Neuroimaging reveals a potential brain-based pre-existing mechanism that confers vulnerability to development of chronic painful chemotherapy-induced peripheral neuropathy (CIPN)

Neuroimaging reveals a potential brain-based pre-existing mechanism that confers a vulnerability towards development of chronic painful chemotherapy induced peripheral neuropathy (CIPN).

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ABSTRACT

Background

Chemotherapy Induced Peripheral Neuropathy (CIPN) is a debilitating condition impacting 30% of cancer survivors. This study is the first to explore whether a brain-based vulnerability to chronic sensory CIPN exists.

Methods

This prospective, multicentre cohort study, recruited from three sites across Scotland. 3T Brain fMRI scans were carried out on chemotherapy naïve patients at a single fMRI centre in Edinburgh. Nociceptive stimuli (with 256mN monofilament) were administered during the fMRI. Development of chronic sensory/painful CIPN (CIPN+) was determined based upon EORTC CIPN20 changes, nine months post chemotherapy, and imaging data analysed using standard software.

Results

Of thirty patients recruited (two lung, nine gynaecological and nineteen colorectal malignancies), data from twenty patients at nine months post chemotherapy was available for analysis. Twelve were classified as CIPN+ (mean age= 63.2 [SD 9.6], six female), eight as CIPN- (mean age 62.9 [SD 5.5], four female). In response to punctate stimulation, group contrast analysis showed that CIPN+ compared to CIPN- had robust activity in sensory, motor, attentional and affective brain regions. An *a priori* chosen region-of-interest analysis focussing on the periaqueductal gray, an area hypothesised as relevant for developing CIPN+, showed significantly increased

response in CIPN- compared to CIPN+. No difference in subcortical volumes between CIPN+ and CIPN- patients was detected.

Conclusions

Before the administration of any chemotherapy or CIPN symptoms, we observed altered patterns of brain activity in response to nociceptive stimulation in patients who later developed chronic sensory CIPN. This suggests the possibility of a pre-existing vulnerability to developing CIPN centred on brainstem regions of the descending pain modulatory system (DPMS).

Key Words:

Descending pain modulatory system (DPMS)
fMRI
Pain
Painful Chemotherapy Induced Peripheral Neuropathy (CIPN)
Prospective, multicentre cohort study.

Introduction:

Chemotherapy induced peripheral neuropathy (CIPN) is a common side effect of chemotherapy[1]. Acutely, CIPN occurs in 68% of patients and over 30% of patients continue to suffer symptoms long term[2, 3]. An estimated four million new cases of chronic CIPN are diagnosed annually in the United States alone[4]. CIPN prevalence is predicted to increase as cancer treatments become more effective, and survival improves.

However, the pathophysiological mechanisms underpinning CIPN development and the pathogenesis of CIPN related pain remain unclear[5, 6]. Effective treatment options are limited and no proven preventative measures exist[7, 8].

To date, efforts to understand CIPN development, prevention and treatment have almost entirely focused on the peripheral nervous system (PNS). Although CIPN is a peripheral neuropathy, with symptoms affecting discrete components of the PNS (sensory, motor and autonomic), the interplay of PNS mechanisms with those of the central nervous system (CNS), in particular the brain where pain itself emerges as a perception, has been clearly demonstrated in non CIPN clinical and pre-clinical neuropathy research[9-11]. The chronic pain literature extensively demonstrates the capacity of the brain to contribute to chronic pain development, maintenance and exacerbation[11].

However, the concept that there might be a vulnerability to developing chronic pain, which can be assessed partially using functional brain imaging, is only recently

gathering traction in the pain field[12-14]. In particular, dysfunction in two systems; the descending pain modulatory system (DPMS) and the reward system have been linked to increased risk of chronic pain development[14]. Pre-clinical and clinical research has shown that the DPMS - a cortico-brainstem-spinal cord network - modulates dorsal horn nociceptive processing to increase or decrease pain experiences and is serotonergic, noradrenergic and opioidergic in nature [15-17]. The relative balance of the inhibitory and faciliatory outputs from the DPMS powerfully influences a person's pain perception. Also, in healthy volunteers pre-stimulus functional connectivity to this region determines acute pain outcomes and relates to trait anxiety and pain vigilance – implying a pre-existing potential vulnerability[18]. Functionality and connectivity within this network as well as between this network and other pain-related brain regions are altered in a number of chronic pain conditions, including diabetic painful neuropathy, osteoarthritis and pelvic pain [10, 19, 20]. Additionally, structure and function within the brain's reward network have been shown as relevant to the chronification of back pain [13, 21, 22], and is important in an individual's response to analgesia [23].

In CIPN, while genetic differences and other factors contribute to its development[24], the brain has been overlooked in CIPN pathophysiology. To date, only two clinical studies have assessed brain structure and function in CIPN[25, 26]. These did not interrogate the DPMS or the reward system, and they did not examine patients prior to commencing chemotherapy treatment. We hypothesised that a difference in DPMS and reward system responses to nociceptive stimuli would occur in patients who develop chronic sensory CIPN (CIPN+) compared to those who do not (CIPN-). Specifically, we hypothesised that these differences could be demonstrated using

functional magnetic resonance imaging (fMRI) prior to chemotherapy treatment; suggesting a vulnerability to the development of chronic sensory CIPN. Based on work in other neuropathic pain conditions showing anatomical changes in the chronic pain state, we also examined whether there are pre-existing anatomical changes in cortical and selected subcortical structures (thalamus, insula, ACC and the PAG).

Patients and Methods:

Ethical Approval:

Ethical approval was obtained from the Scotland A Research ethics committee (13/SS/0201). The study was conducted in accordance with the International Conference on Harmonisation Good Clinical Practice Guidelines (ICH GCP) and the World Medical Association Declaration of Helsinki. All patients gave written informed consent.

