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Tonic Stimulation of Dorsal Root Ganglion Results in Progressive Decline in Recruitment of A α and A β -Fibers in Rats Protocol

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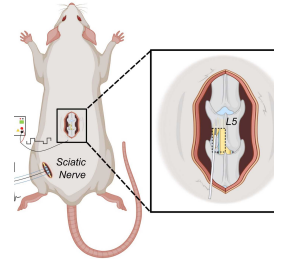
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We use this protocol and it's working

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Abstract

This is a protocol for an experiment to characterize the recruitment and maintenance of action potential firing in A α / β -fibers generated during tonic dorsal root ganglion stimulation (DRGS) applied over a range of clinically relevant stimulation parameters. This protocol will result in data describing the A α / β -fiber activation thresholds and A α / β -fiber recruitment due to each stimulus parameter. Data showing whether the evoked responses changed during extended periods of stimulation and to assess the time necessary for ECAP amplitude to fully recover due to tonic DRGS. As well as data measuring the ECAP latency and width at baseline and at the time point corresponding to a 50% reduction in ECAP amplitude.

Image Attribution

Ting JE, Hooper CA, Dalrymple AN, Weber DJ. Tonic Stimulation of Dorsal Root Ganglion Results in Progressive Decline in Recruitment of A α / β -Fibers in Rats. *Neuromodulation*. 2024 Dec;27(8):1347-1359. doi: 10.1016/j.neurom.2024.06.498.

Materials

	A	B	C	D	E	F	G	H
	Electronic Equipment	Pre-surgery Tools	Surgical Tools	Surgical Accessories	Drugs	Misc. Hardware /Docs	Anesthesia/Vital Signs Monitoring	Implant Tools
	AM-Systems Stimulator Model 4100	Eye lube	Disposable scalpel	Syringes (water, saline)	Isoflurane	Electrical tape	Anesthesia vaporizer	Surgical microscope
	Intan RHD Recording Controller	Surgical tape	Scissors - small pointy	Stainless steel bowl (trash)	Saline	Scissors	Temperature probe	Microrulers
	Intan RHD 16-Channel Bipolar-Input Recording Headstage	Clippers	Scissors - small round tip	Cotton balls		Drug Dosage sheet	Circulating water heater	
	Nerve cuffs	Vacuum	Forceps - small straight	Gauze, large		Notes doc		
	DRG electrodes		Forceps - small curved	Gauze, small		Calipers		
	EMG electrodes		Periosteal elevator	Absorbent triangle points				
	Cooner wire		Rongeurs (2 smallest sizes)	Suction beaker (enzyme)				
			retractors	Suction tube with beaker plug				
			Cauterizer	Suction large tube to vacuum				
			Piezo drill	Suction tip				
			Drill bit	Suction stylet				
			Microscissors	Bone wax				
				Cotton buds				

Experiment Model (Rat Anesthetization)

- 1 Anesthetized adult Sprague Dawley rats (11 female, nine male; >eight weeks old; 200–500 g; Charles River Laboratories, Wilmington, MA) with isoflurane (inhalation, 4%–5%).
- 2 Maintained anesthesia with isoflurane (inhalation, 1.5%–2.5%) for the duration of the experiment.
- 3 Applied lubricant to the eyes and administered glycopyrrolate (intramuscular, 2.5 mL/kg) to reduce salivary, tracheobronchial, and pharyngeal secretions.
- 4 Continuously monitored vital signs including respiratory rate, core temperature, and blink and withdrawal reflexes throughout the experiment.
- 5 After the experiment, euthanized the animal with isoflurane overdose (inhalation, 5%).

Implant DRG and Nerve Cuff Electrodes

- 6 Shaved the lumbar region of the back and dorsal aspect of the hindlimb.
- 7 Made an incision over the L4–L6 lamina and performed a partial laminectomy of the L5 vertebrae to expose the caudal portion of the L5 dorsal root ganglion (DRG).
- 8 Placed a bipolar, cylindrical electrode lead (platinum/iridium, contact length = 0.76 mm, interelectrode spacing = 0.5 mm; Abbott Neuromodulation, Plano, TX) over the dorsal aspect of the L5 DRG to deliver DRG stimulation.
- 9 Exposed the sciatic nerve ipsilateral to the target DRG and bluntly dissected the nerve from the surrounding muscle and connective tissue.
- 10 Placed a tripolar cuff electrode (1.5-mm diameter; Micro-Leads, Somerville, MA) around the sciatic nerve to measure evoked responses.
- 11 Inserted a pair of intramuscular needle electrodes into the triceps surae muscle group for bipolar electromyography, and a ground electrode either between the skin and fascia of the hindlimb or near the base of the tail.

