Frequency Coding Ability of the Somatosensory Thalamocortical System and Its Modulation by Anesthesia Depth

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Abstract

The purposes of the present study were to characterize and compare the mid-tail cortical and thalamic somatosensory evoked potentials (SEPs), and to examine how the depth of the barbiturate anesthesia affected them. After the tail representative locations of sacrococcygeal dorsal root (S2 or S3), thalamus (ventroposterior lateral nucleus, VPL) and primary somatosensory cortex (SI) were set up for recording, the rats were infused serially with diluted sodium pentobarbital solution beginning from light (5 to 10 mg/kg/hr) to deep (30 to 40 mg/kg/hr) and then stop infusion (recovery). The effects of anesthetic depth on SEPs were examined of dorsal root, thalamic and cortical field potentials evoked by mid-tail stimulation of various stimulation intensities (100 μA to 2mA, step 100 μA, at 2 Hz) and frequencies (0.5 to 11 Hz, step 0.5 to1 Hz, at 3T). The depth of anesthesia did not affect the strength-response curves of the SEPs. In contrast, the depth of anesthesia differentially influenced the frequency following capabilities of different recording sites. Under light anesthesia, thalamic SEP was only significantly affected with stimulation frequencies higher than 8 Hz, whereas cortical SEP was significantly affected with 2 Hz or higher. Under deep anesthesia, thalamic SEP evoked by low frequency tail stimulation was not significantly changed. In contrast, cortical SEP was affected much strongly so that under 1 Hz stimulation, a significant difference could be observed. We concluded, therefore, that thalamus was only partially responsible for the limited frequency following capability of the SI, and that the main effect of pentobarbital was on the cortical level. From the data obtained, an exponentially decaying curve could be observed for the cortical SEP under different stimulation frequencies. The decay constant showed a 50% change with a change in anesthesia depth. We propose that the decay constant could be used as a sensitive index for the monitoring of anesthetic depth.

Key Words: primary somatosensory cortex, ventroposterior lateral nucleus, tail, somatosensory evoked potential

Introduction

Coding of stimulation frequency is an important function of a sensory system. It is well known that the primary somatosensory cortex cannot follow high frequency stimulation faithfully (12). A partial explanation of this phenomenon has been attributed to the thalamic gating mechanism (6). That is, under anesthesia or sleep, the thalamic cells are hyperpolarized which switches the thalamic cells into a bursting mode and thereby decreases their frequency following capabilities (5). Accordingly, the major decrease of the frequency following capability should occur at the level of the thalamus in anesthetized condition. We tested this hypothesis with simultaneous recording of sensory transmission in the tail representation areas of the thalamus (the ventroposterior lateral nucleus, VPL) and the primary somatosensory cortex (SI) responding to electrical stimulation of the tail of the rat. Dorsal root potentials were recorded as an independent index of input level.

Somatosensory evoked potential (SEP) has been

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widely used in the clinical ward for the purpose of testing the integrity of the somatosensory system. Due to its large variability, SEP have not been used as a routine index of anesthetic depth during general surgery (4,8). The second objective of the present study was to try to test whether the frequency following profile of the SEP should be modified during a change of the anesthetic depth, and therefore, might be a useful index for the monitoring of anesthesia depth.

Materials and Methods

The experiment was carried out on male Wistar rats (350-450g; n=8). The rats were anesthetized with intraperitoneal sodium pentobarbital (50 mg/kg). Trachea, femoral artery and vein were cannulated for ventilation, blood pressure monitoring and drug administration respectively. From an hour before and during the whole recording period, diluted pentobarbital solution was infused intravenously. Blood pressure, heart rate and rectal temperature was monitored continuously. A feedback controlled thermal blanket was used to regulate the rectal temperature at 37.5 °C.

