Altered cortico-brainstem connectivity during spontaneous fluctuations in pain intensity in painful trigeminal neuropathy.

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Abstract: Chronic neuropathic pain can result from nervous system injury and can persist in the absence of external stimuli. Although ongoing pain characterizes the disorder, in many individuals, the intensity of this ongoing pain fluctuates dramatically. Previously, it was identified that functional magnetic resonance imaging (fMRI) signal covariations between the midbrain periaqueductal gray matter (PAG), rostral ventromedial medulla (RVM), and spinal trigeminal nucleus (SpV) are associated with moment-to-moment fluctuations in pain intensity in individuals with painful trigeminal neuropathy (PTN). Since this brainstem circuit is modulated by higher brain input, we sought to determine which cortical sites might be influencing this brainstem network during spontaneous fluctuations in pain intensity. Over 12 minutes, we recorded ongoing pain intensity in 24 PTN participants, and classified them as fluctuating (n=13) or stable (n=11). Using a PAG seed, we identified connections between the PAG and emotional-affective sites such as the hippocampal and posterior cingulate cortices, the sensory-discriminative posterior insula, and cognitive-affective sites such as the dorsolateral prefrontal (dlPFC) and subgenual anterior cingulate cortices that were altered dependent on spontaneous high and low pain intensity. Additionally, sliding-window functional connectivity analysis revealed that the dlPFC-PAG connection anticorrelated with perceived pain intensity over the entire 12-minute period. These findings reveal cortical systems underlying moment-to-moment changes in perceived pain in PTN, which likely cause dysregulation in the brainstem circuits previously identified, and consequently alter the appraisal of pain across time.

Significance statement: Whilst the intensity of an individual's chronic pain is often measured at a specific point in time, it is known that in a large proportion of individuals, pain intensity fluctuates dramatically from moment-to-moment. In individuals with chronic neuropathic pain, we found that these spontaneous pain intensity fluctuations are associated with neural function fluctuations, specifically of function reflected as neural connectivity between brainstem pain modulatory circuits and cortical regions, including the dorsolateral prefrontal and cingulate cortices. These findings raise the possibility that modulating brain regions such as the dorsolateral prefrontal cortex in individuals with fluctuating chronic pain may provide an avenue for analgesic treatment.

Introduction:

The perception of pain is vital to survival, warning us of danger and motivating us to remove ourselves from it. As such, pain is adaptive. However, if pain persists beyond the period of normal healing and extends for longer than 3 months, it is considered chronic in nature, maladaptive, and pathological. Chronic pain that results from damage to the nervous system is called neuropathic pain and can occur in the absence of any external stimulus. In any one individual, the ongoing intensity of their pain can fluctuate between days, hours, or even from moment-to-moment. Whilst the precise biological mechanisms underlying perceived pain intensity fluctuations in chronic pain patients are unclear, it is well known that the brainstem contains multiple circuits capable of modulating the intensity of perceived pain by modulating noxious information at the level of the dorsal horn and spinal trigeminal nucleus (SpV) (Crawford et al, 2022b; Oliva et al, 2022; Youssef et al, 2016).

Previous work has demonstrated the role of these circuits in chronic neuropathic pain by establishing that signal coupling between the midbrain periaqueductal gray matter (PAG), rostral ventromedial medulla (RVM), and SpV was associated with perceived spontaneous pain intensity fluctuations in PTN patients (Mills et al, 2020). Whilst this circuit is a well-established brainstem pain modulatory network, whether changes in the connectivity of this circuit alone underpin spontaneous changes in pain intensity, or whether the connectivity of this brainstem circuit is also modulated by descending inputs from higher brain centres remains unknown. It is well-known that the PAG receives descending modulatory influences from higher brain centres including areas such as the dorsolateral prefrontal cortex (dlPFC), anterior cingulate cortex (ACC),

4

88 hippocampus, amygdala and the hypothalamus (Eippert et al, 2009; Ongür et al, 1998;

89 Sevel et al, 2015).

90

91 These connections appear to drive changes in pain intensity in situations such as

92 placebo and offset analgesia and nocebo hyperalgesia (Crawford et al, 2023a;

93 Crawford et al, 2023b). Indeed, it was previously shown that signal covariation

94 between these regions and the PAG are critical in determining whether an individual

95 mounts a placebo analgesia (Crawford et al, 2023a). Further, it has also been shown

96 that a connection between the dlPFC and PAG drives pain intensity variability during

97 repeated noxious stimuli of the same intensity in healthy participants (Crawford et al,

98 2022a; Crawford et al, 2023b). Given these findings, it is likely that descending

99 influences from higher brain regions also determine whether an individual displays

100 spontaneous changes in ongoing pain intensity.

101

102 The aim of this investigation was to determine if signal covariations between higher

103 cortical regions and the PAG are associated with moment-to-moment fluctuations in

104 pain intensity in individuals with painful trigeminal neuropathy (PTN). We hypothesized

105 that higher brain regions with well-established pain modulatory ability such as the

106 dlPFC, ACC, hippocampus and amygdala would display increased functional

107 connectivity with the PAG during periods of high relative to low pain intensity and would

108 also display moment-to-moment functional connectivity changes with the PAG that

109 were associated with fluctuations in perceived pain intensity.

110

111 **Materials and methods:**

112 *Participants and Pain Measurements*

5

Twenty-four PTN participants were recruited for the study (8 male; mean±SEM age=47.3±2.9 years; range=25-78 years). Diagnosis of PTN presence was made in accordance with the Liverpool criteria (Nurmikko & Eldridge, 2001) by a clinician in the research group (E.R.V). Inclusion criteria required a primary pain complaint relating to PTN and a pain intensity on the day of scanning >0. Exclusion criteria included typical MRI contraindications (current pregnancy, metallic implants), as well as the presence of any additional chronic pain condition in addition to PTN. Written informed consent was obtained from each participant for all procedures, approved by the [Author University] Human Research Ethics Committee and consistent with the declaration of Helsinki. Data from 24 PTN participants was used in previous studies (Mills et al, 2018; Mills et al, 2020).

