power and up to 50% for the measure ITPL, leaving 37% and 50%, respectively, to be caused by intersubject variability and other unobserved factors (*i.e.*, differences in pharmacokinetics and pharmacodynamics). In addition to standard one-way ANOVA, we also performed repeated measurement ANOVA because we included four channels and six periods for each subject. Results of both of these statistical methods were qualitatively identical such that all significant effects remained significant and all nonsignificant effects remained nonsignificant.

Other Findings

To our knowledge, no studies have investigated the changes in the variability of phase representation and corresponding power of auditory-induced activity accounting for the shape of the BAEP wave V in humans. The mathematical and neurophysiological aspects of our analysis have already been published in a related context. The principle of investigating power and statistical measures of phase coherence are proposed for cortical and subcortical signals.[17,18] For cortical signals, it is common to investigate phase-locking values between different signals.[19–22] In our case, the ITPL as a phase-locking measure catches the statistical variability of the locking of the neuronal signals to the auditory stimulus. We interpret an increase in the ITPL as a more precise locking of neuronal processing to the stimulus and a decrease in the ITPL as a higher variability of locking of the phase to the stimulus.

Table 5. Results of the Analysis of Variance (ANOVA) and Effect Size for the Intertrial Phase-locking (ITPL) Single-sweep Signals Accounting for the Brainstem Auditory-evoked Potential (BAEP) Wave V for Frequency Bands and Anesthetics Over Periods

	7–14 Hz	14–28 Hz	28–57 Hz	57–114 Hz	114–228 Hz	228–457 Hz
P value ITPL						
Isoflurane	2.00E^{-03}	5.72E^{-01}	1.99E^{-01}	8.15E^{-02}	0.00E^{+00}*	2.25E^{-12}*
Sevoflurane	1.14E^{-03}	4.79E^{-02}	1.40E^{-03}	2.93E^{-04}*	1.58E^{-13}*	7.50E^{-06}*
Desflurane	1.19E^{-05}*	4.82E^{-01}	7.56E^{-01}	3.22E^{-03}	0.00E^{+00}*	3.73E^{-11}*
Propofol	4.33E^{-01}	2.31E^{-02}	1.15E^{-02}	5.63E^{-02}	1.45E^{-08}*	2.68E^{-04}*
η^2 ITPL						
Isoflurane	0.0931	0.0197	0.0370	0.0493	0.4305	0.2800
Sevoflurane	0.0674	0.0379	0.0659	0.0774	0.2124	0.1031
Desflurane	0.1700	0.0271	0.0160	0.1031	0.4968	0.2972
Propofol	0.0177	0.0469	0.0529	0.0389	0.1528	0.0830

ANOVA reveals significant differences among periods for all anesthetics for the frequency bands 114–228 Hz and 228–457 Hz. Also significant is the difference over periods for anesthesia with sevoflurane for the frequency band 57–114 Hz and for desflurane for the frequency band 7–14 Hz. Test level was 0.01 (*) throughout; Bonferroni correction was applied for multiple comparison for six frequency bands and four anesthetic drugs. To characterize the effect size for the same analysis of variance, we used η^2, which is calculated as the ratio of the effect variance (SS_{effect}) to the total variance (SS_{total}). The value of η^2 describes the degree of association between the effect and the dependent variable.

* Significant after Bonferroni correction for six frequency bands and four anesthetic drugs (test level 1%).

