The effect of repeated laser stimuli to ink-marked skin on skin temperature – recommendations for a safe experimental protocol in humans

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Background: Nd:YAP laser is widely used to investigate the nociceptive and pain systems, generating perpetual and laser-evoked neurophysiological responses. A major procedural concern for the use of Nd:YAP laser stimuli in experimental research is the risk of skin damage. The absorption of Nd:YAP laser stimuli is greater in darker skin, or in pale skin that has been darkened with ink, prompting some ethics boards to refuse approval to experimenters wishing to track stimulus location by marking the skin with ink. Some research questions, however, require laser stimuli to be delivered at particular locations or within particular zones, a requirement that is very difficult to achieve if marking the skin is not possible. We thoroughly searched the literature for experimental evidence and protocol recommendations for safe delivery of Nd:YAP laser stimuli over marked skin, but found nothing. Methods: We designed an experimental protocol to define safe parameters for the use of Nd:YAP laser stimuli over skin that has been marked with black dots, and used thermal imagine to assess the safety of the procedure at the forearm and the back. Results: Using thermal imaging and repeated laser stimulation to ink-marked skin, we demonstrated that skin temperature did not increase progressively across the course of the experiment, and that the small change in temperature seen at the forearm was reversed during the rest periods between blocks. Furthermore, no participant experienced skin damage due to the procedure. Conclusion: This protocol offers parameters for safe, confident and effective experimentation using repeated Nd:YAP laser on skin marked with ink, thus paving the way for investigations that depend on it.

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23 Introduction

24 Laser stimulation is an important tool for investigating the nociceptive and pain systems,

25 because it allows for the selective activation of nociceptive neurons with a brief, tightly

26 controlled stimulus. Although CO_2 lasers have been widely used, the skin discolouration

27 they produce (Lefaucheur et al. 2012) and difficulties with using them in small spaces and at

28 awkward angles have prompted a shift in favour of solid state lasers (e.g. Cruccu et al.

29 2003). Solid state lasers such as Neodymium Yttrium–Aluminium–Perovskite (Nd:YAP)

30 lasers may be preferable due to their greater flexibility: the machines tend to be less

31 cumbersome, and transmission of the laser through a flexible fibre-optic cable means that

32 these stimuli can be delivered inside an MRI tunnel (Perchet et al. 2008).

33 Many experimental protocols require repeated stimulus application (for example, to

34 measure thresholds, assess acuity, etc.). However, when laser is used for repetitive

35 stimulation there is a risk of progressive temperature increase if the same skin area is

36 heated too often.   Progressive increases in skin temperature over the course of a procedure

37 may (a) elevate the risk of skin damage and (b) compromise the reliability of the outcome

38 being studied – for example, perceptual acuity, perceptual intensity or ERP amplitude. This

39 risk may be elevated in people with chronic pain because there are emerging data that

40 suggest spatially defined dysfunction in thermoregulation and cortical stimulus processing in

41 these groups (e.g. Moseley et al. 2012a; Moseley et al. 2012b; Moseley et al. 2012c). To

42 reduce this risk, the location of the stimulus is usually shifted slightly between trials to

43 prevent heating of the same skin area in successive trials. This shifting of location is also

44 thought to reduce sensitisation or habituation to the stimulus (Iannetti et al. 2003; Wiech et

45 al. 2010).

46 This risk of temperature increase and consequent skin damage presents an obvious problem

47 because, to our knowledge, there are no published data outlining the number or frequency

48 of Nd:YAP laser stimuli that is safe. A further issue is the quandary presented when

49 researchers want to accurately localise stimulation sites: it is generally considered that

50 marking the skin with ink is problematic because darkened skin absorbs the radiant heat

51 more rapidly, exposing it to risk of damage (Leandri et al. 2006). One option would be to

52 mark the skin with white ink. However, pale markings are difficult to see on pale skin,

53 making this approach unsuitable for procedures in which an experimenter must quickly

54 localise stimulation sites. Furthermore, marking stimulation sites with a colour that is

55 difficult to see makes experimenter fatigue more likely, and could thus compromise

56 accuracy. Using black pens for skin markings is common practice in other experiments that

57 deliver stimuli to human skin and would be a convenient and sensible option for studies that

58 use laser stimuli, if such markings can be used without increasing skin temperature enough

59 to compromise reliability or cause skin damage.

