

1 **Tracking small sensory nerve action potentials in human**
2 **axonal excitability studies**

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31 **Highlights**

- 32 • Sensory axons are often more affected than motor in disease. However, the size and
33 latency of the corresponding action potential hampers studies of their properties using
34 threshold tracking.
- 35 • This paper addresses these difficulties, with techniques and an amplifier of simple
36 construction. We demonstrate the tracking of 2- μ V targets without excessive
37 averaging, and thereby increase the applicability of excitability studies.
- 38 • The ability to record sensory potentials in patients with a variety of neuropathic
39 conditions may benefit from the ability to record potentials as small as those recorded
40 here.

41

42 **Abbreviations**

43 CMAP, compound muscle action potential; CMR(R), common-mode rejection (ratio); CSAP,
44 compound sensory action potential; CTS, carpal tunnel syndrome; EMG, electromyogram;
45 IC, integrated circuit; IV, current-threshold relationship; NCS, nerve conduction study; QT,
46 charge-duration; RC, recovery cycle; TE, threshold electrotonus; SNR, signal-to-noise ratio;
47 SR, Stimulus-response.

48

49 **Abstract**

50 **Background:** Excitability studies on normal and diseased human axons *in vivo* have been
51 greatly enhanced by fast non-invasive threshold-tracking techniques, using surface
52 stimulation and recording. Although sensory axons are often more affected in disease, most
53 studies to date have focussed on motor axons, because of technical difficulties in resolving
54 pathologically small nerve volleys in the presence of noise and stimulus artefact.

55 **New Methods:** This paper describes techniques for tracking low-amplitude compound
56 action potentials, using a battery-powered, isolated preamplifier of simple construction with
57 high common mode rejection (>125 dB [balanced inputs]) and low noise (<0.4 μ V referred to
58 inputs [shorted]).

59 **Results:** We demonstrate the preamplifier's capability by tracking targets as small as 2 μ V
60 for a full range of excitability measurements without the usual distortion due to residual
61 stimulus artefact and without the need for clamping, additional filtering or ensemble
62 averaging.

63 **Comparison with existing methods:** In practice, threshold-tracking studies have been
64 unable to study sensory axons when the maximal compound sensory action potential was less
65 than about 15 μ V. The techniques and amplifier in the present study allow measurements to
66 be made from nerve with maximal responses less than half that size, and we present three
67 recordings in patients with pathologically small nerve action potentials ≤ 7 μ V.

68 **Conclusions:** Based on measurements of stimulus artefact distortion, noise and the
69 performance in experiments, we conclude that the techniques described here will facilitate the
70 study of diseased axons for which the sensory potentials have high thresholds and may be
71 only a few microvolts in amplitude.

72 **1 Introduction**

73 Non-invasive threshold tracking techniques have been applied to the study of the underlying
74 mechanisms of a wide range of neuropathologies (reviewed in: Krarup and Moldovan, 2009;
75 Krishnan et al., 2009). As a rapid *in vivo* method, threshold tracking is able to examine the
76 action of a variety of drugs and toxins in human and animal subjects in real time (Kiernan et
77 al., 2005; Krishnan et al., 2005; Kuwabara and Misawa, 2008; Nodera and Rutkove, 2012;
78 Park et al., 2009).

79 In contrast to fixed stimulus studies, where the intensity of both conditioning and test stimuli
80 are set and then kept constant, threshold tracking tests the effect of a conditioning stimulus by
81 automatically adjusting the test stimulus intensity to maintain a response at a pre-determined
82 fraction of the maximal compound action potential. This target response is commonly 40-
83 50% of maximum because this lies on the steepest part of the stimulus-response curve.

84 Even in subjects with only a few surviving motor units, the size and latency of the compound
85 muscle action potential (CMAP) may be sufficient to allow a clean and artefact-free
86 recording. In the study by Kiernan and colleagues (2000) which introduced the technique for
87 the rapid measurement of multiple measures of axonal excitability, and established normative
88 data for clinical testing, the maximal CMAP of thenar muscles to the median nerve
89 stimulation was, on average, 8.4 mV (baseline to peak) and, in a recent report of nerve
90 conduction studies on a large cohort of healthy individuals, it was 9.1 mV with a latency of
91 onset of ~3.6 ms (Benatar et al., 2009).

92 In contrast excitability studies on sensory axons are technically more challenging, primarily
93 because they depend on nerve action potentials which are much smaller and have shorter
94 latencies (Krarup, 2004). The compound sensory action potential (CSAP) is measured in

95 microvolts and recorded antidromically from the surface with lower limits in relatively young
96 subjects of 26 μV (Kiernan et al., 2001) and 18.1 μV (Howells et al., 2012), when measured
97 peak to peak. However, in 100 normal subjects, Benatar and colleagues (2009) reported the
98 maximal median sensory CSAPs of 3.1 to 86 μV , dependent on age, though the measurement
99 was baseline to peak. In threshold tracking studies, the target potential is submaximal,
100 typically 40 – 50% of the maximal CSAP, so that there may be an inadequate signal-to-noise
101 ratio, in normal subjects, particularly the elderly.

102 Currently there is no commercially available system for electromyography and nerve
103 conduction studies (EMG/NCS) with a real time analogue output suitable for tracking the
104 threshold of sensory axons.

105 In order to track small CSAPs efficiently, the preamplifier requires low noise and high
106 common mode rejection. In addition, for small CSAPs close to the noise level, the threshold
107 tracking strategy needs to take account of the fact that a ‘response’ may be due to noise alone.
108 This paper shows how a preamplifier of simple construction can be used to track microvolt
109 potentials just above the noise floor established by the recording electrodes, without
110 significant intrusion of the stimulus artefact into the recording. We show how it is possible to
111 study selectively the most excitable sensory axons, well below previously recorded
112 measurements.

113 **2 Methods**

114 To assess the feasibility of recording axonal excitability with small CSAPs, 14 experiments
115 were performed on three healthy subjects and three subjects with neuropathies resulting in
116 small CSAPs. Recordings of low amplitude targets ($\sim 4 \mu\text{V}$) were performed on one subject

117 on five separate occasions. All studies were performed with the approval of The University of
118 Sydney's Human Research Ethics Committee, and in conformity with the *Declaration of*
119 *Helsinki*.

120 **2.1 Preamplifier**

121 **2.1.1 Design**

122 The preamplifier used in this study is illustrated in the Fig. 1 and broadly consists of an input
123 and output stage galvanically separated by an isolation amplifier (ISO124, Burr Brown). The
124 instrumentation amplifier IC (AD8221BR) has exceptional common mode rejection (CMR)
125 and low noise characteristics and the inputs are protected against 1 kV human body model
126 electrostatic discharge (AD8221 datasheet, Analog Devices). High-pass filtering of electrode
127 offsets is achieved by bootstrapping the output by tying the instrumentation amplifier
128 reference to the low-passed output, thereby preserving the high common mode rejection.
129 Universal active filters (UAF42, Texas Instruments) are used to simplify the filter design; the
130 first UAF is configured as a two-pole high-pass (0.2 Hz) Butterworth filter which also
131 provides the low-pass reference signal for the instrumentation amplifier. The second UAF is a
132 two-pole high-pass (2 Hz) Butterworth filter to remove motion artefact. The final pair of
133 UAFs is configured as a four-pole low-pass (2 kHz) Bessel filter, which has superior step
134 response characteristics and is positioned so as to additionally remove the ripple due to the
135 modulation/demodulation carrier frequency of the isolation amplifier. The instrumentation
136 amplifier is configured with a fixed gain of 250 which is suitable for amplification of CMAPs
137 while still achieving good CMR for sensory potentials. Additional gain (x40) for CSAPs is
138 achieved using the auxiliary amplifier of the second UAF. The input and output stages are
139 each powered by a pair of 9-volt (PP3) batteries, and the integrated circuits (ICs) are placed

140 so that the current drain is balanced on either side of the isolation. The preamplifier was
141 easily constructed on a single-sided circuit board (100x100 mm) with socketable ICs (apart
142 from the surface mount instrumentation amplifier) and through-hole passive components.

