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MRI structural brain changes associated with sensory and emotional function in a rat model of long-term neuropathic pain

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Abstract

In human conditions, chronic pain is associated with widespread anatomical changes in the brain. Nevertheless, little is known about the time course of these changes or the relationship of anatomical changes to perception and behaviour. In the present study, we use a rat model of neuropathic pain (spared nerve injury, SNI) and 7Tesla MRI to determine the longitudinal supraspinal changes associated with pain-like and anxiety-like behaviours. SNI rats and sham controls were scanned at seven time points, one week before surgery, two weeks after, and then once a month for five months. At each time point we performed behavioural tests, including thermal and mechanical sensitivity, and tests of locomotion and exploratory behaviour (open field and elevated plus maze). We found that SNI rats had early and sustained thermal and mechanical hyperalgesia, and developed anxiety-like behaviours several months after injury. Compared to sham controls, SNI rats had decreased frontal cortex volumes several months after surgery, coincident with the onset of anxiety-like behaviours. There was also decreased volume in retrosplenial and entorhinal cortices. We also explored areas that correlated with mechanical hyperalgesia and found increased hyperalgesia was associated with decreased volumes in bilateral S1 hindlimb area, anterior cingulate cortex (ACC, areas 32 and 24), and insula. Overall, our results suggest that long-term neuropathic pain has widespread effects on brain anatomy related to the duration and magnitude of the pain.

Introduction

Several studies have reported that chronic pain in humans is associated with changes in brain anatomy, such as gray matter density and cortical thickness (Mao and others 1993; Mochizuki and others 2003; Willoch and others 2004; Jasmin and others 2004; Apkarian and others 2004b; Schmidt-Wilcke and others 2005; Schmidt-Wilcke and others 2006; Hamani and others 2006; Kuchinad and others 2007; DaSilva and others 2007; Davis and others 2008; Teutsch and others 2008; Schweinhardt and others 2008; Geha and others 2008; Lutz and others 2008; Kim and others 2008). However, important questions remain that may

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be better suited to animal model studies, such as how neuroanatomy changes over time, and how various behaviours relevant to chronic illness might predict these changes.

While the human studies fairly consistently show decreases in gray matter or cortical thickness related to the duration and/or severity of chronic pain, the specific brain regions showing significant effects are not entirely consistent, and often include brain areas not conventionally considered pain-related. In a recent review, May (2008) reported that there were very few studies that showed changes in primary and secondary somatosensory cortices or the thalamus. In contrast, the most common regions to have decreased grey matter were cingulate, orbitofrontal, and insular cortices, regions implicated in the affective dimension of pain and/or affect in general. This pattern is not surprising, considering that chronic pain is a common complaint of patients having a variety of affective disorders, including depression, chronic fatigue, and post-traumatic stress disorder, and that pain considerably affects quality of life (Kewman and others 1991; Haythornthwaite and rud-Larson 2000; Frare and others 2002; Campbell and others 2003; de Gier and others 2003; Petrak and others 2003; Apkarian and others 2004a; Harman and Ruyak 2005; Kalaydjian and others 2007; Logan and others 2008; Daniel and others 2008; Dick and others 2008). Therefore, in determining the functional significance of changes in brain anatomy related to chronic pain, it is important to examine not only pain sensation, but also measure the affective component of the pain.

In the present study, we used a spared nerve injury model (SNI) in rats in order to reveal the temporal development of anatomical changes in the brain related to chronic pain. We chose the SNI model of neuropathic pain described by Decosterd and Woolf (2000) because of its high reproducibility across animals, and its lack of resolution many months after induction (common in many other animal pain models). Further, in addition to measuring pain behaviour, we examined anxiety-related behaviours. Thus, we were able to determine not only when changes in the brain occurred, but also how these changes related to sensory and affective components of the pain experience.

Methods

Animals and surgical procedures

Thirteen male Long-Evans rats (150–180g, Charles River, QC) were housed in pairs (except for one (SNI), who was housed alone) in standard shoebox cages connected to a ventilation rack, in a temperature-controlled (23 ± 1 °C) environment (14h light/10h dark cycle; lights on at 07:00h). The rodents had ad lib access to tap water and were fed 5g of food per 100g of body weight per day per rat (Rodent Chow 5075, Charles River). Animals were randomly assigned to either the SNI (n=8) or sham surgery (opening and exposure of nerve without contacting nerve; n=5) group. SNI involves the transection of two of the three distal branches of the sciatic nerve (tibial and peroneal), while “sparing” the sural nerve. Although others have reported that Long-Evans do not show cold hyperalgesia in the spinal nerve ligation (Yoon and others 1999), we have found reliable mechanical and thermal hyperalgesia in this strain with neuropathic pain models involving injury to the sciatic nerve (Coderre and others 2007). Ethical treatment of animals was ensured, and all procedures were approved by McGill University’s Animal Care Committee.