Study design and participants:

This was a prospective, observational, multicentre, cohort study where chemotherapy naïve patients, scheduled to receive neurotoxic chemotherapy, were recruited to undergo a single brain, structural and functional MRI scan prior to chemotherapy treatment.

Setting:

Patients were recruited from three sites across Scotland: the Edinburgh Cancer Centre, NHS Fife and Forth Valley Royal Hospital. Clinical questionnaires and demographic data were collected in person, or by phone. All brain fMRI scans were carried out at a single centre in Edinburgh, using a Siemens Verio 3T MRI Scanner at

the Edinburgh Imaging Facility, Queens Medical Research Institute, University of Edinburgh. Data collection was completed in April 2016.

Inclusion criteria:

(a) Planned primary bortezomib treatment (for multiple myeloma) or oxaliplatin, paclitaxel, docetaxol, cisplatin (for adjuvant treatment with curative intent of colorectal, testicular, uterine or ovarian cancer).

(b) Aged 18 years or over at study entry.

(c) Patient's usual medical team agreement.

(d) Able to provide informed written consent after explanation of the study protocol.

(e) Able to complete questionnaire assessments in English.

(f) In the opinion of the investigator, the patient was able to complete the various assessments.

Exclusion criteria:

(a) Diagnosed neurological conditions, (such as Multiple Sclerosis or residual signs/symptoms from a previous stroke), which may influence brain fMRI and/or peripheral neuropathy findings.

(b) Pre-existing neurological or chronic pain/neuropathic condition.

(c) Diabetes, a history of alcohol excess or pre-existing chemotherapy.

(d) Skin conditions that prevent assessment of the relevant areas affected by peripheral neuropathy.

(e) Suffered from significant psychiatric illness, which would hinder their completion of the study.

(f) Unstable or rapidly deteriorating general medical conditions.

(g) Who in the opinion of the research team or their usual medical team, would be unable to complete the study protocol for any other reason.

(h) Were contraindicated to undergoing an MRI (eg: metal implants, claustrophobia).

Patient flow:

Patient flow through the study is depicted according to guidance from the: Strengthening the Reporting of Observational studies in Epidemiology (STROBE) guidelines[27] (Figure 1).

Outcome Measures:

Clinical Questionnaire: CIPN classification

The European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Chemotherapy-Induced Peripheral Neuropathy 20 (CIPN20) was used to define chronic sensory CIPN[28]. This questionnaire was validated in a large European cohort and is easily and quickly administered either in person or on the phone. In our study CIPN20 was completed at the following time points: baseline before chemotherapy commencement, before each cycle of chemotherapy, three, six, nine and twelve months after chemotherapy completion. Questionnaires were administered by research nurses trained in its administration and with clinical experience in care of patients with CIPN. We identified patients with chronic sensory CIPN by reviewing changes in the relevant sensory questions of the questionnaire at the nine-month time point as compared to baseline (eTable 2 online only). Each patient controlled for their own pre-chemotherapy status. The nine-month time point

was chosen because our focus was on chronic, as opposed to acute CIPN, and likelihood of CIPN resolving at this point is low[2]. Those with a sustained change of two points or more on the sensory subscale (out of potential change of 24) were classified as CIPN+, and those without change were classified as CIPN-. This was an arbitrary cut-off based on clinical observations. CIPN 20 relates to symptoms in the past week and is therefore not of use in assessing acute toxicity within the latest chemotherapy cycle. Therefore, to assess progression from acute to chronic CIPN, acute CIPN (aCIPN) was defined clinically as any change in the common toxicity score, combined with a full clinical assessment, in those cases requiring a chemotherapy dose reduction (eTable 1 supplementary material online only).

Brain Magnetic Resonance Imaging

Study participants underwent a 3T brain fMRI scan using the same MRI scanner (3T Siemens Magnetom Verio Syngo, located at the Imaging Centre at the University of Edinburgh), at a single time point prior to starting chemotherapy. The following data were acquired; a T1-weighted MPRAGE sequence to assess brain structure; a T2*-weighted gradient echo sequence for blood oxygen level dependent (BOLD) functional activity. For full details of scan parameters see eTable 3 (supplementary material online only).

Functional imaging paradigm:

A nociceptive punctate stimulus using a 256mN von Frey filament was administered. This stimulus was chosen because mechanoreceptors are known to be involved in CIPN development[29]. Moreover, aberrations in response to punctate stimulus have been demonstrated in patients before and after chemotherapy treatment[30, 31].

Participants received 64 punctate stimuli with a mean inter stimulus interval of 15 seconds. The stimuli were administered 2cm above the right medial malleolus in each patient. Pain ratings were not recorded during this scan as patients were free of all neuropathy symptoms at this stage of the study.

Statistical Analysis and Sample Size:

Demographic Data

Statistical analyses were carried out using *IBM SPSS Statistics for Macintosh (V 25.0)*. Group means were compared using a two-sided t test (for normally distributed variables) and a Kolmogorov-Smirnov test (for variables with a skewed distribution), as comparison groups contained fewer than 25 patients each. Categorical variables were compared using Pearson's chi square test or Fisher's exact test when group size did not fulfil expected cell counts. Where possible, 95% confidence intervals (CIs) were calculated to aid interpretation of the results. Sample size was based on previous fMRI studies using this or a similar paradigm design and that had between N=12 and N=16 healthy controls or patients per group. Therefore we aimed to recruit 30 patients in total for this study [10, 19, 32]. Ideally, to ensure statistical robustness we would have aimed for 20 patients per group, however as this was the first study of its type, we aimed to pilot a smaller sample size to ensure feasibility of running the study and completing the scans.

