Strain Differences in Cortical Electroencephalogram Associated with Isoflurane-induced Loss of Consciousness

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ABSTRACT

Introduction: Previously observed increased sensitivity to noxious stimulation in the Dahl salt-sensitive rat strain (SS/JrHsdMcwi, abbreviated as SS) compared to Brown Norway rats (BN/NhsdMcwi abbreviated as BN) is mediated by genes on a single chromosome. The current study used behavioral and electrocortical data to determine if differences also exist between SS and BN rats in loss of consciousness.

Methods: Behavioral responses, including loss of righting, (a putative index of consciousness) and concurrent electroencephalogram recordings, in 12 SS and BN rats were measured during isoflurane at inhaled concentrations of 0, 0.3, 0.6, 0.8, 1.0 and 1.2%.

Results: In SS compared to BN rats, the mean ± SEM EC_{50} for righting was significantly less (0.65 ± 0.01% *vs.* 0.74 ± 0.02% inhaled isoflurane) and delta fraction in parietal electroencephalogram was enhanced 50–100% at all isoflurane levels during emergence. The frequency decay constant of an exponential fit of the parietal electroencephalogram spectrum graphed as a function of isoflurane level was three times less steep (mean ± SEM slope −57 ± 13 *vs.* −191 ± 38) and lower at each level of isoflurane in SS *versus* BN rats (*i.e.,* shifted toward low frequency activity). Electroencephalogram differences between strains were larger during emergence than induction.

Conclusions: Sensitivity is higher in SS compared to BN rats leading to unconsciousness at lower levels of isoflurane. This supports using additional strains in this animal model to study the genetic basis for differences in anesthetic action on mechanisms of consciousness. Moreover, induction and emergence appear to involve distinct pathways.

What We Already Know about This Topic

- There is considerable individual variation in anesthetic sensitivity with regard to transitions in consciousness

What This Article Tells Us That Is New

- Strain differences in response to isoflurane suggest genetically distinct mechanisms for electroencephalographic and behavioral correlates of transitions in consciousness, as well as providing evidence for distinct pathways of anesthetic induction and emergence

THE mutual understanding of unconsciousness during general anesthesia and surgery that exists between patients and anesthesiologists is implicit. However, it is difficult to define and/or reliably measure unconsciousness,[1] and it is even more difficult to characterize it physiologically. Unconsciousness results from anesthetic effects at specific sites, which are numerous and can be both cortical and subcortical.[2] Even more complex is the individual variation in anesthetic sensitivity with regard to responses typically associated with unconsciousness. However, variation in biologic systems, when reproducible, may actually be useful to compare and eventually identify relevant mechanisms that are responsible for the variation. The current study was designed to compare and quantify strain differences in responses that are established and proven to be associated with consciousness.

A number of quantitative electroencephalogram parameters, reflecting anesthetic suppression of the central nervous system, correlate with changes in spontaneous or evoked

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behavior, which are indicative of a change in the level of consciousness. In rat studies, the same electroencephalogram measurements correlate with volatile anesthetic concentrations that caused the animals to lose their righting reflex---a putative but widely used index of loss of consciousness in this species.[3]

We have recently observed that two rat strains (Dahl salt-sensitive rat strain SS/JrHsdMcwi, abbreviated as SS and Brown Norway rat BN/NhsdMcwi abbreviated as BN), which are parental strains for a larger chromosome substitution model, exhibit marked differences in cardiovascular sensitivity[4] and movement response[5] to anesthetics, with SS rats being considerably more sensitive than BN rats. Additional (preliminary) studies suggested that there also were strain differences in anesthetic sensitivity related to loss of consciousness. Our goal for the current study was to investigate this further. The hypothesis was that the SS exhibits behavioral and electroencephalogram signs of loss of consciousness at a lower anesthetic level compared to BN during both induction and emergence. In order to test this hypothesis, we used previously established behavioral testing[6] in conjunction with recordings of spontaneous electroencephalogram activity in the parietal and motor cortex of SS and BN animals during isoflurane administration. We sought to identify quantifiable differences between these two strains in central neuronal changes that were presumably responsible for differences in anesthetic-induced unconsciousness. We reasoned that if SS animals exhibited differences in putative behavioral indices of consciousness compared with BN, then concurrent differences in regional electroencephalogram measurements between the two strains would reflect meaningful differences in central neuronal function and processing that regulate consciousness. Finally, increasing evidence has suggested that anesthetic induction to the point of loss of consciousness involves a mechanism that is separate from emergence to the awake state.[7–9] For this reason, strain differences in anesthetic response were studied separately for each of these processes. If meaningful differences between SS and BN could be established in the current study, then it would be reasonable (in future studies) to compare loss of consciousness in strains from the previously established chromosomal substitution panel based upon the SS and BN parentals.[10] The goal would then be to use a pharmacogenomic approach to further investigate mechanisms of anesthetic-induced loss of consciousness.

Materials and Methods

The animals used in this study were 8- to 12-week old male SS and BN rats, ranging in weight from 250 to 400 g, developed and maintained under the direction of the Human and Molecular Genetics Center at Medical College of Wisconsin (PhysGen program). All protocols were reviewed and approved by the Animal Care and Use Committee at the Medical College of Wisconsin. All rats were maintained on a low (0.4%) sodium chloride diet to minimize the development of hypertension and associated end organ damage in SS rats.[11] All animals were housed in a reverse light-dark cycle room for at least 10 days before surgical implantation, and remained there for the duration of the experiment. The rationale for the reverse light-dark cycle was to maximize the incidence of wakefulness in nocturnal rodents during the time of day that they would be studied. Food and water access was *ad libitum*.

Surgical Preparation

Electrode placement: Anesthesia was induced in 11 SS and 12 BN rats (aged 9–12 weeks) using 2% inhaled isoflurane. The animal's head was secured in a small animal stereotaxic instrument (Model 900, David Kopf Instruments, Tujunga, CA), and a rat gas anesthesia head holder (Model 929-B, David Kopf Instruments) was slipped over the nose/mouth to continue anesthesia at 2% inhaled isoflurane. A temperature monitor (model 73A, YSI, Yellow Spring, OH) was used to measure rectal body temperature, which was maintained at 37°C *via* a water-circulating heating pad (model K-20F, American Pharmaseal Company, Valencia, CA). The dorsal surface of the head was prepared for surgery with betadine and steam-sterilized drapes. Following local skin infiltration with 1 ml of 0.5% bupivacaine using a 25 g needle, a midline incision was made. The skin was retracted, the exposed cranium was gently scraped of connective tissue, and any bleeding was stopped with hydrogen peroxide or cauterization.

