Title: Quantitative and fiber-selective evaluation of dose-dependent nerve blockade by intrathecal lidocaine in rats

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Running title page:

a) Evaluation of nerve blockade induced by lidocaine in rat

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c) number of text pages: 20

number of tables: 0

number of figures: 5

number of references: 34

number of words in the Abstract: 235

number of words in the Introduction: 472

number of words in the Discussion: 1310
d) ABBREVIATIONS.

CST, cutaneous stimulus threshold; CPT, current perception threshold;
e) Category of our manuscript

Neuropharmacology

Toxicology
ABSTRACT

We investigated whether cutaneous stimulus threshold (CST) as determined using a Neurometer could be utilized for quantitative and differential nerve evaluation of reversible and irreversible nerve block following intrathecal lidocaine administration in rats. Rats with intrathecal catheters were randomly assigned to one of 5 groups (saline, or 2%, 5%, 10%, or 20% lidocaine). Prior to and 4 days after drug administration, CST was determined at 5 Hz, 250 Hz and 2000 Hz. In the 2% lidocaine group, CST from end of lidocaine infusion to recovery from anesthesia was also monitored. Skin-clamp testing and gait observation were performed for comparison with CST findings. Behavioral examinations revealed persistent sensory or motor impairment lasting 4 days in groups receiving ≥5% lidocaine, but not in the saline and 2% lidocaine groups. With 2% lidocaine, return to baseline CSTs at 5 and 250 Hz was delayed compared to thresholds at 2000 Hz. Although CSTs in the 5% group at 5 and 250 Hz increased significantly, thresholds at 2000 Hz did not differ from those in rats administered saline. CSTs with ≥10% lidocaine displayed no differences between frequencies. At each frequency, CSTs for rats with ≥5% lidocaine increased in a clearly concentration-dependent manner. These results suggest that CST testing enables evaluation of the different nerve functions for Aβ, Aδ, and C fibers in rats for lidocaine concentrations ≤5%, and allows quantitative assessment of persistent neurological deficit induced by lidocaine in rats.
Introduction

Current perception threshold (CPT) has recently seen wide use in the evaluation of sensory function in humans with peripheral neuropathy resulting from diabetes mellitus, carpal tunnel syndrome, or complex regional pain syndrome (Masson and Boulton, 1991; Katims et al., 1989; Suzuki et al., 1995; Dinh et al., 1997). The device used to measure CPT, the Neurometer CPT/C (Neurotron, Baltimore, MD), delivers a gradually increasing current to the skin surface, allowing both quantification of sensory function in terms of perception threshold and assessment of functions for different types of nerve fiber. This is achieved by stimulation using three sine-wave pulses at frequencies of 2000, 250 and 5 Hz, which in humans stimulate large myelinated (Aβ), small myelinated (Aδ) and small unmyelinated sensory fibers (C), respectively (Pitei et al., 1994). Some investigators have applied CPT measurements to the evaluation of differential sensory blockade following spinal or epidural anesthesia (Liu et al., 1995; Tay et al., 1997; Sakura et al., 1998). Although CPT testing represents an established method in humans, only one report has described the application of CPT in animal studies (Kiso et al., 2001).

In animal studies investigating neural disturbances induced by systemic disease, traumatic injury or administration of anesthetic agents, quantitative evaluation of reversible or irreversible neurological damage is extremely important for elucidating the underlying mechanisms. Until now, damage has commonly been assessed by observing animal escape behaviors following nociceptive stimuli, which can be produced using mechanical (e.g., skin clamp, tail clamp, or Von Frey monofilament test) or thermal (e.g., hot plate or radiant heat test) techniques (Drasner et al., 1994; Kitamura and Doe, 2003, Mogil et al., 2001).
The present study utilized current stimulus threshold (CST; in animal studies, CST represents a more appropriate term than CPT) as a new method for assessing dose-dependent nerve block with lidocaine in animal models. CST studies have not yet been used to assess dose-dependent nerve blockade induced by local anesthesia in animal models of spinal anesthesia. Although anesthesiologists attribute differential nerve fiber blockade at reversible neural block to differences in nerve fiber diameter, conduction velocity or surrounding myelination, the real causes remain unclear (Gasser and Erlanger, 1929; Gissen et al., 1980; Fink, 1989). Irreversible neural injury caused by high concentrations of local anesthetic in spinal anesthesia has become a serious problem over the past 15 years, and the underlying mechanisms are likewise unknown (Rigler et al., 1991; Drasner et al., 1994; Sakura et al., 1995a; Sakura et al., 1995b). If application of the Neurometer in an animal model can be shown to be useful, large amounts of information regarding the mechanisms of differential nerve blocking and neurotoxicity induced by local anesthetics will become available. The present report therefore investigated whether CST could be utilized for quantitative and differential fiber evaluation of reversible and irreversible nerve block caused by intrathecal administration of lidocaine in rats.
Materials and methods