60 An additional 'safety' check that is commonly used in studies involving painful stimuli is to

61 calibrate the stimulus intensity to individual participants according to pain rating (Mancini et

62 al. 2011; Wager et al. 2006; Weiss et al. 1997). This is a reasonable approach for preventing

63 undue suffering that could be caused by the experimental pain percept. However, pain is

64 influenced by a multitude of factors, including attention (Villemure & Bushnell 2002),

65 salience (Wiech et al. 2010), emotion (Wiech & Tracey 2009), task demands (Petrovic et al.

66 2000) and expectations (Atlas & Wager 2012). Consequently, stimulus calibration according

67 to pain percept may not be a useful strategy to ensure safety. The finding that people with

68 chronic pain have alterations in perceptual acuity (Wand et al. 2011) suggests that the

69 stimulus energy-percept mismatch may be even more pronounced in people with pain,

70 emphasising that experimental procedures for studying pain must be tested for safety in

71 those with and those without pain.

72 Here we present data from an experimental protocol in which multiple stimuli were applied

73 to the black-ink-marked skin of 15 people with chronic low back pain and 13 healthy control

74 participants, and thermal imaging was used to evaluate skin temperature before and after

75 stimulation blocks. We quantified the effect of repeated stimulation on skin temperature to

76 ascertain the safety of an inter-stimulus interval and block-rest-block protocol when the

77 locations of stimuli have been marked with black ink.

78 Methods

79 Participants

80 We recruited adult volunteers using flyers and word of mouth. Volunteers were not eligible

81 if they had sensation problems, diagnosed peripheral vascular disease, diabetes mellitus, or

82 psychological or neurological problems, or unusual skin conditions (e.g. dermographism)

83 that might compromise skin safety with laser application. Volunteers with skin too dark for

84   possible erythema to be noticed were also ineligible. Participants had to be pain-free or

85   have chronic back pain, defined as pain between the levels of T12 and S1 (with or without

86   concurrent leg pain)(Merskey & Bogduk 1994). Volunteers with back pain were excluded if

87   they had concurrent neck or arm pain, were pregnant or less than six months post-partum,

88   or had had spinal surgery. Participants were remunerated at AU$20/hr for their

89   involvement. All procedures conformed to the Helsinki Declaration and were approved by

90   the institutional ethics committee.

91   Experimental procedure

92   Participants received laser stimuli to the skin of the forearm and the back. Prior to

93   stimulation, the hair on the dorsal forearm and the back was trimmed using clippers, and a

94   template was used to draw two dot-grids onto the skin with a black Artline®200 FINE 0.4 pen.

95   Dots were spaced 4mm apart in the proximal-distal (forearm) or medial-lateral (back)

96   direction, and 5mm apart in the radial-ulnar (forearm) or cephalo-caudad (back) direction.

97   The forearm grid measured 48mm x 20mm; the back grid measured 80mm x 20mm (see

98   Figures 1 and 2).

99   Stimuli

100   Laser stimuli were delivered using an Nd:YAP laser (Deka: Stimul 1340, wavelength 1340 nm,

101   spot diameter measured at 3.5mm, pulse width 6ms), which has previously been shown to

102   activate Aδ nociceptors specifically (Iannetti et al. 2006). The intensity of laser stimulus for

103   each participant was established, on the basis of an ascending staircase procedure, before

104   testing began. The operator delivered laser stimuli of changing intensity, asking participants

105   to describe what they felt. Once a painful pinprick was elicited, participants rated the pain

106   intensity on a 0-10 numerical rating scale with anchors of "no pain" (0) and "worst pain ever

107   felt" (10). The operator aimed to identify the intensity that consistently elicited pinprick

108   pain of 2-3 out of 10, and this was used for the testing procedure. If a participant reported a