143 **2.1.2 Bench tests**

144 The frequency response was tested by applying a 0.5-mV sinusoidal signal to the
145 preamplifier's inputs and measuring the peak response as a function of frequency between 0.2
146 Hz and 10 kHz.

147 The common mode rejection between 10 Hz and 10 kHz was determined by measuring the
148 amplitude of a sinusoidal signal between the preamplifier's inputs (differential-mode, A_{diff})
149 required to give the same output response as a large sinusoidal signal at both inputs
150 (common-mode, A_{cm}). The common mode rejection ratio (CMRR) is calculated as: $CMRR =$
151 $-20 * \log_{10}(A_{diff}/A_{cm})$.

152 **2.2 Electrodes and skin preparation**

153 Skin impedance was lowered at both the recording and stimulation sites by abrasion of the
154 skin surface (3M Trace Prep, 3M, Ontario, Canada), followed by cleaning with an isopropyl
155 alcohol swab. Sensory potentials were first recorded antidromically from digit 2 using
156 disposable Ag/AgCl ring electrodes (RE-D, The Electrode Store, Enumclaw, WA, USA),
157 with two ring electrodes 4 cm apart (Eduardo and Burke, 1988), with the more proximal
158 electrode around the proximal phalanx. The cathode, anode and ground electrodes were of the
159 disposable Ag/AgCl ECG type (1010M & 4620M, Unilect, Unomedical, Stonehouse, UK).
160 The ground electrode was placed on the dorsal aspect of the hand. The cathode was located at
161 the wrist crease with the anode 10 cm proximally, towards the radial edge of the forearm. The

162 location of the cathode was adjusted to obtain the minimum threshold current so reducing
163 further the stimulus artefact.

164 **2.3 Excitability testing**

165 Axonal excitability was tested with the QtracS threshold tracking software (© Prof Hugh
166 Bostock, Institute of Neurology, UCL, UK) using a stimulus-response paradigm which
167 controlled both the stimulation and recording through a 16-bit multifunction data acquisition
168 device (NI USB-6251).

169 The stimuli were delivered by an isolated bipolar constant current stimulator (DS5, Digitimer,
170 Welwyn Garden City, UK) which followed a real-time command voltage which was
171 synchronised with the recording sweep. The compound action potentials were amplified by
172 the preamplifier (gain x10k) and bandpass filtered (2 Hz to 2 kHz). The amplified waveform
173 had any residual mains frequency noise removed with a HumBug 50/60 Hz noise eliminator
174 (Quest Scientific, North Vancouver, BC, CA) before being digitised at a sampling frequency
175 of 10 kHz.

176 As described in previous studies (Bostock et al., 1998; Howells et al., 2012; Kiernan et al.,
177 2000; Kiernan et al., 2001; Tomlinson et al., 2010) the **TROND** protocol cycles through five
178 measures of axonal excitability: stimulus-response (SR) curve, strength-duration relationship
179 (plotted as charge-duration [QT]), threshold electrotonus (TE), current-threshold (IV)
180 relationship and a recovery cycle (RC).

181 **2.4 Tracking small, noisy action potentials**

182 For most of the CSAPs recorded, the maximal CSAP and the signal to noise ratio (SNR) were
183 sufficient to allow “proportional tracking” (as in the usual TROND protocol; Kiernan et al.,

184 2000; Kiernan et al., 2001). Proportional tracking adjusts the stimulus step size in proportion
185 to the error between the actual and target responses, allowing fast tracking when the stimulus
186 conditions change rapidly and finer tracking control of the stimulus for slow events. For the
187 smallest CSAPs and noisier recordings, a different tracking strategy was adopted, since the
188 lower SNR meant that proportional tracking would have resulted in excessive oscillations
189 about the target response. A more conservative approach was taken where the basic stimulus
190 step size was a fixed percentage (Δ) of the current stimulus. If successive responses fell on
191 the same side of the target then, the step size was increased by Δ , 2Δ , 3Δ and $4\Delta\%$ of the
192 current stimulus until the target was met (and vice versa). On the other hand, when successive
193 responses fell on opposite sides of the target, the step size was halved until it returned to the
194 basic step size of Δ . This method is referred to as ‘arithmetic/geometric tracking’.

195 For low SNR recordings the peak-to-peak measurement accuracy of the biphasic CSAPs was
196 improved by requiring the positive peak to precede the negative peak (i.e. peak[+]-to-peak[-
197]).

198 **2.5 Post digitisation signal conditioning**

199 Two passes of a 1-2-1 binomial smoothing filter (Marchand and Marmet, 1983) were applied
200 to the digitised data in order to remove high-frequency noise acquired after amplification or
201 as a result of the digitisation process. The transfer function for this low-pass Gaussian filter is

202 $H(f) = \cos^4\left(\frac{\pi f}{2 f_N}\right)$, where f_N is the Nyquist frequency. Initial observations of the digitised

203 waveforms showed that the stimulus artefacts were small and the return to baseline was
204 nearly complete before the onset of the CSAP. As such, previously required “clamping”
205 techniques were unnecessary, and the measurement window could be shortened (see
206 Discussion).

207 **3 Results**

208 The circuit diagram of the amplifier is shown in Fig. 1A.

209 **3.1 Amplifier performance**

210 With the preamplifier's inputs shorted and the gain set to x10k the output of the preamplifier
211 was measured with a true RMS multimeter (34401A, Agilent) to be 3.85 mV, or 0.385 μ V
212 when referred to the amplifier's inputs (RTI). There was no appreciable attenuation in the
213 frequency response of the amplifier between 5 and 1000 Hz (Fig. 1B). Beyond 5 kHz the
214 filter roll-off was 22.3 dB/octave. The high-pass filter has an attenuation of 11 dB/octave
215 below 1 Hz but steepens to 14.9 dB below 0.5 Hz due to the ac-coupling arrangement. The
216 equivalent 'noise bandwidth' was calculated from the frequency response (Fig. 1B) and was
217 2.4 kHz which gives a noise density of 7.9 nV/ $\sqrt{\text{Hz}}$ (RTI), in close agreement with the figure
218 in the manufacturer's datasheet.

219 With balanced inputs the common mode rejection was greater than 136 dB below 500 Hz and
220 had a minimum value of 125 dB (Fig. 1C) over the bandwidth of the amplifier (2 kHz).

221 **3.2 Recovery from stimulus artefact during CSAP recruitment**

222 Figure 2 illustrates the recovery from stimulus artefact during the recruitment of sensory
223 fibres by gradually increasing stimulus strengths. Subject #1 had higher recruitment
224 thresholds (right column) and from the greater stimulus artefact and background noise, most
225 probably a greater skin surface-electrode impedance. The peak deflections due to stimulus
226 artefact were 8.5, 5.2 and 2.2 μ V for Subjects #1, 2 and 3 respectively. In all three recordings
227 the stimulus artefact had decayed to a negligible value before the CSAP onset.