For the seven days preceding the day of scanning, PTN participants recorded the intensity of their ongoing pain three times a day using a visual analogue scale (VAS) (0=no pain; 10=worst pain imaginable). The average of these pain ratings was taken as a measure of "diary pain intensity". Participants also described their pain distribution by shading areas of a standard anatomical template of the face, and described the quality of their ongoing pain through completion of the McGill's pain questionnaire (Melzack, 1975).

Scanning Procedures

Magnetic resonance imaging (MRI) was recorded during this study using a 3-Tesla MRI scanner (Achieva, Philips Medical Systems, The Netherlands) at [Author Scanning Location]. Participants lay supine on a scanner bed with their head positioned within a 32-channel transmit and receive head coil. Ear buds and over ear

headphones were provided to participants to reduce scanner noise, and sponges were placed between these headphones and the head coil to limit head motion throughout the course of functional scanning. A high-resolution T1-weighted anatomical image of the entire brain was collected (288 axial slices, repetition time=5600ms, raw voxel size=0.87x0.87x0.87mm thick, acquisition time=3min45sec). Following this, a series of 360 gradient-echo echo-planar brain volumes with blood oxygen level dependent (BOLD) contrast were recorded (37 axial slices, repetition time=2000ms, echo time=30ms, raw voxel size=3.0x3.0x4.0mm thick, acquisition time=12min). Throughout this 12-minute functional MRI (fMRI) scan, participants were instructed to rate their ongoing pain experience using a computerized visual analogue scale (CoVAS, Medoc, Israel). The CoVAS was connected to a digital screen visible to participants whilst inside the scanner, allowing them to report their ongoing pain intensity in real time (0=no pain; 10=worst pain imaginable). Pain intensity values were saved at the conclusion of each scanning session and subsequently averaged into 2-second rating periods overlying each functional volume for future analysis.

MRI and Statistical Analysis

Structural and functional image preprocessing was performed using Statistical Parametric Mapping version 12 (SPM12) within MATLAB version R2023a (Ashburner et al, 2014; Friston et al, 1994). fMRI images were first realigned and movement parameters inspected to ensure that no participant displayed excessive motion throughout the 12-minute scan (>1mm rotational or >0.5 degrees radian translational motion). These movement parameters were then modelled and removed from the fMRI signal through a Linear Modelling of Realignment Parameters (LMRP) procedure (Macey et al, 2004). Cardiac (frequency band of 60-120 beats per minute + 1

163 harmonic) and respiratory (frequency band of 8-25 breaths per minute + 1 harmonic)

164 noise was modelled and removed using the Dynamic Retrospective Filtering

165 (DRIFTER) toolbox (Särkkä et al, 2012). Each participant's fMRI image series was

166 then linearly detrended to remove the effects of global signal intensity changes and

167 co-registered to their corresponding T1-weighted anatomical image. The T1-weighted

168 image was spatially normalized to the Montreal Neurological Institute (MNI) template

169 using the Manual Computational Anatomy toolbox (CAT12) (Seiger et al, 2018) and

170 these same normalization parameters applied to the fMRI image series. This process

171 resulted in fMRI images being resliced into 2x2x2mm voxels. Finally, for the functional

172 connectivity analyses, we applied a temporal high-pass filter with a cut-off frequency

173 of 0.02Hz to the smoothed fMRI images to remove the low-frequency signals that

174 cannot be resolved using 50-second windows (Leonardi & Van De Ville, 2015). Given

175 that our elected seed resided within the midbrain PAG, a small structure with a discrete

176 functional colocalization, all connectivity analyses were first performed using

177 unsmoothed fMRI images to preserve the integrity of seed signal and seed-to-voxel

178 relationships. Resulting connectivity contrast maps were then spatially smoothed

179 using a 6mm Full Width at Half Maximum (FWHM) Gaussian filter.

180

181 Since equal number of PTN participants presented with unilateral left- and right-sided

182 pain, functional images were flipped along the midline so that each participants

183 resulting contrast images represented chronic pain in the left side of the face. Given

184 that ascending pain pathways are largely represented contralaterally (at least in the

185 case of the thalamus, the primary somatosensory and posterior insular cortices), this

186 methodological consideration meant that we could produce a clear representation of

187 the cortical sites receiving input from, or driving the activity to the unilateral (right) PAG

188 seed identified in previous investigations (Mills et al, 2020).

189

190 *Pain Ratings*

191 Inspection of pain intensity ratings across the entire 12-minutes of fMRI scanning

192 revealed a stratification within PTN participants, such that in some individuals ongoing

193 pain fluctuated greatly while in others pain remained relatively stable. In 11

194 participants, pain remained stable with these participants reporting an average

195 difference between minimum and maximum pain of 0.2 out of 10 on the VAS (stable

196 group). In the remaining 13 participants, ongoing pain intensity varied considerably,

197 and in these participants, we observed an average difference in minimum to maximum

198 pain of 3.4 out of 10 on the VAS (fluctuating group). For fMRI analyses, in order to

199 match perceived pain intensity with changes in BOLD signal intensity and connectivity,

200 ongoing pain intensity ratings for each participant were shifted forward two volumes (4

201 seconds). This shift accounted for the approximate hemodynamic delay since fMRI

202 measures the oxygenation demands in discrete brain regions that follow 3-5 seconds

203 after the neuronal response (West et al, 2019).