For subsequent electroencephalogram recording, we placed concentric bipolar electrodes in the primary motor cortex (M1) and stainless steel screw electrodes in the parietal association cortex (PtA). At each site, a 1–2 mm diameter hole was drilled through the cranium, using a dental drill and bur No. FG 1 (Rhino XP, Midwest Dental Products Corp., Des Plains, IL). Two PtA screw electrodes were placed 4 mm posterior and 2.5 mm bilaterally (relative to bregma). The bipolar M1 electrode was placed 2 mm anterior and 1.9 mm right of bregma. Additional stainless steel machine screws were placed in the cranium as anchors. The leads were connected to a 6-contact adaptor (Plastics One Inc., Roanoke, VA) and secured to the cranium with gentamicin enriched bone cement (Palacos R&G, Zimmer Orthopaedic Surgical Products, Dover, OH) and cerebond skull adhesive (Leica Microsystems, Bannockburn, IL). During adhesive application, anesthetic concentration was gradually decreased. When the assembly was completed, anesthetic administration was terminated and the animal was removed from the stereotaxic unit. The rat emerged on the heating pad, and 10 mg/kg enrofloxacin subcutaneously and 0.02–0.05 mg/kg buprenorphine subcutaneously were administered. The animal was returned to the housing cage in the animal facility. Injections of buprenorphine (0.02–0.05 mg/kg subcutaneously, twice daily) continued for 3 days, and injections of enrofloxacin (10 mg/kg subcutaneously, twice

daily) continued for 7 days. The animals were observed for 7–10 days for any infection or other complications.

Arterial and venous catheterizations: An additional group of three SS and three BN rats received chronic indwelling arterial and venous catheters to measure circulating plasma levels of volatile anesthetics and hemodynamic changes between the two groups. Briefly, initial anesthesia induction utilized the drop method, where animals were placed in a plastic chamber with approximately 5 ml of 20% isoflurane in propylene glycol. Upon losing the righting reflex, the animal was quickly placed on a surgical warming pad, and a nose cone was applied to maintain airflow of 2% vaporized isoflurane. Using aseptic techniques, the left hind limb was shaved, cleaned, and sterilized. A 1.5 cm incision was made, and the femoral artery and vein were isolated and separated. Sterile polyethylene catheters (RenaPulse Tubing 040; Braintree Scientific, Braintree, MA.) were passed into the vessels through a small incision of both the artery and vein and tied down with 3-0 silk suture ties where appropriate. Tubing was externalized subcutaneously in the mid scapula area, filled with 100 U/ml heparinized saline and blocked with a stainless steel rod. After 7–10 days of recovery, the stainless steel plug was removed and the arterial line was attached to a saline filled pressure transducer for monitoring mean arterial pressure with a bridge amplifier attached to a personal computer. In addition, 1 ml blood samples were taken to determine plasma concentrations by gas chromatography at 0.8 and 1.0% inspired isoflurane during induction and at 0.6 and 0.8% during emergence. At the 0.8% level of inspired isoflurane, 0.125 ml arterial blood samples were taken to measure blood gases and pH levels. Each rat was studied during induction or emergence on separate days to mimic electroencephalogram studies.

Behavioral and electroencephalogram measurements: Following 7–10 days of recovery from electrode placement, the animals were studied in a custom-built, transparent, Plexiglas®(Evonik Industries AG, Essen Germany), airtight anesthesia enclosure with a volume of 39.3 l, where rats breathed spontaneously in a 30% O_2 environment. Isoflurane was delivered at 5 l/m and inspired agent concentration was monitored with a POET IQ2 (Criticare Systems, Inc., Waukesha, WI). Body temperature was maintained at 37°C. Since loss of consciousness (LOC) was the goal of this study, the loss of righting reflex (LORR) was used as a proxy measurement for LOC, in accord with previous studies,[3] while graded responses to vibrissal stroking, exposure to an offensive odor, and corneal stimulation were also tested. LORR was scored by manually tipping the enclosure 45°, while a cotton-tipped applicator was used to test the corneal and vibrissal responses. The reaction to offensive odor was tested using a highlighting pen (Sharpie permanent marker, fine point; Sanford, Bellwood, IL). The extent of each behavior response was scored on a five-point scale between 0 and 2, according to previously published criteria.[6,12] Concurrently with the behavioral studies, continuous 15-min electroencephalogram recordings were generated at each level of anesthetic. Analog electroencephalogram signals were pass-band filtered between 0.1 Hz and 1 KHz, digitized at 500Hz, notch-filtered at 60 Hz and recorded on a desktop computer using Windaq acquisition software (DATAQ Instruments, Akron, OH).

Experimental Protocols and Data Analysis

Behavior responses and electroencephalogram recordings were examined at 0.3, 0.6, 0.8, 1 and 1.2% isoflurane, with 30 min equilibration between concentrations. Each animal was studied using two protocols in random order on separate days. In the first protocol, isoflurane was adjusted up ("induction"), and in the other protocol isoflurane was adjusted down ("emergence"). The order in which these induction and emergence protocols were performed was randomized. Behavioral tests were performed on SS and BN rats simultaneously in identical, adjacent anesthesia test boxes fed by a common vaporizer. The same technician scored all rat behaviors and video records were kept for review. All replicate behavioral data for each strain were averaged and used to generate sigmoidal dose-response curves (variable slope) with GraphPad Prism v5 for Mac (GraphPad Software, San Diego, CA), where ED_{50} describes the anesthetic level at which behavioral response is half-maximal, and the Hill coefficient describes the steepness of the slope representing the transition between alertness and unconsciousness. Goodness of fit values (R^2) were also calculated for each plot.