228 **3.3 Signal-to-noise measurements *in vivo***

229 Two passes of the binomial smoothing filter resulted in an additional attenuation of high
230 frequency noise (post-digitisation) with a steep roll off and a -3 dB point at 4.4 kHz (Fig. 1B,
231 open circles).

232 With the lowest amplitude targets, 10% of CSAP and 2 μV , a measure of the equivalent noise
233 was obtained in one subject (Fig. 3) by sampling the waveform in a window of the same
234 width, but prior to the stimulus (arbitrarily 50 ms earlier than the peak measurement). The
235 noise measured this way was $0.8 \pm 0.5 \mu\text{V}$ (mean \pm SD; closed circles in Fig. 3), not
236 surprisingly the same for both the 10% ($\sim 4 \mu\text{V}$) and 2- μV targets. The standard deviations
237 for the peak measurements were slightly higher (2 μV , 0.55 μV ; 10%, 0.6 μV) reflecting the
238 additional variation in amplitude in response to a tracking stimulus, which is, of necessity,
239 not constant. The vertical bars in Fig. 3 show one standard deviation from the mean
240 measurements. The 10% target and noise are separated by approximately three standard
241 deviations from their means, while the 2- μV target and noise are separated by a little more
242 than one standard deviation.

243 The signal-to-noise ratio (SNR) was calculated from these data using the formula:

244
$$SNR = \left(\frac{RMS_{peak}}{RMS_{noise}} \right)^2$$
 giving 19.2 and 5.0 for the 10% and 2- μV targets respectively.

245 **3.4 Threshold tracking small sensory action potentials**

246 **3.4.1 Healthy subjects**

247 We found that the smallest practical limit of CSAP size that could be tracked without
248 averaging was 2 μV (except for RC; see Legend to Fig. 4 and Discussion). Using
249 ‘arithmetic/geometric’ tracking of peak[+]-to-peak[-] amplitudes (see Methods), it was

250 possible to obtain all measures of axonal excitability for the 2- μ V target in less than 32 min
251 (Fig. 4 [Subject #3], see also Fig. 3B [Subject #2]). The measures from recordings in the
252 three subjects are shown in Fig. 5 (filled circles), and compared with conventional recordings
253 tracking responses 40-50% of maximal in the same subjects (open circles).

254 These recordings suggest that the low-threshold afferents have a greater inward rectification
255 much as in motor axons (Shibuta et al., 2010; Trevillion et al., 2010). However, unlike motor
256 axons there is a suggestion of a relatively depolarized resting membrane potential in these
257 low-threshold sensory axons.

258 **3.4.2 Neuropathy**

259 Threshold tracking recordings were made in three subjects with neuropathies that resulted in
260 small CSAPs (Kennedy's disease, amyloid neuropathy and chronic inflammatory
261 demyelinating polyneuropathy [CIDP]) as shown in Fig. 6. The maximal CSAPs were:
262 Kennedy's disease, 5.2 μ V; amyloid neuropathy, 6.5 μ V; and CIDP, 7 μ V, and the tracked
263 target CSAPS were 2.1, 2.6, and 2.8 μ V, respectively. Recordings of such low-amplitude
264 sensory potentials were not previously possible without averaging many sweeps.

265 **3.5 Repeatability of low target recordings**

266 Recordings of the 10% target ($4.1 \pm 0.3 \mu$ V) were made in the same subject on five different
267 occasions and are shown in Fig. 7. In the charge-duration plot (QT, Fig 7A) the slope of the
268 lines represents rheobase, and not surprisingly it differs between the individual recordings
269 with differing application of stimulating electrodes. The strength-duration time constant on
270 the other hand was consistent between the recordings ($721 \pm 22 \mu$ s), but longer than the value
271 reported by Kiernan and colleagues (2001) of 527 μ s, which was obtained at a target level of

272 40%. Reproducibility of the other excitability recordings was very good, so that the 5 lines
273 for the 5 different recordings can seldom be distinguished.

274 Variability in the recovery cycle recordings is partly due to a reduction in the SNR inherent in
275 the recording technique for the recovery cycle. The response to the supramaximal
276 conditioning stimulus is first subtracted from the conditioned test response before the peak
277 measurement is made, thereby increasing the noise contribution by $\sqrt{2}$, halving the SNR.

278 **4 Discussion**

279 This paper tackles the difficulty in obtaining reliable measures of the excitability properties
280 of cutaneous sensory axons of low threshold. It is with diseased nerves with pathologically
281 small CSAPs and high thresholds that the true usefulness of the techniques described here
282 may be realised, and the described amplifier has now been used in hundreds of recordings
283 and many of them involving pathologically small CSAPs. The discussion will focus on the
284 optimal recording conditions for small CSAPs, the advantages of the amplifier design, and
285 the physiological and clinical need for better recording techniques.

286 **4.1 Axonal excitability close to the electrode noise floor**

287 *Synchronous mains frequency noise* is to some extent always present in raw biosignals,
288 particularly those acquired from the surface. Notch filters distort action potential
289 measurements and in any case they do not remove the higher harmonics. In-line reduction of
290 mains frequency noise can be achieved in hardware by a device such as the HumBug
291 50/60Hz noise eliminator, or in software by sampling a stimulus free portion of the recording
292 to remove a mains-locked waveform from the response.

293 *Asynchronous noise* due to the impedance of the electrode and skin-electrode interfaces or
294 noise due to the amplification system itself all impact on the signal-to-noise ratio. The
295 amplitude of both synchronous and asynchronous noise can be attenuated by careful skin
296 surface preparation at the recording site and an amplifier with both high common mode
297 rejection and low noise.

298 Because the *stimulus artefact tail* frequently intrudes into the CSAP, so that it rides on the
299 decaying artefact, the standard *TROND* protocol contains a correction for a sloping baseline
300 in CSAPs by “clamping” the digitised data during the measurement window. The sloping
301 baseline is removed by subtracting a straight line that passes through the start and finish of
302 the measurement window at amplitudes corresponding to the mean of the ten samples
303 recorded on either side of the measurement window. This type of clamping assumes that the
304 artefact at the time of the response can be represented as a straight line, whereas it is usually
305 more exponential than linear, so that clamping introduces a systematic and nonlinear
306 distortion of the peak-to-peak measurement. In addition, movement of the CSAP within the
307 measurement window due to changes in its latency alters the amount of distortion. The
308 degree of nonlinearity of the stimulus artefact decay during the measurement window also
309 determines the minimum observable action potential.

310 Alternatives for removing the stimulus artefact after acquisition include artefact subtraction
311 and localised fitting of the baseline; however for threshold tracking of small amplitude
312 potentials these methods have their drawbacks. Artefact subtraction involves the delivery of a
313 fractional stimulus which is subthreshold; the response is then scaled back up to achieve the
314 stimulus artefact alone. Artefact subtraction can be an effective tool for reducing the stimulus
315 artefact (Trevillion et al., 2010); however it doubles the recording time and halves the SNR.
316 Further, because the artefact does not scale linearly with stimulus intensity, the artefact

317 removal is not complete. Another technique is to fit a curve to the decay of the stimulus
318 artefact (Wagenaar and Potter, 2002). Axonal excitability studies, however, involve
319 conditioning the test stimulus with sub- and supra-threshold pulses, in which the conditioning
320 artefact or response may extend beyond the test stimulus.

321 The *arithmetic/geometric tracking* applied to the 2- μ V potentials in this study, was slower at
322 tracking sharp transitions in stimulus conditions; on the other hand it was less susceptible to
323 noise and overall it resulted in faster tracking of low signal-to-noise-ratio potentials.