204

205 *PAG Functional Connectivity:*

206 Since a previous investigation (Mills et al, 2020) revealed the PAG, via relay with the

207 RVM and SpV, to be an integral component of the descending brainstem circuitry

208 responsible for spontaneous pain fluctuations in PTN (Mills et al, 2018) , we were

209 interested in determining the cortical inputs which first make contact with this

210 brainstem circuitry to drive these effects. As such, we performed functional

211 connectivity using the PAG cluster previously identified in brainstem-isolated space as

212 a seed region for whole brain voxel-by-voxel functional connectivity analyses (Figure

213 1A). In the present study, the PAG seed comprised eight contiguous voxels, four

214 voxels at two rostro caudal levels extending z=-8 to -10 in MNI space. The fMRI signal

215 from each voxel within the seed was extracted and averaged to represent the mean

216 PAG seed fMRI signal.

217

218 To study the functional cortical connections to the PAG, we performed separate

219 functional connectivity analyses. We first calculated PAG connectivity within pre-

220 defined time periods (blocks) corresponding to low and high pain intensities (Figure

221 1B). Secondly, we discerned additional temporal information about these connections

222 by using a sliding-window dynamic functional connectivity analysis to assess the

223 relationship between PAG connectivity and pain intensity throughout the 12-minute

224 scan period (Figure 1C).

225

226 *PAG High versus Low Pain Block Functional Connectivity*

227 First, we aimed to determine how PAG connectivity with cortical sites was different in

228 PTN participants during periods of spontaneously high relative to spontaneously low

229 perceived pain. To achieve this, for each of the 13 fluctuating pain participants, we

230 calculated PAG connectivity in two separated 50-second (25 volumes) time periods

231 (blocks) – one during the lowest perceived pain period across the 12-minute fMRI scan

232 and the other during the highest perceived pain (Figure 1B). Importantly, to minimize

233 any motion-related artefact relating to moving the CoVAS slider, we isolated blocks

234 where pain remained relatively stable for an entire 50-second period and avoided any

235 periods when a large (>1cm) volume-to-volume movement along the CoVAS occurred.

236 For each block, using each participant's fMRI images we conducted first-level

10

237 analyses with timeseries of PAG seed signal within the specified block attached as a

238 condition, similar boxcar models traditionally used in task-related fMRI analyses. This

239 first-level resulted in the generation of two contrast images for each participant, one

240 where voxels of greater beta-value represented greater PAG connectivity during

241 spontaneously low pain, and the other during spontaneously high pain. These images

242 were spatially smoothed with a 6mm FWHM gaussian filter. These contrast images

243 reflecting connectivity map effects were entered into a second level, paired, random-

244 effects analysis of all 13 fluctuating pain participants. We set an initial threshold of

245 $p<0.001$ uncorrected for multiple comparisons, with a cluster defining threshold of 10

246 contiguous voxels, and each resulting cluster was subject to small volume correction

247 (svc) for multiple comparisons to reduce the likelihood of type 1 errors.

248

249 Within each resulting cluster, we extracted PAG connectivity strength values for both

250 lowest- and highest-pain blocks in each fluctuating pain participant and plotted mean

251 (±SEM) functional connectivity strengths to provide a measure of connectivity direction

252 (i.e. correlated or anti-correlated). These connectivity strengths were then entered into

253 post-hoc paired t-tests to determine the direction and degree of significance in change

254 of PAG connectivity between low- and high-pain blocks (Bonferroni corrected for

255 multiple comparisons). In addition, because the majority of participants with fluctuating

256 pain showed not one single VAS movement but several throughout the scan, which

257 corresponded to pain intensity values which were between the lowest and highest

258 blocks, we further assessed whether the sites we identified in the initial paired analysis

259 also displayed a "dose response" with PAG connectivity and perceived pain. That is,

260 additional blocks were included where possible where PAG signal was extracted, and

261 contrast maps generated during 50-second periods of pain closer to the lowest

262 reported intensity (middle-low) or closer to the highest reported intensity (middle-high).

263 This process resulted in a maximum of four PAG connectivity blocks for each cluster

264 that represented fluctuating pain during lowest (block 1) to highest (block 4) pain in

265 each participant.

266

267 To assess PAG connectivity over time in the 11 participants who displayed stable pain

268 over the 12-minute scanning period, we calculated PAG connectivity in four equally

269 spaced 50-second blocks (Figure 2A). For each significant cluster identified in our

270 fluctuating pain participants low- versus high-pain block analysis, we extracted and

271 plotted the mean PAG connectivity for all four blocks within the stable pain participants.

272

273 *PAG Sliding-Window Functional Connectivity*

274 Since it is likely that cortical structures engage pain modulatory circuits of the

275 brainstem not only when pain is spontaneously high or low but also across a

276 continuum, we determined if there was an ongoing relationship between fluctuating

277 and spontaneous pain intensity and PAG connectivity strength. Eleven of the 13

278 fluctuating pain participants displayed multiple (>3) pain intensity changes throughout

279 the 12-minute scanning period. Using the Dynamic BC toolbox (Liao et al, 2014), in

280 each of these 11 participants, fMRI image sets were divided into 50-second (25

281 volume) windows with a repetition time of 2 seconds, resulting in 336 sliding windows

282 (Figure 1C). For each of these windows, a Pearson's correlation coefficient was

283 calculated between the PAG seed and each cortical voxel, resulting in a series of 336

284 dynamic PAG connectivity maps per participant. After undergoing spatial smoothing

285 (6mm FWHM), these dynamic PAG connectivity maps were entered into a first-level

286 linear regression analysis in which PAG dynamic functional connectivity was

12

287　compared with 336 corresponding sliding-window pain intensity ratings. This resulted

288　in a single contrast image where more positive beta-values represented voxels where

289　ongoing coupling with the PAG closely followed spontaneous pain fluctuations – that

290　is, when voxel-PAG connectivity was higher, so too was perceived pain and vise versa

291　for low pain.