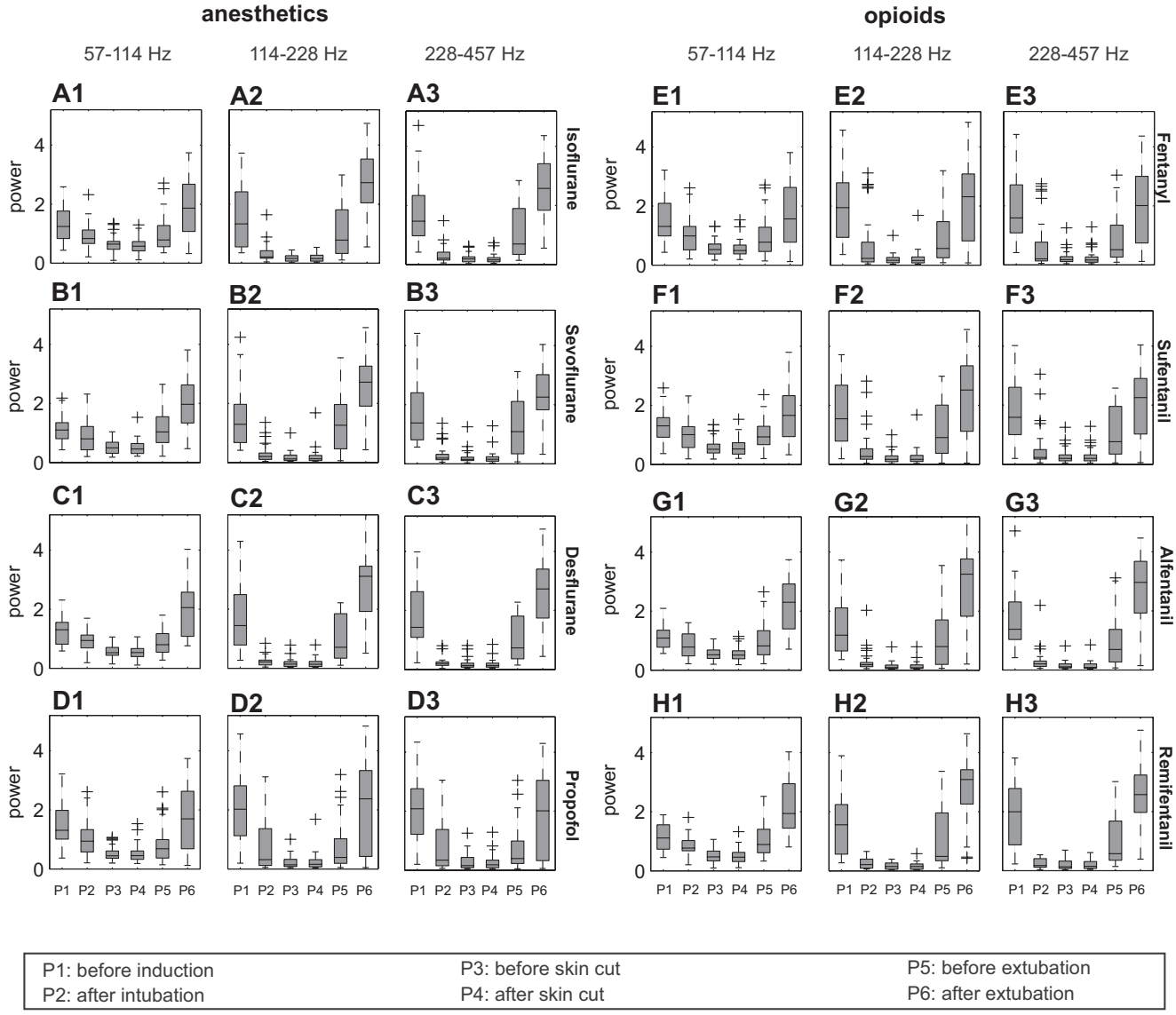

Fig. 5. Population statistics for the induced power over periods for the frequency bands 58–114 Hz, 114–228 Hz, and 228–457 Hz for the groups of anesthetics and opioids separately. There are significant differences in induced power of single sweeps accounting for the brainstem auditory-evoked potential (BAEP) wave V between periods for all kinds of anesthetics and opioids used for the frequency bands 57–114 Hz, 114–228 Hz, and 228–457 Hz. The pattern as expressed by the population statistics (median, upper and lower quartile [*box*], 1.5 times interquartile range [*dotted line*], and outliers [*crosses*]) resembles for the anesthetics and opioids used. There is a decrease in induced power and variability for the periods that account for general anesthesia and an increase towards the end of anesthesia again. Differences between drug groups consist in a differing variability at certain periods. Please note that group sizes are different as indicated in table 1.

Limitations

A limitation of the study is the operative setting in which electroencephalographic data were collected; the potential influence of artifacts is a critical issue for the measures chosen and the kind of analysis performed. Power and phase measures could be substantially changed by superposition of signals of nonbiological origin and biologic origin. For a systematic discussion of artifacts of biologic origin we will divide these kinds of artifacts in artifacts that are timely locked to the stimulus and artifacts that might occur independent of the auditory stimulation.