MRI Data

Structural data for calculation of subcortical brain volumes were analysed using tools from *FMRIB's Software Library* (FSL): FAST[33] for T1 image segmentation and FIRST[34] for extraction of subcortical structures and volume calculation. These analysis tools

implement a model based subcortical brain region segmentation. Segmentation is unique for each structure and uses laterality if this exists https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FIRST/UserGuide. Between group volume differences were compared using a factorial repeat measures ANOVA in SPSS. Analyses were adjusted using the Bonferroni correction for multiple comparisons across both sides of the brain. Whole brain volume was also used as a comparative adjustment measure in these analyses.

Functional data were analysed using *Statistical Parametric Mapping* (SPM12) software within MathWorks MATLAB R2016a (http://www.mathworks.com). fMRI volumes were reconstructed into NIfTI format and realigned to the mean volume. The structural image was segmented and warped to MNI space. The mean fMRI and structural volumes were co-registered, and the normalisation parameters applied to the whole fMRI dataset which was then smoothed using an 8mm FWHM gaussian kernel and resampled at 2mm isotropic resolution. Data were high pass filtered with 128s cutoff, and serial correlations modelled using a first-order autoregressive model. At the first level, the punctate events were modelled as delta functions convolved with the hemodynamic response function (HRF). Motion parameters were included as nuisance regressors. Contrasts of punctate onsets were progressed into a second level random effects flexible factorial analysis, modelling the factors of subject, CIPN group, and other experimental factors of no interest (see supplementary materials). The group contrasts of CIPN+ greater than CIPN-, and its inverse, were evaluated, and clusters achieving a FWE-corrected significance of $p < 0.05$ deemed statistically significant.

To enhance sensitivity for periaqueductal grey (PAG) activation with our sample size, an *a priori* anatomic mask was applied to the data, in addition to performing whole-brain analysis. The mask was functionally defined as detailed in previously published literature[35, 36].

Results:

Demographic Data:

Thirty patients were recruited to the study and all underwent brain fMRI prior to commencing chemotherapy. Ten patients were unavailable for follow up for the following reasons: two had disease progression, two died, four withdrew from the study, there was one scan acquisition failure, and one patient had non CIPN chemotherapy related toxicity. Of the 20 patients where data were available at nine months, 12 patients developed chronic sensory CIPN. There were no differences in age, sex, operation status, or cancer type between CIPN+ and CIPN- patients (Table 1). Data showing the progression of individual patients from no CIPN, through acute CIPN during chemotherapy and then either to resolution or to CIPN+ at nine months are presented in the supplementary material (eTable 4, Supplementary Materials).

Brain Structure:

No statistically significant differences in the volumes of the four brain regions tested were found between CIPN+ and CIPN - groups (Table 2).

Brain Function:

Group contrast of CIPN+ compared to CIPN- patients had increased brain activity in response to punctate stimulation in the following regions: superior parietal lobule and

angular gyrus bilaterally, occipital cortex bilaterally, left somatosensory cortex (region correlated to sensory innervation of the ankle), right precentral gyrus extending to the dorsal anterior cingulate cortex bilaterally, bilateral supplementary motor cortex, anterior and posterior insula subdivisions bilaterally, left caudate nucleus, left thalamus, left medial lemniscus, left middle and inferior frontal gyri, right medial superior frontal gyrus, right anterior cingulate gyrus, right inferior pons, right superior medulla, right inferior and middle temporal gyri, right precuneous, and finally the right cerebellum.

In comparison, group contrast of CIPN- compared to CIPN+ patients had increased brain activity in response to punctate stimulation in the following regions: occipital cortex bilaterally, left fusiform gyrus, left superior temporal gyrus, right superior parietal lobule and right angular gyrus, left postcentral gyrus and the PAG bilaterally (Figures 2 and Figure 3).

Discussion:

Our main finding is that brain response to nociceptive punctate stimuli differed between CIPN+ and CIPN- patients before any chemotherapy was administered or any CIPN symptoms were present, suggesting a pre-disposing state. Notably, CIPN+ patients had more brain activity in response to punctate stimulation, manifest in core pain processing regions including the insula, thalamus, somatosensory cortex and cerebellum ahead of developing CIPN symptoms [37]. We note that some of these regions, discussed below, are described in a myriad of neuropathic pain states.[38-44] What is fascinating is that here they are activated more in pain free patients prior to CIPN development when compared to pain free patients who did not develop CIPN. To

highlight these similarities between pre CIPN brain activations, to those identified in established neuropathic pain, we have generated a meta-analyzed z-score brain map of neuropathic pain using NeuroQuery and overlayed this with our CIPN+ activation maps (figure 4).[45] The NeuroQuery toolbox allows users to generate a predictive MRI-derived spatial distribution, based on very large-scale meta-analyses of functional MRI studies. Table 3 also shows the comparison of our results and those of other fMRI studies in established Neuropathic pain.

The insula, a structure subdivided into anterior, mid and posterior divisions has been shown in meta-analyses as the region most frequently associated with pain processing[46]. Pain related connectivity of the various insula subdivisions to the somatosensory cortex (primary and secondary), as well as to the thalamus, is robustly documented[47]. The cerebellum is recognised as an active contributor to pain perception, albeit rarely mentioned[48]. The anterior cingulate cortex (ACC), which is part of the descending pain modulatory system (DPMS) as well as serving broader roles in affective and cognitive aspects of pain processing, was also more active in the CIPN+ group.