- 12 After the experiment, measured the distance along the sciatic nerve from the stimulation site on the DRG to the recording site on the sciatic nerve to determine the conduction velocity of evoked responses in each animal.

Data Acquisition and Stimulation

- 13 NOTE: Performed all processing and analysis of the data in MATLAB (MathWorks, Natick, MA).
- 14 Delivered bipolar electrical stimulation to the L5 DRG using symmetric, biphasic waveforms of varying current-amplitude, pulse width, and frequency, depending on the experimental paradigm (see Experimental Paradigms section below).
- 15 Recorded evoked compound action potentials (ECAPs) from the sciatic nerve cuff electrodes while DRG stimulation was delivered.
- 16 Electroneurogram (ENG) signals were sampled from the sciatic nerve cuff electrode at 30 kHz and digitized using an Intan headstage and recording system (RHD2216, Intan Technologies, Los Angeles, CA).
- 17 Used conduction velocities to determine which afferent fiber types were contributing to each identified ECAP ($A\alpha/\beta$: >14 m/s; $A\delta$: 2.2–8 m/s; C: <1.2 m/s).
- 18 Calculated conduction velocities of the ECAPs using the distance between the DRG stimulation and cuff electrodes and the latency of the first peak of the ECAP.
- 19 Bandpass-filtered (300–3000 Hz) the ENG data before further processing to identify ECAPs.
- 20 Blanked stimulation artifacts over a time window ranging from 100 μ s to 1 ms in duration, depending on the pulse width applied in a particular trial.

Experimental Paradigms

- 21 **Recruitment:**
- 21.1 Varied stimulus pulse width and frequency around the typical clinical parameters (300 μ s, 20 Hz)^{1,2}, to test the effects of varying each parameter on $A\alpha/\beta$ -fiber activation thresholds (AT), or the charge required to elicit a detectable ECAP, and $A\alpha/\beta$ -fiber recruitment.

- 21.2 Delivered DRGS at 20 Hz with pulse widths of 50, 150, 300, and 500 μ s when testing for pulse width effects.
- 21.3 Applied DRGS at frequencies of 5, 20, 50, and 100 Hz using 300- μ s pulses when testing for frequency effects.
- 21.4 Randomized the amplitude of DRG stimulation current-pulses to generate recruitment curves of ECAPs recorded from the sciatic nerve for each trial.
- 21.5 Applied 50 to 300 pulses (3–15 seconds) to the DRG with a 5- to 10-second interval between different amplitude levels for each amplitude level.
- 21.6 Measured motor thresholds (MTs) for each pulse width and frequency parameter combination.
NOTE: An MT is the charge level at which a muscle contraction was visible in the hindlimb.
- 21.7 Used a bootstrapping method, described in detail in prior studies^{3,4}, to detect A α / β -fiber ECAPs.
- 21.8 Bootstrapping Method Summary:
 - 21.9 a. Stimulus-triggered averages (STA) were generated from random samples of the ENG recorded during 80% of the total stimulus repetitions (50–300).
 - 21.10 b. Depending on DRG stimulation frequency, we produced 50 random STA samples for trials with 5-Hz DRG stimulation and 100 samples for trials with 20-, 50-, or 100-Hz DRGS for each trial.
 - 21.11 c. Calculated the root mean square (RMS) of each averaged ENG signal using a 100- μ s sliding window with 33- μ s overlap with the previous window.
 - 21.12 d. Defined the ECAP detection threshold as one standard deviation greater than the upper bound of the 99% confidence interval (CI) of the baseline RMS amplitude during a 1-millisecond period before each stimulus.
 - 21.13 e. Successfully identified an ECAP in a given trial if the same 100- μ s RMS window contained suprathreshold values for 95% of the random samples.
 - 21.14 f. Validated automatically detected ECAPs by visual inspection.