292

293　These contrast images were then entered into a second-level one sample random

294　effects analysis to determine cortical sites in which dynamic PAG functional

295　connectivity significantly correlated with perceived pain in each of the 11 fluctuating

296　pain participants. We set an initial threshold of p<0.001 uncorrected for multiple

297　comparisons, with a cluster defining threshold of 10 contiguous voxels and each

298　resulting cluster was subject to small volume correction (svc) to reduce the likelihood

299　of type 1 errors.

300

301　**Results:**

302　*PTN Participant Characteristics*

303　Individual PTN participant characteristics are displayed in Table 1. Mean (±SEM) pain

304　in the 7-days preceding the MRI session (diary pain) was 3.8±0.4 out of 10 with a

305　mean PTN pain duration of 5.2±1.3 years. All 24 PTN participants recorded current

306　pain on the day of scanning, frequently selecting words to describe their pain such as

307　throbbing (39%), sharp (26%), and exhausting (37%). Across the 12-minutes of

308　scanning, 13 participants reported multiple fluctuations in pain intensity, with 11

309　participants reporting relatively stable pain intensity – altering VAS slider position a

310　maximum of once within the entire 12-minute period (Figure 2A). Variable and

311　consistent PTN participants did not differ significantly in terms of age (*mean±SEM*

13

312 *years*: Variable=43.7±4.0; Consistent=51.5±4.0; p=0.20), pain diary intensity

313 (*mean±SEM pain*: Variable = 4.3±0.6; Consistent = 3.1±0.4; p=0.15), or years lived

314 with PTN pain (mean±SEM years: Variable = 4.6±1.8; Consistent = 5.9±1.8; p=0.66)

315 (two-sample t-test). Furthermore, the previous 7-days mean pain intensities were not

316 predictive of pain during the scan which is revealed by the lack of correlation (R = 0.22)

317 and no statistical significance (p=0.33) between these measures. Of the 13 variable

318 PTN participants, 6 reported predominantly left-sided pain, and 7 predominantly right-

319 sided pain. In the 11 consistent PTN participants, 3 reported predominantly right-sided

320 pain, 5 left-sided pain, and 3 reported bilateral pain.

321

322 During the 12-minute fMRI scan, however, compared with PTN participants with

323 consistent pain ratings those with variable ratings demonstrated significantly greater

324 ongoing pain intensity ratings (*mean±SEM VAS pain*: Variable = 3.9±0.7; Consistent

325 = 1.9±0.6; p<0.05), as well as pain intensity variability (*mean±SEM VAS variability*:

326 Variable = 7.1±1.3; Consistent = 0.4±0.2; p<0.05) (two-sample t-tests) (Figure 2B).

327 Despite this, after performing linear regression analyses in all 24 PTN participants, no

328 significant interaction was observed between ongoing pain intensity and variability

329 across the entire 12-minute scanning period (R=0.27, p=0.20) (Figure 2C). For the 13

330 fluctuating PTN participants, mean (±SEM) VAS pain intensity during the 4 pain blocks

331 used for subsequent connectivity analyses were: Block 1 (lowest pain; n=13) 2.9±0.7,

332 Block 2 (n=11) 3.7±0.4, Block 3 (n=10) 4.0±0.8, Block 4 (highest pain; n=13) 5.0±0.7.

333

334 *PAG High versus Low Pain Block Functional Connectivity*

335 Our voxel-by-voxel analysis comparing PAG functional connectivity during periods of

336 spontaneously high versus low pain in our fluctuating PTN participants revealed

14

several cortical regions displaying pain intensity related changes. Significantly greater PAG connectivity strength during high relative to low pain was identified in the ipsilateral posterior insular cortex (mean±SEM PAG connectivity Beta-value lowest pain vs highest pain: 0.011±0.009 vs 0.037±0.013), ipsilateral hippocampus (-0.011±0.005 vs 0.028±0.010), ipsilateral dlPFC (-0.007±0.004 vs 0.007±0.003), and the posterior cingulate cortex (PCC) (-0.005±0.004 vs 0.021±0.006). The inverse contrast revealed a single cluster satisfying correction in the subgenual anterior cingulate cortex (sgACC) which displayed significantly greater PAG connectivity during periods of spontaneously low compared to high pain (0.023±0.008 vs -0.001±0.005) (Figure 3, Table 2) (paired t-tests, all p<0.001 uncorrected for multiple comparisons, small volume corrected). No significant linear relationship was observed between PAG connectivity within each of these clusters during low or high pain periods and either mean pain diary intensity or variability in the seven days preceding the scan (Table 1).

For each of these clusters, PAG connectivity strength was also extracted from additional blocks of increasing pain throughout the scan for a maximum of four graded pain blocks (block 2=moderate-low pain, block 3=moderate-high pain). Within the ipsilateral hippocampus (mean±SEM PAG connectivity Beta-value: block 2, 3: 0.007±0.007, 0.014±0.009), dlPFC (0.005±0.005, 0.013±0.008), and PCC (0.005±0.005, 0.013±0.008) clusters, we observed a graded response of PAG connectivity change such that PAG connectivity closely followed spontaneous pain as it moved higher/lower throughout the scan. Conversely, clusters within the ipsilateral posterior insula and sgACC did not clearly show this graded response effect. Visual inspection of connectivity patterns within these clusters showed a strikingly similar

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362 effect between each of our 13 fluctuating PTN participants, with 12 of the 13

363 participants showing increases between low and high pain periods in PAG-

364 hippocampus, PAG-posterior insula, and PAG-dlPFC. Likewise, 12 of the 13

365 participants showed decreases between low and high pain periods in PAG-sgACC

366 connectivity.