324 *Recovery cycle measurements* in sensory axons with low SNR were further improved with a
325 hybrid recording protocol. For intervals shorter than 30 ms (which allows for possible M-
326 responses and F-waves), the response to the supramaximal conditioning stimulus needs to be
327 subtracted from the conditioned test. To overcome the resultant halving of the SNR,
328 ensemble averaging of 4 responses was performed for these intervals only (see RC2 in Fig.
329 4).

330 **4.2 Conclusion**

331 Unlike nerve conduction studies which study the conduction of action potentials along a
332 segment of nerve, threshold tracking studies probe the excitability under the stimulating
333 cathode. The ability to track CSAPs as small as 2 μ V opens up the possibility of documenting
334 the properties of nerves and nerve branches not commonly studied, in addition to allowing
335 studies documenting the progression and response to treatment of patients with sensory
336 involvement in polyneuropathies such as kidney disease, (Krishnan et al., 2006), diabetes
337 (Krishnan et al., 2008) and chemotherapy (Park et al., 2011). It is likely that the amplifier will
338 be important in clinical trials.

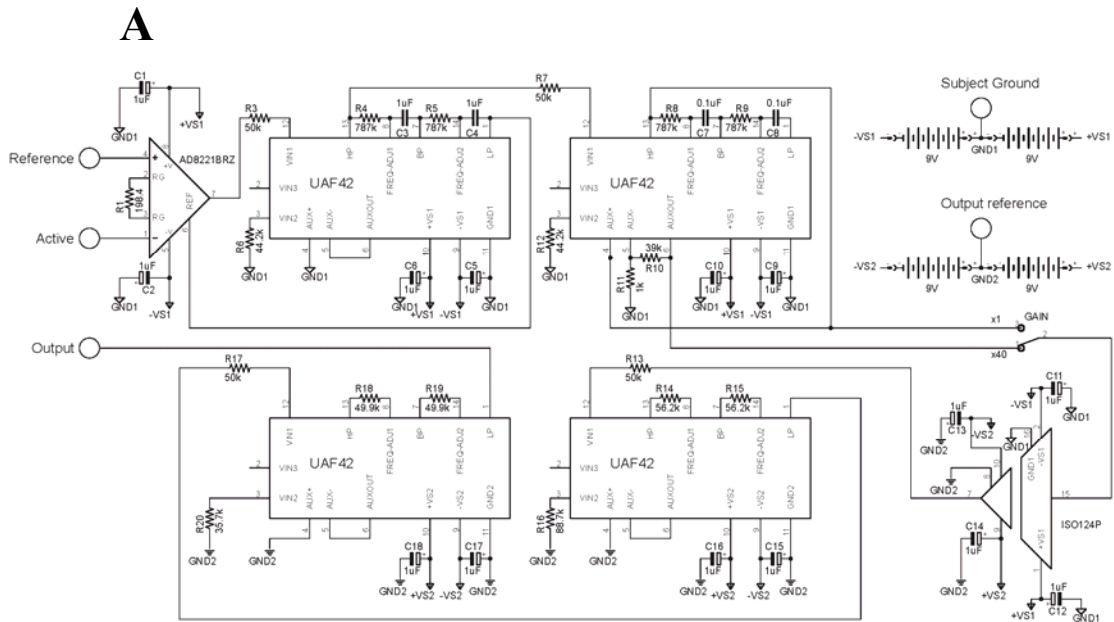
339 **Disclosures**

340 H.B. receives a share of the royalties for the sales of the Qtrac software.

341 **Acknowledgments**

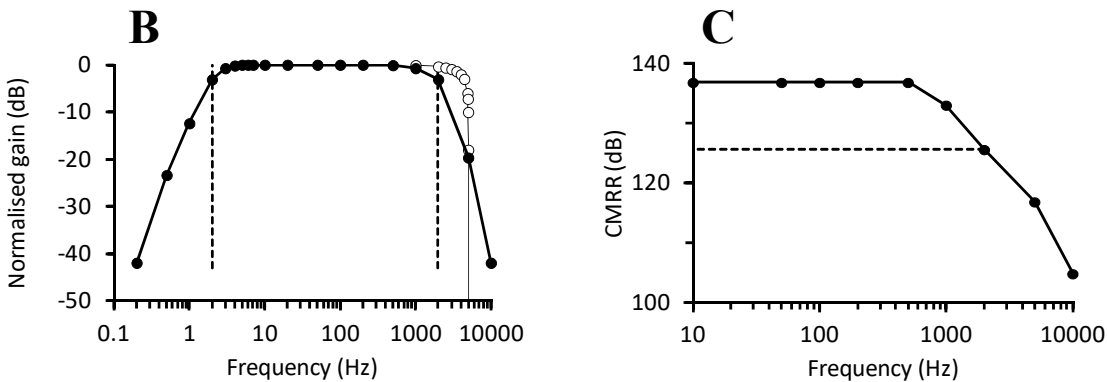
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Figure 1. Battery-powered isolated preamplifier for small sensory potentials.

351

A. Circuit schematic. The circuit is powered by two pairs of 9-volt batteries and the subject

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is galvanically isolated from the data acquisition system by the isolation amplifier (ISO124P).

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The subject ground is tied to the midpoint (GND1) of the pair of batteries on the subject's

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side of the isolation amplifier, whereas the amplifier's output is referenced to the midpoint

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(GND2) of the pair of batteries on the data acquisition side of the isolation amplifier. The

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instrumentation amplifier (AD8221BRZ) provides the critical first stage of amplification and

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is set to a gain of x250 with a 1% 200Ω resistor (matched as close as possible to 198.4Ω).

358

The first active filter chip decouples any dc-offset potentials. The second active filter is a 2-

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pole Butterworth high-pass filter ($f_c=2\text{Hz}$) for the removal of motion artefact. The 3rd and 4th

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active filter chips combined provide a 4-pole low-pass Bessel filter ($f_c=2\text{ kHz}$) to limit the

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measurement bandwidth. **B.** Normalised frequency response measured with a 0.5-mV sine

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wave. The gain is normalised to the maximal bandpass response. The dotted lines indicate the

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band-pass (2 Hz to 2 kHz). The open circles indicate the low-pass filter characteristics of two