Lidocaine hydrochloride was purchased from Sigma (St. Louis, MO). All lidocaine solutions were prepared using physiological saline.

Animal preparation

This study was approved by the Saga Medical School Committee on Animal Research. Male Sprague-Dawley rats (Japan SLC, Hamamatsu, Japan; 200-250 g body weight) were housed for one week in the animal facilities of our school under a 12-h light-dark cycle before use in the study. After inducing anesthesia by intraperitoneal injection of pentobarbital sodium (50 mg/kg), an intrathecal polystyrene catheter (external diameter, 0.30 mm; Hakko Medical, Nagano, Japan) was implanted using a modification of the method described by Yaksh and Rudy (1976). External diameter of the catheter was about half that of the PE10 catheter (external diameter, 0.58 mm) used conventionally for minimizing rat spinal injury during and after intrathecal catheterization (Sakura et al., 1996). The intrathecal catheter was passed through an incision in the atlanto-occipital membrane and advanced about 10 cm, so that the tip reached the lower lumbar area (Drasner et al., 1994). Rats with normal neurological function and behaviors at 7 days postoperatively were used for the rest of the experiment.

Cutaneous current stimulus threshold testing

Animals were randomly assigned to one of 5 groups (n=6 each), to receive either saline or 2%, 5%, 10% or 20% lidocaine. CSTs at 5 Hz, 250 Hz and 2000 Hz were determined using a Neurometer CPT/C. The day after eliminating leg hair using a depilatory cream (Epilat, Kanebo, Tokyo, Japan), a pair of stimulation electrodes was placed on the skin of the hindpaw. One electrode was at the palm and the other was at the posterior of the ipsilateral thigh in all rats, at sites corresponding to the L5 and L6
dermatomes in rat, respectively (Takahashi and Nakajima, 1996). Current was delivered to the skin via a 1-cm diameter gold surface electrode covered by a thin layer of electro-conductive gel. Each rat was placed in a Ballman cage (Natsume Seisakusyo, Tokyo, Japan) with suitable restraints. After rats were placed and kept still in the cage, measurement of CST was initiated. Stimulus intensity was gradually increased from 1 to 999 CST units (0.01-9.99 mA), if necessary, and CST was defined as the minimum current required to elicit squeaking or withdrawal of the hindpaw, at which point current stimulus was immediately stopped. Absence of rat responses is easily determined by defects of rat vocalization or leg movement within a maximum of 1 s after stimulus, because responses to current stimuli in rats arise instantaneously when gradually increased current stimuli is delivered by a smoothly wheeled rotor. An examiner who was blinded to the delivery of current stimulus assessed behaviors induced by the stimulus. In a preliminary experiment to evaluate whether use of depilatory cream affects CST measurement, no differences in CST values were observed between rats with leg hair removed using cream or hair clippers. Measurements were performed before and for 4 days after drug administration in all groups, to examine whether irreversible neurological impairments were produced. CST was measured three times at each frequency at 5-min intervals, and means were obtained for except CST measurement several hours after injection in the 2% lidocaine group. In the 2% lidocaine group, to test application of the Neurometer to reversible block induced by lidocaine, CST was measured from the end of lidocaine administration until CST returned to baseline, in addition to CST measurement before and during drug administration. This was done because clinical experience indicates that 2% lidocaine is more likely to result in reversible rather than irreversible nerve block. Thresholds at the
three frequencies in this group were determined every 10 min, with measurements completed within 2 min, in accordance with the literature (Kiso et al., 2001). CST values were then converted into a CST ratio ($\frac{\text{CST}_{\text{post-administration}}}{\text{CST}_{\text{pre-administration}}}$) and normalized to clearly indicate differences in CST among the three frequencies, with the exception of CSTs for determining distributions for the three frequencies before drug administration. CST$_{\text{pre-administration}}$ was defined as the mean CST measured before drug administration in each group. All solutions were administered intrathecally using a micro-infusion pump at an infusion rate of $1 \mu l \cdot min^{-1}$ for 80 min (total injected volume, 80 $\mu l$).