367

368 Within each of these five clusters, measures of PAG connectivity in stable pain PTN

369 participants were extracted within four evenly distributed scan windows as described

370 previously. In no single cluster did we identify changes in PAG connectivity throughout

371 the scan duration. That is, within the 11 stable PTN participants, PAG connectivity with

372 the ipsilateral posterior insula (mean±SEM PAG connectivity Beta-value Blocks 1, 2,

373 3, 4: 0.016±0.010, 0.006±0.012, 0.011±0.010, 0.016±0.010), the PCC (0.002±0.008,

374 0.004±0.008, 0.001±0.009, 0.005±0.008), the ipsilateral hippocampus (0.008±0.018,

375 0.009±0.014, 0.021±0.015, 0.013±0.013), the ipsilateral dlPFC (0.005±0.008,

376 0.010±0.006, 0.006±0.007, 0.010±0.004) nor the sgACC (-0.003±0.005, 0.005±0.007,

377 0.004±0.005, 0.006±0.006) were significantly altered between any of the four pain

378 blocks (one-way ANOVA, p>0.05). Moreover, post-hoc two-factor repeated ANOVA

379 analyses revealed a significant interaction between group assignment (i.e. "stable" or

380 "fluctuating" PTN pain) and PAG connectivity strength with each of these five regions

381 between each increasing intensity pain block (Figure 3-1).

382

383 *PAG Sliding Window Connectivity*

384 Dynamic functional connectivity analysis revealed that in our group of fluctuating PTN

385 participants, PAG dynamic functional connectivity was inversely correlated with

386 sliding-window pain intensity over the entire scan in a region of the contralateral (to

16

387   side of predominant pain) dlPFC (Figure 4, Table 3). We extracted and temporally

388   smoothed the PAG dynamic connectivity values within the dlPFC cluster, identifying a

389   significant inverse relationship between ongoing pain intensity ratings and PAG-dlPFC

390   connectivity in 9 of the 11 participants ($p < 0.05$). That is, as pain was spontaneously

391   high throughout the 12-minute scanning period, PAG-dlPFC connectivity was more

392   anticorrelated, and vice versa. Two individual participant plots of pain versus PAG-

393   dlPFC connectivity are shown in Figure 4. In no cortical region did PAG dynamic

394   connectivity positively correlate with pain intensity.

395

396   **Discussion:**

397   The results of this study highlight that in individuals with chronic neuropathic pain,

398   fluctuations in ongoing pain intensity are associated with altered communication

399   between cortical areas with known roles in pain-processing and modulation, and pain-

400   modulatory circuitry of the brainstem. That is, when pain was spontaneously high, we

401   observed higher PAG coupling with the hippocampus, as well as the posterior insula,

402   dlPFC, and PCC compared to periods where pain was spontaneously low. Conversely,

403   when pain was spontaneously low, greater PAG coupling with the sgACC occurred

404   compared to when pain was spontaneous high. These coupling changes were isolated

405   to the fluctuating PTN participant group, with no overall changes in PAG coupling

406   within these sites in PTN participants where pain remained stable. Additionally, our

407   sliding-window analysis revealed that throughout the entire 12-minute scanning

408   window, PAG-dlPFC coupling was negatively associated with ongoing pain intensity

409   ratings. That is, the higher the pain the lower the PAG-dlPFC coupling and vice-versa.

410   These data show that there are moment-to moment changes in cortical signalling to,

411   or reception of signals from, brainstem pain-modulatory circuits residing in the

17

412   midbrain PAG, and these likely contribute to fluctuations in spontaneous pain intensity

413   in individuals suffering chronic neuropathic pain.

414

415   It is well-established that the PAG is capable of altering the perception of pain either

416   by descending input to the spinal cord dorsal horn (for the body) or SpV (for the face)

417   via relay with the RVM, or by ascending inputs to the cortical sites responsible for the

418   pain percept (Fairhurst et al, 2007; Heinricher et al, 2009; Ong et al, 2019; Zambreanu

419   et al, 2005). In pain-free individuals, the PAG-RVM descending pathway is believed

420   finely balanced to manage nociceptive load, enabling analgesia when pain is

421   unnecessary (such as when fighting or fleeing), or hyperalgesia to enable rest and

422   healing behaviours. However, in individuals with chronic neuropathic pain, it has been

423   suggested that this pathway is shifted, driven by cortical input, to promote an baseline

424   pro-nociceptive state (Chen et al, 2018; Henderson & Keay, 2018; Ossipov et al,

425   2014). Indeed, it was previously established that this PAG-RVM circuitry was

426   modulated in this group of PTN patients, with altered connectivity patterns between

427   the PAG-RVM-SpV in those where pain fluctuated considerably over time. Here we

428   establish the likely top-down cortical inputs which contact this network to drive pain

429   intensity fluctuations.

430

431   It has been previously proposed that the dlPFC acts as a cortical interface between

432   cognitive processing and pain modulation, performing this role by communicating with

433   the PAG both directly and via the anterior cingulate cortex (Eippert et al, 2009; Sevel

434   et al, 2015). Directed connectivity analyses previously identified decreases in dlPFC-

435   PAG coupling as predictive of greater placebo analgesia in a healthy population (Sevel

436   et al, 2015), and non-invasive stimulation of the dlPFC can both attenuate and amplify

18

437 endogenous pain modulatory responses (Krummenacher et al, 2010; Tu et al, 2021).

438 Moreover, stimulation of the dlPFC in chronic pain patients can enhance pain

439 tolerance and alleviate pain symptoms (Graff-Guerrero et al, 2005; Leung et al, 2018).