Electroencephalographic measures are highly sensitive to artifacts generated by electrical devices (infusion pumps, ventilators, *etc*) used in the operating theaters and by surgical manipulations on the patients, including high-frequency electrical cauterizing (a high-frequency electrical device used to close small bleeding vessels). For these artifacts of nonbiological origin, we expect our artifact detection to detect and subsequently exclude most of the contaminated trials due to their properties in power and frequency. We emphasize that we eliminated all trials contaminated by artifacts in this study. This is different from other techniques, which eliminate or reject contaminations by artifacts but still use the according sweeps.

Possible Limitations Resulting from Artifacts of Neuronal Origin. Artifacts of biologic origin pose a serious challenge, especially in a scalp-electrode mon-

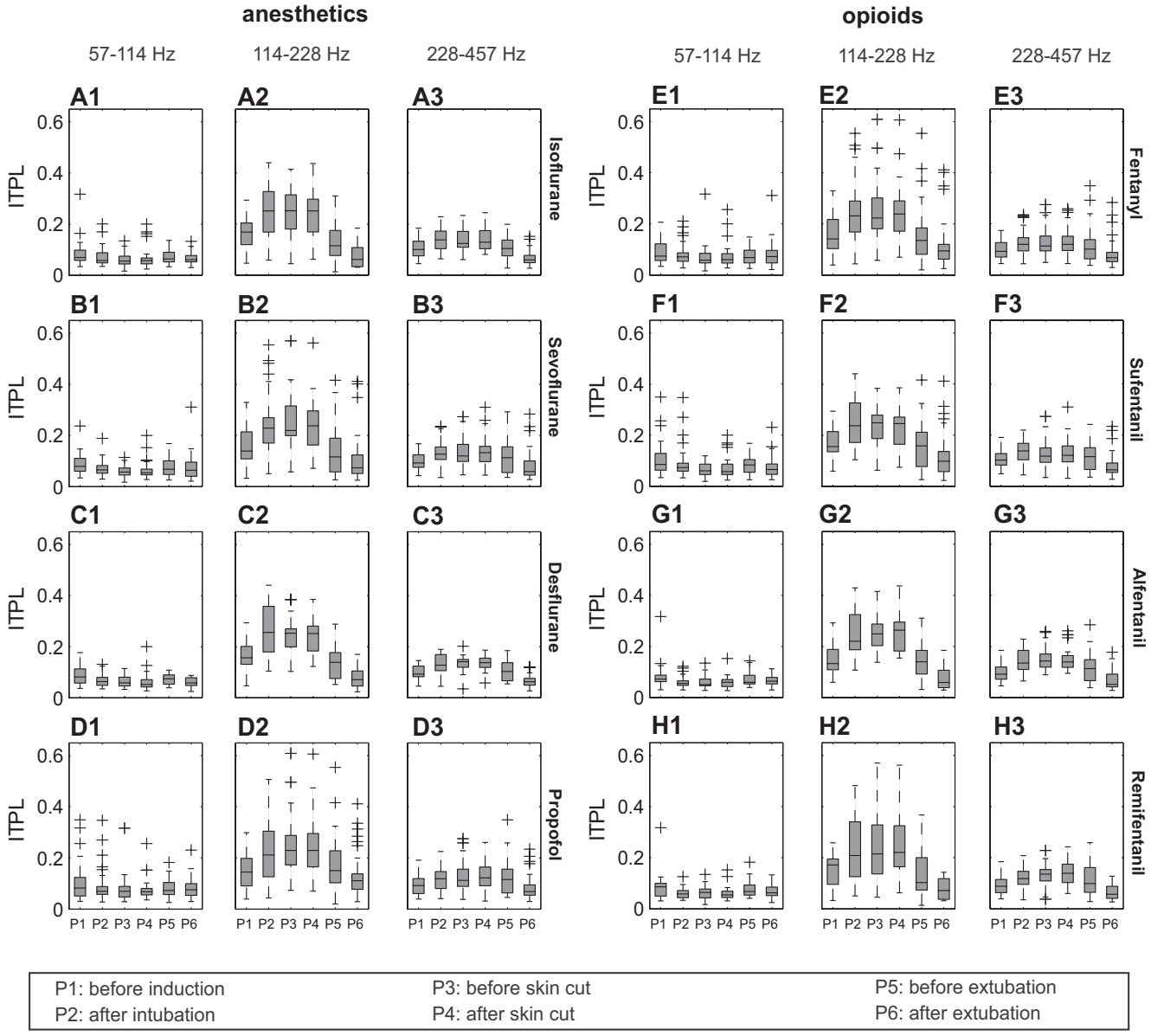

Fig. 6. Population statistics for the intertrial phase-locking (ITPL) over periods for the frequency bands 57–114 Hz, 114–228 Hz, and 228–457 Hz for the groups of anesthetics and opioids separately. There are significant differences in the ITPL value of single sweeps accounting for the brainstem auditory-evoked potential (BAEP) wave V between periods for all kinds of anesthetics and opioids used for the frequency bands 114–228 Hz and 228–457 Hz. The pattern as expressed by the population statistics (median, upper and lower quartile [*box*], 1.5 times interquartile range [*dotted line*], and outliers [*crosses*]) resembles for the anesthetics and opioids used. There is an increase in the ITPL for the periods that account for general anesthesia up to twofold and a decrease towards the end of anesthesia again. Differences between drug groups consist in a differing variability at certain periods. Please note that group sizes are different as indicated in table 1.

tage. These electrodes catch the resulting potential of a superposition of possibly many different potentials caused by different sources that can be functionally active ensembles of neurons generally anywhere in the central nervous system but in muscle activity as well. The aspect that the brainstem-evoked oscillations are so-called far-field potentials in contrast to near-field potentials arising in the proximity of the electrode (signals of cortical and subcortical origin) might aggravate the problem of separating brainstem-evoked oscillations from signals superimposed by other generators.