Compared to CIPN-, the CIPN+ group also showed increased activity in the right superior frontal gyrus (SFG). Interestingly, two other CIPN studies investigating brain structure and function both report the SFG as an area of change. Nudelman et al followed women diagnosed with brain cancer and carried out brain scans before and after chemotherapy comparing them to women who had cancer but not chemotherapy. The study looked at brain perfusion, showing increased perfusion in the SFG bilaterally in patients with painful acute CIPN symptoms [26]. Boland and

colleagues showed decreased activity in the right SFG in response to painful stimuli in chronic CIPN patients[25]. The design of both studies differs from ours in that they looked at symptomatic patients with diagnosed CIPN. It is nonetheless interesting that both identified the same region of vulnerability as our study. The SFG has been implicated in deciphering mismatch between expected and actual pain[49]. The area is also important in the cognitive aspect of pain encoding[50], and is indicated as one of the brain regions showing decreased volume in neuropathic pain[51]. Our findings suggest this region might play a role in susceptibility to chronic painful CIPN, but further work is needed to better understand its precise role in pain.

In contrast, prior to chemotherapy treatment the CIPN- group compared to the CIPN+ group had significantly lower overall brain activity in response to punctate stimulation. However, the periaqueductal gray (PAG), a key region of the DMPS, had increased activity in response to punctate stimulation in the CIPN- group. The PAG along with outputs from the rostral ventromedial medulla (RVM) is known both to inhibit (via OFF cells) and facilitate (via ON cells) nociceptive processing in the dorsal horn of the spinal cord[15-17, 52]. As such, the PAG, as a core part of the DPMS that comprises the ACC, RVM and hypothalamus (amongst other regions), controls how much nociceptive input comes to the brain and, therefore, the resultant pain experienced. Lately, it has been postulated as playing a role in determining susceptibility and/or resilience to developing persistent chronic pain[14]. Our results support the idea that CIPN- patients have an increased ability to engage the descending inhibitory aspects of the DPMS. This engagement probably explains the overall decreased brain activity in pain processing regions seen in this group compared to CIPN+. It also probably confers a resilience towards developing CIPN.

This interpretation is corroborated in experiments, discussed above, where increased PAG activity pre- delivery of a nociceptive stimulus in humans was predictive of subsequent behaviour classifying the stimulus as non-painful[18]. Further, animal models of neuropathic pain suggest that direct PAG stimulation is effective in alleviating established neuropathic pain symptoms[53]. Additionally, healthy human experimental models, predating our study, show that aberrance in the DPMS, demonstrated using cerebral blood flow analysis, correlated with subsequent pain expression[54]. More recent neuroimaging work in babies highlights extraordinary variation in DPMS strength of connectivity right from birth[55]. Understanding what developmentally (nature and nurture) produces such variances in DPMS function is an active area of current research, both preclinical and increasingly clinical [14].

Interestingly, some of the regions highlighted in our results (figure 2) are part of the default mode network (DMN). The DMN is important in attention, prospection, memory and self-processing and is altered in chronic pain conditions including neuropathic pain[56, 57]. More recently it has been correlated with processing of neuropathic pain in real time and shown to have reversibility in aberrant connectivity in response to analgesic treatment[58, 59]. In our context it is plausible that a vulnerability to developing CIPN is also detectable in DMN connectivity. We have not assessed this explicitly in this analysis but have data and hope to do so in the future.

Contrary to our expectations, we did not see any reward related regions as more or less active in response to punctate stimulation in any of the group contrasts. We had anticipated that this network would be important in CIPN development, based on research showing reward system involvement in conversion from acute to chronic

pain[13, 21, 22, 60-63]. These studies examine transitions from an acute pain state (back pain) to chronicity, and so there is the possibility that the reward system differences are better related to a resistance to analgesia (that in itself will hold the person in a persistent pain state), which has been suggested as an alternative explanation[64]. Consistent with these functional observations, our structural analysis of the NAc, Amygdala, Thalamus and Brainstem similarly found no differences between CIPN+ and CIPN- patients, in contrast to the findings of our preliminary analysis investigating aCIPN, which showed the NAc was significantly smaller in the aCIPN+ group[65] . Further research with more time points for neuroimaging data acquisition is required to better understand the transition from acute to chronic CIPN [66].

Strengths and limitations of this study

The main limitation of this study is the sample size. Ideally, we would have wanted to recruit 20 participants per group to improve statistical power[67, 68]. Our small sample size increases the risk of false negative as well as false positive errors. These results serve as a pilot for other larger studies of this nature. Defining and quantifying chronic CIPN remains a problem, with mixed evidence regarding the benefits of using neurophysiological, clinical or questionnaire-based measures[69, 70]. One possible limitation of this study is that we used only one measure – the EORTC20 questionnaire for our chronic sensory CIPN definition. Recent evidence, however, shows that this is a reliable and repeatable diagnostic test of CIPN[71]. Our choice of this measure was based on the ease of its administration across three sites at multiple time points, as well as on its reliability to differentiate sensory, motor and autonomic PNS dysfunction. In addition, we know from clinical practice that patients often do not describe distressing sensory CIPN symptoms very well using standard pain

questionnaires. We lost a third of our patient population at follow-up – more than anticipated in the power-calculation, and so going forwards recruiting a larger cohort is recommended.

To date, CIPN severity has been deemed proportional to the type, duration, and dose of chemotherapy as well as the damage that treatment induces in peripheral nerves. Our results suggest that pre-chemotherapy, a brain-based vulnerability for developing chronic sensory CIPN exists. Thus, clinicians may need to consider certain patients as having a baseline risk for sensory CIPN development alongside the specifics of chemotherapy dose and duration. This might be done using an increasing array of bedside tools to help stratify patients[72].