440 Previously, the function of the dlPFC was extended to be involved in moment-to-

441 moment pain intensity changes in in healthy humans, with the dlPFC demonstrating

442 reduced activation, alongside altered coupling patterns with pain processing areas

443 such as the posterior insula and primary somatosensory cortex, in individuals who

444 express greater variation in perceived pain intensity during a series of identical noxious

445 stimuli (Crawford et al, 2022a). The results of the present investigation suggest that

446 this dlPFC role is also operating in chronic pain patients - via descending input to the

447 PAG - with both block and sliding window connectivity analyses showing dlPFC-PAG

448 connectivity is associated with moment-to-moment fluctuations in pain intensity.

449 Additionally, top-down connections from the dlPFC to PAG are known to not only

450 encode pain modulation but also threat responses to an individual's immediate

451 environment. Wang and colleagues demonstrated the functional connectivity patterns

452 of distinct PAG columns during threat processing, showing that more negative PAG-

453 dlPFC coupling during high threat relative to low threat states (Wang et al, 2022).

454 Given that greater pain, especially when inside the confined environment of an MRI

455 scanner, likely imposes a high threat to body homeostasis, ongoing and updating

456 signals from the dlPFC to the PAG could encode such a threat.

457

458 Similar to the dlPFC, we identified that coupling between the PAG and posterior insula

459 increased with perceived pain intensity. The posterior insular appears to encode

460 sensory components of pain, and shows functional activation correlates with pain

461 intensity in healthy humans (Wager et al, 2013). Uniquely, the posterior insular is one

462   of the only cortical sites where direct stimulation can trigger pain, and where cortical

463   injury can cause the manifestation of neuropathic pain symptoms (Bergeron et al,

464   2021; Mazzola et al, 2012). Further, viral tract tracing in non-human primates

465   demonstrates the posterior insula as the primary target for projection neurons arising

466   from the spinothalamic tract (Dum et al, 2009), consistent with a role in detecting and

467   encoding the magnitude of incoming pain signals. Since our fluctuating group

468   displayed significantly higher variability of ongoing pain and greater overall pain

469   intensity, the posterior insula may code both overall pain intensity and modulate the

470   PAG to alter moment-to-moment pain intensity fluctuations.

471

472   It has been proposed that brain regions that code the emotional-affective aspects of

473   pain, such as the hippocampus and posterior cingulate, play a more central role in

474   chronic pain then they do during acute pain (Garcia-Larrea & Peyron, 2013). In

475   humans, the connection between the hippocampus and PAG is believed to encode

476   the memory and retrieval of pain and threat responses (Mokhtari et al, 2019; Wang et

477   al, 2022), and in experimental animals, chemogenic modulation of hippocampal

478   function can suppress neuropathic pain behaviours via direct modulation of sensory

479   processing sites (Xuhong et al, 2018). Further, human chronic pain patients

480   demonstrate increased ongoing activation in the PCC when compared with controls,

481   and in fibromyalgia patients, this increased activation has shown to correlate with pain

482   catastrophizing, or negative affect toward pain symptoms (Hsieh et al, 1995; Lee et al,

483   2018). Our data indicate that spontaneous changes in pain intensity in PTN patients

484   are also encoded in these emotional-affective cortical regions, which may play a direct

485   role in modulating the intensity of ongoing pain.

486

487   The sgACC was the only cortical region in which we found PAG connectivity to

488   decrease as pain intensity spontaneously increased. The sgACC was originally

489   proposed by Vogt and colleagues to rarely encode acute pain responses, and instead

490   is integral for producing emotional responses to pain via direct autonomic projections

491   to subcortical structures such as the central nucleus of the amygdala and the PAG

492   (Vogt, 2005). These connections however have shown not only to encode emotional

493   responses, but the degree of pain modulatory capacity in healthy humans. Specifically,

494   in a large human cohort Cheng and colleagues showed that greater sgACC and

495   brainstem coupling reduced the intensity of temporal summation (Cheng et al, 2015).

496   Similarly, in a longitudinal study, Bingel and colleagues found that activation of the

497   sgACC related to the magnitude of habituation over time, suggesting a core role of this

498   site in suppressing nociceptive responses through recruitment of antinociceptive

499   circuits (Bingel et al, 2008). Our data not only support the role of the sgACC in

500   inhibiting pain responses over time, but further suggest that in PTN participants where

501   pain fluctuates, the sgACC-PAG connection is inhibited to facilitate an increase in pain

502   intensity.

503

504   *Limitations:* This study comes with several caveats. Firstly, we reflected some

505   participants images so that the left side of the brain corresponded to the side of "most

506   pain". Whilst this approach was used to keep the pain side consistent, we appreciate

507   that some higher brain regions display laterality and thus some information may have

508   been diluted by this process. Secondly, since participants were required to operate the

509   VAS manually by sliding their fingers along its extent, cortical activation of motor

510   networks may have been induced that could alter our interpretation. To counter this,

511   when conducting our pain block analysis, we isolated scan periods where pain

remained relatively stable within the 25-volume period of interest. Additionally, only the dlPFC survived correction in the dynamic functional connectivity analysis, which is not likely to be involved in directing hand movements and any motion effects would be averaged across the 50-second period comprising each of the 336-time windows. Thirdly, though our sample can detect a medium effect size with a power of 0.95, the relatively small sample size in both our fluctuating and stable pain groups could limit overall generalizability, although these sample sizes are similar to previous studies investigating ongoing pain fluctuations in chronic pain disorders (Baliki et al, 2011; Baliki et al, 2006; Foss et al, 2006; Geha et al, 2007). Finally, the choice to employ sliding-window connectivity over a "static connectivity" measure using each scan volume independently is worth noting. Whilst it is unclear whether averaging seed-to-voxel signal in short (50 second) periods measures similar processes observed across a total scan period, this technique has previously demonstrated utility in tying behavioural variables such as ongoing shifts in vigilance and respiration with physiological ones such as brain network dynamics (Chang et al, 2013; Thompson et al, 2013). Indeed, this investigation utilized a similar linear regression approach to Chang and colleagues, assessing whether PAG connectivity with the cortex tracks pain intensity over time.

