

The Effect of Tramadol on the Rat Sciatic Nerve Conduction: A Numerical Analysis and Conduction Velocity Distribution Study

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The aim of this study was to document the effect of tramadol as an opioid on individual fibers of rat sciatic nerve. To accomplish this objective, compound action potentials (CAPs) were recorded from isolated nerves treated with tramadol from five different concentration levels. Then recorded CAPs and the control group were analyzed by numerical methods namely Conduction Velocity Distribution (CVD) and Fast Fourier Transform (FFT). The results show that the area under CAP and the time derivative of CAP curves decreases, and the excitability of the nerve trunk falls as well (rheobase and chronaxie increases) with increasing tramadol concentration. CVD deduced by model study was divided into subgroups as SLOW (8–26 m/s), MODERATE (26–44 m/s), MEDIUM (44–60 m/s) and FAST (60–78 m/s). The decrement in percentage relative contribution of these conduction velocity groups starts with a concentration of 0.25 mM tramadol, especially in the subgroup named FAST. The power spectrum shifts from higher frequency region to lower frequency region as the tramadol concentration increases. These findings show that fast conducting fibers are more susceptible to tramadol than medium and moderate groups and tramadol possibly acts on channel activity rather than passive properties (such as space and time constant) of nerve fibers.

Key words—compound action potential; conduction velocity distribution; tramadol; rat sciatic nerve

INTRODUCTION

Tramadol as an opioid is a clinically used, orally active analgesic drug. It is considered to act in the central nervous system by activating μ -opioid receptors. Opioids are also postulated to exhibit a local anaesthetic effect in the peripheral nervous system.^{1,2)}

Compound action potentials (CAPs) recorded from nerve trunk contains information concerning the number of active fibers and the propagation velocities of their action potentials. For the functional investigation of nerves *in situ*, the CAP has widespread application in basic research and in clinics; such as to determine the functional state of that particular nerve, diagnose any nervous disease, observe the nerve's growth, or deduce the conduction velocity distribution.^{3,4)}

One of the commonly used measures of nerve function is the conduction velocity distribution (CVD) of the nerve bundle. Using an appropriate CVD estimation model, the functional state of a given fiber group may be monitored before and after a certain event was applied so that quantitative comparison between

the groups is possible.^{5–9)}

In myelinated fibers there is definite delay of about 0.1 ms at each node, which represents the time necessary for Na⁺ ions to move through the membrane at the node in a quantity sufficient to discharge the membrane capacity.¹⁰⁾ This is closely related with the density of sodium channel in the membrane patch. The time constant ($\tau = R_m C_m$) is determined by the membrane capacity (C_m) and membrane resistance (R_m) while the space constant ($\lambda = \sqrt{R_m/R_o}$) is designated by membrane (R_m) and extracellular resistance (R_o). These two passive parameters (τ and λ) of the nerve also play an important role in determining the time delay.¹¹⁾ The longer the delay, the slower the conduction velocity is. Even, ease of excitation of nerve fibers is the index of the rapid depolarization of the membrane, so the fast conduction.

There exist many studies in the literature concerning tramadol, yet complete effects of it on peripheral nerve conduction are unclear. This study aims to determine the effect of tramadol on CVD and passive conduction parameters of rat sciatic nerves.

MATERIALS AND METHODS

All animals were cared for in accordance with the

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National Institutes of Health's (NIH) Guide for the care and use of laboratory animals. These protocols were reviewed and approved by the animal use committee. Due to sex dependency of CVD, 6 male Sprague-Dawley rats 12–14 weeks of age and weighing 250–300 g were used for the experiments. After birth, the animals were separated based on sex and three rats were housed per cage at ambient temperature and a 12-h light/dark cycle. They were fed with standard rat diet and water without restrictions for the duration of the experimental protocol.

All chemicals were purchased from Sigma (Sigma-Aldrich Chemie, Steinheim, Germany).

Experimental procedure Under light anaesthesia (30 mg/kg, i.p. sodium pentobarbital), sciatic nerves were dissected from the back foot of the Sprague Dawley rats sacrificed by cervical dislocation and exsanguinations. Nerves were then rapidly transferred to the recording chamber, which was superfused with a fresh modified Locke's solution (mmol/l): 140 NaCl, 5.6 KCl, 2.2 CaCl₂, 1.2 MgCl₂, 10 glucose and 10 Tris-(hydroxymethyl-aminomethane), pH 7.4 at a constant rate of 5 ml/min at fixed 33.2°C temperature then monitored online by BiosigW software.