We hope that our findings prompt further discussion and research into the pre-chemotherapy brain. Improved understanding of who is more vulnerable to developing CIPN will usefully inform patient care at all stages in the cancer journey. Future benefits could include allowing people to make informed choices around their oncological treatment options; early identification of high-risk individuals allowing targeted preventive strategies; and using a stratified approach, with more intensive monitoring for development of CIPN in high-risk individuals. There is the opportunity for pain specialists to work as part of the wider multidisciplinary team so that early prevention and treatment can be directed towards those most at risk.

Author Contributions:

Conceived and designed the experiment: MS, LR, IT, LC, MF, SL. Performed the experiments: MS, LR, KS, NR. Analysed the data: MS, LR, KS. Contributed materials/analysis tools: MS, LR, CEW, NR, SL. All authors wrote the paper.

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Declaration of Interest:

The authors do not declare any conflict of interest.

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	CIPN- n= 8 (SD) or (% of n)	CIPN+ n= 12 (SD) or (% of n)
Age (mean years)	62.9 (5.5)	63.2(9.6)
Sex (Male)	4(50%)	6(50%)
Primary management with surgery Yes No	 6 (75%) 2 (25%)	 12 (100%) 0 (0%)
Cancer Type Lung Gynaecological Colorectal	 2 (25%) 2 (25%) 4 (50%)	 0 (0%) 3 (25%) 9 (75%)
Pre-chemotherapy Pain Score (0 to 10)	1.1 (1.8)	0.3 (0.8)

Table 1: Baseline characteristics of study participants according to chronic CIPN classification.

STRUCTURE	MEAN VOLUME in mm^3 (95%CI) CIPN-	 CIPN+	Unadjusted Significance	Adjusted Significance for WBV
L Thalamus R Thalamus	7605 (7071 to 8139) 7469 (7927 to 7455)	7332 (6986 to 7679) 7283 (6888 to 7979)	p=0.40	p=0.48
L Accumbens R Accumbens	580 (491 to 669) 473 (411 to 535)	599 (507 to 690) 429 (324 to 533)	p=0.82	p=0.90
L Amygdala R Amygdala	1377 (1123 to 1631) 1485 (1294 to 1677)	1419 (1286 to 1554) 1470 (1254 to 1687)	p=0.90	p=0.70
Brainstem	21455 (19794 to 23115)	21184 (19865 to 22503)	p=0.78	p=0.98
Grey Matter	555537 (504677 to 606396)	548068 (525108 to 571028)	p=0.73	p=0.85

Table 2: Group differences in mean volumes. 95%CI = 95% confidence interval. L= left. R=right. WBV= Whole brain volume. Multiple comparisons (because of bilateral structures) adjusted with Bonferroni correction.