109   diminution or an increase of pain intensity during the procedure, the operator adjusted the

110   laser stimulus intensity accordingly, with a maximum permitted laser intensity of 2.0J. This

111   level was established during piloting as a limit that would prevent skin damage, and thus

112   became an additional exclusion criterion (if a laser stimulus intensity of 2.0J produced less

113   than 2/10 pinprick pain). Participants received stimuli in pairs. Three blocks of 14 stimulus

114   pairs were delivered to the forearm and three blocks of 16 pairs to the back, with at least 30

115   seconds between pairs and 3-8 minutes between blocks (See Figure 3). The interval

116   between pairs was selected to reduce perceptual habituation, and the break between

117   blocks was chosen to allow participants relief from the sustained concentration required by

118   the perceptual acuity task. Blocks were randomly ordered. Each stimulus was delivered

119   over a dot in the dot-grid. The two stimuli of each pair were delivered 4-44mm (at the

120   forearm) or 4-76mm (at the back) apart. This approach was part of a wider plan to evaluate

121   perceptual acuity at each location. It was impossible for any one location to be stimulated

122   twice without a break of at least 120 seconds. The operator monitored skin colour visually,

123   so as to detect any localised erythema that could indicate undue skin heating.

124   Outcome

125   We used infrared thermal imaging (FLIR SC620 camera, FLIR systems, Oregon, USA;

126   sensitivity <40 mK, field of view = 24 x 18°) to record the average skin temperatures within

127   the demarcated zones before and after each stimulation block. This camera produced an

128   image that is colour-coded by infrared radiation. The ThermaCAM Researcher Professional

129   software (version 2.9, FLIR systems, Oregon, USA) allows the user to delineate a certain area

130   and calculate absolute temperature parameters for that area. In this study, the demarcated

131   stimulation zone (forearm grid 48mm x 20mm; back grid 80mm x 20mm) was delineated

132   and the calculation was made as an average for that area. Skin condition was visually

133   monitored throughout the procedure.

134   Statistical Analysis

135   Temperature data were analysed using two analyses of variance (ANOVA): (1) temperatures

136   before and after each block were compared using repeated-measures ANOVA with Time

137   (before/after block) and Site (forearm/back) and Block (1/2/3) as within-subject factors, and

138   Group (patient/healthy) as the between-subject factor, and (2) forearm skin temperatures

139   before each block were compared using repeated-measures ANOVA with Block (1/2/3) as

140   the only factor. Planned comparisons were used to investigate significant effects, and

141   Bonferroni adjustments were applied to correct for multiple comparisons. Alpha was set at

142   0.05. Where the assumption of sphericity was violated, adjusted values are reported, with

143   degrees of freedom also adjusted accordingly.

Results

144

145 In this experiment, each participant received 84 stimuli at the forearm and 96 stimuli at the

146 back, delivered over a period of ~70 minutes.

147 We recruited 29 adult volunteers, one of whom was excluded for dermographism. Of the 28

148 adults tested, 15 of them had chronic low back pain (mean age ± SD = 43 ± 17 years; 8

149 female) and 13 were pain-free (mean age ± SD = 49 ± 14 years; 8 female).

150 Overall, skin temperature was greater at the back than at the forearm (main effect of Site,

151 $F(1,26) = 9.23$, $p = .005$). Skin temperature increased over each block (main effect of Time,

152 $F(1,26) = 19.45$, $p < .001$) and the extent of the increase was greater in the forearm than it

153 was in the back (Time x Site interaction, $F(1,26) = 30.028$, $p < .001$). Comparisons of the Time

154 x Site interaction showed that forearm skin temperature increased over a block ($p < .001$,

155 mean change ± SE = 0.40 ± 0.07°C, 95% CI 0.27 - 0.54) and that back skin temperature did

156 not change over a block ($p = .098$, mean change ± SD = -0.08 ± 0.05°C, 95% CI -0.02 – 0.18).

157 There was no main effect of Group ($p = 0.938$) or Block ($p = 0.626$) on skin temperature and

158 there were no other significant interactions.