Dose ranges of 0.25; 0.50; 1.0; 2 and 4 mM tramadol were chosen, for the reason that IC₅₀ value is given as 2.3 mM in literature.¹⁾ Tested doses of tramadol were applied to perfusion medium cumulatively and sciatic nerves were exposed to each dose for 20 minutes which is the time required for maximal decrease in CAP amplitude.¹⁾ Square-shaped supra-maximal pulses of 0.2 ms duration at a frequency of 1 Hz were applied from the proximal end of the nerve trunk through a stimulus isolation unit (Model SIU5, Grass Instruments) connected to a stimulator (Model S88K, Grass Instruments) and distal end of the nerve was inserted in suction electrode for recording in order to guarantee recording from the same number of fibers activated at any point along the nerve. Supra-maximal pulses were determined as the stimuli of intensity approximately 20% greater voltage than required for getting maximal CAP amplitude. CAP recordings were performed through a suction electrode from the tibia branch of the nerve trunk. Amplified ($\times 1000$) and filtered (1 Hz to 10 kHz) (CP511 A.C. Amplifier, Grass Instruments) CAP signals were digitized by 12 bit A/D converter (Advantech PCL-1710LG) with 0.02 ms sampling period

and 1024 time samples per CAP record. Computer software called BiosigW was used to control the A/D converter and to store data in a hard disk for further analysis. Since recorded signals were clear, signal averaging was not needed.

Analysing Procedure To investigate the status of neural function before and after the tramadol treatment, the strength-duration curves were plotted and mathematical procedures were conducted on all CAP recordings. Conduction velocities of the FAST and MODERATE group that constitute the majority of nerve fibers in a bundle were measured/computed by means of the difference in time between stimulus artifact and the first onset point of CAP ($\Delta t_{\text{latency}}$), and the point at which peak amplitude of CAP (Δt_{peak}) occurs, respectively. Upstroke velocity (\dot{V}_{max} : the maximum time derivatives of CAPs (dV/dt)_{max}) and the area under the CAP were also computed. Maximum time derivatives corresponding to the maximum rate of change in rising phase of CAP are used as an index of conduction activity for fastest nerve fibers in a bundle. The area under CAP is proportional to the number of excited active nerve fibers.

Conventionally, nerve conduction velocity (CV) can be determined by dividing the "distance" (Δx) that CAP travels along the nerve by the "time difference" (Δt) that it takes for this travel. Therefore, we can formulate the CVs for the fastest and relatively slower fibers group as follows;

$$CV_{\text{latency}} = \Delta x / \Delta t_{\text{latency}} \quad (1)$$

$$CV_{\text{peak}} = \Delta x / \Delta t_{\text{peak}} \quad (2)$$

where $\Delta t_{\text{latency}}$ and Δt_{peak} are the time differences from stimulus artifact to onset time and to peak time of CAP respectively. Both conduction velocities carry information on the functional state of the fastest and relatively slow fiber groups in the excited nerve.¹⁷⁾ Conduction velocities were estimated by using Eq. (1) and Eq. (2), where Δx was taken as 35 mm. The percentage decreases in CV_{latency} and CV_{peak} for each tramadol concentration as compared to control value have been also calculated.

Analysing the CAPs recorded at certain distances from the stimulus site, over a suitable mathematical model, one can compute fiber diameter distribution of that nerve. To obtain the individual nerve fiber activity, a conduction velocity distribution histogram was developed using the mathematical model that we have enhanced.^{6,12)} Our model based on the model

proposed by Cummins *et al.*^{7,8)} The basic principle of the model based on the statements of CAP can be expressed as

$$\text{CAP}(t) = \sum w_i f_i(t - \tau_i) \quad (3)$$

where CAP(t): the observed compound action potential as a function of time; N: number of fiber classes; w_i : the amplitude weighing coefficients for class i ; $f_i(t)$: single fiber action potential in class i .⁶⁻⁸⁾ The weighing coefficients (w_i) are general parameters to account for all influence on the contribution of each fiber class to the observed CAP. In order to estimate the individual fiber activity from CAPs, distribution of conduction velocity (CVD) for both control and tramadol treated nerves were obtained.

For the sake of visually augmented effect of tramadol regarding the difference and ease of interpretation four conduction velocity groups were defined intentionally, by dividing CVD into subgroups. The CV subgroups are as, SLOW: 8–26 m/s; MODERATE: 26–44 m/s; MEDIUM: 44–60 m/s; FAST: 60–78 m/s.