Behavioural testing

We tested for mechanical and thermal hyperalgesia: tests for cold sensitivity (acetone test) were performed as described by Choi et al. (1994), and tests for mechanical sensitivity (von Frey test, VF) were adapted from Chaplan et al. (1994). We also performed tests of anxiety, locomotion, and exploratory behaviours (elevated plus maze, EPM, and open field, OF). The EPM consisted of four arms of equal length (110cm from end-to-end), two of which had high walls. We recorded time spent in the closed and open arms as well as the middle (which was scored when the forelimbs were outside of the closed arm), the number of times rats exited the closed arms, and the number of rears. The OF was a 90×90cm box with high walls. We scored the time spent in the perimeter (within 15cm of the wall) and centre, the number of rears, and the distance travelled (which was scored for every 15×15cm square the rat traversed). The EPM and OF tests lasted 10 minutes each, and the same blind observer (A.L.L.) performed all the testing and scored all the videos. For the sensory testing, although the observer was still blind, it was sometimes obvious which animals had SNI because of the everted position of the affected hindpaw (Decosterd and Woolf 2000).

Surgery was performed at age 6 weeks, and behavioural tests were conducted within the week prior to surgery and at post-surgical weeks 2, 5, 9, 14, 19 and 24.

Behavioural analysis—We used repeated measures general linear model (GLM) to look for group by time point (weeks relative to surgery) interactions (within subjects), as well as overall main effects for group (between subjects), followed by posthoc tests (alpha 0.05) at each time point between groups. For sensory tests, the additional factor of side (ipsilateral (left), contralateral to injury) was included. Separate analyses were done for each behaviour (VF, acetone, EPM, OF), and post-hoc tests were carried out using one-sided t-tests at each time point when significant omnibus effects were found. For the rare instances where data were missing for a single time point, that data point was calculated as the mean of the group for that time point, so that all cases could be included in the repeated measures GLM. Analyses were performed using SPSS (SPSS Inc. version 16.0.1).

MRI and Deformation Based Morphometry (DBM)

Animals were anaesthetized with 1–2% isoflurane and were wrapped in a heating pad during scanning. Core temperature and respiration rate were monitored throughout the scans. We used a 3D inversion recovery rapid acquisition with relaxation enhancement (RARE) T1-weighted MRI sequence in a 7T Bruker BioSpin, Pharmascan 300 rodent scanner, with the following parameters: TR = 3000ms, TE 7.3ms, flip angle 180°, 0.35mm isovoxel resolution, matrix 128×128×80, total scan time 48 minutes. We scanned the animals at seven time points, during the same weeks as behavioural tests were performed. All rats were scanned at each time point, yielding a dataset of 91 brain scans.

Automated DBM was applied to the MRI data to examine differences in gray matter volume across brain structures. The detailed methods for this procedure are described elsewhere (Kovacevic and others 2005; Lau and others 2008). The tools used are available from the McConnell Brain Imaging Centre (http://www.bic.mni.mcgill.ca/software). In brief, the method involves linear rigid-body (translation, rotation) registration of brains to a template

brain (a brain chosen from the dataset), correction of images for non-uniformity using the N3 algorithm (Sled and others 1998), pairwise (between a brain and each other brain in the dataset) affine registration to create a reference template, and a 12-step nonlinear registration of each brain to that template, which was updated at each step (Collins and others 1994; Kovacevic and others 2005). Deformation fields were created for each image, and Jacobian determinants were calculated at each voxel for each vector in the deformation field (Chung and others 2001; Janke and others 2001). For the linear model analyses, Jacobians were scaled for overall brain size and log-transformed so that values were centred around zero, and ranged from −1 to 1. All DBM results are plotted in terms of Jacobian determinant values, which reflect the relative expansion (values greater than 1) or contraction (values less than 1) at each voxel relative to the reference space. Images are displayed on the template created from the average of all 91 brains in the dataset.

We performed two sets of analyses: the first (linear mixed-effects) to determine the longitudinal changes associated with SNI compared the sham group; and the second (linear fixed effects) to identify the brain areas associated with the level of hypersensitivity in the SNI animals.