**Behavioral examination**

To assess sensory and motor functions, skin-clamp testing and gait observation were performed, respectively, and compared with Neurometer findings. Sensory function was assessed as the presence or absence of an area of analgesia. Briefly, rats were examined by pinching the skin from hind paw to tail using an artery clip, exerting a force of 500 g as previously described (Takagi et al., 1966). An area of analgesia was considered present when the rat did not squeak or withdraw the hindpaw (or tail) within 4 s of starting skin clamping. Motor function of rats was defined from walking behaviors as: “normal”, no abnormality; “moderate”, limited movement of hindpaw; or “severe”, no movement of hindpaw. Neurological impairment lasting for 4 days after lidocaine administration was defined as irreversible, in accordance with the literature (Ready et al., 1985; Kalichman et al., 1992; Sakura et al., 1995b; Hashimoto et al., 1998). CST measurement and behavioral examinations were performed by examiners blinded to the administered intrathecal solution. A schema of the study design is shown in Figure 1.

**Data analysis**
Data were analyzed using Dunnett’s test for multiple comparisons of changes in CST ratio over time at each frequency in the 2% lidocaine group, using mean CST before administration of 2% lidocaine as a control. This analysis was adapted for comparisons of the 2%, 5% and 10% lidocaine groups with the saline group at each frequency. Analysis of variance followed by Scheffe’s test for post hoc comparisons was used to compare CST ratios between the 3 frequencies in the 10% lidocaine group. Analysis was performed using Statview 5 software (SAS Institute, Cary, NC). Values of P<0.05 were considered statistically significant.

Results

CST values in response to the three frequencies as measured before drug administration in 24 rats displayed a narrow distribution, indicating that CST was consistent in rats (Fig. 2). Before drug injection, no rats displayed any areas of analgesia as detected by skin-clamp testing and abnormal gait behaviors.

After drug administration, neither respiratory arrest due to excessive extension of spinal block nor insufficient spinal block were observed, and all animals survived and satisfied study protocols. With the exception of rats in the saline group, both hind limb and tail paralysis were initially seen in all animals within 10 min of drug administration, and block area expanded simultaneously. After the end of drug administration, although level of sensory block regressed hourly and disappeared within a few hours in the 2% lidocaine group, neurological deficits in rats receiving ≥5% lidocaine remained for 4 days. Rats receiving ≥5% lidocaine displayed the worst neurological dysfunction at the end of lidocaine infusion. Neurological impairments displayed partial improvement, but became fixed within 1-2 days after lidocaine administration and remained present until the day 4 as shown by behavior tests (Fig. 4). CST data confirmed these findings (data
not shown). No neurological deficits were apparent in the saline group during or after infusion.

**Assessment of reversible blockade**

CST recovery profiles in the 2% lidocaine group at each frequency are shown in Figure 3. CST measured at 2000 Hz stimulation was the first to return to baseline, 40 min after the end of drug administration, with CSTs recorded at 5 Hz and 250 Hz returning to baseline levels at about 70 and 80 min after ending drug administration, respectively. Mean period from end of drug administration to complete recovery of baseline CST for all three frequencies was 80 min. According to skin clamp and gait behaviors, all rats in the 2% lidocaine group displayed complete recovery from anesthesia within about 100 min after drug administration (data not shown).

**Assessment of irreversible neurological impairment**

Neurological deficit lasting for 4 days after intrathecal drug administration was found in rats from the 5%, 10% and 20% lidocaine groups, which displayed varying sensory and/or motor impairments according to skin clamp and gait observations (Fig. 4).

CST ratios at all frequencies increased in a manner that was clearly concentration dependent (Fig. 5). In both saline and 2% lidocaine groups, all CST ratios measured up to 4 days after drug administration were almost at unity for each of the three frequencies, confirming normal neurological function for rats in those groups. Comparing saline and 5% lidocaine groups, no differences in CST ratio were elicited at 2000 Hz, but CST ratios at 5 Hz and 250 Hz were significantly increased in the 5% lidocaine group. Although CST ratios at all three frequencies in the 10% and 20% lidocaine groups were markedly higher in a concentration-dependent manner, no significant differences were observed between the three frequencies in the 10% lidocaine group. In the 20%
lidocaine group, most CST values at the three frequencies reached the limit of electrical stimulation output (9.99 mV).