Valuable information may be gained by the application of Fast Fourier Transform (FFT) on biological signals.¹³⁾ According to Fourier theorem, any waveform can be constructed by the superposition of a number of independent sine, cosine, and constant functions, and hence this waveform consists of different powered signals of different frequencies.^{14,15)} By using FFT procedure, we have also calculated power spectrums of CAPs and the changes in power spectrums with tramadol concentration were determined, and the results were compared. Our problem is whether the power spectrum would reveal information about the relative contribution of fiber groups that conduct in different velocities before and after tramadol application.

In spectral analysis, we determined the window time as 5.12 ms. For the FFT analysis the number of data points should be power of two, and sampling period is 0.02 ms (sampling frequency is 50 kHz, so it easily becomes adequate for the Nyquist frequency), 256 data points (2^8) are enough to comprise from the stimulus artefact to end of compound action potential (stimulus artifacts were cleared before FFT analysis). FFT analysing procedure was conducted on one window for each CAP recording. Since detailed resolution did not give valuable information, frequency axis is divided to 16 bins and each represents a 200 Hz bandwidth.

Statistics Unless otherwise specified, the comparison of different doses (in mM) of tramadol effect for measured parameters was done with one-way analysis of variance (ANOVA), followed by Duncan post-hoc test for multiple comparisons when analysis of variance indicated significant results. Measured parameters compared with Student's *t*-test to recognize whether effect of tramadol application is reversible or not. Additionally, $p < 0.05$ values were taken as significant. Data are presented as mean \pm SEM throughout the text.

RESULTS

We have observed that treating the rat sciatic nerve with tramadol depresses the associated CAPs in a dose-dependent and reversible manner. As shown in Fig. 1, increasing the tramadol concentration decreases peak amplitude of CAPs and prolongs the latency periods.

The strength-duration curves that may be the assessment tool for the ease of excitability of a given nerve trunk are given in Fig. 2 for the control and five different concentrations of tramadol. As it can be traced from Fig. 2, the strength-duration curves of five different concentrations are shifted upward away from the control as the concentration increases. From these curves, rheobase and chronaxie values were determined and given in Table 1. Rheobase and chronaxie values for each tramadol concentration suggest that as the tramadol concentration increases excitability of nerve fibers decreases in.

Since maximum depolarization value (MD) of

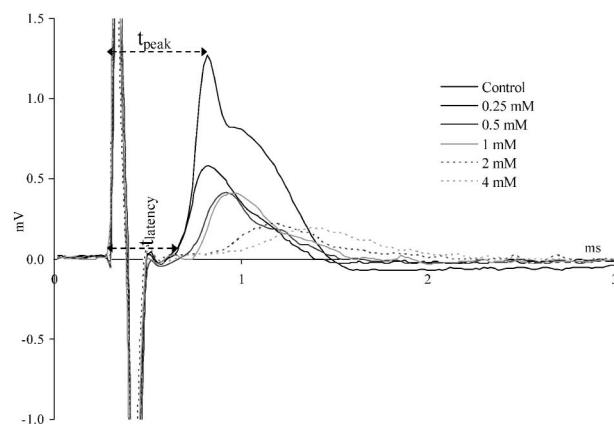


Fig. 1. Typical CAP Signals Recorded 35 mm Away from the Stimulating Electrodes

The graph legends represent tramadol concentration. As the tramadol concentration is increased, CAP amplitude decreased and latency is increased.