Electroencephalographic data were analyzed in two different ways. First, the earliest 30-s electroencephalogram epoch from the first noise-free segment immediately following behavioral stimuli, was transformed into a power density spectrum using custom scripts in MATLAB v. 7.5 (MathWorks Inc., Natick, MA). A 30-s segment was chosen to reduce variability between electroencephalogram desynchronization following behavioral arousal and return to spontaneous electroencephalogram activity. Cursory examination indicated 49.2 ± 7.3 s was the mean minimum time between stimulus and return to spontaneous electroencephalogram at the transitional dose of 0.8% isoflurane during induction or emergence. To verify whether nonstationary variability was stable over the interval of interest, a Morlet wavelet analysis was performed.[13] Morlet analysis transforms complex electroencephalogram signals into a two-dimensional integral of power and frequency over time, where episodic phase transitions could be detected. Wavelet transformation of single, raw electroencephalogram recordings involved convolving the signal with complex wavelets with central frequency ranging from 1 to 60 Hz in 1 Hz increments. Since the region of interest appeared to be stable, a spectral analysis was performed. Data segments were analyzed using Welch's power spectral estimation method with a 250-point window and an 80% overlap. Relative band power ratios were then calculated by comparing average dBm/Hz in a specified range to the entire frequency spectrum up to 60Hz (a limit imposed by the notch filter). Average band powers

were calculated for δ-(2–4 Hz), θ_2-(4–8 Hz), θ_1-(9–12 Hz), β- (13–30 Hz) and γ-waves (30–55 Hz). Second, the same 30 s-electroencephalogram epochs were Fast Fourier Transformed in a Hanning window for the entire frequency range from 1 to 59 Hz, plotted on a log-log grid using Axograph X for Macintosh (Axograph Scientific**), and visually fit to a double exponential function of the following form:

$$y = a_1 e^{(-f/\tau_1)} + a_2 e^{(-f/\tau_2)} + c$$

Where a is amplitude of the power, f is the frequency, and τ is the frequency decay constant. Two exponentials were necessary when the periodograph inflected away from the $1/f$ inverse relationship between power and frequency,[14] while single exponential fits were chosen where the second exponential was identical or the polar opposite of the first. Since the purpose of this analysis was to express the frequency distribution, a weighted average of decay constants was estimated with the following function:

$$weighted\,\tau = \frac{(a_1 \times \tau_1) + (a_2 \times \tau_2)}{a_1 + a_2}$$

The higher the value in weighted τ, the less synchronization between the various frequency elements, while lower numbers represent more synchronization. Finally, the time course of dynamic changes in the frequency spectrum was of interest. Average weighted τ values were plotted against the administered isoflurane level and then were fit to a linear function to compare the changes in decay constants between strains over the entire experiment.[15]††

Statistical Analysis

Mean plasma isoflurane concentrations resulting from 0.6 and 0.8% inhaled isoflurane in SS and BN strains were analyzed with one-way ANOVA and mean arterial blood pressure measurements comparing SS rats to BN rats were analyzed with unpaired *t* tests. Behavioral data comparing the log of volatile anesthetic gas concentrations to the fractional effects of anesthetic concentrations on behavioral responses were expressed as the 95% upper- and lower-confidence levels for EC_{50} and Hill Slope factors. Analyses of plasma isoflurane concentrations, blood pressures and behavioral data were all performed using GraphPad Prism. Since grouped observations for electroencephalogram data appeared normally distributed, as shown by D'Agistino-Pearson computations, two separate multivariate ANOVA with repeated measures tests were applied using Statistica 8 (Statsoft Inc., Tulsa, OK) or Graphpad Prism. The between factors were rat strain and averaged band power in the first analysis, and rat strain and average weighted τ values in the second analysis. In both cases, the within factor was the isoflurane level. Bonferroni posthoc tests were used only in groups where significant interactions were found. Additionally, for each strain (SS and BN), recording site (PtA and M1) and protocol (induction and emergence), trends in the magnitude of τ measurements at different isoflurane doses were fit to linear equations by plotting averaged τ values as a function of the isoflurane level. Dose-response effect of isoflurane on the electroencephalogram was determined by calculation of goodness of fit of linear regression and performing an F test to determine whether or not the corresponding slopes were different from zero. The slopes between strains were also compared with unpaired, two-tailed Student *t* tests. All regression analysis was performed using Graphpad Prism. For discrete, independent measurements of blood plasma concentrations and mean arterial pressures, unpaired, two-tailed Student *t* tests were computed in Microsoft® Excel® for Mac 2004 (Microsoft Corporation, Redmond, WA). All results are expressed as mean ± SEM, and $P \leq 0.05$ was considered significant.

Results

Mean electroencephalogram power between 1 and 59 Hz at baseline (0% isoflurane) did not differ between SS and BN rats (mean difference range +0.02 to −0.09 dB/Hz, $P > 0.05$ with one-way ANOVA). During the emergence protocol there was no difference between BN and SS rats in the mean plasma ± SEM isoflurane concentrations resulting from 0.6 and 0.8% inhaled isoflurane (0.35 ± 0.05 and 0.45 ± 0.07 mM respectively for BN *vs.* 0.30 ± 0.09 and 0.39 ± 0.07 mM for SS). Likewise during induction, the plasma isoflurane concentrations for BN and SS rats under 0.8 and 1.0% inhaled isoflurane, respectively, were virtually identical (0.41 ± 0.02 and 0.50 ± 0.01 mM for BN *vs.* 0.40 ± 0.06 and 0.50 ± 0.01 mM for SS). However, mean arterial blood pressures were generally higher in SS rats compared to BN. For example, under 0.8% inhaled isoflurane, mean arterial pressure was 113.7 ± 5.5 and 107.7 ± 8.4 mmHg during emergence and induction, respectively, for BN rats, while mean arterial pressure for SS rats at the same concentration was significantly higher at 149.7 ± 6.6 and 160.6 ± 4.9 mmHg.

