

Test of Drug X in Teased Fiber Model

Purpose

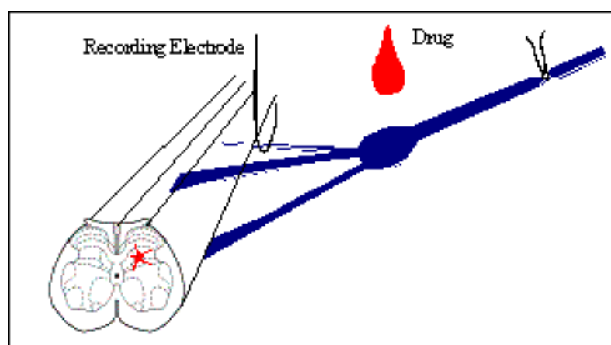
To determine the effect of a drug X on peripheral afferents activities in response to innocuous and noxious mechanical and thermal stimuli.

Surgery

Adult Sprague-Dawley rats are initially anesthetized with pentobarbital sodium (50 mg/kg ip). A catheter is placed in the jugular vein for continuous administration of anesthetic and a tracheotomy allows insertion of a tracheal cannula for artificial ventilation. A 4-cm-long laminectomy is performed over the lumbosacral enlargement to expose the spinal cord and dorsal roots. The rat is held in a stereotaxic frame to prevent any movement during recording. The skin over the laminectomy is elevated to form a pool that is filled with light mineral oil. Continuous anesthesia is accomplished by giving a mixture of 50 mg sodium pentobarbital in 9 ml 0.9% NaCl at a rate of 1.0 ml/h. The end tidal CO₂ is maintained at around 30 mmHg. The animal's body temperature is maintained at 37°C by a feedback-controlled electric heating blanket.

Single teasing fiber recording

Extracellular single-fiber recordings from the distal stump of the dorsal root. For



electrophysiological recordings, a silver wire hook electrode is used to record extracellular single-unit discharges in filaments of the L4 or L5 dorsal roots. A small strand of the dorsal root is teased distally from the main trunk and is further separated into a filament containing a single

active fiber (Fig.). The dorsal root filament is wrapped around the recording electrode. Electrical stimulation is applied at the receptive field for recordings of orthodromic spikes to determine the conduction velocity. Single fibers can be classified into A_α-, A_β-, or C-fiber according to its conduction velocity. Mechanical and thermal stimulation of a receptive field located in the skin is delivered. In these units, single fiber is recorded in response to graded intensities of

mechanical stimuli delivered to the receptive field. Background activity and responses to brush, pressure, and pinch stimuli are recorded for 10 s each with a 20-s inter-stimulus interval. Two types of mechanical stimuli will be applied to the receptive field. Innocuous stimulation (brush) is delivered by repeated brushing in a stereotyped manner with a camel's hair brush. Noxious stimulation (pinch) is applied with an arterial clip. The response to heat stimulation is tested by a feedback controlled Peltier device that is lightly placed on the receptive field. The size of the thermal probe is 10 mm in diameter. The baseline temperature will be set at 30 °C. Neuronal responses to increasing temperatures between 37 °C and 51 °C will be recorded in increments of 2 °C. The stimulus duration is 10 s with an inter-stimulus interval of 30 s.

After drug application (local or intraperitoneal injection of certain concentration in certain volume), same series of mechanical and thermal tests will be repeated at several time points.

The SPIKE2 computer software program and CED 1401Plus, a multichannel data-acquisition system by Cambridge Electronic Design, are used to record the timing and amplitude of action potentials and to analyze data on- or off-line. The advantage of this program is that it can differentiate between action potentials of different dorsal root units by

amplitude and assign a colored and numbered template to each spike. Each channel can be duplicated to demonstrate various plotting modes simultaneously.

Data analysis

The stored digital record of unit activity is retrieved and analyzed off-line. For single-fiber recordings, responses to mechanical stimuli applied to the receptive field for 10 s will be calculated by subtracting the preceding 10 s of background activity to yield a net increase in discharge rate. Statistical significance was tested using one-way ANOVA followed by Dunn's test. A change was judged significant if $P < 0.05$. All values are presented as means \pm SE.

Groups

There will be only one group of animals per fiber group. Comparison will be within group, i.e., before and after drug administration. We can only inject one drug (possibly at ascending series of 3 doses) and measure repeatedly. Most importantly, we can get only one recording for each rat. Huge variation of responses among fibers would require larger sample size. Thirty would be our best-estimated number.