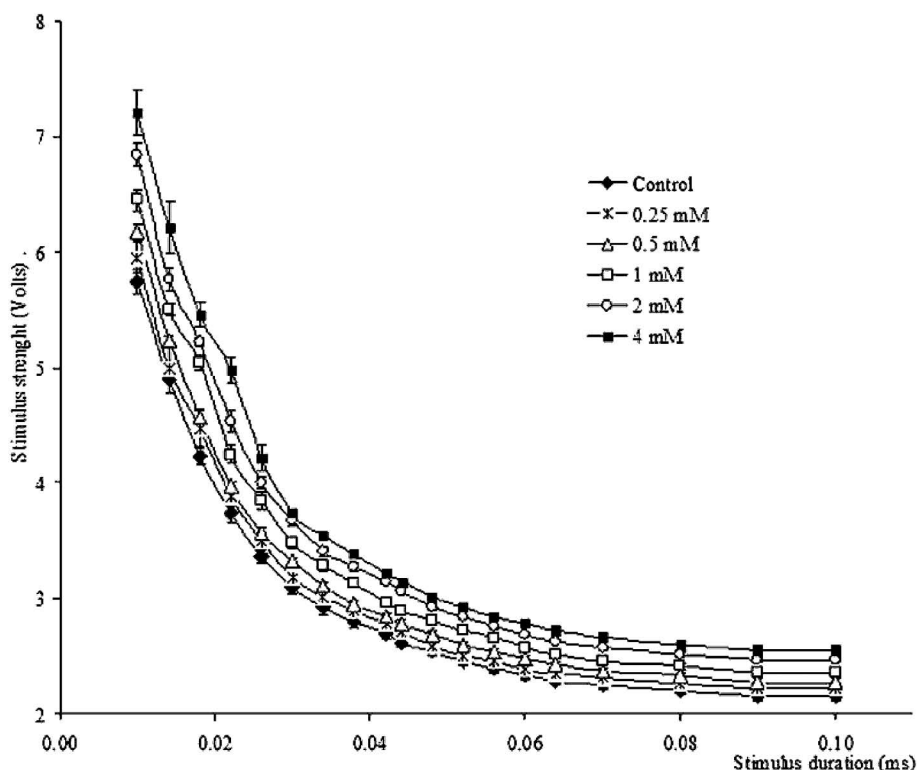


Fig. 2. The Strength-duration Curve for Direct Stimulation of Rat Sciatic Nerve before and after Treated with Different Tramadol Concentration

Rheobase and chronaxie values for each curve are given in Table 1 ($n=6$).

Table 1. Change in General Parameters of Nerve Excitability before and after Treated with Different Tramadol Concentrations

	Tramadol concentration					
	Control	0.25 mM	0.5 mM	1 mM	2 mM	4 mM
Rheobase (V)	2.21 ± 0.03	2.26 ± 0.02	$2.34 \pm 0.03^*$	$2.39 \pm 0.03^*$	$2.50 \pm 0.01^*$	$2.56 \pm 0.04^*$
Chronaxie (μ s)	16.88 ± 0.18	17.25 ± 0.58	17.26 ± 0.28	$18.55 \pm 0.25^*$	$18.65 \pm 0.38^*$	$19.62 \pm 0.77^*$
MD (mV)	1.82 ± 0.35	1.28 ± 0.18	$0.98 \pm 0.20^*$	$0.90 \pm 0.18^*$	$0.72 \pm 0.17^*$	$0.51 \pm 0.16^*$
Area (mV.ms)	0.70 ± 0.10	0.55 ± 0.07	$0.45 \pm 0.09^*$	$0.46 \pm 0.06^*$	$0.39 \pm 0.05^*$	$0.32 \pm 0.08^*$
\dot{V}_{\max} (V/s)	16.34 ± 3.61	11.21 ± 1.42	$7.53 \pm 1.75^*$	$6.68 \pm 1.49^*$	$4.55 \pm 1.43^*$	$3.50 \pm 1.06^*$

Rheobase represents the minimum voltage required to elicit an action potentials and chronaxie represents time required for two times the rheobase values. MD is the maximum depolarization value of CAP, \dot{V}_{\max} represents maximum value in rate of change of CAP with time. Values are given as mean \pm SEM ($n=6$) and $*p < 0.05$ represents the significant levels as compared with control value.

CAP and the area under the CAP are proportional to the number of excited nerve fibers in that particular nerve, MD values and areas under CAPs were measured/calculated and given in Table 1. The change in upstroke velocity (\dot{V}_{\max}) can also be used as an index of conduction activity of nerve fibers in a bundle.¹⁶⁾ Upstroke velocities of rising phase of CAPs were also calculated and are given in Table 1.

The time differences were measured by taking both the onset time ($\Delta t_{\text{latency}}$) of CAP and the peak time (Δt_{peak}) of CAP as reference (Fig. 1).

Using Eqs. (1) and (2), conduction velocities for CV_{latency} and CV_{peak} were computed and given in Table 2 for whole experimental groups. Statistically significant change in CV starts for both CV_{latency} and CV_{peak} at 0.5 mM ($p < 0.05$) and marked with “***”. In order to make proper assessment on the occurrence of statistically significant change, relative changes (in %) on CV_{latency} and CV_{peak} with respect to control value were calculated and given in “Change” column of the table.