Behavioral Responses

Isoflurane EC_{50} values for LORR tests were significantly lower for SS compared to BN rats during induction (0.65 ± 0.01% *vs.* 0.74 ± 0.02% inhaled isoflurane) and emergence (0.42 ± 0.03% *vs.* 0.68 ± 0.02% % inhaled isoflurane). Fitted Hillslope factors were significantly steeper for SS compared to BN rats during induction (8.6 ± 1.1 *vs.* 6.8 ± 1.1) but shallower during emergence (3.9 ± 0.5 *vs.* 7.5 ± 1.4), indicating that (compared to BN), the SS rats had slightly earlier attenuation of behavioral responses as isoflurane concentrations were increasing but prolonged attenuation and delayed recovery of these responses as isoflurane concentrations were decreasing

** http://axograph.com. Accessed November 6, 2012.

†† A similar approach using the entire electroencephalogram spectrograph on a log-log scale with two linear fits received the Best New Technical Innovation Award at the 2010 Annual Meeting of the Society for Technology in Anesthesia.[16]

(fig. 1). Vibrissal response scores during induction for SS, like LORR, were significantly lower than BN (0.69 ± 0.01% *vs.* 0.75 ± 0.02%) at the EC_{50} for isoflurane. Hillslope factors for the vibrissal response were much steeper (18.8 ± 7.4 for BN *vs.* 14.9 ± 2.0 for SS during induction) than those for righting, displaying a behavior highly sensitive to anesthesia. Olfactory and corneal responses were better preserved during anesthesia. During emergence and induction, EC_{50} values and Hillslope factors were lower in SS compared to BN rats, showing greater sensitivity to anesthesia, but maximal best fit values using logistic regression were lower for BN compared to SS rats, suggesting partially conserved olfactory and corneal responsiveness in BN rats even at the highest concentrations of isoflurane (fig. 1). Out of a possible 2, maximal isoflurane-induced attenuation during induction was 1.8 ± 0.04 for olfactory response and 1.9 ± 0.1 for corneal response in SS rats compared with 1.4 ± 0.1 and 1.5 ± 0.1 olfactory and corneal responses, respectively, in BN. During emergence, the maximal attenuation of responses were 1.6 ± 0.2 and 1.8 ± 0.5 in BN compared with 2.0 ± 0.3 and 2.0 ± 0.3 in SS rats.

Electroencephalogram Recordings

Raw electroencephalogram recordings indicated a predominance of slow wave activity in the 0–10 Hz range in an SS rat compared to a BN (fig. 2, A and B). Therefore, we compared normalized electroencephalogram power in five frequency bands, δ-(2–4 Hz), θ_2-(4–8 Hz), θ_1-(9–12 Hz), β (13–30 Hz) and γ-waves (30–55 Hz) between SS and BN rats during induction and emergence (fig. 2C). At specific isoflurane levels, power in the δ and θ_2 bands recorded from the PtA were significantly higher in the SS group compared to BN during emergence. Conversely, γ activity recorded from the PtA was significantly lower in the SS group compared to BN during emergence at specific isoflurane levels. At the M1 recording site, no differences in band power were observed in the δ- θ_1- or β- range between SS and BN strains during induction or emergence.

Traditional comparisons of discrete power bands in the frequency spectrum fail to depict transitions in power across all frequencies as the brain succumbs to anesthetic unconsciousness. Therefore, we obtained estimates of global electroencephalogram activity (fig. 3A) by fitting one- or two-exponential functions to frequency-binned power spectra from a 30-s, artifact-free epoch chosen from 5 min of continuous recording after behavioral testing (figs. 3B and 4 typical traces). As expected with exponential relationships in electroencephalogram power spectra, the majority (>70%) of electroencephalogram recordings were fit with a single exponential at 0% isoflurane, indicative of long-range, critically balanced dynamic interactions between cognitive processes,[14,17] whereas the majority (>75%) required two exponentials at 0.8% isoflurane as various mental processes were gradually impaired to the point of loss of consciousness.[18] Trends in global electroencephalogram activity from 0 to 1% isoflurane during induction and emergence from anesthesia were compared between SS and BN rats using the averaged exponential slopes (τ) at each concentration (fig. 4). As illustrated in Figure 5, τ increased (indicative of a greater fraction of higher frequency activity) with decreasing isoflurane levels and vice versa. Values tended to be lower in SS compared to BN rats at all levels of isoflurane (fig. 5). When averaged for all SS and BN animals, linear slopes of averaged τ values were significantly flatter (*i.e.,* closer to 0) in SS compared to BN during emergence, especially in the PtA where specific τ values in SS were smaller at 0.3–0.8% (fig. 5A). A flatter trend was also observed in the M1 recording site in SS compared to BN during the induction protocol, with specific differences in τ values at 0–0.3% inhaled isoflurane (fig. 5D). The trend in M1 was also flatter in SS compared to BN rats during emergence. However, (in contrast to induction), no specific doses were different in averaged τ values (fig. 5B). Finally, in the PtA recording site during the induction protocol, discrete amplitudes in averaged τ values were smaller in SS compared to BN rats at individual 0.3 and 0.6% inhaled isoflurane but linear slopes of τ as a function of isoflurane level were not compared between the two strains because frequency distributions did not follow a linear trend in the BN group (fig. 5C).

Such nonstable transitions to unconsciousness and prolonged behavioral effects during recovery may reflect a hysteresis between induction and emergence. Therefore, we compared the exponential slopes (τ) of the electroencephalogram at each level of inhaled isoflurane within species to determine whether a difference exists between induction and emergence (fig. 5, A–D). Regression analysis indicated that there was a dose effect of τ plotted as a function of the administered isoflurane level such that it was linear in all groups, except for parietal recordings in BN rats during induction. In this group, the main effect (τ as a function of isoflurane level) was highly significant over the course of induction, but (as stated above) the R^2 value of the regression line was much lower than the other groups (0.37 *vs.* between 0.89 and 0.95) and the slope of averaged τ values *versus* isoflurane level was not statistically different from zero. In BN rats, the transition from consciousness to unconsciousness was marked by a higher τ at 0.3 and 0.6% isoflurane followed by a smaller τ as rats began to lose the righting response. This finding suggests that a barrier exists to the induction of anesthetic unconsciousness, while recovery of consciousness is a stable process. In contrast, this hysteresis in electroencephalogram pattern suggestive of persistent increased activity during induction (but not emergence) is suppressed in the SS rat strain.