The rat was mounted on a stereotaxic apparatus and a right-side craniotomy was performed overlying the parietal bone. The dura mater was removed, and tail representative areas of the cortical surface and the thalamus were mapped with monopolar silver ball electrode (diameter 0.5 mm) and glass electrode (tip diameter 10 to 20 μm) respectively. Glass micropipettes were filled with artificial cerebrospinal fluid and 1% potamine sky blue for marking the recording site. The tail representative area of the SI was usually located at approximately 2.5 mm caudal to the bregma and 2.5 mm lateral to the midline; that of the thalamic VPL nucleus was usually located 3.5 mm caudal and 3.5 mm lateral. A laminectomy was carried out on L1-S1 vertebrae to expose the sacrococcygeal cord and its dorsal roots. A pair of stainless hook electrode was used to record the S2 or S3 dorsal roots. The selected dorsal root was identified by tapping and stroking its cutaneous receptive field over the tail. Biocompatible silicone glue (Wacker 604) was used to cover the cortical and the dorsal root electrodes to stabilize the preparation and also to prevent the tissues from dehydration. The general design of the experiment is diagrammatically represented in Figure 1A.

The stimulation was delivered as a 2 ms duration constant current square wave pulse to the left side of the middle part of the tail. The distance between the positive and the negative poles of stimulation needle electrode was about 5 mm and the negative pole was placed rostrally. Grass P511 AC amplifier was used to record the cortical evoked potential and dorsal roots CAP with band pass filter set at 1 to 300 Hz and a gain of 5,000. The setting for the thalamic evoked potentials were divided into two parts, one for low frequency component (1 to 300 Hz), the other for high frequency component (300 to 3K Hz), gain was 10,000.

Sodium pentobarbital infusion rates were adjusted from light to deep and then returned to light anesthetic stage. In order to achieve a distinctive difference in anesthetic depths, after the completion of all surgical procedure and electrode arrangements, the rats were infused first with a slower rate of 5 to 10 mg/kg/hr for at least an hour. After obtaining the first set of evoked potentials (light stage), the infusion rate was increased to 30 to 40 mg/kg/hr. This infusion rate was kept for at least half an hour. The faster rates of infusion were used if the animal did not show a deepening in anesthetic level. The criterion to judge this was a lowered heart rate of more than 5 bpm. After the second set of SEPs was obtained (deep stage), the infusion of the anesthetics was stopped for half an hour and resumed afterwards at a slower rate (about 5 to10 mg/kg/hr) for an hour (recovery stage). Data were used for those rats whose heart rates returned to their original level under light anesthesia. Within each stage, a complete set of intensity-response and frequency-response tests was performed. This was done by varying the current intensities from 100 μA to 2 mA (in increments of 100 μA, at a stimulation frequency of 2 Hz), and varying frequencies from 0.5 to 12 Hz (in increments of 0.5 or 1 Hz, at a stimulation intensity of 600 μA (about 3x A β threshold, 3T). Each evoked potential was obtained by averaging traces from 50 cycles to enhance signal-to-noise ratio. A 12-bit analog-to-digital converter card (AT-MIO-16E-2, National Instruments) was used to digitize the evoked potentials at a sampling rate of 6 KHz. A total of 512 points of data was collected for each stimulation cycle. The data was logged to computer hard disk and analyzed off-line with a program developed with LabVIEW (National Instruments). Parameters analyzed were initial latency, peak latency and area under curve in channels of dorsal roots SEP and the high frequency component of thalamic SEP. For the cortical SEP channel, we analyzed the peak latency and peak-to-peak amplitude (Figure 1C). The relative fluctuation of the slow frequency component of the thalamic channel was found to be too large to be useful. Friedman repeated measures ANOVA on ranks was used to examine the significance of the differences among the light, deep and recovery groups. Pair-wise comparisons were made with nonparametric Student-Newman-Keul test. Data are expressed as mean ± standard error of the mean.