Linear mixed effects model to test group differences over time—To assess longitudinal changes, we used a mixed effects model with the factors subject (random effect) and group-by-age (fixed effect). For the group factor, all animals were considered controls at the pre-surgery time point, and at subsequent time points SNI rats were in the SNI group, and sham rats were in the control group. The group-by-time point interaction allowed us to assess for differences between groups over time. For analyses we used RMINC (http://launchpad.net/rminc), which operates via the R statistical package (http://www.r-project.org/). Correction for multiple comparisons were made using false discovery rate (FDR) at a q=0.05 (Genovese and others 2002), which corresponded to an uncorrected p=0.0008, $t_{(78)} > 3.5$. For significant clusters (contiguous voxels), we extracted mean Jacobian values for the entire cluster for each rat. For one of the significant clusters (PFC), we ran a repeated-measures ANOVA in SPSS on the mean Jacobian values with posthoc tests for group differences at each time point.

Linear fixed effects to test effects of mechanical hyperalgesia magnitude—To determine brain regions whose volume was associated with the level of mechanical hyperalgesia, we used a general linear model with von-Frey 50% threshold (VF) as the predictor in SNI rats at all time points post-surgery. We used Gaussian random field theory-based cluster analysis (Worsley and others 2002) to identify significant clusters that passed a corrected threshold of p=0.05. We performed ROI analyses on each significant cluster as was done for the previous analysis. Because age and log-VF scores are correlated, we ran partial correlations in SPSS for each ROI controlling for age. We did this as well for the ROIs identified in the linear mixed effects analysis above.

In keeping with results from studies in humans, we restricted our search to areas within brain gray matter, including cortical and subcortical regions. Brain coordinates are based on the atlas by Paxinos and Watson (2005), and brain regions are labelled according to current

literature and close approximations to human anatomy with particular attention to pain and nociceptive processing.

Results

Behavioural results

For the von Frey test there was a significant group x time point effect ($F_{6,132}$ = 4.123, p<0.005) and a significant main effect for group x side ($F_{1,22}$ = 12.739, p<0.005). Posthoc tests revealed significantly lower thresholds in SNI than Sham on the ipsilateral paw at post-surgery weeks 2, 9, 14, 19, 24 and marginally significant for 5 (p=0.060), as well as a significant difference at weeks 14 and 19 contralateral to the injury (Figure 1A). For acetone, there was a significant effect for time point x group x side ($F_{6,132}$ = 4.379, p<0.001), and a significant main effect for group x side ($F_{1,22}$ = 21.008, p<0.001). Posthoc tests revealed significantly elevated response times for the SNI animals at all ages ipsilaterally, but no differences on the contralateral side (Figure 1B). Overall, these tests demonstrate that the SNI model was associated with early and sustained cold and mechanical hyperalgesia in the paw.

For EPM and OF tests, in addition to significant effects, we report posthoc tests with trends at the alpha 0.1 level. In all of the EPM and OF measures, the only significant within-subjects main effect interaction (time point x group) was for total movement in the OF ($F_{6,66}$ = 3.750, p<0.005), but there was no significant main effect of group ($F_{1,11}$ = 0.639, p>0.440, Fig 1C). Posthoc tests revealed less locomotor activity in SNI than Sham animals at post-surgery weeks 5 and 9 (p<0.1). SNI also performed fewer rears in the OF at weeks 5 and 9 (p<0.05), and spent proportionally less time exploring the centre of the OF at week 9 (p<0.05). In the EPM, SNI rats made fewer exits from the closed arms at 19 (p<0.05) and 24 (p=0.05) weeks post-surgery (Fig 1D). There was also a trend that at 19 weeks post-surgery, SNI rats did fewer rears in the EPM (p<0.1), and spent proportionally less time in the centre of the EPM (p<0.1). Note that most of the EPM differences occurred at late time points – times when there seemed to be no differences between SNI and control rats in locomotion. Altogether, these tests suggest that anxiety-like behaviours appear late – around 4 months after nerve injury – in the SNI model.

MRI results

MRI data were analyzed with deformation based morphometry, which detects differences in the relative volume of structures. Age-related decreases in overall grey matter were seen for all rats, regardless of group.