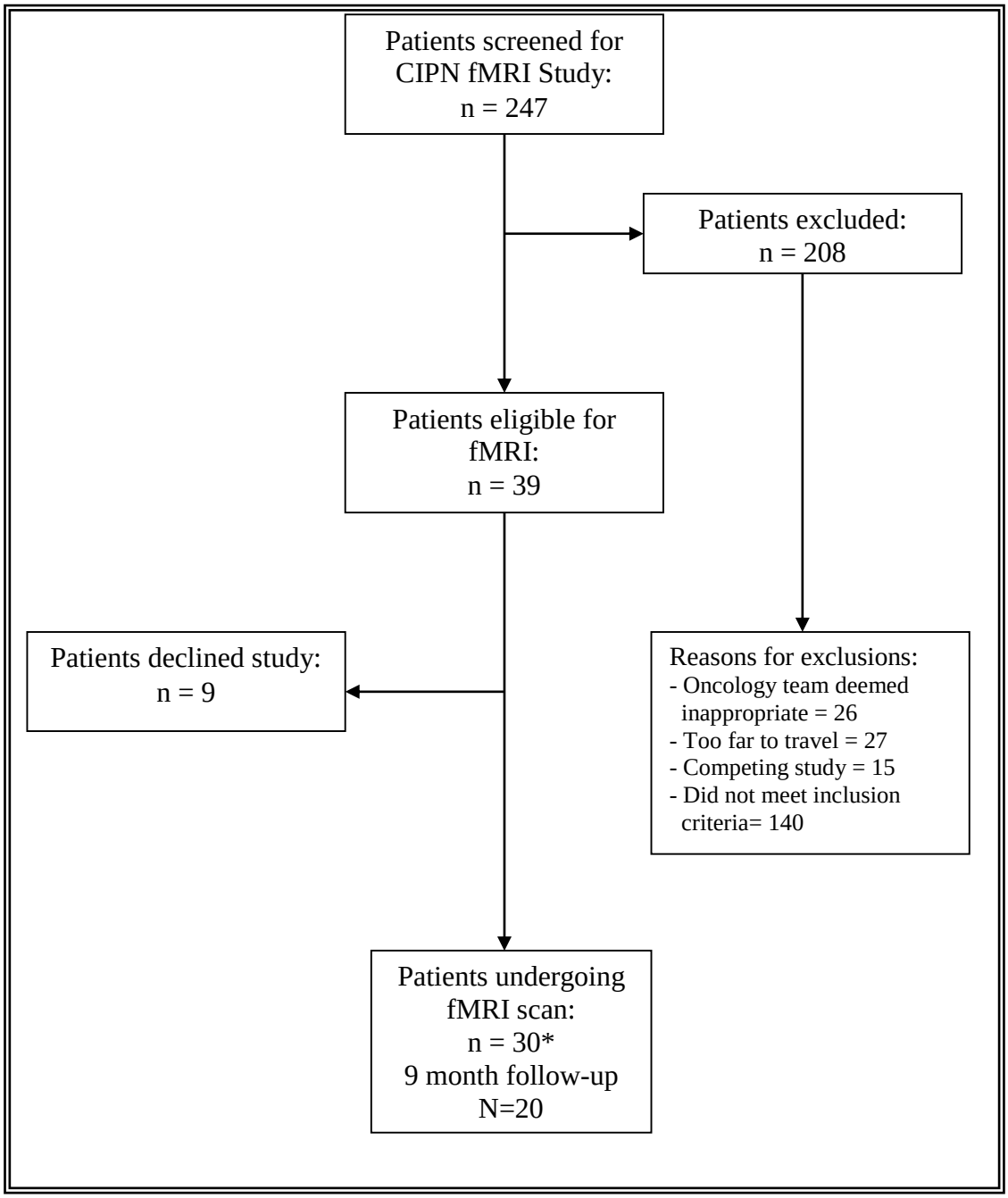

Figure 1: STROBE diagram depicting patient recruitment and flow. Numbers included in each individual analysis are specified in the results section.

Figure 2: Group differences in response to punctate stimulation. Z = z axis (top to bottom) position of slices with z=65 being most superior and z= -15 most inferior. Green = increased activation in the group contrast of CIPN+ compared to CIPN-. Red= increased activation in the group contrast of CIPN- compared to CIPN+. Results displayed are those found significant from a whole brain analysis with Family-wise Error (FWE)-corrected $p < 0.05$.

Figure 3: An *a priori* defined region of interest analysis identified significantly increased bilateral PAG activation to punctate stimulation in CIPN- compared to CIPN+ ($p < 0.05$ Family-wise Error (FWE)-corrected). There was no statistically significant activation in this region in the group contrast of CIPN+ compared to CIPN-.

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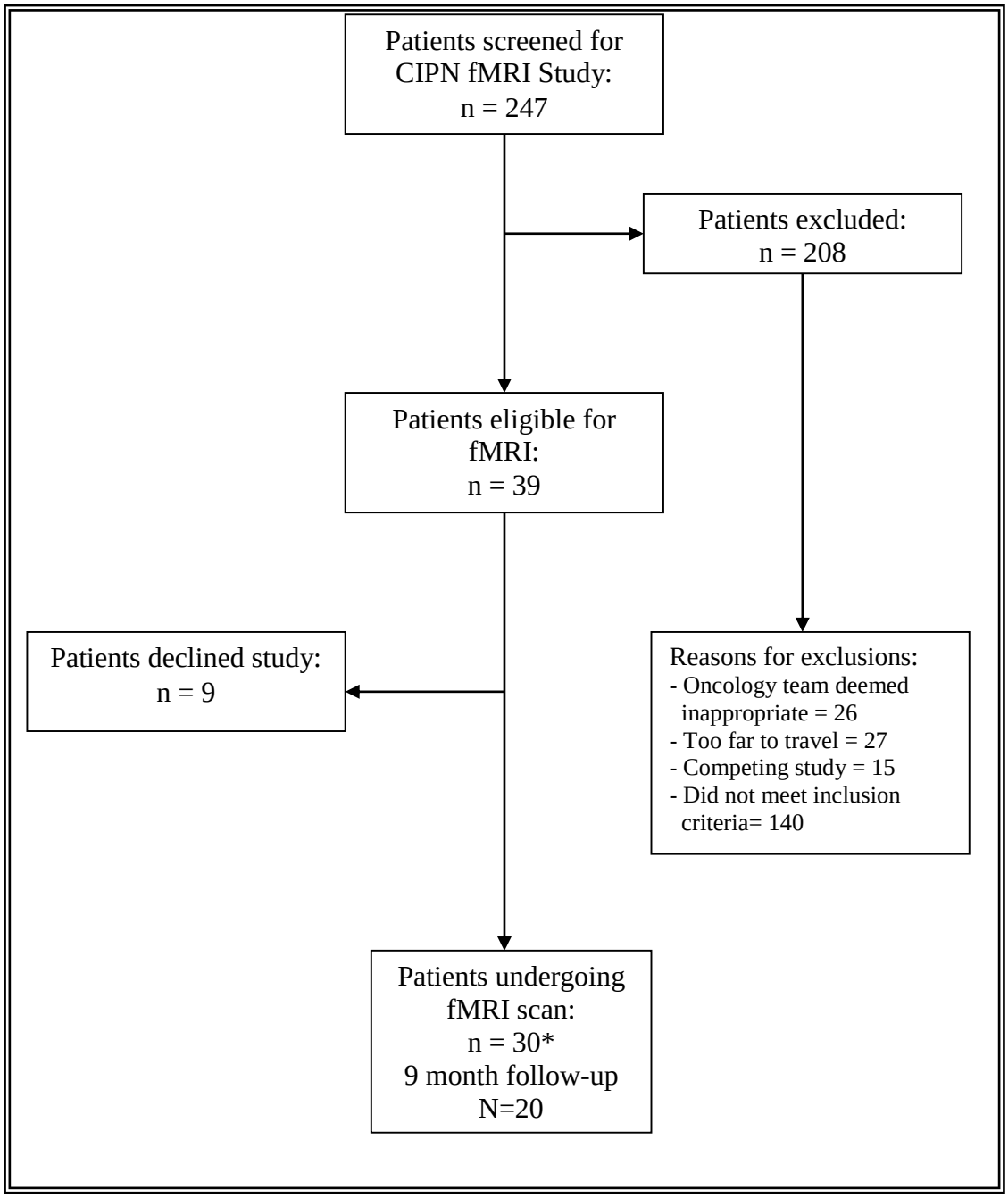
Regions of Brain Activation in (CIPN+) > (CIPN-) to punctate stimuli	**MNI coordinates**	**Activated Brain Regions in Neuropathic Pain to heat[10,25,42] brush or cold[41,44] or no stimulus[26,39,43]**	**Reported coordinates**
L superior parietal lobule, angular gyrus	-38 -54 58	L posterior parietal lobe[41]	32 -58 62 (Talariach)
L somatosensory cortex (ankle)	-14 -44 60	R Primary and B Secondary Somatosensory Cortex [10,42,44]	24 -39 69 (MNI) 39 -15 18 (MNI)
R precentral gyrus, extending to the B dorsal anterior cingulate cortex, B supplementary motor cortex, B anterior and posterior insula, L caudate, middle cingulate, L IFG & L thalamus	42 -8 62	Posterior Insula [10,43] Insula[44] Thalamus[39]	40 -34 14 (MNI) 42 4 16 (MNI)
L middle frontal/ inferior frontal gyri	-46 12 34	L area opercularis and L middle frontal gyrus[25]	-54 2 12 (Talariach) -34 40 22 (Talariach)
R medial superior frontal, anterior cingulate gyrus	8 50 0	B Superior frontal and cingulate gyri[26]	4 18 56 (MNI)
R precuneus	14 -36 46	R precuneus[42]	6 -63 36 (MNI)
R cerebellum	12 -78 -30	R cerebellum [10, 42]	3 -30 -36 (MNI)