Conclusions

Overall, our data reveal that cortical sites interact with the brainstem circuits responsible for ongoing pain intensity fluctuations in patients with chronic orofacial neuropathic pain. Whilst it is well known that areas like the dlPFC and ACC are involved in pain modulation in pain-free humans, we extend this idea and show that in individuals with ongoing pain, perceived intensity can fluctuate moment-to-moment

22

537   and we show here such fluctuation is associated with descending analgesic circuitry

538   function. Given that areas such as the dlPFC are accessible to manipulation through

539   techniques such as transcranial direct current stimulation (tDCS), transcranial

540   alternating current stimulation (tACS), or repetitive transcranial magnetic stimulation

541   (rTMS), these findings may provide the platform for testing the analgesic ability of the

542   dlPFC in chronic pain, particularly in those individuals that display variations in ongoing

543   pain intensity. Indeed, we have recently shown that tACS of the dlPFC can engage

544   subcortical circuitry involved in the generation of sympathetic outflow to muscle and

545   skin, likely via the PAG (Sesa-Ashton et al, 2022; Wong et al, 2023).

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685

686

Figure Legends:

688 **Figure 1. Midbrain periaqueductal gray (PAG) seed region, pain block, and**

689 **sliding window connectivity design. A.** With coordinates derived from a previous

690 investigation utilizing this same data set, a 1mm sphere surrounding the PAG was

691 generated and used as a seed region for subsequent connectivity analyses. **B.** Visual

692 representation of the bock analysis used to assess cortico-brainstem functional

693 connectivity during periods of spontaneously high versus spontaneously low pain. In

694 this analysis, connectivity between the PAG seed and each cortical voxel was

695 assessed within a 50-second period overlying participants lowest reported pain (blue

696 shaded bar) against a 50-second period overlying participants' highest reported pain

697 (orange shaded bar). Pain intensity was assessed using a visual analogue scale (VAS)

698 from 0=no pain to 10=worst pain imaginable. **C.** In addition to the block design, a

699 sliding window analysis was performed where PAG connectivity was assessed during

700 a 50-second period (window 1 light grey box) which was then shifted forward by one

701 volume (window 2 dark grey box). This resulted in 336 contrast images representing

702 50-second mean PAG connectivity values across the entire 12-minute scan.

703

704 **Figure 2. Ongoing pain intensity ratings and pain intensity variability in**

705 **fluctuating (n=13) and stable (n=11) pain groups. A.** Trace of VAS responses

706 recorded throughout the entire 12-minute fMRI scan series. All participants fit the

707 diagnostic criteria for trigeminal neuropathic pain, however, note that in only our

708 fluctuating pain group (left plot) are multiple spontaneous shifts in perceived pain

709 observed. **B.** Mean pain intensity and pain rating variability in fluctuating (orange bars)

710 and stable (blue bars) pain groups across the 12-minute scan. Fluctuating pain

711 participants exhibited significantly higher mean intensity and variability of pain than

stable participants. **C.** Linear regression analysis comparing participant mean pain

intensity and variability throughout the 12-minute scan period. No significant

interaction between these variables was observed despite variable participants

demonstrating both greater intensity and variability than stable pain participants.

Figure 3. Significant differences in PAG connectivity strength during periods of

high- versus low-pain in fluctuating pain participants (n=13). Across the 12-

minute scanning window, the 25 volumes where participants rated their pain as most

intense were entered into a second-level, paired design with the 25 volumes where

participants rated their pain the least intense. The upper panel shows significant

cortical clusters with greater midbrain periaqueductal gray matter (PAG) connectivity

during high versus low pain (red colour scale) and vice versa (blue colour scale). The

slice locations in Montreal Neurological Institute space are shown at the top right of

each slice. The middle panel shows plots of mean (±SEM) PAG connectivity extracted

from the above clusters and compared between fluctuating (high- and low-blocks) and

stable (evenly dispersed blocks) participants. Note that the magnitude of PAG

connectivity change in these sites only differs in those participants where pain

fluctuates. Within the bottom panel is a post-hoc analysis of the dose-dependent

response within these clusters, as across the scan course our fluctuating pain group

exhibited periods of perceived pain between the lowest and highest reported blocks.

Note that in the hippocampus, dorsolateral prefrontal, and posterior cingulate cortex

PAG connectivity appears to particularly track with the magnitude of pain only in those

participants displaying fluctuating pain. Please see Figure 3-1.

736 **Figure 4. Cortical region demonstrating ongoing PAG connectivity change**

737 **relating to fluctuations in pain intensity perceived across the 12-minute scan**

738 **period.** The left overlay shows firstly the midbrain periaqueductal gray matter (PAG)

739 PAG seed (upper box) sectioned within a render also showing a significant cluster in

740 the contralateral (to side of pain) dorsolateral prefrontal cortex where PAG-connectivity

741 was anticorrelated with perceived pain throughout the entire scanning period in

742 fluctuating pain patients. The right panel provides visual examples of two fluctuating

743 pain PTN participants where PAG-dorsolateral prefrontal cortex connectivity (orange

744 line) was continuously anticorrelated with perceived pain (blue line) over the 12-minute

745 scanning window. FC = functional connectivity; VAS = visual analogue scale; dlPFC =

746 dorsolateral prefrontal cortex.