159 Despite the small change in forearm temperature over each block, there was no difference

160 between skin temperatures at the start of each block (no effect of Block, $p = .248$),

161 indicating that the rest periods between blocks were sufficient for the forearm skin

162 temperature to return to baseline.

163 None of our participants had localised erythema sufficient to warrant termination of the

164 procedure, none had any visible indications of skin damage on completion of the

165 experiment, and none reported adverse effects afterwards.

Discussion

166

167 In this study, Nd:YAP laser stimuli were applied to the ink-marked skin of 28 participants

168 without resultant skin damage or lasting heating of the skin. No participant reported skin

169 damage due to the procedure. The thermal imaging data showed no overall increase in skin

170 temperature across the course of the experiment. Skin temperature at the back did not

171 change within blocks. Skin temperature at the forearm increased within each block, but

172 that increase was reversed during the rest periods between blocks such that skin

173 temperatures at the start of each forearm block did not differ.

174 These results demonstrate that it is possible to use Nd:YAP laser stimuli repeatedly over ink-

175 marked skin without inducing skin damage, provided that strict safety parameters are

176 observed.

177 There were four factors that ensured the safety of this design. The first factor was strict

178 exclusion criteria. We excluded participants with neurological or sensation problems, and

179 we ensured that skin tone was pale enough for the operator to detect any laser-induced

180 change in skin colour. Although Nd:YAP does not produce the same discoloured skin lesions

181 that CO_2 laser is known to, whether it produces lesions of deeper layers of the skin is

182 unknown (Iannetti et al. 2006). We therefore considered the visual monitoring of skin

183 colour a necessary precaution in this study, and planned to exclude any volunteer in whom

184 this would not be possible.

185 The second factor was the selection of a conservative upper limit to the intensity of laser to

186 be used in the experiment. This limit was based on pilot work in our lab, during which two

187 participants (both males with Fitzpatrick skin type III, 50 and 24 years old) developed minor

188 skin lesions after repeated application of stimuli over 2J, despite reporting less than 2/10

189 pain at such intensities. Those pilot subjects would not have been excluded on the basis of

190 the other exclusion criteria. We therefore recommend a conservative upper limit to the

191 intensity of laser to be used in an experiment, so as to identify and exclude participants who

192 may be at risk of skin burn during a procedure in which the laser stimulus intensity is

193 determined according to subjective ratings. Our results also indicate that calibration of

194 stimulus intensity according to pain report was not dangerous in this group of participants

195 with and without chronic back pain. Considering that people with chronic pain may display

196 a greater energy-percept mismatch than their healthy counterparts (Wand et al. 2011),

197 there is reason to expect that report-based calibration of stimulus intensity may be unsafe.

198 However, this study did not find evidence of skin damage with the stimuli used, which may

199 be attributable to the 2J safety limit on stimulus energy.

200 The third factor that ensured participants' safety was the use of break periods, which

201 allowed for recovery of skin temperature towards a baseline level between stimulation

202 blocks. The fourth factor that ensured the safety of this design was that the ink markings

203 used in this study were small dots made with a 0.4mm-diameter pen. As such, the

204 blackened skin area constituted a very small fraction of the area over which the laser

205 stimulus was delivered (mark diameter 0.4mm; beam diameter 3.5mm), probably resulting

206   in a differential heating effect over the area of surface exposed to the beam. Blackened skin

207   is expected to absorb Nd:YAP laser more superficially, leading to quicker absorption and

208   dissipation of heat, and more selective activation of Aδ fibres than would be expected in un-

209   blackened skin (Leandri et al. 2006). In this study, the rate at which skin temperature

210   recovered from laser stimulation was likely linked to the comparatively small area over

211   which the skin was blackened. Any future work based on this report will need to consider

212   these four factors in order to achieve equivalent safety.