Group differences—Results from the mixed effects model for the interaction between time point and group are summarized in Fig 2 and Table 1. Several areas showed a relative decrease in the SNI group compared to shams. These areas included prefrontal cortex (comprised of secondary motor, anterior cingulate cortex (ACC areas 24 and 32, labelled “cg1” and “prelimbic,” respectively, in Paxinos and Watson (2005))), retrosplenial cortex (agranular and dysgranular), entorhinal cortex, S1 forelimb area, and a cluster including S1 jaw area, frontal cortex area 3, and dorsal agranular insula. Plots of the data, shown in Fig 2,

demonstrate that the group differences appear to be driven by the late time points, when the above regions are relatively smaller in SNI than sham rats. It should also be noted that these areas that showed group-by-age interactions had overall strong effects of age alone; i.e. the age effects were generally large and consistent for both sham and SNI groups, while the age-by-group interactions were relatively more subtle. For the PFC cluster shown in Fig 2b, the repeated measures ANOVA showed a significant main effect within-subjects (time point; $F_{6,66}$=5.00, p<0.0005), but not for the group x time point interaction ($F_{6,66}$=1.00, p=0.463). Tests for simple effects showed significant differences between groups only at time points 19 and 24 weeks post-surgery (p<0.05). Partial correlations between mean Jacobian value for each ROI and log-VF, controlling for age, were all non-significant.

Effects of mechanical hyperalgesia magnitude—Here, we correlated VF thresholds of the SNI rats with the log-Jacobian values of the DBM maps. Significant clusters are presented at a corrected p<0.05, corrected for multiple comparisons using random field theory. VF thresholds had a significant negative correlation –i.e., increased mechanical sensitivity was associated with decreased volume – with bilateral S1HL/M2, bilateral ACC area 24 (ACC (24)), ACC area 32 (ACC (32)) cortex, and left insula cortex. The bilateral ACC (24) region was slightly caudal to and did not overlap with the ACC region identified in the group analysis, but both regions were within area 24b (Vogt and others 2004), while the ACC (32) cluster was included in the group analysis PFC cluster. Results, including partial correlations controlling for age, are summarized in Fig. 3 and Table 2.

Discussion

We showed here that rats with a chronic painful injury developed anxiety-like behaviour weeks to months after the pain began. Further, we showed a decreased volume in prefrontal and retrosplenial cortices that began at approximately the same time as the anxiety-like behaviour. Finally, we showed that the decrease in cortical volume in somatosensory, anterior cingulate cortex, areas 32 and 24 and insular cortices correlated with the magnitude of mechanical hyperalgesia. Together, these findings suggest that anatomical changes in the brain are related to both affective and sensory aspects of altered pain perception.

Results from the sensory tests confirm findings of other studies that rats sustaining SNI injury have early and sustained hypersensitivity to mechanical and to cold stimuli throughout the duration of the study (Decosterd and Woolf 2000). The development weeks later of decreased exits from the closed arm of the elevated plus maze suggests that these rats may have begun to experience more anxiety-like behaviours over time. Although one might interpret the reduced exits from the closed are as possibly being related to depression, the elevated plus maze is a commonly used model of anxiety-like behaviour and has been validated to assess the anti-anxiety effects of pharmacological agents and steroid hormones, and to define brain regions and mechanisms underlying anxiety-related behaviour (Pellow and others 1985; see also Walf and Frye 2007). Validated depression models, in contrast, include tail suspension, forced swim and sucrose consumption (Cryan and others 2002).

A number of studies of neuropathic pain in rats have examined the effects on anxiety-like behaviour, and results have been inconsistent. In an early study, Kontinen et al. (1999) found

no anxiety –like effects in the elevated plus maze for rats two weeks after receiving a spinal nerve ligation (SNL; Kim and Chung 1992). On the other hand, using an HIV-induced painful peripheral neuropathy, Wallace and colleagues (Wallace and others 2007a; Wallace and others 2007b; Wallace and others 2008) found that at 2–3 weeks post-infection entries into and time spent in the middle of an open field, interpreted as anxiety-like behaviour were reduced, whereas total movement was not affected. Similarly, rats with herpes zoster-associated neuropathy showed anxiety-like behaviours in an open field test at 2-weeks post treatment. In rats with a chronic constriction injury (CCI; Bennett and Xie 1988), Roeska et al. (2009) reported anxiety-like behaviour in the elevated plus maze three weeks after the injury, but did not find these effects using the partial sciatic nerve ligation (PSNL) model of Seltzer and colleagues (1990). LaGraize et al. (2004) used the light/dark chamber test of anxiety in rats with an L5 spinal nerve ligation model of neuropathic pain and found enhanced escape/avoidance three days following the lesion. Mice studies also show contradictory results. Hasnie et al (2007) tested mice up to 28 days post PSNL and found no change in elevated plus maze or open filed behaviour. Similarly, Urban et al. (2008) tested mice for up to 7 weeks post-SNI and found no anxiety-like effects in open field or elevated plus maze testing. In contrast, two studies have reported anxiety-like effects in the elevated plus maze and light/dark test at four weeks post-SNI, but not earlier (Narita and others 2006; Matsuzawa-Yanagida and others 2008). Nevertheless, none of these studies has tested either rats or mice at later time points, when we began to see changes in anxiety-like behaviour. More research is needed to determine if in fact the probability of developing anxiety-like behaviour increases with time post-injury.