Pressure injection of a small amount of

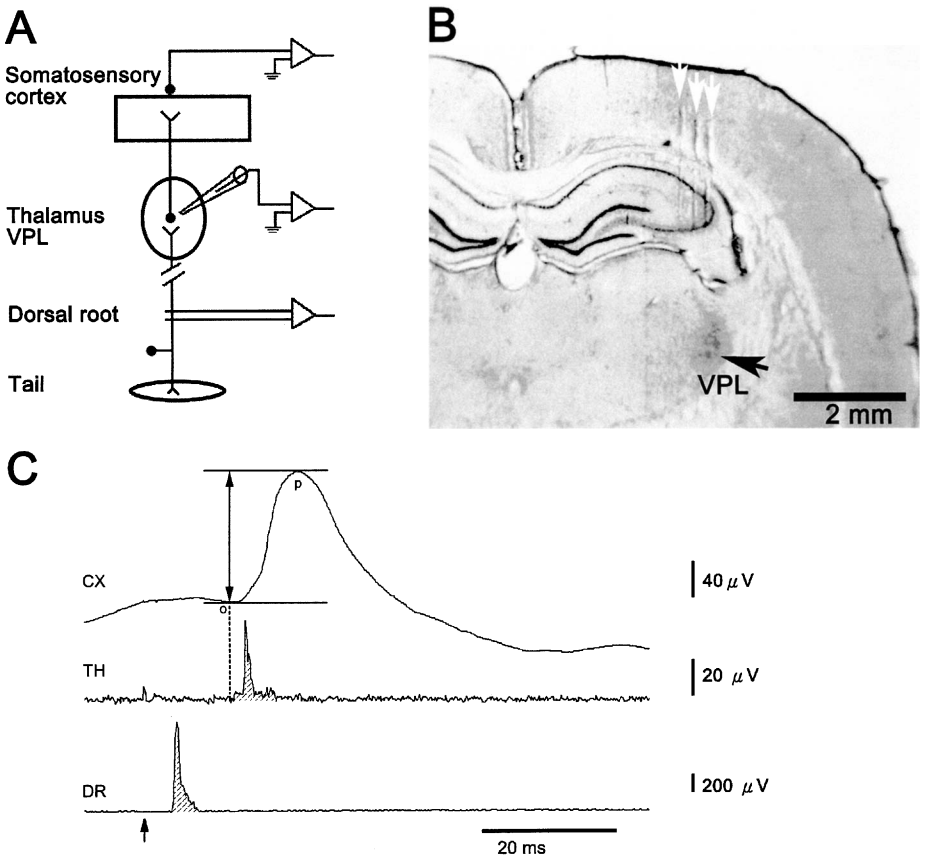

Fig. 1. Basic experiment design (A) Three recording electrodes were used to record mid-tail-evoked field potentials. These were from bottom up: a pair of hook electrode under a sacrococcygeal dorsal root (S2 or S3); a glass microelectrode in the ventroposterior lateral nucleus (VPL) of the thalamus; and a ball electrode on the primary somatosensory cortex. (B) An example of VPL recording site (black arrow), as revealed by the potamine sky blue mark. The three white arrows point to the recording tracks made during the search of the tail representation area in the VPL. (C) An example of mid-tail evoked SEPs. At the point of the arrow, a constant current square wave pulse of 2 ms duration was given to the left side of the mid-tail. The dorsal root compound action potential (DR) and the high frequency component of the VPL field potential (TH) has been rectified and integrated. Area-under-curve of their respective SEPs is marked with oblique slashes. The peak-to-peak value of the cortical SEP (CX) was taken of the most prominent short latency positive peak (arrow line). The o point was the starting point of the VPL SEP (dotted line).

pontamine sky blue was used to mark the thalamic recording site at the end of the recording session (Fig. 1). The rats were sacrificed under deep anesthesia and perfused intracardially with normal saline following by 4% formalin solution. The brain was taken out and store in 30% sucrose formalin solution for at least a week in the refrigerator. Fifty-micron thick serial frozen sections were cut on a sliding microtome to reveal the location of the thalamic recording site (Fig. 1B).

Results

General Condition

SEPs were successfully recorded in 8 rats. Blood pressure and heart rate of these rats were affected by the depth of anesthesia. These values (n=8) were 122 ± 4 mmHg and 412 ± 13 bpm for light condition, 112 ± 4 mmHg and 358 ± 13 bpm for deep condition, and 123 ± 4 mmHg and 402 ± 11 bpm for recovery condition. Blood pressure and heart rate during deep condition were significantly lower than those of the light and recovery conditions.

There were no spontaneous movements of all animals at all times. Pinching of the distal paws of the animal produced only the slightest flexor reflex rarely in rats under light or recovery conditions. This was immediately supplemented with a small dose (0.1 ml) of diluted pentobarbital solution (10 mg/kg).