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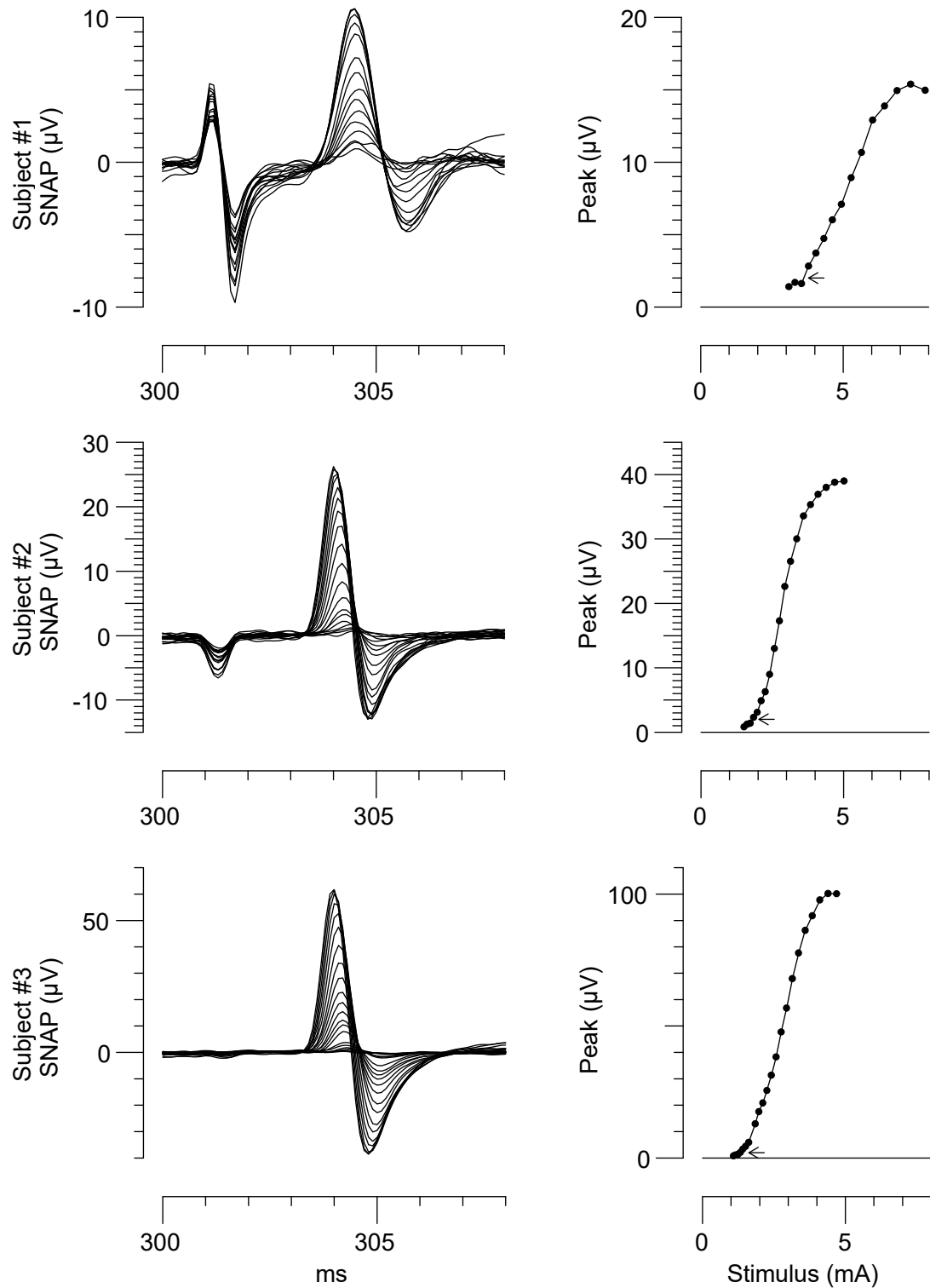
applications of a 1-2-1 Gaussian filter for data sampled at 10 kHz. **C.** Common mode

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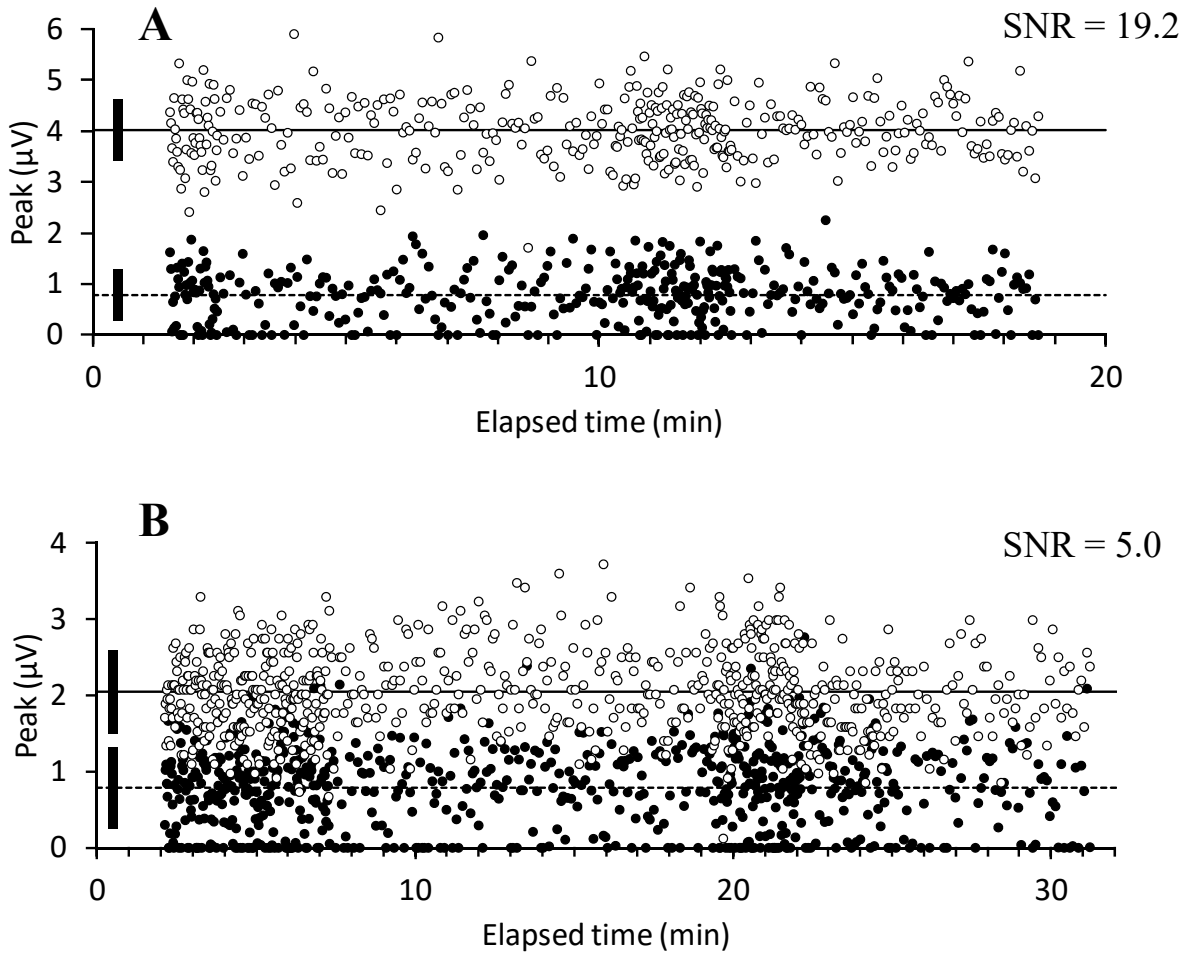
rejection vs. frequency. The dotted line corresponds to the -3dB frequency of the low pass

366

filter, and shows a minimal CMRR of 125 dB over the amplifier's bandwidth.