People who suffer from chronic neuropathic pain often complain of other affective and cognitive symptoms, so our findings of decreased exploratory behaviour suggest that the model effectively mimics multiple aspects of human pain. There is also evidence that a decrease in anxiety-like behaviours can be reversed with pain treatment (Wallace and others 2008; Roeska and others 2009) and our experimental design will be useful for determining in future studies the brain changes associated with the reduction of anxiety-like behaviours.

The decrease in size of prefrontal cortex became significant at approximately four months after surgery, although the trend began by nine weeks post-surgery. While all animals exhibited clear mechanical and cold hyperalgesia at the first post-surgical test, they initially showed normal behaviour in both the open field and elevated plus maze tests. At 16 weeks post-surgery, SNI rats exited the closed arm of the elevated plus maze less frequently than shams. This change in anxiety-like behaviour shows a strikingly similar temporal pattern to the anatomical changes in prefrontal cortex, suggesting that the anatomical changes and increased anxiety are related.

A number of human studies have implicated the prefrontal cortex in pain-related affect. Whereas the PFC is not always activated by short-lived escapable acute pain in healthy subjects (Apkarian and others 2005), it is highly activated during periods of sustained inescapable chronic pain in patients with long-term back pain (Baliki and others 2006). Recently, Geha et al. (2008) showed PFC atrophy in patients with chronic complex regional pain syndrome (CRPS) and found that the strength of connectivity between atrophied frontal areas related to the anxiety experienced by the patients. Similarly, Apkarian et al. (2004a;

2004b) showed atrophy in the dorsolateral PFC in chronic back pain patients, and these patients also had deficits in emotional decision-making. Another study showed a correlation between levels of trait anxiety and activity in rostral lateral PFC when a healthy individual watched another experiencing pain (Ochsner and others 2008). Finally, the dorsolateral PFC is activated during mood-related pain modulation, in which pain affect is preferentially altered (Villemure and Bushnell 2009), as well as during expectation-related placebo analgesia (Wager and others 2004). Thus, it is not surprising that atrophy of PFC could alter emotional responses related to a chronic pain state.

We also report decreased size of the retrosplenial cortex in SNI versus sham groups. Several studies suggest that the restrosplenial cortex (RSC) and (in humans) posterior cingulate cortex (PCC, areas 31 and 23) have important roles in pain processing. For example, some neurons in the human PCC are responsive to nociceptive stimuli (Lenz and others 1998; Bentley and others 2003; Schlereth and others 2003). In the rabbit, some RSC neurons respond to visceral and cutaneous nociceptive stimuli, and since rats and rabbits do not have a PCC, the functions of RSC in these species might be the same as those of dorsal PCC in humans (Sikes and others 2008). Several studies have reported decreased gray matter density in the PCC in chronic pain from headache (Schmidt-Wilcke and others 2005), phantom-limb (Draganski and others 2006), and fibromyalgia (Kuchinad and others 2007). Other evidence suggests that the PCC is involved in signaling threat in the context of fear of pain (Ochsner and others 2006) and in the empathic response to pain (Danziger and others 2009). The RSC (or PCC in humans) thus may play an important role in pain perception, and the decreased volume we reported in this region suggests that its role is altered in a model of chronic pain.

A second major finding was that S1 hindlimb region and ACC (areas 24 bilaterally and 32 midline) had decreased volume correlating with the extent of mechanical hyperalgesia. These findings suggest that S1 and ACC are involved in either the sensory experience of the heightened pain perception or are involved in modulatory circuits that contribute to the hyperalgesia. Substantial evidence suggests that S1 activity is more related to pain perception than to modulation (Bushnell and others 1999; Apkarian and others 2005). However, mid (MCC) and anterior cingulate cortices are probably involved in both. Some cells in both the human MCC (Hutchison and others 1999) and the rabbit ACC, MCC, and PCC (Sikes and Vogt 1992; Sikes and others 2008) have nociceptive responses, and other animal studies have reported that the ACC is important for descending facilitation of pain and mediation of supraspinal (i.e. non-reflexive) pain behaviours (Zhang and others 2005; Cao and others 2008; Xu and others 2008). In rats, electrical stimulation of the ACC facilitated nociceptive behaviour (tail flick, paw withdrawal latency) and C-fibre-mediated activity in the spinal cord, an effect that appears to be mediated via the brainstem dorsal reticular nucleus (Calejesan and others 2000; Zhang and others 2005). Conversely, ACC stimulation could also reduce (aversive) responses to pain via the PAG in a rat model of neuropathic pain (Labuda and Fuchs 2005). Some area of ACC or MCC is activated in about 80% of PET, SPECT, and fMRI pain studies of healthy subjects (Apkarian and others 2005). Kulkarni and colleagues (2005) reported that increased cerebral blood flow (CBF) in ACC occurred during attention to the pain unpleasantness, while MCC CBF increased with attention to pain localization. Similarly, increasing perceived pain intensity and unpleasantness via hypnosis increases the BOLD fMRI signal in MCC and ACC,