- 21.15 g. Defined the A α / β -fiber AT for a given recruitment trial as the lowest DRG stimulation charge level (current-amplitude $\times$ pulse width; nC) with a detected ECAP.
- 21.16 To compare initial ECAP amplitudes elicited at AT, averaged the amplitudes from each tested frequency (5, 10, 20, 50, and 100 Hz) to yield one value per frequency for each animal.
- 21.17 Normalized the mean amplitudes from each animal to the mean value at 20 Hz for the corresponding animal.
- 21.18 Performed one-way analysis of variance (ANOVA) and Tukey–Kramer post-hoc tests with α -level of 0.05 to assess differences in ATs due to DRG stimulation frequency or pulse width.
- 21.19 Performed paired, two-tailed *t*-tests with α -level of 0.05 to compare A α / β -fiber AT and MT in corresponding trials.
- 21.20 Performed one-way ANOVA and Tukey–Kramer post-hoc tests with α -level of 0.05 to evaluate differences in initial ECAP amplitudes due to DRG stimulation frequency.

22 **Tonic DRG Stimulation:**

- 22.1 Delivered DRG stimulation continuously in 180-second intervals at pulse frequencies of 5, 10, 20, 50, and 100 Hz to study whether evoked responses changed during extended periods of stimulation and to assess the time necessary for ECAP amplitude to fully recover.
- 22.2 Determined the recovery time through turning off DRG stimulation for an interval of varying duration (30–120 seconds; recovery phase) before turning it on for an additional 30 seconds to measure ECAP amplitudes after the recovery phase.
- 22.3 Used 300- μ s pulses and a current amplitude equaling 120% of AT, which was determined for each tested frequency before conducting tonic DRG stimulation experiments.
- 22.4 Measured ECAP amplitudes throughout each 180-s bout of tonic DRG stimulation.
- 22.5 Identified ECAPs occurring during a time window corresponding to the latency of A α / β -fiber responses:

- 22.6 a. Calculated the peak-to-peak amplitude of each ECAP elicited during 1-second windows immediately after the onset of DRG stimulation (ie, 0–1 seconds after onset for 0 second time point) and at 1-second intervals throughout each 180-second trial.
- 22.7 Normalized each ECAP measurement by subtracting the peak-to-peak of the baseline ENG activity (measured in a 0.5-millisecond window occurring before each DRG stimulation pulse) from the peak-to-peak of the ECAP to account for any changes in the baseline noise level during trials.
- 22.8 Calculated the mean and standard deviation of the ECAP amplitudes in each 1-second window and used these values for further processing.
- 22.9 Averaged across amplitudes in 1-second windows in all trials, regardless of DRG stimulation frequency, meaning that DRG stimulation at 5 Hz resulted in five amplitude measurements per 1-second interval, whereas DRGS at 100 Hz yielded 100 measurements.
- 22.10 Fit the ECAP amplitude (A_{ECAP}) measurements obtained from each trial with exponential decay functions (ie, $A_{\text{ECAP}} = b_1 e^{b_2 t} + b_3$, where t is time during DRG stimulation) and obtained the reported decay time constants (ie, b_2) from each fit.
- 22.11 Excluded fits that were not statistically significant ($p > 0.05$) and fits in which the value of the decay constant fell outside of three standard deviations of the mean decay constant for that particular frequency group.
- 22.12 Pooled the time constants across all trials and animals to yield a single mean value and standard deviation for each tested frequency.
- 22.13 Calculated the final ECAP amplitude for each trial as the mean of the last 15 seconds of amplitude measurements from the exponential fit.
- 22.14 Compared the peak-to-peak amplitude measured in the first 1-second interval of DRG stimulation after the variable-length recovery phase with the ECAP amplitude measured immediately after onset of the initial tonic DRG stimulation period (ie, baseline level) to measure the rate and extent of ECAP recovery.
- 22.15 Performed linear regression to model the recovery rate of ECAPs during the 180-second interval following cessation of tonic DRG stimulation for each frequency group:
- 22.16 a. A linear model of the form $y = mx + b$ was used, where x is the time in seconds since cessation of DRG stimulation and y is the amplitude of the ECAP, measured as a percentage of the initial ECAP amplitude.
- 22.17 b. The slope (m) in this model represents the recovery rate, and the intercept (b) represents the ECAP amplitude at the end of the 180-second period of tonic DRG

stimulation.