Table 3 Regions of Brain Activation in (CIPN+) > (CIPN-) Compared to Regions of Brain Activation in other Neuropathic Pain Studies. Reported clusters for this study achieved a whole-brain cluster-wise significance level of $p < 0.05$ Family wise Error (FWE) -corrected for multiple comparisons. MNI coords = Montreal Neurological Institute brain atlas coordinates. L= left, R= Right, B= Bilateral. References from other neuropathic pain studies reported alongside each comparable region of activation. Please note some studies reported MNI and others Talairach coordinates resulting in some disparity.



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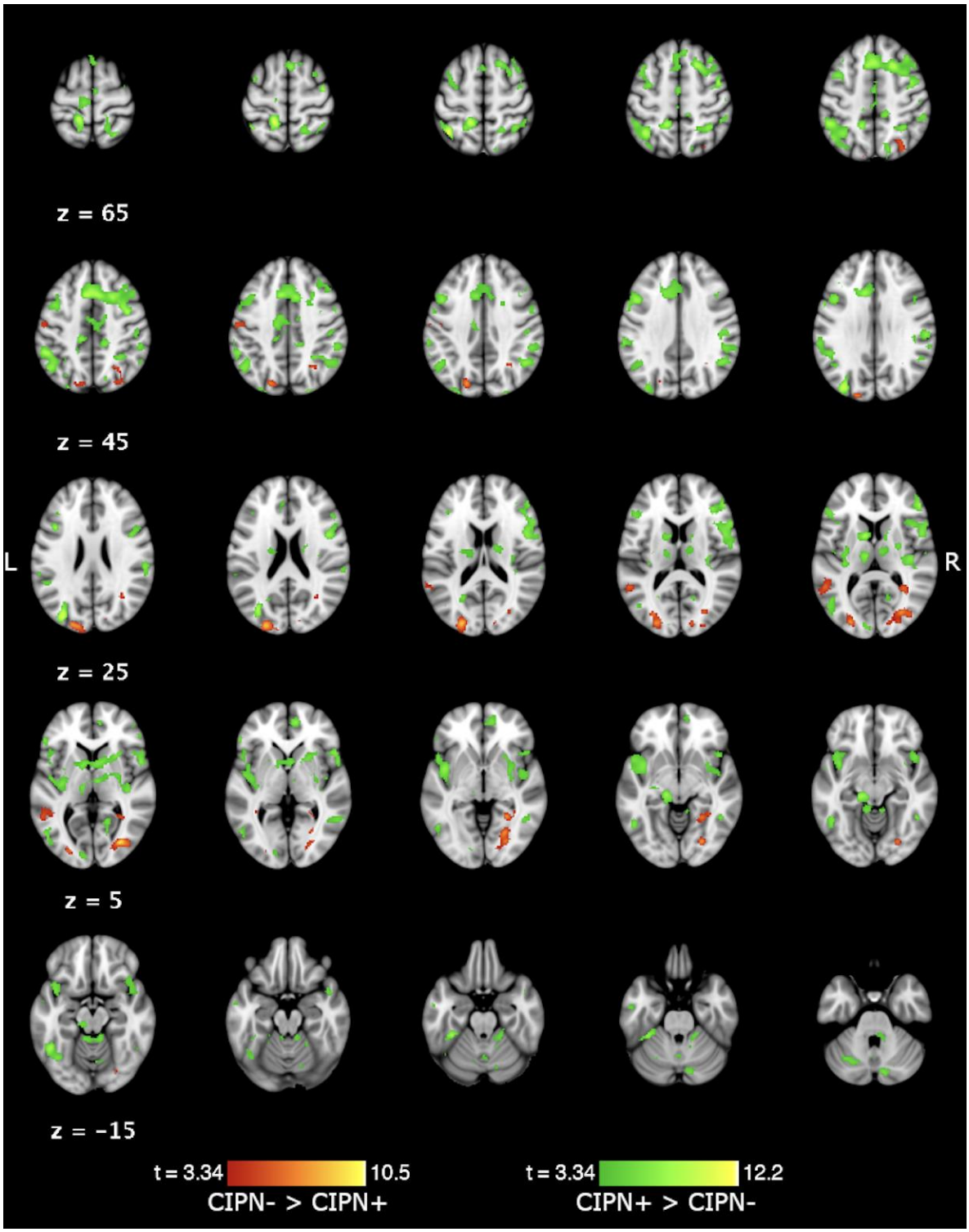