Cortical, Thalamic and Dorsal Root SEPs under Light Anesthesia

An example of cortical, thalamic and dorsal root SEPs evoked from the middle part of the tail is illustrated in Figure 1C. We were analyzing the most prominent fastest component of the SEPs. The

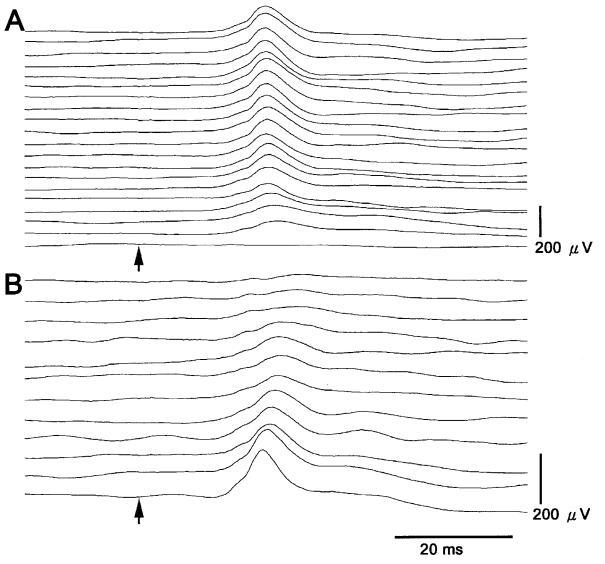

Fig. 2. A representative example of cortical SEP after mid-tail stimulation (arrow) of different intensities (A) and frequencies (B). Each trace represents an average of 50 stimulation cycles. (A) The twenty traces from bottom to top are SEPs obtained by varying intensities from 100 μA to 2 mA in steps of 100 μA respectively. The stimulation frequency was 2 Hz. (B) The twelve traces from bottom to top are SEPs obtained by varying frequencies from 0.5 Hz, 1 Hz to 11 Hz (in steps of 1 Hz) respectively. The stimulation intensity was 600 μA.

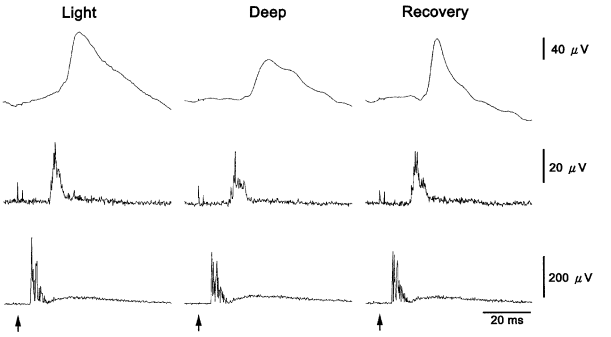

Fig. 3. A representative example of the effect of anesthesia depth on SEPs. Stimulation parameters were 3 Hz, 600 μA and 2 ms duration. Note DR and TH SEPs were not affected significantly by the depth of anesthesia; whereas the amplitude and the slope of the CX SEP were depressed.

threshold intensity for these SEPs was about 200 μA. At low frequency stimulation rate (2 Hz) and maximal stimulation intensity (2 mA), the peak latencies for these SEPs (n=8) were 24.7 ± 0.7 ms, 16.3 ± 0.6 ms and 7.0 ± 0.6 ms for SI, VPL and S2-3 dorsal roots respectively.

The cortical, thalamic and dorsal root SEPs were differentially sensitive to varying stimulation intensity and frequency. Amplitudes of cortical and thalamic SEPs grew rapidly to their maximal with an increase in stimulation intensity to about 3T (Figures 2A and 4A). With further increases in the stimulation intensity, further increases of the dorsal root CAP were still noted (Figures 4A, 4B and 4C). It's an indication that the cortical and thalamic SEPs components we recorded were induced by the largest afferent fibers in the tail. At 100 or 200 μA, there was no trace of the SEPs. But as soon as they were evoked at 200 or 300 μA, their latencies stayed relatively constant at supra-maximal intensities (Figure 5A). The cortical SEP showed the largest variation with intensity and frequency. This is illustrated in Figure 2B. Cortical SEP became smaller and slower as the stimulation frequency increased. The frequency effect on the amplitude and latency of the SEPs is quantitatively shown in Figures 4D and 5D respectively. Cortical SEP was significantly smaller and slower at 2 Hz and higher, whereas thalamic SEP was affected at stimulation frequencies higher than 8 Hz.