**Discussion**

Until now, distinguishing between spontaneous movement and responses elicited by current stimuli in rat behaviors has represented a matter of concern. However, the two were easily differentiated using CST measurements, as spontaneous paw movements or vocalizations were transient, whereas responses to stimuli were continuous during stimulation. In addition, no deviations in CST baseline at the three frequencies were noted among rats with similar birth dates, same family line and sex (Fig. 2). Another consideration is that nociceptive stimuli at frequencies >0.3 Hz may produce central sensitization (Schouenborg, 1984; Herrero et al., 2000). However, CSTs measured within 2 min at 10 min intervals display a small distribution over a period of 3 h in rats (Kiso et al., 2001). This indicates that central sensitization in rats is not produced by the electro-stimulation generated by the Neurometer.

**Assessment of different sensory fibers**

The period needed for CST to return to baseline in the 2% lidocaine group, in which nerve block was reversible, was basically the same as the recovery time defined by skin-clamp testing and gait behaviors. This result is in agreement with the clinical effects of 2% lidocaine, as 2% lidocaine used for spinal anesthesia in humans is well known to produce reversible nerve blockade. CST testing in animal models thus seems comparable to conventional nociceptive testing for evaluation of local anesthetic effect. During reversible nerve block induced by spinal anesthesia in humans, larger diameter nerve fibers recover more rapidly from anesthesia (Fink, 1989), where recovery profiles
for cold sensation (C), pain by pin prick (Aδ) and tactile sensation (Aβ) correspond to those of individual CPTs at 5, 250 and 2000 Hz, respectively (Liu et al., 1995). Our study of rats receiving 2% lidocaine, in which blockade was reversible in all cases, likewise demonstrated that CSTs for lower frequency stimulation took longer to return to baseline. This result, as indicated by human studies (Liu et al., 1995; Tay et al., 1997), suggests that sensory function in rats can be assessed using the Neurometer in a fiber-selective manner. Anatomical similarities between rats and humans are supported by anatomical evidence that mammalian neurophysiology is fundamentally conserved between mammalian species at the infratentorial level (Myers and Sommer, 1993). Neurometer findings in the present study of rats appear to confirm the feasibility of selectively evaluating different sensory fibers using electrical stimuli of 5 Hz, 250 Hz and 2000 Hz to stimulate C, Aδ and Aβ fibers, respectively. Kiso et al. reported the first application of the Neurometer in rats, and suggested that this device was useful for sensory fiber-selective evaluation (2001). Their study utilized vanilloid capsaicin, a specific desensitizer of small-diameter afferent nerve fibers (Aδ and C fibres) (Magerl et al., 2001). Capsaicin raised CSTs at 5 Hz and 250 Hz stimulus without changing those at 2000 Hz, indicating the possible utility of Neurometer functions for differential nerve evaluations in rats (Kiso et al., 2001). The present study reinforced this suggestion by demonstrating different tolerances in the three types of nerve fibers against local anesthesia (C < Aδ < Aβ) (Fink, 1989), in accordance with the basic theory that, as previously mentioned, each type of nerve fiber displays a different recovery profile following spinal anesthesia.

**Quantitative assessment of lidocaine neurotoxicity**

Following the recognition of cauda equina syndrome after continuous spinal
anesthesia with 5% lidocaine in several reports (Rigler et al., 1991; Schell et al., 1991; Gerancher, 1997; Loo and Irestedt, 1999), local anesthetic neurotoxicity has remained an area of great concern. However, the mechanisms underlying this phenomenon remain unclear. Numerous animal studies have thus been performed to clarify the mechanisms of irreversible neurological impairment induced by local anesthesia (Drasner et al., 1994; Sakura et al., 1995b; Hashimoto et al., 1998; Sakura et al., 1995a). In animal studies of neurotoxicity following local anesthesia, many investigators regard neural damage that continues for 2-4 days as representative of persistent neurological injury or neurotoxicity (Ready et al., 1985; Sakura et al., 1995b; Hashimoto et al., 1998). The suitability of this definition has been confirmed by histopathological examinations (Kalichman and Calcutt, 1992; Hashimoto et al., 1998; Kalichman et al., 1989). The present study used this definition and regarded neurological impairment continuing for 4 days as irreversible. In vivo animal studies have demonstrated that concentrations of lidocaine ≥5% administered continuously, rather than as a bolus, can produce persistent neurological impairment due to restricted distribution of lidocaine (Drasner et al., 1994; Sakura et al., 1995b; Hashimoto et al., 1998). In the present study, irreversible neurological sequelae were produced in rats intrathecally administered 80 µl of ≥5% lidocaine, a volume comparable to that used by Drasner and colleagues in their rat model (1994). These results confirm that the rat model of spinal anesthesia used in our study is appropriate for investigating lidocaine neurotoxicity.