An impact of cortical activity onto our results based on filtered single-sweep signals is possible when the cortical activity, especially the power and phase of induced or ongoing activity changes systematically with different states of anesthesia. A second potentially influential effect on the measures power and phase precision of the signals can be imagined for different levels of noise across different states of anesthesia. In such a scenario, *e.g.*, the measured ITPL of a potentially very precise stimulus-locked signal could be systematically altered by varying signal to noise ratios. In other words, *i.e.*, the

increased phase-locking in a certain frequency range may result from relative prominence of BAEPs after suppression of cortical activity by anesthesia. The partly influence of spontaneous brain activity on our measures power more than phase seems to be likely for the frequency bands 7–14 Hz, 14–28 Hz, and 28–57 Hz. The significant changes in the power might picture the well-known shift in ongoing spontaneous brain activity towards lower frequencies for anesthetized states.[23–27] For the higher frequencies, amplitudes of prestimulus electroencephalogram oscillations are diminished in the graphs of figure 3, particularly for the scale 114–228 Hz. However, figure 3 contradicts the theory that spontaneous high-frequency oscillations influenced by general anesthesia explain the results of our analysis. In such a case, we would expect the single-sweep oscillations to be much more out of phase for the time period analyzed. For latencies past 15 ms, figure 3 illustrates such a possible behavior of single sweeps. Furthermore, figure 3 shows the applicability of the wavelet filtering to separate signal contents, which have been published before.[8,28]

Cortical/subcortical evoked responses in higher-frequency bands have mainly been reported for somatosensory-evoked potentials but also for auditory-evoked responses. However, these signals, which have all been described to be influenced either by general anesthesia for midlatency auditory-evoked potentials[12] or by arousal for somatosensory evoked potentials,[29,30] the latencies at which these high-frequency oscillations occur are much later than the time domain we investigate. For somatosensory-evoked potentials, these high-frequency oscillations have been shown to be superimposed on the primary cortical response, which is the N20 with a latency of 20 ms.[31,32] High-frequency oscillations have been detected in midlatency auditory-evoked potentials at a latency between 15 ms and 30 ms.[12]

To exclude that changes in signals of cortical origin might have induced the changes observed in our signals within the first couple of milliseconds after the auditory stimulus, we compared our observed stimulus locking in the first milliseconds to the stimulus locking right before the next sweep.