The estimated CVD for control group and for 5

Table 2. Nerve Conduction Velocity and the Change in the Number of Fibers *versus* Tramadol Concentration

	Conduction velocity				% Change in relative number of fibers			
	CV _{latency} (m/s)	Change (%)	CV _{peak} (m/s)	Change (%)	CV groups			
					FAST	MEDIUM	MODERATE	SLOW
Control	78.4±3.8		55.7±2.6					
0.25 mM	70.0±2.9	10.7	48.5±2.5	12.9	-84.9*	-28.1*	-4.1	-33.5*
0.50 mM	66.1±1.5	15.7*	46.0±2.9	17.5*	-96.9*	-66.3*	-8.9	-50.2*
1.0 mM	63.3±2.4	19.2*	40.1±1.7	28.1*	-88.8*	-73.2*	15.1	-56.4*
2.0 mM	54.0±3.1	31.1*	32.9±0.9	40.9*	-99.3*	-96.5*	-1.1	10.2
4.0 mM	53.3±3.9	32.0*	30.0±1.7	46.2*	-96.5*	-96.7*	-33.0*	77.2*

Values are given as mean ± SEM (n=6) and *p<0.05 represents the significant levels as compared with control value.

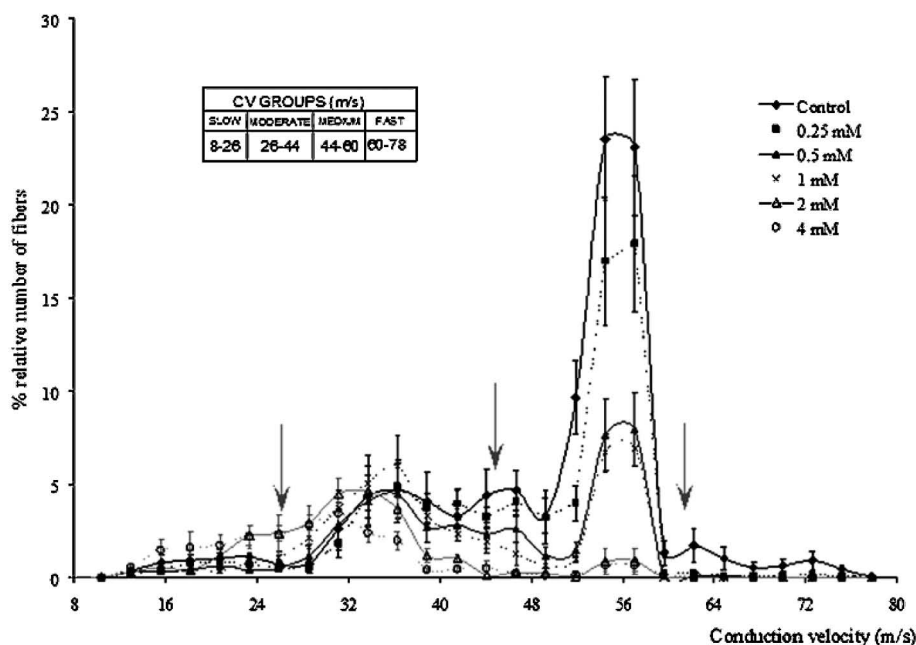


Fig. 3. Conduction Velocity Distributions Estimated by the Model for Control and Tramadol Treated Nerve Groups are Given as Smooth Lines

(◆), (■), (▲), (x), (△), and (o) represent control and 0.25; 0.5; 1.0; 2.0; 4.0 mM tramadol concentration, respectively. The arrows show the borders of intentionally defined conduction velocity groups. CV ranges of each group are given in the inset table (n=6).

different tramadol concentrations with the results are plotted in the same graph as smooth lines (Fig. 3). The three arrows in the figure show the borders of these CV groups where remarkable changes take place. CV group names and corresponding CV ranges are given in the inset of the figure. Relative number of active fibers were recalculated according to newly defined CV groups (SLOW, MODERATE, MEDIUM and FAST) and shown separately in Fig. 4 as bar graphs for control and for each tramadol concentrations.

The Fast Fourier Transform (FFT) has been applied to CAP waveforms related to nerves under the influence of tramadol for rapid and efficient spectral

analysis. Relative power (%) values of control and five different concentrations *versus* frequency bin graphics are given in Fig. 5. In all graphs, frequency band ranges between 0–3000 Hz, but 0–1000 Hz interval constitutes most of the spectrum. As seen in the graph, a high frequency component of the spectrum decreases while the tramadol concentration increases.