213   Limitations

214   This report provides safe parameters for Nd:YAP laser stimulation using a 3.5mm spot

215   diameter. Further work would be required to determine safe parameters for other spot

216   sizes, because the area over which the stimulus is delivered affects the extent of skin

217   heating. If researchers require moment-by-moment information on skin temperature

218   changes, real-time thermal imaging will be necessary. We used imaging before and after

219   blocks, and are therefore unable to provide data on skin temperature between stimuli

220   within a block.

221   A reasonable concern about this report is that we did not personally reassess participants'

222   skin condition in the days following the procedure. It is possible that participants developed

223   delayed signs of skin damage, particularly considering that any strongly absorbent regions of

224   tissue could be subject to greater risk of heat-induced damage due to an unequal heating

225   effect. However, participants were explicitly asked to report any signs of skin damage, and

226   we received no such reports. Furthermore, the intensity limit of 2J was established on the

227   basis of pilot testing that showed no skin damage in pilot participants, according to visual

228   assessments made immediately after the procedure and in the following days. We are

229   therefore confident that the parameters presented here did not result in visible skin

230   damage in this group of 28 participants.

Conclusion

231

232   This procedure offers parameters for safe and effective experimentation using Nd:YAP laser

233   and black ink skin markings when delivering stimuli to the back and the forearm of healthy

234   participants and participants with chronic back pain.

235

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251 # References

252 Atlas LY, and Wager TD. 2012. How expectations shape pain. *Neuroscience Letters* 520:140-

253 148. http://dx.doi.org/10.1016/j.neulet.2012.03.039

254 Cruccu G, Pennisi E, Truini A, Iannetti GD, Romaniello A, Le Pera D, De Armas L, Leandri M,

255 Manfredi M, and Valeriani M. 2003. Unmyelinated trigeminal pathways as assessed by laser

256 stimuli in humans. *Brain* 126:2246-2256. 10.1093/brain/awg227

257 Iannetti GD, Truini A, Romaniello A, Galeotti F, Rizzo C, Manfredi M, and Cruccu G. 2003.

258 Evidence of a Specific Spinal Pathway for the Sense of Warmth in Humans. *J Neurophysiol*

259 89:562-570. 10.1152/jn.00393.2002

260 Iannetti GD, Zambreanu L, and Tracey I. 2006. Similar nociceptive afferents mediate

261 psychophysical and electrophysiological responses to heat stimulation of glabrous and hairy

262 skin in humans. *The Journal of Physiology* 577:235-248. 10.1113/jphysiol.2006.115675

263 Leandri M, Saturno M, Spadavecchia L, Iannetti GD, Cruccu G, and Truini A. 2006.

264 Measurement of skin temperature after infrared laser stimulation. *Clinical Neurophysiology*

265 36:207-218. http://dx.doi.org/10.1016/j.neucli.2006.08.004

266 Lefaucheur JP, Ahdab R, Ayache SS, Lefaucheur-Ménard I, Rouie D, Tebbal D, Neves DO, and

267 Ciampi de Andrade D. 2012. Pain-related evoked potentials: A comparative study between

268 electrical stimulation using a concentric planar electrode and laser stimulation using a CO2

269 laser. *Neurophysiologie Clinique/Clinical Neurophysiology* 42:199-206.

270 http://dx.doi.org/10.1016/j.neucli.2011.12.003

271 Mancini F, Longo MR, Iannetti GD, and Haggard P. 2011. A supramodal representation of the

272 body surface. *Neuropsychologia* 49:1194-1201.

273 http://dx.doi.org/10.1016/j.neuropsychologia.2010.12.040

274 Merskey H, and Bogduk N. 1994. *Classification of chronic pain: descriptions of chronic pain*

275 *syndromes and definitions of pain terms*: IASP press Seattle.

276 Moseley GL, Gallace A, and Iannetti GD. 2012a. Spatially defined modulation of skin

277 temperature and hand ownership of both hands in patients with unilateral complex regional

278 pain syndrome. *Brain* 135:3676-3686. 10.1093/brain/aws297

279 Moseley GL, Gallace A, and Spence C. 2012b. Bodily illusions in health and disease:

280 Physiological and clinical perspectives and the concept of a cortical 'body matrix'.

281 *Neuroscience & Biobehavioral Reviews* 36:34-46.