If high cortex–related ITPL values would result as an influence of the preceeding sweep, we would expect the ITPL value for a given frequency band to remain high throughout the sweep. The ITPL decreases towards values of 0.1 for the datapoints at the end of the sweeps. This clearly indicates that the observed values of ITPL in the beginning of the sweep are evoked by the stimulus in the beginning of the sweep. This in turn makes a major contribution of cortical signals to the measured ITPL values due to the early time during the sweep very unlikely.

Possible Limitations Resulting from Artifacts of Muscular Origin. Spontaneous muscle activity is known to be affected by general anesthesia in the sense that the occurrence of muscle activity diminishes during general anesthesia and might almost disappear as soon as pharmacological muscle relaxation is used. Our expectation for the influence of spontaneous muscle activity on single-sweep oscillations is that the power calculated from wavelet coefficients increases, whereas the phase of the single sweep is distorted and such the measure of ITPL heavily drops for awake patients. In a first approach to our results, illustrated in figure 5, this is exactly what we see. However, the illustration of the wavelet-filtered single sweeps (fig. 3) is not in accordance with the hypothesis that our results are based on spontaneous muscle activity and the impact of general anesthesia hereupon. More, figure 3 supports the theory that single-sweep oscillations are altered in power and ITPL by general anesthesia. For the measures power and ITPL, figure 5 A4 and B4 show that power changes quite strongly with periods, whereas ITPL does not. In combination with the power changing in A5 nearly identically to in A4 but ITPL changing more strongly in B5, this gives strong evidence that our results are not due to changes of the signal to noise *i.e.*, introduced by spontaneous muscle activity as a major underlying effect.

In awake patients, the reflex of the Musculus postauricularis to auditory stimulation has been described as stimulus triggered myogenic response.[33] This myogenic response might have a frequency representation that is close to that of the BAEP or even overlapping and has been described to vanish with sedation and general anesthesia.[33] However, the latency at which the myogenic response of the Musculus postauricularis can be observed is later (12.5–15 ms after stimulus) than the BAEP and has to our knowledge only been described to interfere with the BAEP for stimulation rates of 65 Hz and higher when subsequent single sweeps closely overlap. In this study, the auditory stimulation rate was 9.1 Hz, and we exclude this myogenic response as the major factor influencing our results.

Conceptual Limitations. Unfortunately, the estimation of the signal to noise ratio is as to our knowledge not a feasible approach for noninvasively measured single-sweep evoked oscillations, because it is impossible to separate neuronal and myogenic artifacts from the real signals as soon as all share similar properties in a time frequency space. The signal to noise ratio is a critical factor for the calculation of ITPL and wavelet power. Whereas myogenic artifacts will increase wavelet power, ITPL will drop heavily because the measure is dependent on a statistically fairly stable representation of phase values at one time point to achieve numbers above 0.05. The values for the ITPL obtained in our study are good for data collection from scalp electrodes, which is an indirect sign of data quality of the artifact-controlled and

-filtered signals. To resolve the individual peaks of the brainstem auditory-evoked response, following each other at about 1-ms intervals, and their spectral estimates, respectively, higher sampling rates than those used in our study are recommended. This is most likely the reason for a slightly increased latency of around 7 ms for the BAEP wave V compared to traditionally reported values of 5–6 ms. However, the chosen sampling rate of 4 kHz is sufficient to study power and intertrial phase-locking in a frequency range up to 457 Hz as reported here. Surprisingly, brainstem postsynaptic potentials, occurring at approximately 100 Hz,[34] seem not to be affected in the ITPL measure. This may be explained by the fact that our measures are based on a window between 5 and 9 ms, summarizing ITPL for the whole window in a single value. With other words, the temporal precision of the analysis is not suited to distinguish between pre- and postsynaptic activity of the brainstem.

As a general limitation, we need to emphasize that the recording montage of four electrodes does not allow a source localization of the recorded segments of the electroencephalogram into the brainstem. Furthermore, the temporal accuracy of our method, which is limited mainly by the sampling rate of 4 kHz does not allow the separation of wave V at 7 ms from the earliest cortical auditory activations, which have been described to occur as early as 8 ms after the onset of an auditory stimulus.[35,36] However, there is strong support either from neurophysiologic knowledge or from the results themselves to exclude anesthesia-dependent changes of the signals of origins other than the brainstem.