Discussion

The findings in this study suggest that SS exhibits greater sensitivity to behavioral and electroencephalogram effects of isoflurane anesthesia than BN. The righting reflex in SS compared to BN rats was attenuated at lower levels of isoflurane during induction and did not return until lower levels of isoflurane were reached during emergence in SS

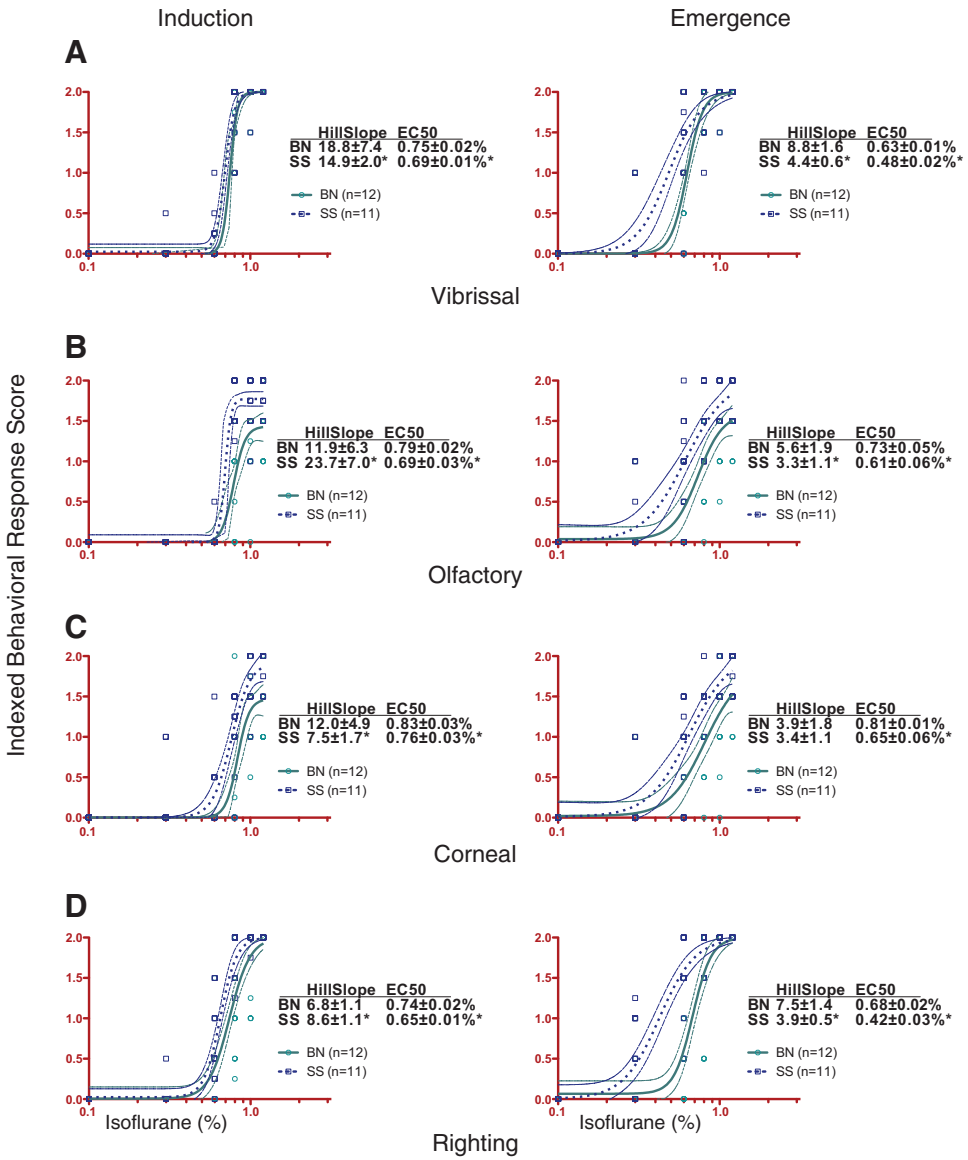

Fig. 1. Indexed behavioral response scores (range 0–2) as a function of percent isoflurane level (on a log scale) for Dahl salt-sensitive (SS, *dashed line*) and Brown Norway (BN, *solid line*) rats are illustrated with upper and lower confidence levels. SS behavior was more sensitive to isoflurane than BN as shown by lower half-maximal concentrations during all behavioral tests. (*A*) Vibrissal stroking responses disappear early and rapidly during induction with isoflurane, but vibrissal responses were obtunded at higher concentrations in BN compared to SS rats during induction and emergence from isoflurane anesthesia. (*B, C*) Olfactory and corneal responses were more resistant to isoflurane, yet SS rats were more likely to lose these responses, and the half-maximal isoflurane was significantly less than BN. (*D*) Loss of righting reflex was more resistant to isoflurane during induction and more compliant during emergence, and the half-maximal concentration of isoflurane was significantly less in SS compared to BN rats, especially during emergence. *$P \leq 0.05$ SS *versus* BN.

compared to BN. Since the righting reflex is an accepted surrogate measure of consciousness[3] this finding indicates that SS animals are more likely to be unconscious at a given level of isoflurane than corresponding BN animals. This is further supported by the other behavioral indices, all of which showed greater suppression at lower doses of isoflurane in SS compared with BN rats. Similar to LORR, vibrissal stroking and olfactory avoidance are characterized by cortical processes.[6,12] Enhanced cortical sensitivity was not only reflected in behavior differences during induction and emergence but also in the corresponding reductions in strength and complexity of electroencephalogram patterns. Enhanced theta activity in SS compared with BN rats during emergence from isoflurane corresponds to an exaggerated shift to the sedated state.[19,20] Along with an increased delta fraction, this agrees with the behavioral observation that SS rats remained sedated or in an altered state longer than BN rats. Moreover, these findings were not attributable