For the control spectrum, relative power comprises 63.35% in 0–1000 Hz and 25.35% in 1000–3000 Hz bands. As the concentration increases relative power contribution in these frequency band shifts to lower frequency bands; at the concentration of 0.5 mM, it is 74.30% in 0–1000 Hz and 18.0% in 1000–3000 Hz bands; and 77.30% in 0–1000 Hz and 13.8% in 1000–

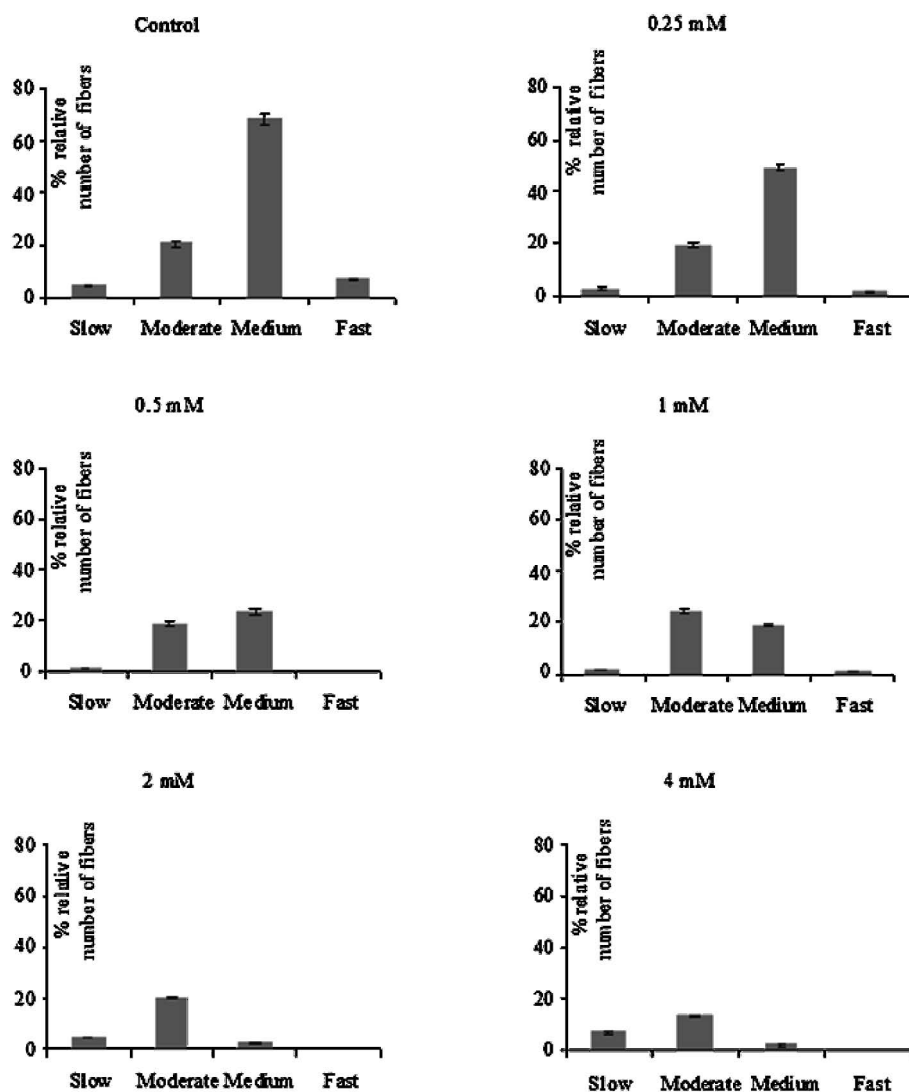


Fig. 4. Recalculated Conduction Velocity Distribution Histograms according to the Defined Conduction Velocity Groups
Values are given as mean \pm SEM ($n=6$).

3000 Hz bands for 4.0 mM (Fig. 5).

DISCUSSION

It is known that opioids inhibit excitatory synaptic transmission by activating opioid receptors in the central nervous system.¹⁸⁻²⁰ Opioids are also known to exhibit a local anaesthetic effect in the peripheral nervous system. Although Yuge *et al.*²¹ mentioned that there was no significant change in the amplitude of CAPs, others reported that conduction of action potential in peripheral nerve is generally blocked by opioids.^{2,22} Such an inhibition on CAP by a non-narcotic opioid tramadol, (1RS; 2RS)-2-[(dimethylamino) methyl]-1-(3-methoxyphenyl)-cyclohexanol hydrochloride was also reported.^{1,23} With this study, we have intended to investigate how the conduction

parameters of rat nerve trunks are affected when the nerve is treated with different concentrations of tramadol.

Tramadol as an opioid has an agonist effect and exhibits its effect by blocking the nerve conduction. As we pointed out in the results section, this blockage is reversible. Because its reversibility, we did not investigate the effects of a medicine which is antagonist of tramadol.