Conclusions and Outlook

From a simple clinical point of view, the influence of general anesthesia on the functioning of structures of the brainstem is not surprising. Deep anesthesia goes along in most cases with abolished reflexes mediated by brainstem structures (the so-called adverse-effects reflexes, *i.e.*, breathing and swallowing reflex, gag reflex, *etc.*). Therefore, it seems reasonable to assume that general anesthesia exhibits a possible effect on signal generation, conduction, and/or processing in the brainstem.

The electrical activity sampled at the electrodes consists of summed potentials that result from ongoing spontaneous brain activity, stimulus-induced activity, and stimulus-evoked activity (for an explanation, see Tallon-Baudry and Bertrand[17]) from different brain regions on different temporal and local scales. The time-frequency representation shows a high impact of general anesthesia on a temporal scale close to the stimulus in the frequency bands 57–114 Hz, 114–228 Hz, and 228–457 Hz for the power and in the frequency bands 114–228 Hz and 228–457 Hz for the ITPL value. We do not know the mechanisms that lead to a power decrease and an increase in the measure of phase coherence. However, the changes in the high-frequency spectrum indicate changes of the activity of large number of neurons on temporal scales of a few milliseconds up to a few tens of milliseconds. These timescales are compatible with those known from neuronal synchronization of assemblies of neurons that have been associated with perception, attention, and consciousness.[37–39]

With the results showing no significant differences among combinations of drugs used for general anesthesia, one might speculate whether these findings are common to the phenomenon of general anesthesia, representing an underlying mechanism. For the hypothesis that groups of neurons are activated by the auditory stimuli, the decrease in power might simply indicate that fewer neurons are activated. In this case, the measure of phase coherence would be enhanced due to a less broad variability in induced activity. A more sophisticated approach might speculate about the induction of altered brain states by general anesthesia and resulting changes in top-down processing.[40,41] With our study, we can neither support nor reject any such hypotheses or even a combination of these. Our results demonstrate first of all that the segments of the electroencephalogram related to BAEP wave V are altered on the level of single sweeps regarding their power and stimulus-locking by different stages of anesthesia. Despite these changes in a single sweep, the averaged auditory-evoked brainstem response wave V remains unchanged. Second, the specific nature of power and stimulus-locking are only correlated to different stages of anesthesia but independent of the specific combinations of opioids and anesthetic drugs administered (in this study, 16 possible combinations). Third, changes in power and stimulus-locking are specific for different frequency bands. In the case of stimulus-locking, mainly the high frequencies express strong modulations across different stages of anesthesia. In case of power, changes are different in their direction for low frequencies compared to high frequencies.

We interpret our results in two ways. First, the increase in stimulus-locking for high frequencies during stages of deeper anesthesia supports the idea that the temporal coordination of neuronal activity and the temporally precise responses from a large number of neurons in the brainstem might play a crucial role in encoding and passing sensory information to higher subcortical and cortical areas of the brain. Second, the averaging procedure might mask strong simultaneous antidromic changes of power and stimulus-locking on the single-sweep level. The current study highlights the possible loss of information and danger of oversimplification when using averaged responses to describe neuronal activity. At the same time, it shows the great analytical potential of single-sweep analysis for the investigation of BAEPs.

The investigation of power and phase properties of single-sweep auditory-evoked activity might be of high interest for groups investigating changes in amplitude

and latency of BAEP wave V possibly induced by diabetes,[42,43] newborn hyperbilirubinemia,[44,45] chronic obstructive pulmonary disease,[46] or hypothyroidism[47] or for those developing monitoring devices used during neurosurgical procedures[48,49] or for the assessment of hearing ability in newborn and early-born infants.[50]