282 http://dx.doi.org/10.1016/j.neubiorev.2011.03.013

283 Moseley GL, Gallagher L, and Gallace A. 2012c. Neglect-like tactile dysfunction in chronic

284 back pain. *Neurology* 79:327-332. 10.1212/WNL.0b013e318260cba2

285 Perchet C, Godinho F, Mazza S, Frot M, Legrain V, Magnin M, and Garcia-Larrea L. 2008.

286 Evoked potentials to nociceptive stimuli delivered by CO2 or Nd:YAP lasers. *Clinical*

287 *Neurophysiology* 119:2615-2622. http://dx.doi.org/10.1016/j.clinph.2008.06.021

288 Petrovic P, Petersson KM, Ghatan PH, Stone-Elander S, and Ingvar M. 2000. Pain-related

289 cerebral activation is altered by a distracting cognitive task. *Pain* 85:19-30.

290 http://dx.doi.org/10.1016/S0304-3959(99)00232-8

291 Villemure C, and Bushnell MC. 2002. Cognitive modulation of pain: how do attention and

292 emotion influence pain processing? *Pain* 95:195-199.

293 Wager TD, Matre D, and Casey KL. 2006. Placebo effects in laser-evoked pain potentials.

294 *Brain, Behavior, and Immunity* 20:219-230. http://dx.doi.org/10.1016/j.bbi.2006.01.007

295 Wand BM, Parkitny L, O'Connell NE, Luomajoki H, McAuley JH, Thacker M, and Moseley GL.

296 2011. Cortical changes in chronic low back pain: Current state of the art and implications for

297 clinical practice. *Manual Therapy* 16:15-20. http://dx.doi.org/10.1016/j.math.2010.06.008

298 Weiss T, Kumpf K, Ehrhardt J, Gutberlet I, and Miltner WHR. 1997. A bioadaptive approach

299 for experimental pain research in humans using laser-evoked brain potentials. *Neuroscience*

300 *Letters* 227:95-98. http://dx.doi.org/10.1016/S0304-3940(97)00320-0

301 Wiech K, Lin C-s, Brodersen KH, Bingel U, Ploner M, and Tracey I. 2010. Anterior Insula

302 Integrates Information about Salience into Perceptual Decisions about Pain. *The Journal of*

303 *Neuroscience* 30:16324-16331. 10.1523/jneurosci.2087-10.2010

304 Wiech K, and Tracey I. 2009. The influence of negative emotions on pain: Behavioral effects

305 and neural mechanisms. *NeuroImage* 47:987-994.

306 http://dx.doi.org/10.1016/j.neuroimage.2009.05.059

307

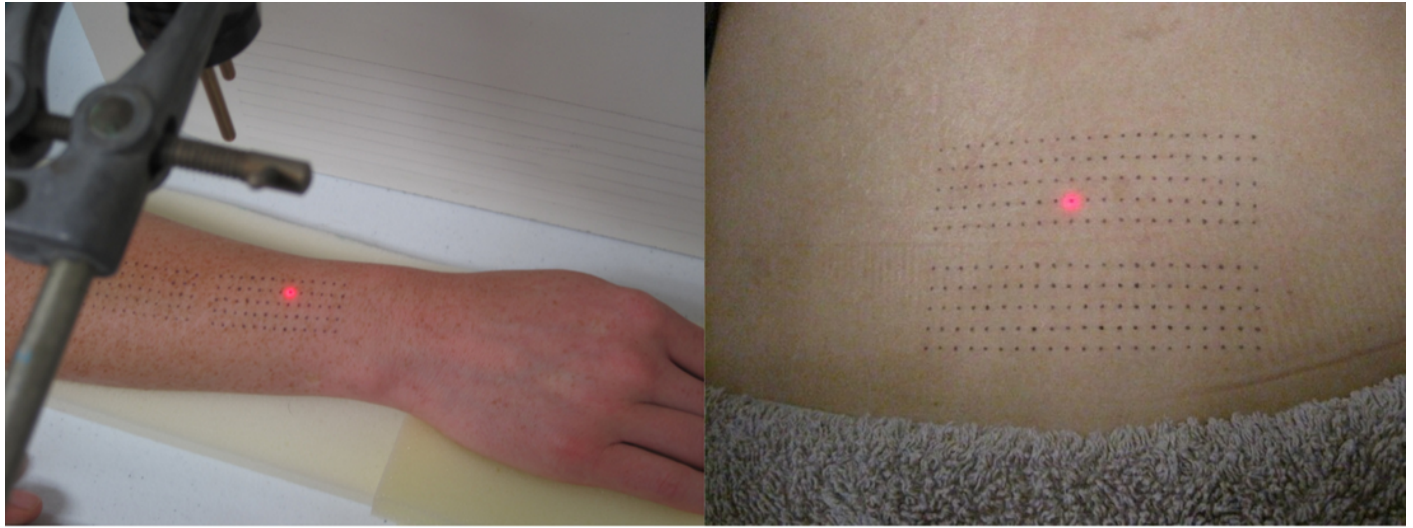
Figure 1: Dot-grids as drawn onto forearm (a) and back (b). Two dot-grids are pictured at each site: the second was used for tactile stimulation (data not presented here).