The results of this study indicate that direct tramadol application results in dose dependent inhibition on nerve conduction (Fig. 1), which is consistent with the literatures.^{1,23,24} There is a decrement in peak amplitude of CAP and prolonged latency in both $\Delta t_{\text{latency}}$ and Δt_{peak} in a dose-dependent manner.

In general, the strength-duration curve is the index

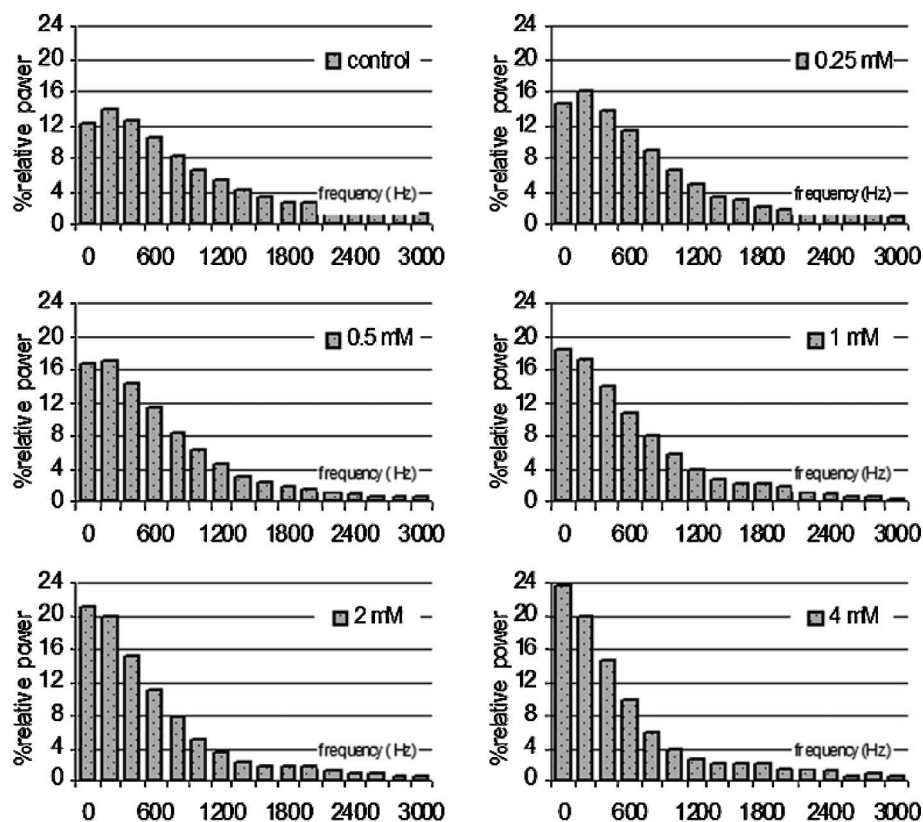


Fig. 5. The Effect of Tramadol on CAP Waveform Power Spectra

Graphs show the power vs. frequency relationship related to CAPs for control and five different tramadol concentrations. The powers are given as % relative values. Frequency axis is divided to 16 bins and each represents a 200 Hz bandwidth.

of the nerve fibers excitability; the ease in excitability, so the fastest the nerve fibers are.¹¹⁾ Our findings show that (Fig. 2 and Table 1) as the tramadol concentration increases; rheobase value gets higher while the chronaxie value prolongs. Since these parameters show ease of excitability of nerve fibers, increase in tramadol concentration results in difficulty of excitability of the nerve fibers. Difficulty in excitability means blockage in conduction in some nerve fibers. This difficulty does not arise from passive membrane properties such as time and space constant, but from the channel activity in axon membrane. This results in support of other's findings.¹⁾ Significant change in rheobase (in V) begins at 0.50 mM, while in chronaxie (in ms) begins at 1.0 mM concentration when compared with control ($p < 0.05$) (Table 1).

Since the area under a CAP waveform is proportional to the number of activated nerve fibers in that nerve, it can be used as a measure of the conduction block.²⁵⁾ One can see from Table 1 that, starting from the control value the area under CAP decreases as the concentration increases. This means that the number

of non-conducting nerve fibers increases with increasing tramadol concentrations. But significant decrease in CAP area starts at the 0.5 mM concentration of tramadol ($p < 0.05$). This conduction block also reflects itself as a decrement in MD values. The dose that significant decrease starts for MD value gets along well with CAP area changes ($p < 0.05$).