respectively (Rainville and others 1997; Hofbauer and others 2001). Furthermore, human and animal studies suggest that ACC has a role in the affective-motivational component of pain: activation of ACC has been found to be associated with the affective dimension of pain in humans (see Vogt 2005). Together, our findings suggest that plasticity in both ACC and S1 is related to the degree of hyperalgesia, consistent with findings in several studies in humans that pain magnitude (duration, intensity, unpleasantness) is associated with a greater extent of neuroanatomical changes (Apkarian and others 2004b; Schmidt-Wilcke and others 2006; Kuchinad and others 2007; Geha and others 2008; Lutz and others 2008; Kim and others 2008). Here, we reported decreased volume in areas whose anatomy is most similar to human ACC. However, the ACC in rat has corticospinal projections (Miller 1987), whereas in primates these projections originate in MCC (reviewed in Vogt and others 2004). Thus, we suggest the decrease in volume in ACC in our model might reflect both an affective response to pain, as well as a sensorimotor response more commonly seen in human studies showing MCC involvement.

One should note that the degree of hyperalgesia was not directly related to the duration of neuropathic injury, and thus the group and correlation analyses in the present study give different results. This raises two important issues that warrant further study: first, it suggests that duration of pain and pain intensity affect the brain differently; second, it implies that the brain may be capable of changing on a relatively short time-scale, and that correlations with behaviour need to be included in such longitudinal studies. The behavioural findings of the present study – that the onset of anxiety-like behaviours and the timeline for mechanical hyperalgesia do not correspond – highlight this latter point.

Anatomical changes related to neuropathic pain

Our study is the first to show anatomical changes in the rat cortex following a peripheral nerve injury. Other studies have shown that a peripheral nerve injury leads to cell death in the spinal cord dorsal horn in humans (Watson and others 1991) and animals (see Zimmermann 2001). Rats that had received a chronic constriction injury of the sciatic nerve (CCI) had increased numbers of neurons with signs of degeneration in the lumbar dorsal horn on the ipsilateral side compared to the contralateral side (Sugimoto, 1990), and peripheral nerve transection also affected dendritic structure of neurons in spinal dorsal horn (Sugimoto, 1984). However, despite these and other papers reporting neuronal death in CCI (Hama and others 1994; Kawamura and others 1997; Whiteside and Munglani 2001) and SNI (Moore and others 2002) others have reported an absence of neuronal loss in dorsal horn laminae I–III in rats with SNI or CCI (Polgar and others 2004; Polgar and others 2005). It is possible that the dying cells reported in the above papers were actually glial.

The presence of transsynaptic degeneration in the spinal dorsal horn suggests that central anatomical abnormalities may play a role in abnormalities of pain perception. It seems logical to assume that such changes in the spinal cord would also extend to the brain. However, until now there was no direct evidence that higher-order anatomical changes in the brain follow a peripheral nerve injury model of neuropathic pain. Our findings of widespread gray matter decreases, and particularly the relationship between these decreases and the magnitude of hyperalgesic responses, suggest that at least some aspects of chronic

pain involve degenerative processes within the cortex. However, it is uncertain what underlying cellular changes are responsible for the volume decrease. Some possibilities include neuronal or glial cell loss, decreased dendritic arborisation, as recently reported (Metz and others 2009), or changes to the structural matrix or blood vessels. Further work is required to establish the underlying cellular and extracellular processes related to morphometric changes as detected by MRI.