367
 368 **Figure 2: Recovery from stimulus artefact during CSAP recruitment. Left column.**
 369 Averaged CSAP response waveforms to six consecutive stimuli (0.5-ms wide) of
 370 progressively increasing intensity for three subjects. **Right column. Peak-to-peak**
 371 **measurements for each of the waveforms in the left column. The arrows indicate the 2- μV**
 372 **target for each subject.**



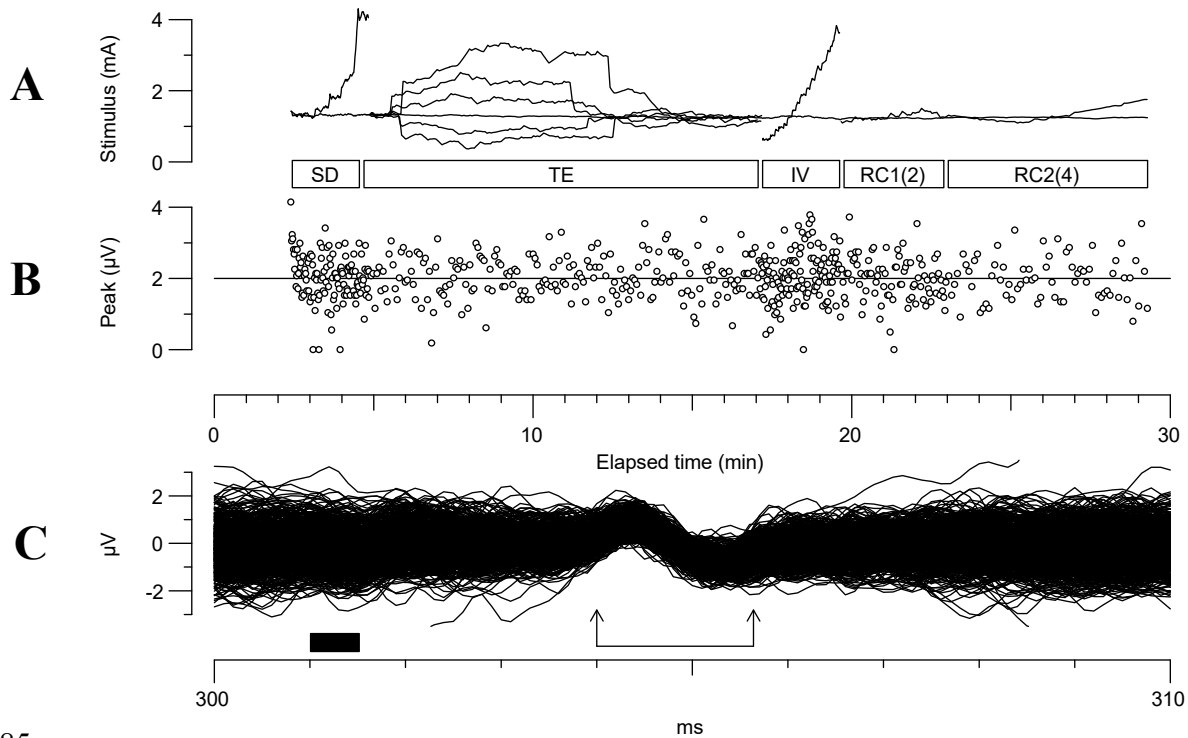
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374 **Figure 3: Signal-to-noise ratio (Subject #2 in Fig. 2).** The data represents the peak-to-peak
 375 amplitudes of the control channel (unconditioned test; open circles) and the equivalent noise
 376 (closed circles). The equivalent noise was measured in an earlier window which preceded the
 377 test stimulus. The solid lines indicate the mean amplitude and the dashed lines the mean
 378 noise. The vertical bars indicate one standard deviation from the mean. **A.** 10% target (~ 4
 379 μV) of maximal CSAP. **B.** Target 2 μV . Note that some of the variability in the test data
 380 (open circles) is because the stimulus was changing to track the threshold for the target
 381 CSAP.