Effect of Anesthesia Depth on SEPs

An example of the effect of deepening barbituate anesthesia on the SEPs is illustrated in Figure 3. Dorsal root and thalamic SEPs did not seem to be affected much by the change of anesthesia depth, whereas cortical SEP became smaller and slower in the deep condition. This suppression returned to control level after a period of recovery.

The effects of anesthesia depth on SEP amplitude and latency were quantitatively analyzed. Figure 4 shows the effects on the amplitude of the SEPs with different stimulation intensities (left panel) and frequencies (right panel). In addition to the frequency dependent change of SEP amplitude, it is clear that under deep condition, the cortical SEP was suppressed even more by the increase in stimulation frequency (Figure 4E). In contrast, thalamic and dorsal root SEPs were not affected any stronger than they were under light or recovery conditions.

Quantitative analysis of effects of anesthesia depth on SEP latencies is presented in Figure 5. A deepening of barbituate anesthesia caused little change in the intensity-response curve (5A, 5B and 5C) as can be seen also in Figures 4A, 4B and 4C. A deepening of anesthesia depth did not change dorsal root SEP latency but caused a large prolongation of cortical SEP latency (5E). There seems to be a change in thalamic SEP latency, but due to large variations, this is not statistically significant.

From a relative point of view, anesthesia depth did not change the intensity-dependent amplitude or latency of the cortical SEP, but it did affect the frequency-dependent amplitude change significantly. Therefore, we fitted the stimulation frequency -

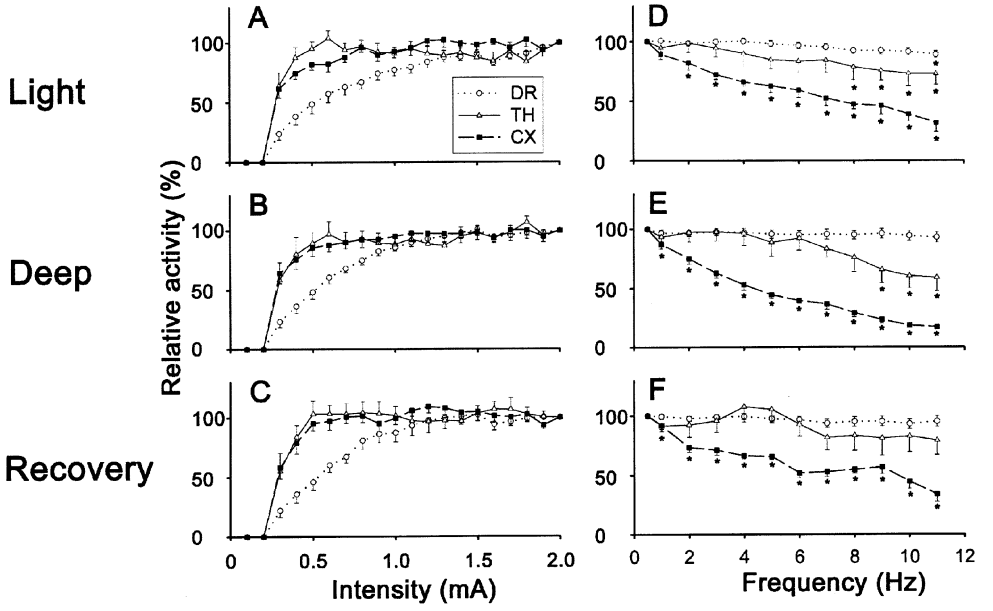

Fig. 4. Effects of varying stimulation intensity (left panel) or frequency (right panel) on relative amplitude of dorsal root (open circles), thalamic (open triangles) and cortical (solid squares) SEPs. Note that the relative amplitude of cortical SEPs diminished significantly with the increasing of stimulation frequency under light (D) or recovery (F) conditions. That of the thalamic SEPs diminished only slightly in the same conditions. Note also that the relative amplitude of the cortical SEP depressed much quickly and strongly with the increasing of stimulation frequency under deep anesthesia (E). The intensity-response curves were similar under all conditions (figures A, B, and C). A star denotes significance level P<0.05.