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Appendix

Sample Size Determination and Randomization Procedure

Sample Size

To determine the necessary sample size of the study, we used the definition of the effect-size (δ), which measures the effect by the differences of the largest mean and the smallest mean across groups in units of one group SD

$$\delta = \frac{\omega_{max} - \omega_{min}}{\sigma_{within\ group}}.$$

The effect-size (δ) is usually classified to be large for values of $\delta \approx 1.25$, medium for $\delta \approx 0.75$, and small for $\delta \approx 0.25$. Given the ANOVA Null Hypothesis $H_0 : T_1 = T_J = GM$ and the alternative Hypothesis $H_1 : T_1 = GM - \delta/2$ and $T_2 = \ldots = T_J = GM$, 25 subjects are needed to reach a test power higher than 80% for a large effect, more than 60 for a medium effect, and more than 150 for a small effect. The effect size of anesthesia on power and phase properties was unknown at the planning of the study; therefore, we planned to use data from 150 patients to be able to detect even small effects. In addition, we accounted for rejection of datasets of individual patients based on standardized artifact detection in electroencephalographic signals. Given our experience with such recordings in the extremely electromagnetically noisy environment like operation theaters and during surgery, we expected that up to 10% of the recordings are expected to be rejected by a *post hoc* artifact detection and rejection. Therefore, we determined the sample size to be more than 190 patients.

Randomization—Sequence Generation

For each of the four hospitals, the randomization list was generated as follows: a Microsoft Excel table (Microsoft Corporation, Redmond, WA) was generated with the corresponding groups (16 groups with 3 patients, with 12 patients for each hypnotic and 12 patients for each opoid) in column 1. In column 2, a number was added using the random number function of Microsoft Excel. Next, the tables were sorted by values in column 2 (in ascending order), which rearranged the group assignments according to the randomly generated numbers. In all four hospitals, 48 patients were in one group for each opioid and 48 patients were in one group for each hypnotic.

Randomization—Implementation

According to the computer-generated list, envelopes with group assignments were sealed and arranged in the order of the randomization list. This order was maintained during patient enrolment.

Randomization—Allocation Concealment

After written informed consent had been obtained, the patients were randomly assigned to 1 of the 16 anesthetic regimens as the responsible anesthesiologist opened the next envelope.

Blinding

Only patients were blinded to the anesthetic regimen. After comprehensive medical evaluation by the visiting anesthetist, patients were informed about the study and asked to give their informed consent. At that time, the patient and the visiting anesthetist were blinded to the anesthetic combination to be used. Anesthetic regimens for single patients were kept in closed envelopes. Random allocation to the definitive drug combination was performed in the operating theater at the time when the patient was scheduled for surgery by drawing the next envelope out of a box. By then, the patient was blinded to the anesthetic regimen, and the attending anesthetist was not.

Schematic Illustration of the Data Flow

Figure 7 illustrates the data flow for analysis, based on synthetic signals where described (fig. 7, S2, S3, W1, W2, I1, I2). To avoid boundary effects for the wavelet-filtering procedures, each of the unmarked sweeps of the original data were flanked by the preceding and by the following sweep (fig. 7, S1).

Figure 7S2 shows a possible single-sweep response (synthetic); to visualize the concept of induced oscillation, we jittered the synthetic signal, *i.e.*, we introduced a small time delay for each response (dotted line). In figure 7S3 we added white noise to these jittered single-sweep responses, resulting in a signal to noise ratio of 0.4. In figure 7, I1 and I2 show the ITPL for the two wavelet-filtered frequency bands 57–114 Hz and 114–228 Hz of 500 synthetic signals; 40 of these were plotted in figure 7W1 and figure 7W2. The wavelet analysis is a time frequency analysis, which in this case is performed as a multi-scale analysis. Like every time frequency analysis, there is a tradeoff between time and frequency resolution. Both are defined by the sampling rate in combination with the properties of the according wavelet and scaling filter. The time resolution for the highest frequency band as used in this analysis (sampling rate 4 kHz, highest frequency band 228–457 Hz), the Daubechies 4 wavelet (8-point digital filter) yields a time resolution of 2 ms. This time resolution increases by steps of the factor 2^n for the next scales or frequency bands, respectively.