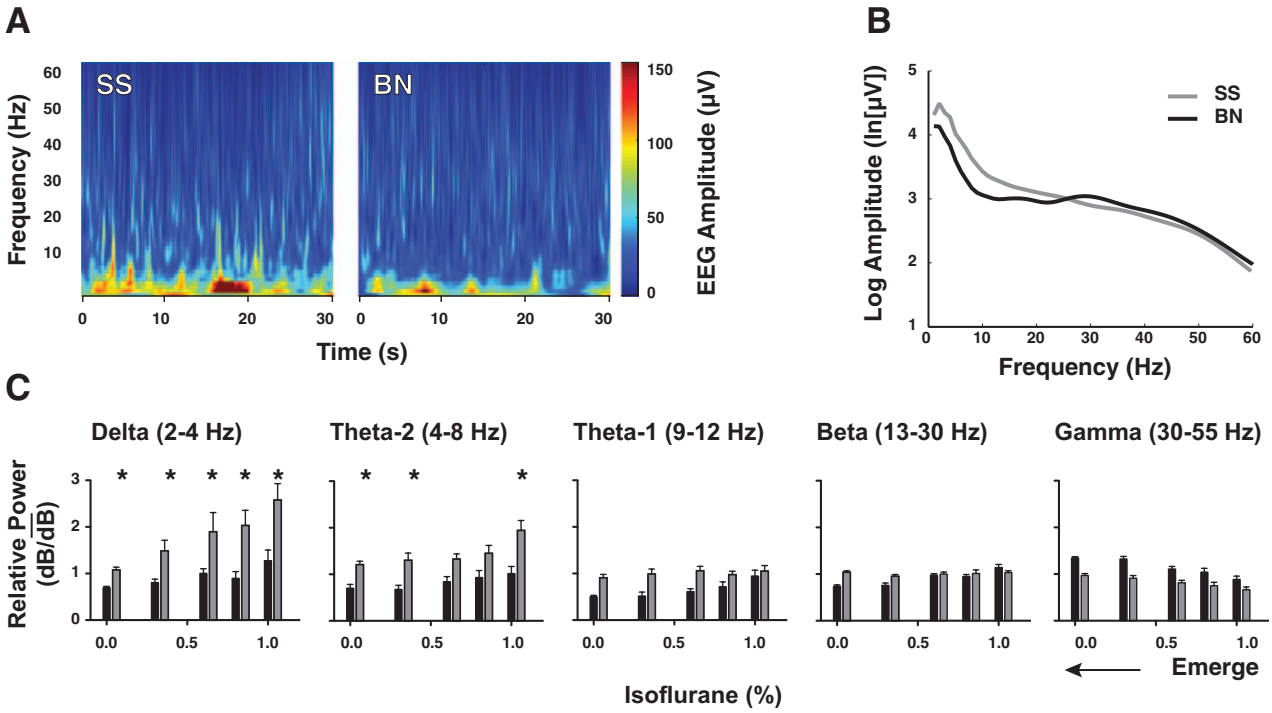

Fig. 2. Increase in low-frequency (δ, θ2) band power in Dahl Salt Sensitive (SS) compared to Brown Norway (BN) rats in the parietal association cortex (PtA) during emergence. (*A*) Frequencies, which were decomposed over time using a Morlet wavelet transform, show that nonstationary electroencephalogram variability was stable during a representative 30-s region of interest immediately after behavioral stimulation; (*B*) Spectral analysis from the same 30-s region illustrates the difference in power in the low-frequency region; (*C*) Summary data showing significant differences in the low-frequency range during emergence in the PtA. Induction and motor cortex data not shown. (*$P \leq 0.05$ *vs.* BN, n = 11 for SS [*shaded bars*] and 12 for BN [*dark bars*]). EEG = electroencephalogram.

to greater hemodynamic depression or anesthetic concentration in SS rats.

In addition to traditional frequency ratios, assessment of global electroencephalogram activity using the novel τ calculation exhibited a greater overall proportion of activity at lower frequencies in SS rats (compared to BN) during emergence from isoflurane. Despite expectations that electroencephalogram activity in the primary motor cortex would correlate with behavioral loss of righting reflex, isoflurane induced shifts to lower frequencies were most prominent in the PtA recording site of SS rats compared to BN. Other studies have shown a prolonged latency between the intention to move (observed in parietal cortical electroencephalograms) and the actual command to move originating in the motor cortex under anesthesia.[9,21,22] Indeed, rat and human studies have shown that unconsciousness ensues when the parietal cortex is inactivated.[1,23] A less prominent shift towards lower frequencies was observed in the M1 recording site during both induction and emergence. This finding may suggest a partial dissociation between immobility and loss of consciousness whereby SS animals were unconscious but not incapable of responding due to an anesthetic suppression of motor responses at M1.[24] Furthermore, complete correlation between electroencephalogram responses and behavioral responses in the anesthetized setting may not be a valid expectation because motor (behavioral) responses involve many steps, including spinal cord transmission, peripheral neuronal function and muscle response.

The other major finding from these results was that the strain difference between SS and BN rats in the isoflurane-induced shift in electroencephalogram pattern to lower frequencies was large and significant during emergence but less prominent during induction. Moreover, while the electroencephalogram pattern did not differ between induction and emergence using isoflurane within the SS group, it did exhibit a greater shift to the slower frequencies in emergence compared to induction within the BN group. These findings are in agreement with others[7,8] who suggest that the central nervous system mechanisms underlying these two processes (anesthetic induction *vs.* emergence) are distinct.

Behavioral Responses during Isoflurane Administration

Anesthetic-induced attenuation of simple movement response to noxious stimulation is regarded as being mediated at the level of the spinal cord independent of higher central nervous system input,[25] though central nervous system facilitation can increase anesthetic requirements at the spinal level.[26] In previous experiments, significantly lower levels of volatile anesthetic inhibited movement response to tail clamp in SS compared to BN rats.[5] However, more complex responses involve cortical processing. The behavioral

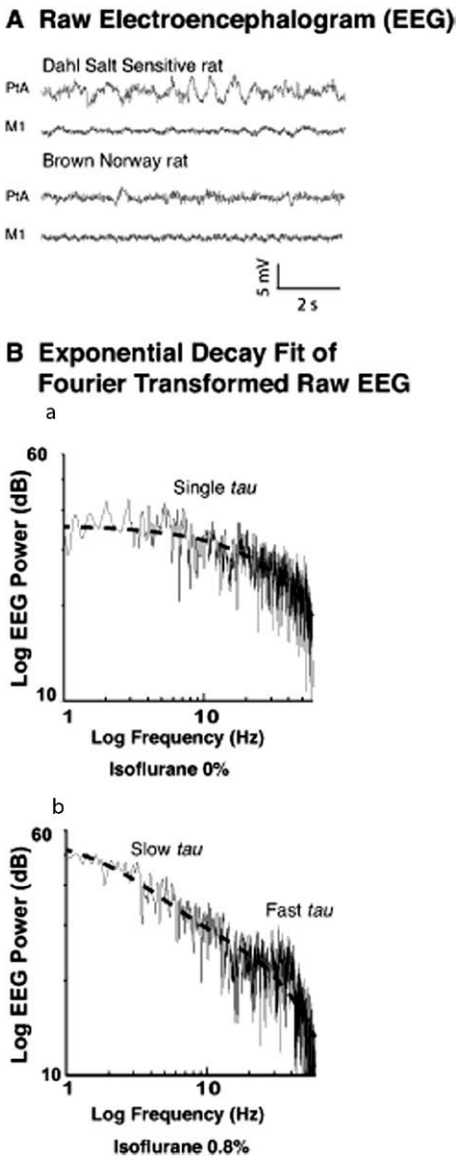

Fig. 3. (*A*) Example of raw electroencephalogram traces at 0.3% isoflurane during an emergence protocol in the parietal association cortex recording site, which illustrates the predominance of slow-frequency, high amplitude waves in Dahl salt sensitive compared to Brown Norway rats. (*B*) Examples of traces used to determine single (a) and double (b) τ values. Electroencephalogram frequency was plotted as a function of electroencephalogram power on a log-log scale and used to generate the exponential rates (τ) of shift in the total power of frequency changes between 1 and 59 Hz. As described in the text, data were best fit to a single or double exponential expression. A more negative value of τ indicated a shift toward lower frequencies while a less negative value of τ indicated a shift toward higher frequencies. (Note: scales truncated to region of interest from 0 to 60 Hz).