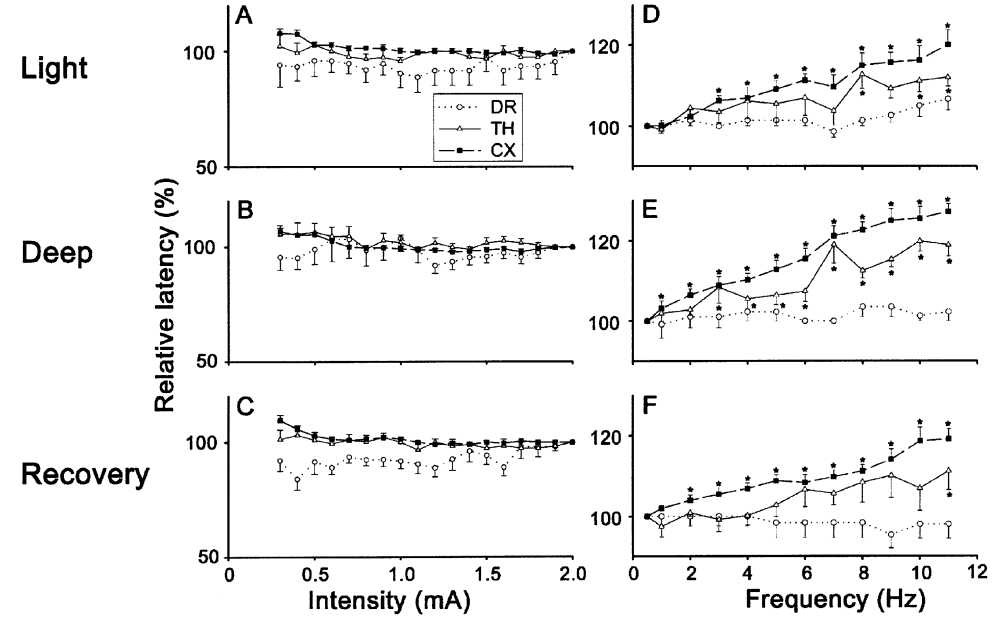

Fig. 5. Effects of varying stimulation intensity (left panel) or frequency (right panel) on relative latency of dorsal root (open circles), thalamic (open triangles) and cortical (solid squares) SEPs. Neither intensity nor anesthesia depth affected any of the SEP latencies significantly. In contrast, TH or CX SEP latencies prolonged as the stimulation frequency increased. These prolongations were further exaggerated with the deepening of the anesthesia (Figure E). A star denotes significance level P<0.05.

amplitude data under the three conditions, namely, light, deep and recovery, to an exponential decay function as illustrated in Figure 6. The regression coefficients of the three curves were 0.99, 0.99 and 0.94 respectively (P values were <0.0001, all highly significant). What we think most interesting is the

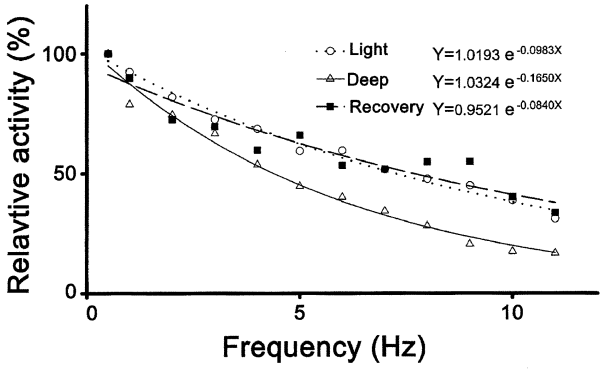

Fig. 6. Fitting of the frequency dependent CX SEP amplitude values to an exponential decaying curve, $Y = a \times \exp(-bX)$, where $a \approx 1$, and b is a anesthetic depth dependent value. Data points were the same as in Figure 4D, E, and F.

change in the b value. A deepening of the anesthesia level changed the b value from 0.0983 to 0.165, a 68% increase. This returned to 0.084 in the recovery condition.