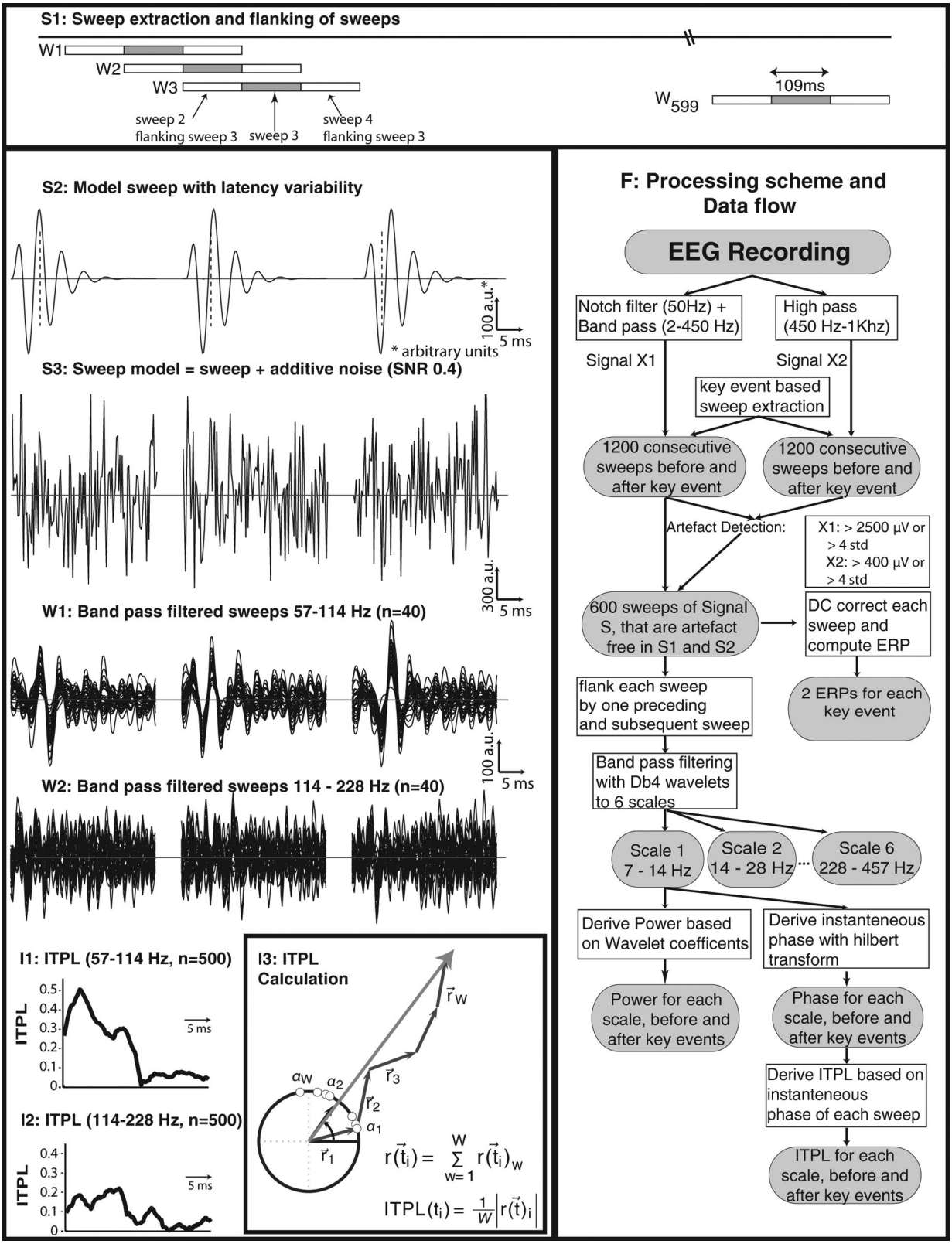

Fig. 7. Visualization of data flow and measures calculation, based on surrogate data. (*S1*) The sweep extraction of the 3 sweeps from the electroencephalogram (EEG); the middle sweep was used for calculating the measures. (*S2*) Synthetic single-sweep oscillations shifted forward and backward in time, visualizing the concept of induced oscillations. (*S3*) The same oscillations as S2 with additive white noise (signal-to-noise [SNR] ratio 0.4). The wavelet-filtering leads to bandpass-filtered signals, here shown for 40 single-sweep signals like in S3 for the frequency bands 57–114 Hz (*W1*) and 114–228 Hz (*W2*). For these frequency bands, the intertrial phase-locking (ITPL) based on 500 sweeps is shown in *I1* and *I2*, respectively. (*I3*) Concept of the ITPL. (*F*) Data flow for analysis schematically from the processing of the EEG to the evoked responses (ERP) and to the measures power and ITPL.