assessments used in the current study (LORR, corneal reflex, olfactory reflex and vibrissal reflex) have all been applied previously to measure avoidance responses. Such purposeful responses have been recognized as basic criteria for conscious function.[27] Although the cortical pathways of these purposeful responses are distinct from each other,[28–30] they were also all attenuated at significantly lower levels of isoflurane in SS compared with BN rats in the current study. This suggests that the enhanced anesthetic sensitivity in SS rats includes conscious behavior involving cortical networks rather than simple movement responses mediated at lower levels of the central nervous system.

Electroencephalogram Responses during Isoflurane Administration

The shift toward delta and theta fractions (of total electroencephalogram) during emergence from isoflurane in SS rats compared to BN is consistent with previous observations of the effects of general anesthesia.[31] This isoflurane-induced change in electroencephalogram patterns was more prominent in SS where the decrease in τ value was observed in both induction as well as emergence in contrast to the BN rats where it was observed only during emergence. During induction, the transient enhancement in τ values at lower isoflurane levels in the BN rats corresponded with what has been described as a "biphasic effect."[32] This apparent hysteresis in electroencephalogram between induction and emergence was not present in the SS rats and so the two strains appear to have different mechanisms leading up to LOC during isoflurane administration. Since there was no assessment tool in the current study to compare behavior before LOC, it is possible that baseline (preanesthesia) levels of consciousness may be different in SS *versus* BN rats. Anecdotally, SS animals tended to appear agitated and partially disinhibited in the wakeful state while (by comparison) the BN animals appeared docile. The recognition of an intermediate "excitement phase" or "phase II" during the transition from consciousness to unconsciousness has been well described for decades.[33] In the current study, the BN rats began somewhat subdued and clearly exhibited such a response during isoflurane before LOC. However, the SS rats began active (as if already further along in the process of anesthetic-induced LOC) and proceeded directly to unconsciousness.

A shift toward slower frequencies in the electroencephalogram implies a global or regional tendency toward neuronal hyperpolarization with consequent reduction in neuronal firing.[1] The electroencephalogram changes that were observed in this study were correlative (and concurrent) with behavioral responses that were indicative of altered consciousness (although causation will require additional verification). The overall electroencephalogram pattern in either animal strain below 0.6% isoflurane and above 1% isoflurane demonstrated relatively little difference before, during and after the behavioral testing. Below 0.6%, the animals generally behaved as though they were awake and this was reflected in the electroencephalogram. Conversely, above 1%, the behavior and the electroencephalogram were indicative of an anesthetized (unconscious) state. In neither case (below 0.6% isoflurane nor above 1% isoflurane) did the formal

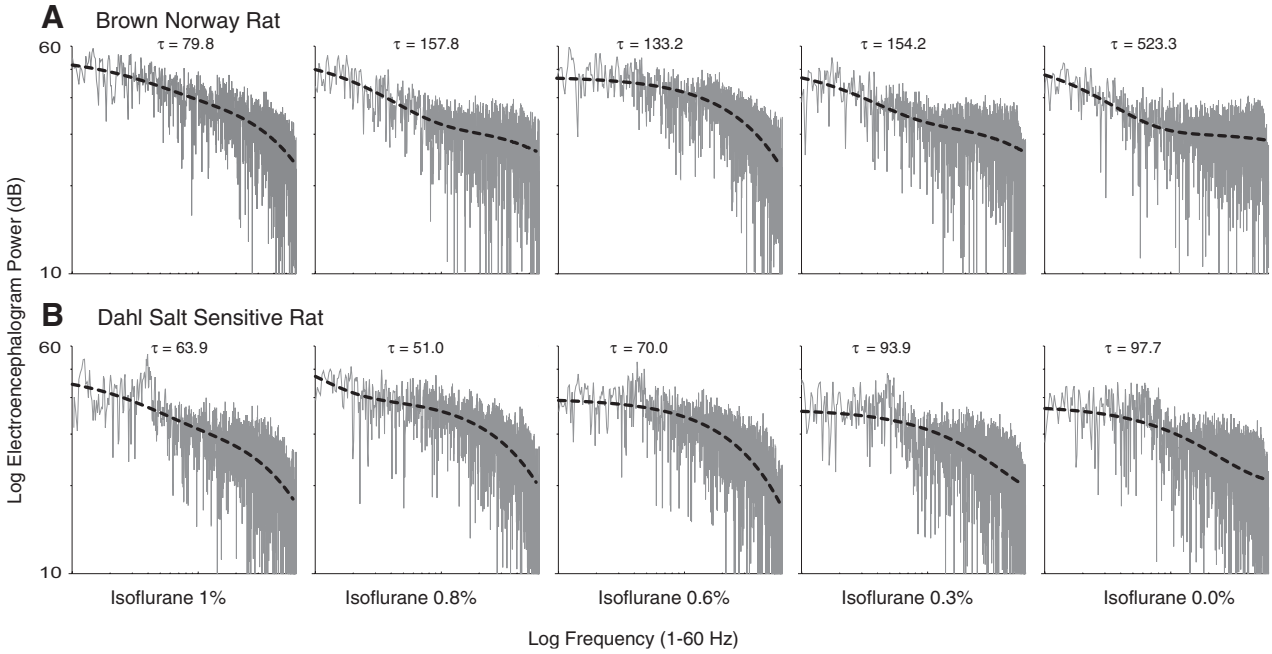

Fig. 4. Examples of how Fast Fourier Transformation graphs (used to generate τ) change with administered isoflurane level in emergence experiments for a Brown Norway (BN) (*A*) and a Dahl salt sensitive (SS) animal (*B*). In both cases, τ increases (indicative of a greater fraction of higher frequency activity) as isoflurane level is reduced. However, at all isoflurane levels, τ tended to be lower in SS compared to BN.

behavior test have an appreciable effect on the pre- and post-test electroencephalogram state. The biggest temporal effect occurred between 0.6 and 0.8% isoflurane, which seemed to represent the transition range between consciousness and unconsciousness. Over this period, the behavior testing and the consequent electroencephalogram pattern suggested arousal, which returned to a quiescent or anesthetized pattern within 1 to 2 min.