Neurometer testing indicated that CST ratios significantly increased in rats administered ≥5% lidocaine for 4 days after lidocaine administration. Skin-clamp testing, which is a simple but reliable method, was used to assess the sensory disturbance (Drasner et al., 1994; Kitamura and Doe, 2003). However, demonstrating
the concentration-dependent nature of sensory impairment is difficult. We therefore considered that a combination of sensory assessment using skin-clamp testing and motor assessment using gait behaviors would enable closer assessment of the severity of neurological impairment, including sensory function. The present experimental protocol was thus designed. As can be easily demonstrated using a combination of skin-clamp examination and gait observation, higher concentrations of lidocaine cause more severe neurological sequelae, including sensory impairment.

The concentration-dependent characteristics of local anesthetic neurotoxicity have been supported by numerous histopathological and electrophysiological in vitro studies (Ready et al., 1985; Kalichman et al., 1989; Lambert et al., 1994), and CST assessments for lidocaine neurotoxicity have yielded similar results. CST thus correlates well with severity of neurological sequelae induced by high concentrations of lidocaine. Neurometer testing appears suitable for use as a means of quantitatively assessing sensory impairment caused by local anesthetic agents in animals.

Compared with rats administered saline, a significant increase in CST ratios at 5 Hz and 250 Hz was found in rats administered 5% lidocaine, which was the minimum concentration needed to cause irreversible neurotoxicity. Conversely, CST ratios at 2000 Hz in the 5% group did not differ from those in the saline group. This indicates that larger nerve fibers are more resistant to irreversible neurotoxicity arising from highly concentrated local anesthetics than small fibers, a fact that is widely recognized and supported by clinical observations that the sequelae of cauda equina syndrome induced by highly concentrated local anesthetics (5% lidocaine or 10% procaine) are predominantly due to dysfunction of the small sensory fibers, as opposed to motor fibers (Rigler et al., 1991; Schell et al., 1991; Gerancher, 1997). CST differences among
the three frequencies regarding tolerances against neurotoxicity induced by 5% lidocaine also support the notion that the Neurometer can be used for fiber-selective assessments. However, no differences were observed among CST ratios at the three frequencies for groups with ≥10% lidocaine. This may indicate that when lidocaine concentrations exceed 5%, all three kinds of nerve fiber experience equivalent levels of injury. Although fiber-selective evaluation of neurological damage produced by higher concentrations of lidocaine becomes more difficult, the Neurometer appears to offer a useful means for assessing lidocaine-induced neurotoxicity in rats due to the concentration-dependent nature of CST changes.

In conclusion, we investigated whether CST could be utilized for quantitative and differential nerve evaluation of reversible blockade and persistent damage caused by administration of intrathecal lidocaine in rats. This is the first report in which the Neurometer has been applied to studies of local anesthetic nerve blockade in an animal model. CST measurements appear useful for quantitatively assessing both reversible blockage and neurotoxicity induced by local anesthesia in animals. In addition, although further investigations are needed, our results suggest that the Neurometer allows investigators to assess the function of different sensory fibers in both animals and humans.