2

Diagram depicting experimental procedure and main results. CLBP: people with chronic low back pain.

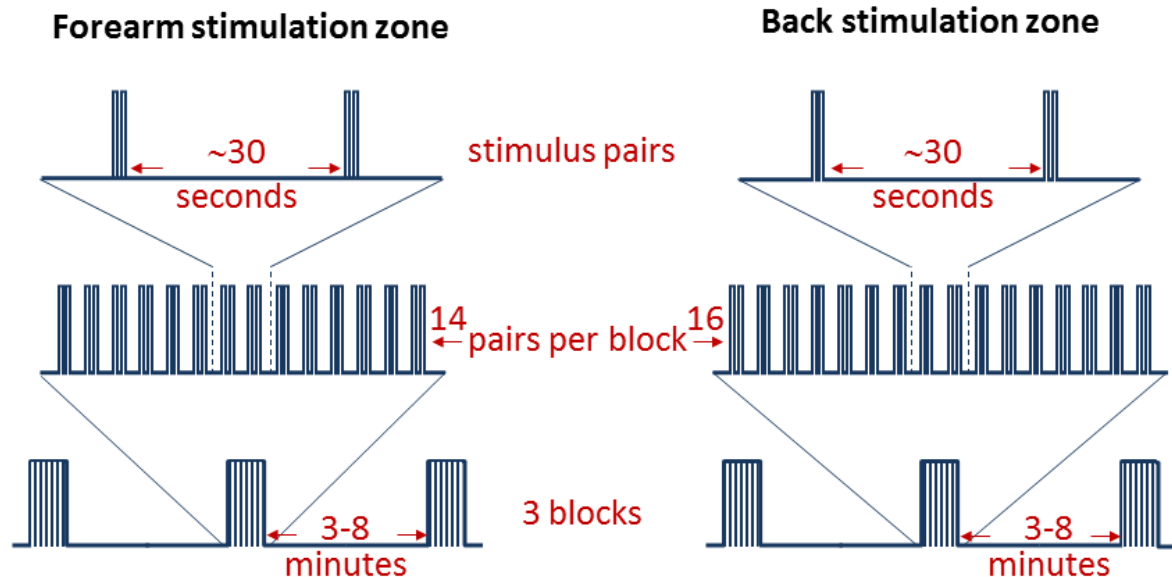
Procedure

Forearm stimulation zone

Back stimulation zone

~30 seconds — stimulus pairs — ~30 seconds

14 ← pairs per block → 16

3-8 minutes — 3 blocks — 3-8 minutes

Results

Average skin temperature in stimulation zones over time

Forearm   Back

- Healthy
- CLBP

Before | After — Block 1
Before | After — Block 2
Before | After — Block 3

Before | After — Block 1
Before | After — Block 2
Before | Before — Block 3