Induction versus *Emergence*

In the current study, in both SS and BN rats, isoflurane altered behavioral manifestations indicative of loss of consciousness during induction as well as emergence. However, the more prominent effect was during emergence. Within each of the strains, the EC_{50} for LORR and vibrissal response was significantly lower for emergence compared to induction. Concurrently, correlate shifts to slower electroencephalogram patterns were only observed during emergence in BN rats but were observed during both induction and emergence in SS. These observations support growing evidence that, while the transition between consciousness and unconsciousness occurs during both anesthetic induction and emergence, the two appear to be separate processes involving distinct pathways.[7–9]

Rationale for the Comparison of SS and BN Rat Strains

The SS and BN rats used in the current study are parental strains for production of consomic (chromosomal substitution) and congenic (partial chromosomal substitution) strains.[5,34] Such animals are effective tools to study the genetic basis for mechanisms of anesthetic action, such as loss of consciousness, because they are they are genetically identical except for the chromosomal segment that has been substituted. Thus, any difference between the SS and one of the consomic strains should be exclusively attributable to the chromosomal substitution of interest. Ultimately, the goal of such studies is to identify cellular processes that explain mechanisms of neuronal function, which account for anesthetic effects on conscious behavior, as has been done for other pharmacogenomic strain differences related to anesthetic administration.[35–37] One limitation of the current study is that parental strain comparisons do not (in and of themselves) identify mechanistic causes and are, in fact, several steps away from this. Instead, they validate that additional screening studies using consomic and successively smaller congenic sub-strains are indicated. Almost certainly, anesthetic-induced unconsciousness is a multi-factorial process of which the SS and BN rat differences likely represent only a portion. Nevertheless, if one or a few small enough chromosomal substitution segments of interest can be identified using congenic strains, there is a good chance that a meaningful component of the mechanism of anesthetic-induced unconsciousness can be clarified based on the function of the related genes.

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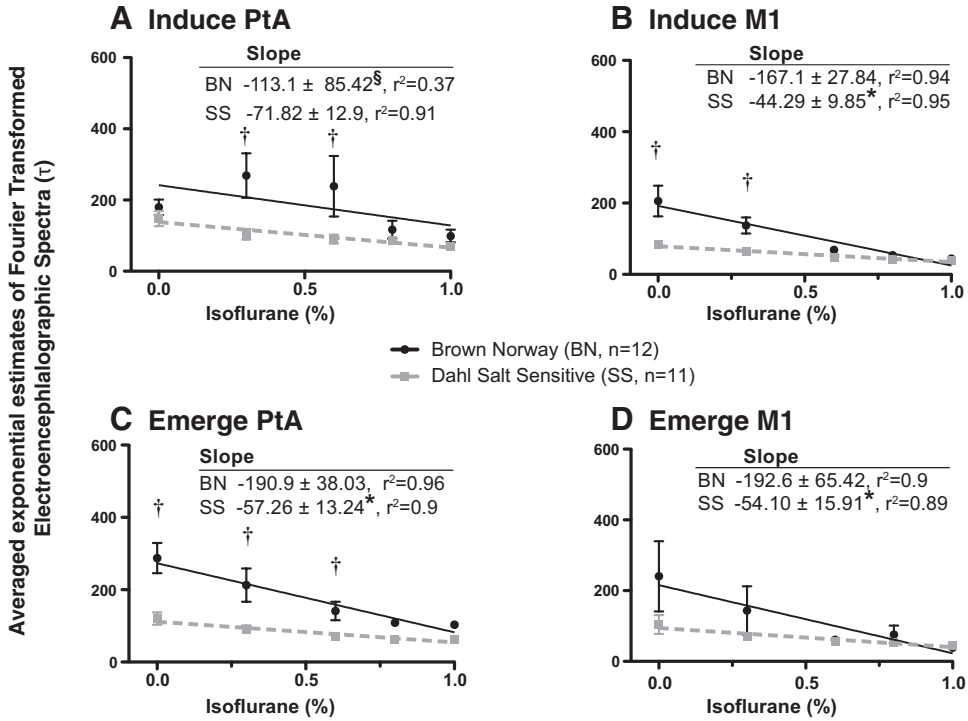

Fig. 5. Global shifts in electroencephalogram activity corresponded to behavioral findings. Data illustrated are mean ± SEM τ as a function of inhaled isoflurane level for Dahl Salt Sensitive (SS, *dashed line*) and Brown Norway (BN, *solid line*). (*A*) During induction, amplitudes of τ were larger, indicative of early excitation in BN compared to SS between 0.3 and 0.6% inhaled isoflurane. Therefore, in contrast to other all other isoflurane dose-response patterns, averaged τ measurements did not follow a linear trend in BN and the slope of the regression line was not statistically different from zero. (*B*) During induction, averaged τ measurements in the primary motor cortex (M1) of SS rats were lower compared to BN below 0.3% inhaled isoflurane, and low-frequency synchrony persisted longer in SS compared to BN rats. (*C*) During emergence, averaged τ measurements in the parietal association cortex (PtA) of SS rats were lower compared to BN below 0.8% inhaled isoflurane (the dose at which one-half of BN rats recovered the righting reflex) and low-frequency synchrony persisted longer in SS compared to BN rats. (*D*) Similar to PtA, low-frequency synchrony persisted longer in SS compared to BN rats at M1 recording sites, but, unlike PtA, average amplitudes of τ were not different at individual inhaled isoflurane concentrations. * $P \leq 0.05$ for the slope of the linear fit in SS *versus* BN; † $P \leq 0.05$ for the amplitude of τ in SS *versus* BN, § slope not different from zero at $P \leq 0.05$, n = 11 for SS and 12 for BN.

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