Pros and cons of animal pain models in neuroimaging research

While there are several shortcomings of animal models in pain research, there are a number of important benefits to using animal models in brain imaging research, both for the generation of hypotheses in clinical research, and for testing such hypotheses. First, pain is an individual experience, a point that is essential to understanding a person's pain condition, but one that also makes studying pain in humans very difficult. Here, we are able to control many variables, including diet, the extent of the injury, genetics, environment, and so on, that allow us to focus on how particular behaviours reflect brain changes. Second, the lifespan of a rat and the potential to use invasive procedures allow us to run prospective longitudinal studies that would be much more difficult in human populations. Longitudinal MRI can also reveal the progression of brain damage evolution in ways that histology alone cannot (Onyszchuk and others 2007).

Conclusions

We found that in a rat model of long-term neuropathic pain, frontal cortex volume decreased several months after nerve injury, coincident with the onset of anxiety-like behaviours. Furthermore, the degree of mechanical hyperalgesia is associated with decreased volume in areas involved in the sensory and affective dimensions of pain. This study supports the hypothesis that pain can affect higher cognitive function through late changes to prefrontal cortex and that pain severity predicts changes in primary somatosensory areas and the ACC, which are related to pain modulation. Our model of longitudinal analysis could be useful for translation of animal to clinical work, and could have applications in drug discovery and other types of treatments for chronic pain.

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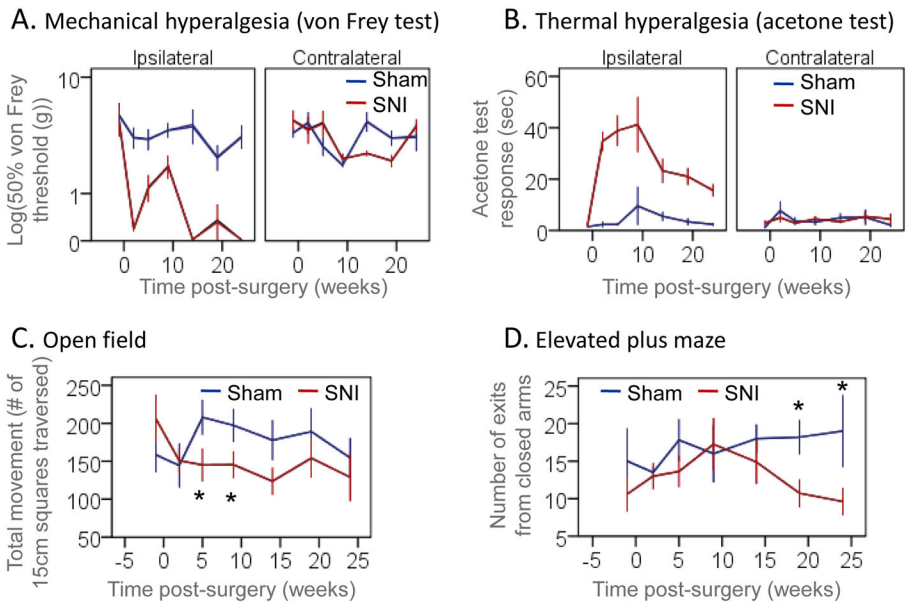

Figure 1.

Behavioural results. SNI rats showed pronounced immediate and sustained mechanical (A) and thermal (cold) (B) hyperalgesia. There was a significant group x time point interaction for locomotion in the open field, with SNI rats showing relatively less movement during middle, but not late time points (C). SNI rats also began to display anxiety-like behaviours several months after injury, demonstrated here (D) as decreased exits from the closed arms of the elevated plus maze. * $p < 0.05$ simple effects tests (shown only for C and D).

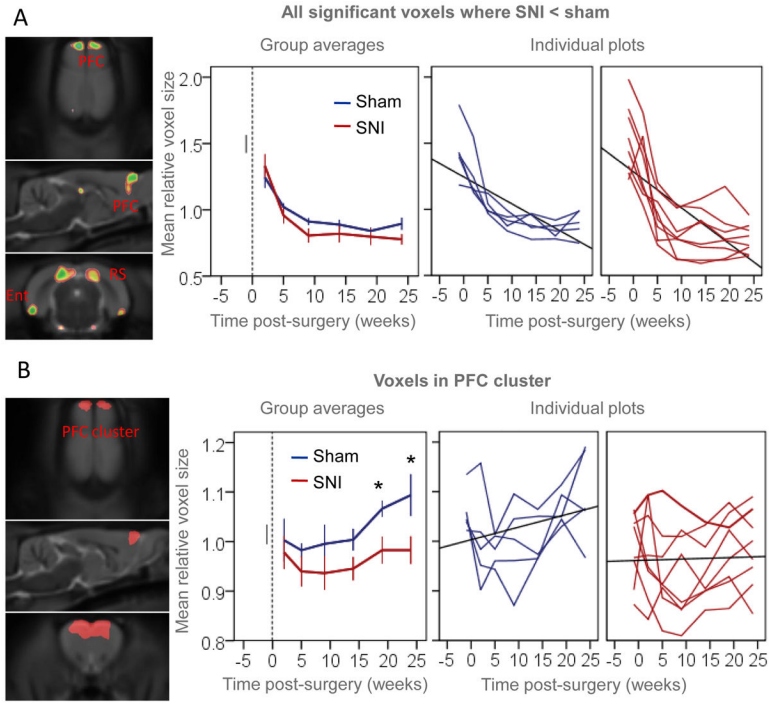

Figure 2.