Upstroke velocity (\dot{V}_{max}) of the CAP can also be used as a sign of individual fastest fiber activity in bundle.^{26,27)} Upstroke velocity decreases with increasing tramadol concentration, and the significant decrement occurs at 0.5 mM ($p < 0.05$) concentration (Table 1). That means fastest fibers start to be effected at this concentration.

Conduction velocities for both $CV_{latency}$ and CV_{peak} decrease with increasing tramadol concentration (Table 2). The percentage decrement ("Change" column in Table 2) in both $CV_{latency}$ and CV_{peak} as compared with control value increase by increasing tramadol concentration, but dominantly in CV_{peak} . The significant change starts in both CV at 0.50 mM. This conventional CV measurement method shows the

effect of tramadol on conduction velocity globally, but not in detail.

Determination of the conduction velocity distribution (CVD) is a numerical method and when applied to the recorded CAP one may gather information on the relative number of active fibers for discrete CV values in a nerve trunk.^{5-9,28)} Hence with CVD, the functional state of a given fiber group may be monitored before and after a certain event, so that making quantitative comparison between groups is possible. Our question is if CVD would reveal information about the relative contribution of fiber groups before and after tramadol treatment. Our CVD findings suggest that increase in tramadol concentration decreases relative contribution of fibers into the groups as compared with control histogram. This means that tramadol induces decrement and blockage in conduction of many group of fibers as seen smoothed lines graph of CVD (Fig. 3).

By determining previously suggested CV subgroups (SLOW, MODERATE, MEDIUM and FAST), we noticed that the most contributing fibers groups to CAP are MEDIUM (~70%) and MODERATE (~20%) in control. Contribution of FAST and SLOW (~6% and ~4%) are relatively small (Fig. 4). Beginning with the 0.25 mM tramadol concentration, relative number of fibers starts to decrease with increasing tramadol concentration; falling is seen in all groups. Going further, an increase in tramadol concentration decrease the amount of contributing fiber of all suggested groups, but FAST group has completely vanished at 0.5 mM. SLOW group starts to increase again at 2 mM. This is possibly because of that the decrements in MODERATE and MEDIUM help to increase SLOW group. At 4.0 mM concentration, most of the groups (approximately 90% of the whole nerves) are depressed.

The Fast Fourier Transform (FFT) analysis results give the relative power (%) values of frequency bin of CAP signals.^{14,15)} From Fig. 5 we see that in all groups including the control, the CAP's frequency components appear to be in 0-3000 Hz interval. The power spectrum shifts from higher frequency to lower frequency region as the tramadol concentration increases. The relative power values that belong to 16 different frequency intervals change with tramadol concentration, and a shift is observed to the subsequent smaller or higher ranked frequency interval, as a general tendency. Since the single fiber action poten-

tial's (SFAP) contribution to CAP changes with changing in tramadol concentration (Eq. (3)), these shifts occur.

It is known that there exists an inverse proportion between SFAP duration and the nerve conduction velocity.²⁹⁾ Due to this fact, fast conduction fibers' SFAP durations are smaller, gaining high frequency components, while the SFAP duration of slowly conducting fibers are relatively longer and hence become low frequency components. Shifting from high frequency to lower frequency component with increasing tramadol concentration shows blockage in conduction. These findings support our CVD findings.

Sciatic nerves consist of axons that have different diameters, so the different conduction velocity. Decrement in CAPs amplitude and area mean that different conduction groups are blocked by different tramadol concentrations. Significant blockage starts mostly at 0.5 mM concentration. Although the mechanism of tramadol effect on peripheral nerve has not been explained clearly yet, influence of it on Na⁺ channel is stated in literature.²³⁾ So, the decrease in ease of excitability with tramadol may suggest that tramadol acts on the channel kinetics rather than passive conduction parameters such as space (λ) and time (τ) constant. And, also these two parameters affect conduction velocity but not the excitability of the nerve.

In our previous study³⁰⁾ bupivacaine as a local anaesthetic agent, first affects the smallest myelinated (motor) fibers, then the fast conducting (neurosensory) fibers. Although we expected similar results in this study our results show that fast conducting fibers are more susceptible to tramadol than medium and moderate groups (Table 2 and Fig. 4). In literature, it is stated that tramadol may act on the peripheral nerve's Na⁺ channels by way of different molecular mechanism than local anaesthetic agents do.²³⁾ Therefore, Na⁺ channel in fast conduction fiber may be more susceptible to tramadol than Na⁺ channel in slow fiber do.

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