747

748 **Table Legends:**

749 **Table 1. Painful Trigeminal Neuropathy Participant Classification.** Participants

750 1-13 were placed in the fluctuating pain group, and participants 14-23 in the stable

751 pain group. *PEA = Palmitoylethanolamide; VAS = visual analogue scale; SD =*

752 *standard deviation.*

753

754 **Table 2.** Montreal Neurological Institute co-ordinates, cluster sizes, and significance

755 values for clusters showing midbrain periaqueductal gray matter connectivity

756 differences during spontaneous low- vs high-pain intensity blocks in fluctuating pain

757 participants (n=13).

758

759 **Table 3.** Montreal Neurological Institute co-ordinates, cluster sizes, and significance

760 values for clusters showing correlations pain intensity and PAG connectivity across

761 the entire 12-minute scan length in fluctuating pain participants (n=13).

762

Table 1. Painful Trigeminal Neuropathy Participant Classification. Participants 1-13 were placed in the fluctuating pain group, and participants 14-23 in the stable pain group. *PEA = Palmitoylethanolamide; VAS = visual analogue scale; SD = standard deviation.*

	Age (Years)	Sex	Side of Pain	Diary Pain Intensity (VAS)	Diary Pain Variability (SD)	Pain Duration (Months)	Current medication Use
1	29	F	Left	5.3	1.5	49	-
2	45	M	Left	3.1	0.4	21	-
3	35	M	Right	1.5	0.7	96	PEA
4	78	F	Left	1.9	0.7	314	Paracetamol, Ostelin, Pritiq, Somac
5	47	F	Right	2.1	0.4	13	PEA
6	34	M	Right	3.7	1.7	10	PEA, Mersyndol
7	66	M	Left	3.9	0.6	48	-
8	44	F	Right	-	-	5	Valium, Panadine forte
9	47	F	Right	4.1	1.6	7	-
10	44	F	Left	8.1	0.4	75	PEA
11	46	F	Left	7.5	2.5	27	Endep
12	28	F	Right	5.8	0.3	52	-
13	25	M	Right	4.5	1.7	8	Panadine forte

14	69	F	Right	3.9	0.4	259	Endep, Lyrica, Oroxine, Crestor, Effexor, Folic acid, Prednisone
15	45	F	Bilateral	2.8	2.8	24	Duloxetine
16	66	M	Right	1.8	0.7	81	Endep
17	36	F	Left	1.9	0.6	37	PEA
18	40	F	Left	5.8	0.3	33	-
19	58	F	Left	4.0	0.4	11	Namipril, Zoloft, Panadol, PEA
20	35	M	Left	4.2	0.7	60	-
21	67	M	Bilateral	2.0	0.9	96	Endep
22	51	F	Left	1.5	0.3	13	Mobic, PEA
23	35	F	Right	3.4	1.0	15	Amitriptyline
24	64	F	Bilateral	-	-	144	-

767

768

769

770   **Table 2.** Montreal Neurological Institute co-ordinates, cluster sizes, and significance

771   values for clusters showing midbrain periaqueductal gray matter connectivity

772   differences during spontaneous low- vs high-pain intensity blocks in fluctuating pain

773   participants (n=13).

Region	MNI Coordinate			Cluster size (kE)	t-value	z-value
	X	Y	Z			
highest > lowest Pain						
ipsilateral posterior insula	-36	-32	8	21	6.08	4.03
posterior cingulate cortex	-6	-36	30	18	5.16	3.68
ipsilateral hippocampus	-52	0	2	11	3.86	3.05
ipsilateral dorsolateral prefrontal cortex	-20	34	28	11	3.77	3.00
Highest < Lowest Pain						
subgenual anterior cingulate cortex	6	-28	-6	12	3.86	3.05

774

775

776

777 **Table 3.** Montreal Neurological Institute co-ordinates, cluster sizes, and significance

778 values for clusters showing correlations pain intensity and PAG connectivity across

779 the entire 12-minute scan length in fluctuating pain participants (n=13).

Region	MNI Coordinate			Cluster size (kE)	t-value	z-value
	X	Y	Z			
Negative Correlation						
contralateral dorsolateral prefrontal cortex	32	40	16	21	4.55	3.27

780

781

782

A
sagittal +2
axial -10

coronal -32

PAG seed

contra

B

pain intensity (VAS)

10

0

0   720

█ highest pain
█ lowest pain

fMRI signal (% change)

2

-2

━ PAG signal
━ voxel signal

0   time (s)   720

C

pain intensity (VAS)

8

0

window 1
window 2

0   720

fMRI signal (% change)

2

-2

0   time (s)   720

PAG seed

axials

sagittals

-38   -32   +34   +6   -6

contra

↑ Pain ↑ PAG conn

1 *t-score* 6

↑ Pain ↓ PAG conn

hippocampus   posterior   dorsolateral   posterior   subgenual anterior
                     insula   prefrontal cortex   cingulate cortex   cingulate cortex

cluster - PAG conn.

0.1

-0.05

F   S   F   S   F   S   F   S   F   S

cluster - PAG conn.

0.05

0.025

0.05

-0.025   -0.025   -0.025

1 2 3 4

F   S

Fluctuating Pain (F) (n=13)   *Stable Pain (S) (n=11)*

■ 1 lowest pain ■ 2 mid-low pain ■ 3 md-high pain ■ 4 highest pain   ■ evenly spaced blocks

PAG seed

contra

↓PAG conn
↑pain intensity

1 ▬▬▬ 6
t-score

+16

dorsolateral
prefrontal
cortex

PAG-dlPFC conn
pain intensity (VAS)

participant 1: R = -0.30

participant 2: R = -0.26

pain intensity
(VAS)

PAG-dlPFC
FC strength

sliding window number

1   336