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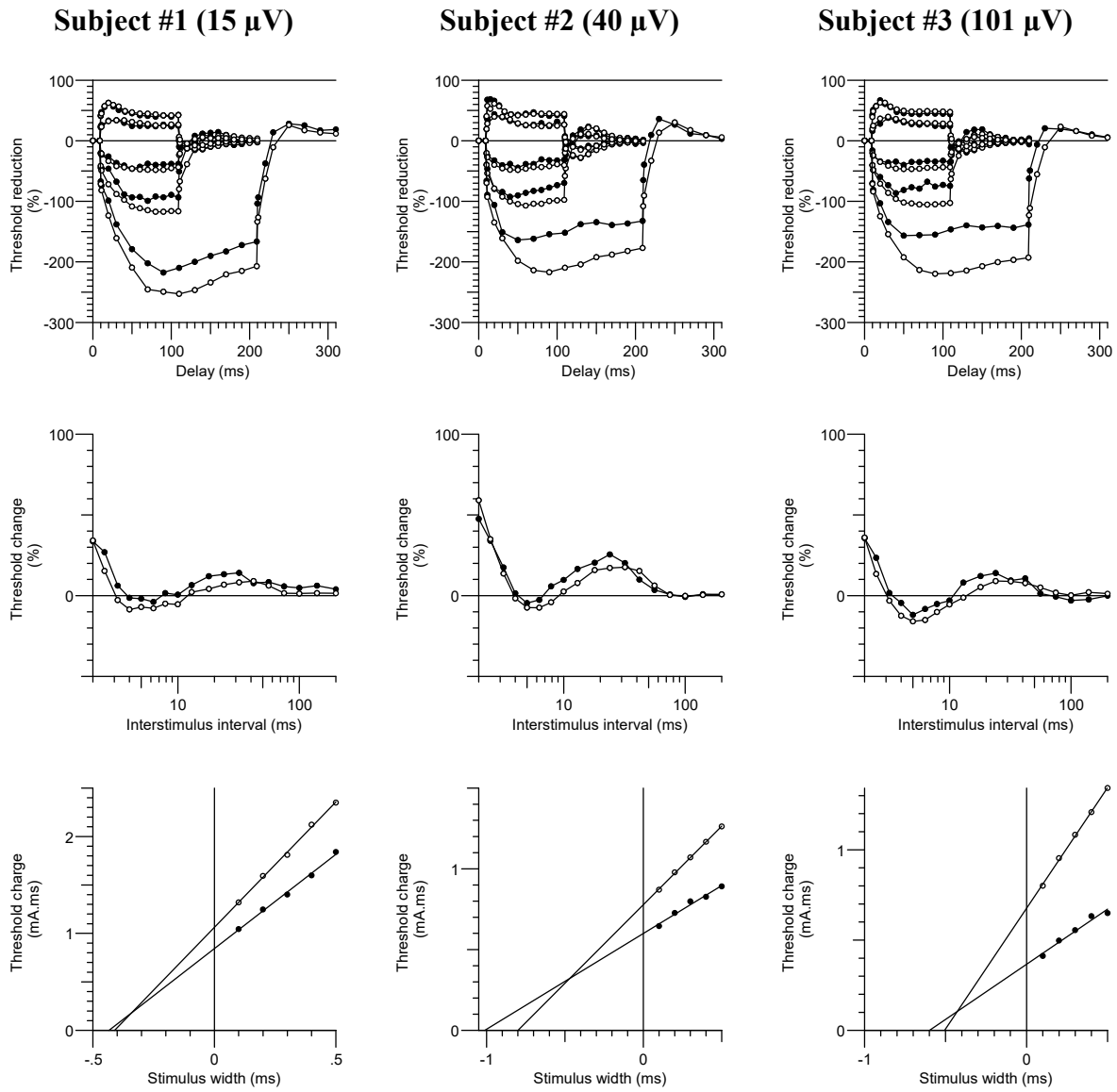
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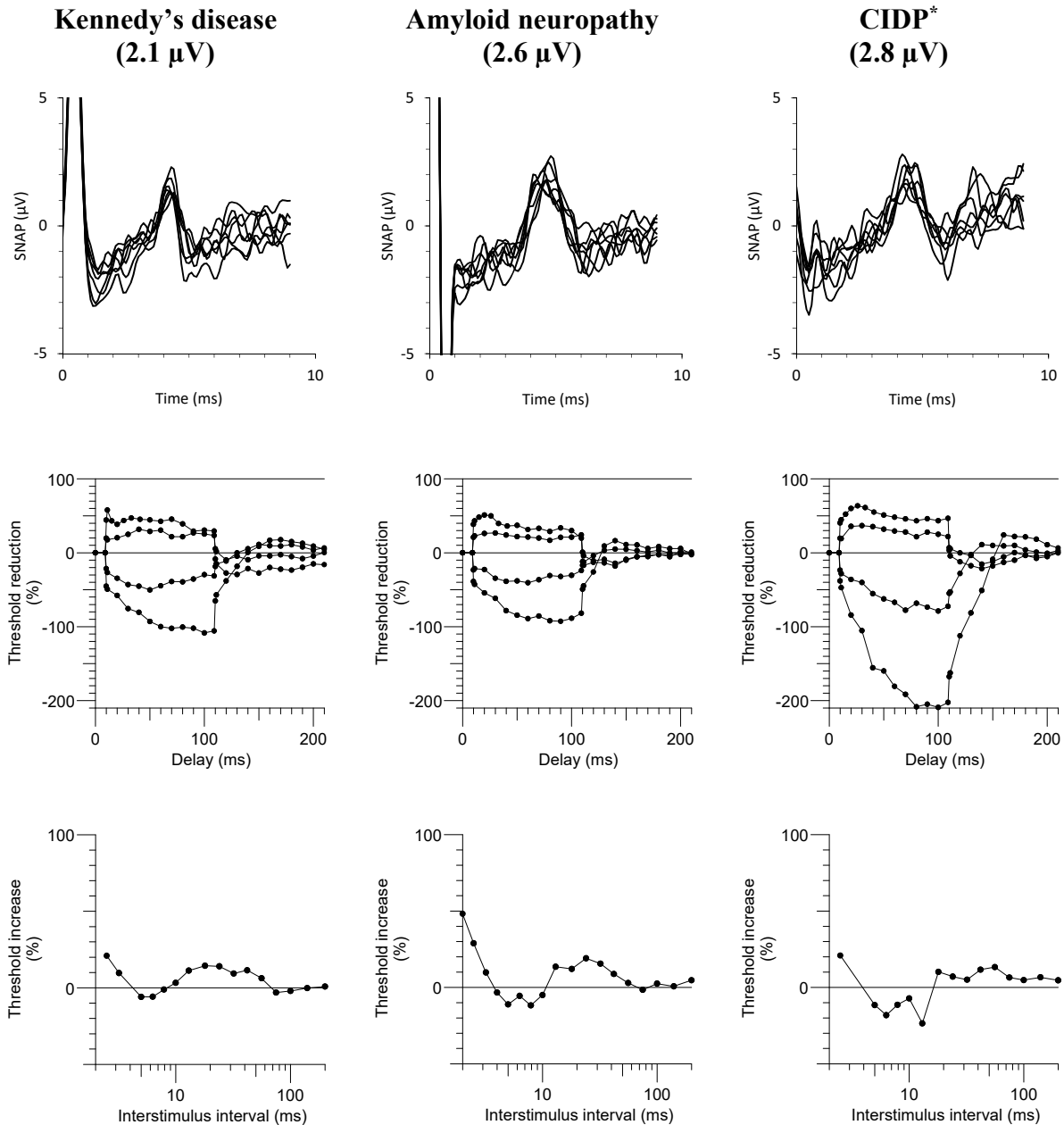
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387 **Figure 4:** Threshold tracking a 2 μV target potential (Subject #3). The various components
388 of the recording are marked below the data in A: SD, strength-duration relationship; TE,
389 threshold electrotonus; IV, current-threshold relationship; RC1, recovery cycle part 1 with 2
390 averages per measurement; RC2, recovery cycle part 2 with subtraction of the response to the
391 conditioning stimulus and with 4 averages per measurement. A. The control stimulus tracked
392 the 2- μV target with a virtually constant value of 1.3 mA for the entire recording. B. The
393 peak-to-peak amplitudes for the unconditioned test potential (2- μV target marked by
394 horizontal line). The variability of the CSAP is due to the intrinsic variability of the recording
395 and the variability of the tracking stimulus. C. Superimposed responses to the unconditioned
396 test stimulus (timing indicated by the solid bar) for the entire recording. Peak-to-peak
397 measurements of the response were made within the interval marked by the arrows, and
398 correspond to the values displayed in B.
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Figure 5: Excitability of low-threshold cutaneous afferents. The maximal CSAP is indicated after the subject. The filled circles denote the low target threshold ($2\ \mu\text{V}$) recordings, and the open circles the standard (40-50%) targets. **Extended threshold electrotonus (top row).** The reduction in threshold to conditioning currents of $\pm 20\%$ and $\pm 40\%$ and -70% of the target threshold. **Recovery cycle (middle row).** The recovery of excitability following a supramaximal stimulus. **Strength-duration properties (bottom row).** Plotted here as charge versus stimulus width. The slope represents rheobase, and not surprisingly is steeper for the standard targets. The absolute value of the X-intercept is the strength-duration time constant, which is longer for the $2\text{-}\mu\text{V}$ targets.



411

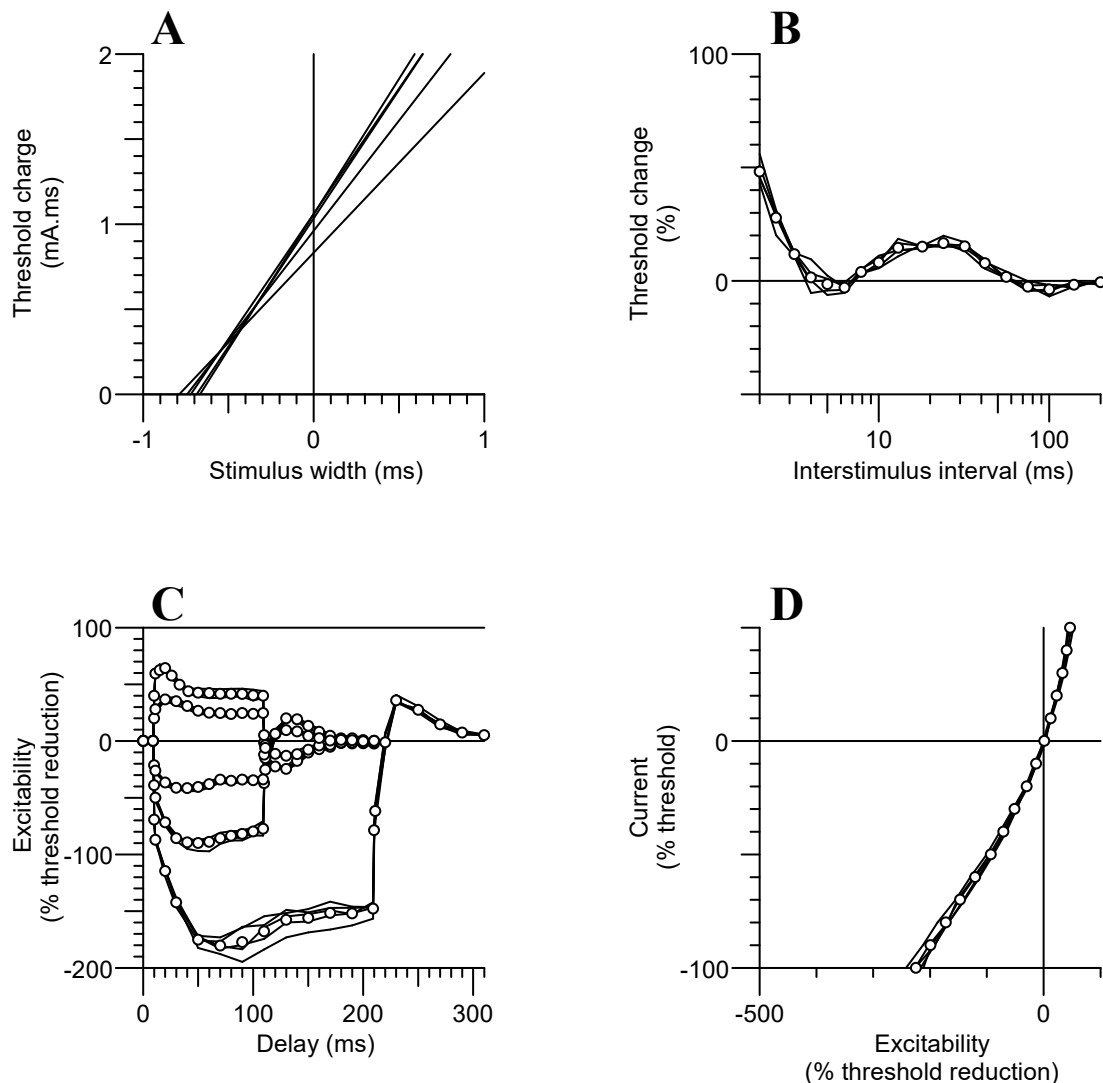
412 **Figure 6: Threshold tracking in patients with neuropathies and pathologically small**
 413 **maximal CSAPs.**