Acknowledgments

The authors wish to thank Dr. Taku Sakurada, Dr. Takashi Miyabe and Dr. Noriaki Miyazaki (staff anesthesiologists, Department of Anesthesiology, Saga Medical School, Nabeshima, Saga, Japan) for their help in the preparation of animals, and Dr. Katims (Neurotron, Baltimore, MD) for his helpful comments and feedback.
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Footnote:

This work was supported by Grants-in-Aid 11770856 (to M.O.) and 12671478 (to N.K.) for Scientific Research from the Ministry of Education, Science and Culture, Tokyo, Japan, and was presented in part at the annual meeting of the American Society of Regional Anesthesia & Pain Medicine, Chicago, IL, April 25 to 28, 2002.
Legends For Figures

**Figure 1:** Schema of the study design. CST and behavior testing was performed before and during drug administration and every day for 4 days in all rats. In the 2% group, CST and skin-clamp tests were performed during drug infusion and within 2 min at 10-min intervals from the end of drug infusion to complete recovery from neural block. Measured CSTs were normalized using CST before drug administration as a control, with the exception of CSTs for determining distributions for the three frequencies before drug administration.

**Figure 2:** CST distributions for the three frequencies of stimulation in the 24 rats used in this study before intrathecal drug administration. Distributions in each frequency occupy a narrow range, indicating that CST is consistent in rats. Data are presented as means ±SEM.

**Figure 3:** Recovery profiles of CST ratio following reversible local anesthesia in 2% group. CST was measured within 2 min at 10-min intervals from the end of intrathecal administration of 2% lidocaine until values returned to baseline (n=6). CST ratio at 2000 Hz was the first to return to baseline, at 40 min after the end of drug administration, followed by values at 5 Hz and 250 Hz (at 70 min and 80 min after the end of drug administration, respectively). Compared to CST before drug administration, *, † and ‡ indicate significant increases in CST ratio at 2000 Hz, 250 Hz and 5 Hz, respectively. Data are shown as means ±SEM.

**Figure 4:** Number of rats exhibiting neurological sequelae for 4 days after intrathecal
drug administration. Motor function was classified as: “normal”, no abnormality; “moderate”, limited movement of hindpaw; or “severe”, inability to move hindpaw. Sensory function was assessed as presence or absence of an area of analgesia, defining abnormal or normal, respectively. Presence of an area of analgesia was indicated when the rat did not squeak or withdraw the hindpaw (or tail) within 4 s after initiating skin-clamping. Although both CST and behavior assessment in the saline group did not differ from control after lidocaine injection, all rats in groups that received ≥2% lidocaine displayed the effect of local anesthesia within 10 min after the start of lidocaine injection. Neurological disturbance in ≥2% lidocaine groups was worst at the end of lidocaine injection. Nerve blocking of rats in the 2% group was reversible, resulting in recovery from blocking with in a few hours after lidocaine injection. Rats in ≥5% groups displayed various degrees of neural impairment for 4 days, indicating irreversible neural damage. In the clinical setting, intrathecal administration of 2% lidocaine (spinal anesthesia) is well known to produce reversible nerve block. Test results for the 2% lidocaine group are in agreement with clinical experience. Nerve damage of rats in ≥5% groups became stable by day 1 (10% and 20% groups) or day 2 (5% group) after lidocaine injection and continued for 4 days. Finally, neither motor nor sensory deficits were found in the saline or 2% lidocaine groups. However, sensory impairment was observed in groups receiving ≥5% lidocaine. Gait abnormality was noted in half of the 5% lidocaine group, with grade and frequency of motor deficit increasing in a concentration-dependent manner.

**Figure 5:** Relationship between CST ratio (\(\frac{\text{CST}_{\text{pre-administration}}}{\text{CST}_{4 \text{ days after administration}}}\)) and lidocaine concentration at the three frequencies as of 4 days after drug
administration. CST ratios at the three frequencies in the 2% lidocaine group resembled those in the saline group. Comparing saline and 5% groups, CST ratios at 5 and 250 Hz were significantly increased in the 5% group, but CST ratio at 2000 Hz displayed no difference. Furthermore, no differences in CST ratio were observed between the three frequencies in groups administered ≥10% lidocaine. Data are presented as means ±SEM.
Figure 1

CST and behavior test

- saline 5, 10, 20%
group
- 2% group

Drug administration

0 20 40 60 80 (min) 1 2 3 4 (day)
Figure 2

![Graph showing CST (mA) against different frequencies.](image)
Figure 4

**motor assessment**

- normal
- moderate
- severe

**sensory assessment**

- normal
- abnormal

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Figure 5

- 2000Hz (A\(\beta\))
  - P < 0.01
  - N.S.

- 250Hz (A\(\delta\))
  - P < 0.01
  - N.S.

- 5Hz (C)
  - P < 0.01
  - N.S.

Graphs showing CST ratio against percentage for different frequencies.