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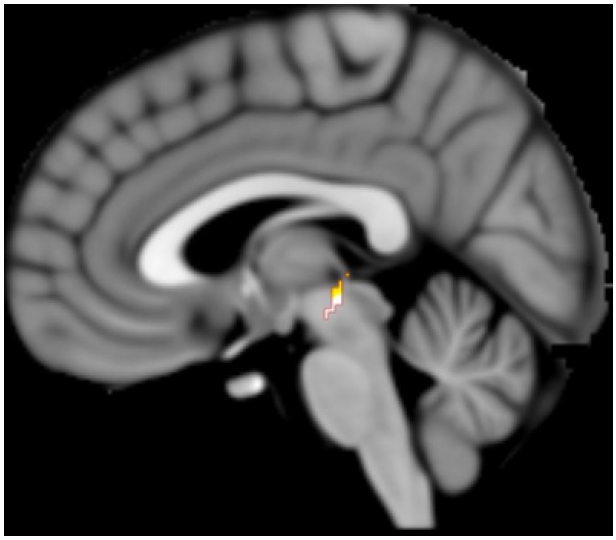

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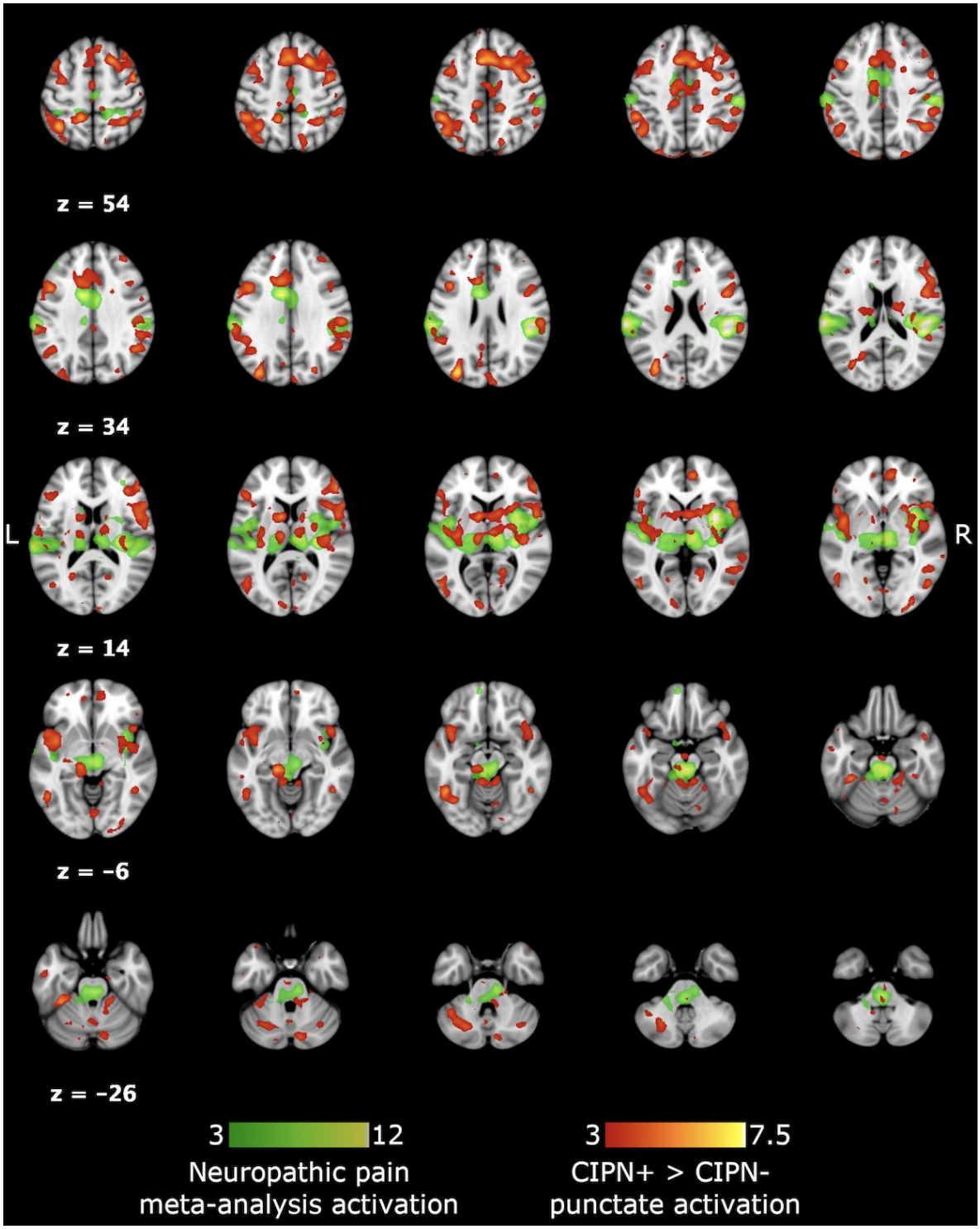

Figure 4: NeuroQuery meta-analysis derived z-score brain activation maps showing neuropathic pain study findings (in green) with CIPN+>CIPN- z-score brain activation maps, in response to punctate nociceptive stimuli, overlayed (in red). NeuroQuery is a toolbox that allows users to generate a predictive MRI-derived spatial distribution for any term, in our case neuropathic pain; based on very large-scale meta-analyses of functional MRI studies https://neuroquery.org/query?text=neuropathic+pain. It is again important to highlight that the red results occurred in pain free patients prior to CIPN development, while the green meta-analyses results show established neuropathic pain states.