414 **Top row:** For each patient, 10 consecutive traces of the tracked 40% target potential are
 415 shown. The target potential is clearly distinguishable from the background noise.

416 **Middle row:** Threshold electrotonus waveforms for each of the neuropathic conditions. The
 417 upward deflection at the start of depolarizing threshold electrotonus is probably due
 418 inadvertent activation by the conditioning stimulus.

419 **Bottom row:** Recovery of excitability following activation by a supramaximal stimulus.

420 *CIDP = chronic inflammatory demyelinating polyneuropathy



422 **Figure 7: Repeatability of small target recordings (Subject #2).** Axonal excitability
 423 recordings in the same subject on five different occasions, tracking a 10% CSAP (4.1 ± 0.3
 424 μV). The open circles represent the mean data and the lines the recordings from different
 425 occasions. **A.** Charge-duration plot (QT) of strength-duration properties. The slope of the
 426 lines is rheobase, and the X-intercept magnitude corresponds to the strength-duration time
 427 constant (SDTC). **B.** The recovery cycle (RC) plots the recovery of an axon following a
 428 supramaximal discharge. **C.** Extended threshold electrotonus (TE) recording with
 429 conditioning levels of ± 20 , ± 40 and -70% of the unconditioned test threshold. **D.** Current-
 430 threshold (IV) relationship plots the threshold reduction versus current strength from $+50$ to $-$
 431 100% of threshold. *The mean data are not displayed for the QT plot (A), because that may*
 432 *lead to the erroneous interpretation of the X-intercept magnitude as the mean SDTC.*
 433

434 **Figure Legends**

435 **Figure 1. Battery-powered isolated preamplifier for small sensory potentials.**

436 **A. Circuit schematic.** The circuit is powered by two pairs of 9-volt batteries and the subject
437 is galvanically isolated from the data acquisition system by the isolation amplifier (ISO124P).
438 The subject ground is tied to the midpoint (GND1) of the pair of batteries on the subject's
439 side of the isolation amplifier, whereas the amplifier's output is referenced to the midpoint
440 (GND2) of the pair of batteries on the data acquisition side of the isolation amplifier. The
441 instrumentation amplifier (AD8221BRZ) provides the critical first stage of amplification and
442 is set to a gain of x250 with a 1% 200 Ω resistor (matched as close as possible to 198.4 Ω).
443 The first active filter chip decouples any dc-offset potentials. The second active filter is a 2-
444 pole Butterworth high-pass filter ($f_c=2\text{Hz}$) for the removal of motion artefact. The 3rd and
445 4th active filter chips combined provide a 4-pole low-pass Bessel filter ($f_c=2\text{ kHz}$) to limit
446 the measurement bandwidth. **B.** Normalised frequency response measured with a 0.5-mV sine
447 wave. The gain is normalised to the maximal bandpass response. The dotted lines indicate the
448 band-pass (2 Hz to 2 kHz). The open circles indicate the low-pass filter characteristics of two
449 applications of a 1-2-1 Gaussian filter for data sampled at 10 kHz. **C.** Common mode
450 rejection vs. frequency. The dotted line corresponds to the -3dB frequency of the low pass
451 filter, and shows a minimal CMRR of 125 dB over the amplifier's bandwidth.

452

453 **Figure 2: Recovery from stimulus artefact during CSAP recruitment. Left column.**

454 Averaged CSAP response waveforms to six consecutive stimuli (0.5-ms wide) of
455 progressively increasing intensity for three subjects. **Right column.** Peak-to-peak
456 measurements for each of the waveforms in the left column. The arrows indicate the 2- μV
457 target for each subject.

458

459 **Figure 3: Signal-to-noise ratio (Subject #2 in Fig. 2).** The data represents the peak-to-peak
460 amplitudes of the control channel (unconditioned test; open circles) and the equivalent noise
461 (closed circles). The equivalent noise was measured in an earlier window which preceded the
462 test stimulus. The solid lines indicate the mean amplitude and the dashed lines the mean
463 noise. The vertical bars indicate one standard deviation from the mean. **A.** 10% target (~ 4

464 μV) of maximal CSAP. **B.** Target 2 μV . Note that some of the variability in the test data
465 (open circles) is because the stimulus was changing to track the threshold for the target
466 CSAP.

467

468 **Figure 4: Threshold tracking a 2 μV target potential (Subject #3).** The various
469 components of the recording are marked below the data in **A:** SD, strength-duration
470 relationship; TE, threshold electrotonus; IV, current-threshold relationship; RC1, recovery
471 cycle part 1 with 2 averages per measurement; RC2, recovery cycle part 2 with subtraction of
472 the response to the conditioning stimulus and with 4 averages per measurement. **A.** The
473 control stimulus tracked the 2- μV target with a virtually constant value of 1.3 mA for the
474 entire recording. **B.** The peak-to-peak amplitudes for the unconditioned test potential (2- μV
475 target marked by horizontal line). The variability of the CSAP is due to the intrinsic
476 variability of the recording and the variability of the tracking stimulus. **C.** Superimposed
477 responses to the unconditioned test stimulus (timing indicated by the solid bar) for the entire
478 recording. Peak-to-peak measurements of the response were made within the interval marked
479 by the arrows, and correspond to the values displayed in **B.**

480

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483 recordings, and the open circles the standard (40-50%) targets. **Extended threshold**
484 **electrotonus (top row).** The reduction in threshold to conditioning currents of $\pm 20\%$ and
485 $\pm 40\%$ and -70% of the target threshold. **Recovery cycle (middle row).** The recovery of
486 excitability following a supramaximal stimulus. **Strength-duration properties (bottom**
487 **row).** Plotted here as charge versus stimulus width. The slope represents rheobase, and not
488 surprisingly is steeper for the standard targets. The absolute value of the X-intercept is the
489 strength-duration time constant, which is longer for the 2- μV targets.

490

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492 **maximal CSAPs.**

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499 *Chronic Inflammatory Demyelinating Polyneuropathy

500

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