Discussion

There were three major findings of the present study. Firstly, the frequency following capability of the VPL and the SI differed greatly. Tail representation area of the VPL followed mid-tail stimulation faithfully to 8 Hz, while that of the SI followed to 2 Hz. Secondly, a deepening in anesthesia depth changed mid-tail evoked VPL activity only slightly whereas it produced a profound but reversible cortical depression. And thirdly, the frequency-dependent amplitude change of the cortical SEP might be a useful index of anesthesia depth during anesthesia.

Transferring frequency information faithfully is an important requirement of the sensory system. Based on electrophysiological studies of single units, neurons in the dorsal column nuclei and thalamus are known to follow afferent stimulation at a frequency higher than 100 Hz or more (1,9). This type of neurons with high frequency following capability is rare in the primary somatosensory cortex. Cytological studies have shown that single medial lemniscal fiber terminates in the VPL with large terminal boutons that form complicated, sometimes glomerular type of synapses (10,14). On the other hand, thalamocortical fiber terminates in the somatosensory cortex in simple synapses. Therefore, it is not surprising that thalamic neurons are able to follow afferent stimulation at higher frequency range than cortical neurons. The contribution of the present study was to record rat thalamic and cortical field potentials at the same time and to examine their frequency following capability systematically with afferent input frequencies.

Our finding that cortical SEP diminished significantly at 2 Hz while thalamic SEP diminished at 8 Hz is in agreement with data from cat (16) and human patient (7), both studies included data obtained separately in many individuals in many recording places. In conscious human patient, the recommended stimulation rate for cortical SEP is 5 Hz (2), reflecting the fact that the "light" condition in the present study was nevertheless an anesthetized condition.

In a continuous intravenous infusion study of pentobarbital of the rat, 20 mg/kg/hr resulted in no apparent accumulation of the anesthetic agent in the plasma for 120 min (17). Therefore, the anesthesia depth we chosed as light (5-10 mg/kg/hr) and deep (30 to 40 mg/kg/hr) stages should have been under different anesthesia depths. At the "light" stage, the metabolic rate of the pentobarbital would be faster than the infusion rate, the plasma pentobarbital concentration would be decreasing. In contrast, at our "deep" stage, pentobarbital might have been accumulating.

A large body of literature deals with the effect of anesthetics on the SEP. In comparison to conscious condition, moderate dose of barbituate increases the amplitude of the short latency SEP (3,11), while long latency SEPs in the cortex and other brain structures are abolished or severed depressed (11,15). By recording forepaw-evoked cortical SEP of the rat from a deep barbituate anesthesia to a light condition (showed spontaneous movements), Shaw and Cant (13) showed that the initial latency of the short latency cortical SEP component (P2 component) gradually shortened. The present study showed that under deeper barbituate anesthesia, the SEP amplitude would be suppressed and its latency prolonged. What is more interesting is the result on the change of the frequency following curve of the thalamic and cortical SEP under different anesthesia conditions. Under our "light" condition, the cortical SEP was significantly suppressed by frequency higher than 2 Hz, and 50% suppression was not reached until about 9 Hz. Under "deep" condition, the cortical SEP was influenced much more by the increase in the stimulation frequency, so that at 1 Hz, a significant suppression was observed, and the 50% suppression was reached much sooner, at about 4 Hz. This is in sharp contrast of the thalamic SEP which showed only slight change under deeper anesthesia condition. Coupled with the result that under "light" condition, thalamic SEP was significantly influenced by stimulation frequency higher than 8 Hz, it is clear that the major site where the low cortical frequency following capability occurs must be within the cortical level. It could be hypothesized that within the somatosensory cortex there are many complicated synaptic connections after a thalamocortical fiber comes in. Multiplication of

functions of these synapses made them incapable of transferring high frequency afferent inputs from the periphery.

Cortical SEP can be monitored easily in the clinical ward. The waveform, amplitude, and latency of the cortical SEP depend greatly on the recording site, the goodness of the contact, the type and depth of anesthesia and the stimulation parameters (4). It is clear, therefore, that a single waveform parameter of the SEP will be of limited utility in assessing the anesthesia depth of the animal or human patient. We found in the present study that shifting the infusion rate of the sodium pentobarbital solution from 10 mg/kg/hr to 30-40 mg/kg/hr brought about a change in the b value of the frequency following curve of the SI SEP by almost 70% (Figure 6). Of particular note is that the b value is obtained from a regression line of many individual points. Although there were fluctuations of the individual points, the b value should be more resistant to local variations. Therefore, we propose that the b value may be investigated further in the future as a candidate index of anesthetic depth.