22.18 Considered the ECAP amplitude to be recovered fully when its difference from the baseline amplitude was not statistically significant.

22.19 Performed one-way ANOVA and Tukey–Kramer post-hoc tests with α -level of 0.05 to evaluate differences in decay constants, final ECAP amplitudes, and recovered ECAP amplitudes after 120 seconds due to DRG stimulation frequency.

23 Mechanisms of ECAP Suppression:

23.1 Measured ECAP latency and width at baseline and at the time point corresponding to a 50% reduction in ECAP amplitude to explore potential mechanisms underlying the effects of tonic DRG stimulation on A α / β -fiber recruitment.

23.2 Obtained ECAP latency and width measurements from the average ECAP trace in 1-second windows during DRG stimulation.

23.3 Defined the ECAP latency as the time from stimulus onset to the time of the positive peak of the ECAP identified within the A α / β -CV window.

23.4 Identified both peaks of the ECAP and defined the start of the negative peak as the midpoint between the positive and negative peak amplitudes to measure ECAP width.

23.5 Determined the halfway point of the negative peak between the start of the peak and full negative peak amplitude and used this amplitude to calculate the width.

23.6 Identified the point at which the ENG signal crossed this half-amplitude point (*intersect* function, MATLAB) and defined the ECAP width as the time difference between these two intersection points.

23.7 ECAP peaks and intersection points used to calculate width were plotted and visually inspected.

23.8 Traces that did not contain identifiable ENG intersection points were excluded from further analysis. Specifically, trials were excluded if fewer than ten ECAP measurements could be obtained from a 15-second window around the time point corresponding to a 50% reduction in amplitude.

23.9 Performed paired, two-tailed *t*-tests with α -level of 0.05 to compare ECAP latencies or ECAP widths at baseline and at 50% of ECAP amplitude.

- 23.10 Performed experiments to determine whether fibers contributing to the ECAP were undergoing changes in their excitation thresholds during tonic DRG stimulation.
- 23.11 Delivered a preconditioning period (3 seconds) of DRG stimulation, followed immediately (~1 second) by tonic DRG stimulation for 180 seconds to evaluate ECAPs before and after tonic DRG stimulation.
- 23.12 Applied DRG stimulation during a postconditioning period (3 seconds) ~1 second after turning tonic DRG stimulation off.
- 23.13 ECAPs detected during the preconditioning period served as a baseline for comparing responses detected during the postconditioning period.
- 23.14 Delivered tonic DRGS at a range of frequencies (5, 20, 50, and 100 Hz) using 300- μ s pulses and current amplitudes equaling 130% of AT.
- 23.15 Delivered pre- and postconditioning periods of DRG stimulation with the same frequency and pulse width as tonic DRG stimulation; however, the current amplitudes for the pre- and postconditioning periods were incremented by 1 μ A in each subsequent trial to determine the current level at which pre- and postconditioning ECAPs were approximately equal in size.
- 23.16 Generated an STA to yield a mean ECAP waveform for each of the 3-second pre- and postconditioning phases of DRG stimulation to compare ECAP amplitude in the Threshold Change Experiments.
- 23.17 Calculated the peak-to-peak amplitude from the mean ECAPs for the pre- and postconditioning phases of each trial.
- 23.18 Used these amplitudes to calculate the ratio of postconditioning ECAP amplitude to preconditioning ECAP amplitude as a percentage. If this value was $\geq 90\%$, the pre- and postconditioning ECAP amplitudes were considered to be approximately equal.
- 23.19 Performed a one-way ANOVA and Tukey–Kramer post-hoc test with α -level of 0.05 to evaluate differences in the current level required to generate an average ECAP in the postconditioning phase that was at least 90% of initial ECAP amplitude due to DRG stimulation frequency.
- 23.20 Performed a paired *t*-test of both the ECAP latencies and ECAP widths, comparing the values measured during the pre- and postconditioning phases; values obtained across all frequencies were grouped.

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