In summary, we reported herein a systematic study of the frequency and intensity change of the dorsal root, VPL thalamic and SI SEPs under two different barbituate depths. The frequency following capability of the VPL and the SI differed so much that it is unlikely that thalamus is the major sensory gate of the afferent input. A deepening of the anesthesia depth changed differentially the frequency following capabilities of the thalamic and the cortical SEPs. This indicates again that the cortical circuitry must be the major target.

Acknowledgements

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References

1. Blum, P., Bromberg, M.B. and Whitehorn, D. Population analysis of single units in the cuneate nucleus of the cat. *Exp. Neurol*. 48: 57-78, 1975.
2. Chiappa, K.H. and Ropper, A.H. Evoked potentials in clinical medicine, short-latency somatosensory evoked potential. *New Eng. J. Med.* 306: 1205-1211, 1982.
3. Dafny, N. Neurological approach as a tool to study the effects of drugs on the central nervous system: dose effect of pentobarbital. *Exp. Neurol.* 59: 263-274, 1978.
4. Guerit, J.-M. Neuromonitoring in the operating room: why, when, and how to monitor? *Electroenceph. Clin. Neurophysiol.* 106: 1-21, 1998.
5. Guido, W., Lu, S.-M., Vaughan, J., Godwin, W. and Sherman, S.M. Receiver operating characteristic (ROC) analysis of neurons in the cat's lateral geniculate nucleus during tonic and burst response mode. *Vis. Neurosci*. 12: 723-741, 1995.
6. Jones, E.G. Thalamus. New York, NY: Plenum Press, 1985.
7. McLaughlin, D.F. and Kelly, E.F. Evoked potentials as indices of adaptation in the somatosensory system in humans: a review and prospectus. *Brain Res. Rev.* 18: 151-206, 1993.
8. Nuwer, M.R. Fundamentals of evoked potentials and common clinical applications today. *Electroenceph. Clin. Neurophysiol*. 106: 142-148, 1998.
9. Poggio, G.F. and Mountcastle, V.B. The functional properties of ventrobasal thalamic neurons studied in unanesthetized monkeys. *J Neurophysiol*. 26: 775-806, 1963.
10. Rainey, W.T. and Jones, E.G. Spatial distribution of individual medial lemniscal axons in the thalamus ventrobasal complex of the cat. *Exp. Brain Res*. 49: 229-246, 1983.
11. Shaw, F.-Z., Chen, R.-F., Tsao, H.-W. and Yen, C.-T. Comparison of touch-and laser-heat evoked cortical field potentials in conscious rats. *Brain Res*. 824: 183-196, 1999.
12. Shaw, N.A. The effects of stimulus rate on the cortical somatosensory evoked potential in the rat. *Electromyogr. Clin. Neurophysiol*. 27: 235-241, 1987.
13. Shaw, N.A. and Cant, B.R. The effect of pentobarbital on central somatosensory conduction time in the rat. *Electroenceph. Clin. Neurophysiol*. 51: 674-677, 1981.
14. Spacek, J. and Lieberman, A.R. Ultrastucture and three dimensional organization of synaptic glomeruli in rat somatosensory thalamus. *J Anat*. 117: 487-516, 1974.
15. West, M.O. Anesthetics eliminate somatosensory-evoked discharges of neurons in the somatotopically organized sensorimotor striatum of the rat. *J. Neurosci.* 18: 9055-9068, 1998.
16. Wiederholt, W.C. Recovery function of short latency components of surface and depth recorded somatosensory evoked potentials in the cat. *Electrocenceph. Clin. Neurophysiol*. 45: 259-267, 1978.
17. Yang, C.C.H., Kuo, T.B.J. and Chan S.H.H. Auto- and cross-spectral analysis of cardiovascular fluctuations during pentobarbital anesthesia in the rat. *Am. J. Physiol*. 270(Heart Circ. Physiol. 39): H575-H582, 1996