(A) Results from the linear mixed effects model showing areas of relative decreased volume in SNI rats compared to controls. Decreases were found in several cortical regions, summarized in Table 1. The plots to the right show the mean relative voxel sizes (or the Jacobian determinant mean values, which indicate an expansion (>1) or compression (<1) relative to the reference space) for all the significant (above a threshold of t=3.5) voxels, for each group plotted over time. Plots on the far right show the relative voxel size for each rat over time. (B) The results for one significant cluster from the whole brain analysis (prefrontal cortex, PFC, including secondary motor cortex (M2), anterior cingulate cortex area 24 (ACC (24)), and anterior cingulate cortex area 32 (ACC (32)). There were significant differences between SNI and sham at time points 19 and 24 weeks post-surgery, but not at earlier time points. RS, retrosplenial cortex. Ent, entorhinal cortex. Error bars are standard errors of the means. The vertical line indicates the approximate time the surgeries were performed. * $p < 0.05$ simple effects tests.

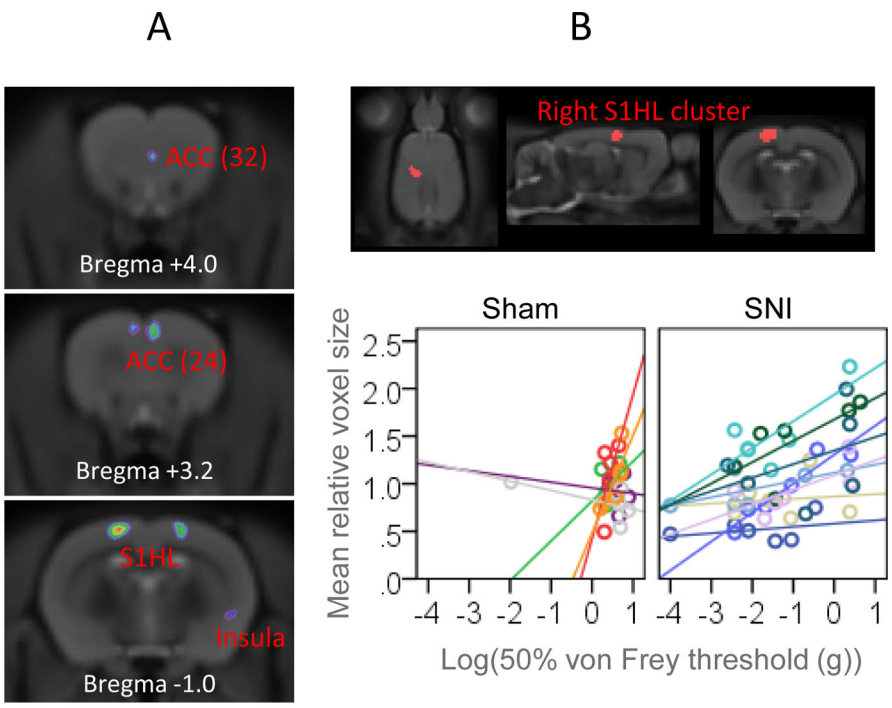

Figure 3.

Results from the von Frey (mechanical hyperalgesia) analysis, showing (A) significant clusters where increasing mechanical hypersensitivity predicted decreased volume in rats with SNI. Six significant clusters were identified in the analysis and are shown in the figure. (B) The scatter plot for one region (right S1HL) is shown (mean relative voxel sizes, or the Jacobian determinant mean values, indicate an expansion (>1) or compression (<1) relative to the reference space) and a regression line is added for each rat. The von Frey scores for all time points post-surgery are included. For abbreviations see Table 2. Error bars are standard errors of the means.

Table 1

Interactions between time point and group (mixed effects model results).

Cluster or ROI name	Side	Coordinates of peak(s) voxel[¥]	Peak region	Peak t-value	Effect direction
PFC	Bilateral/Midline	1.2, 4.2, 8.8 −1.6, 4.3, 8.5	M2 M2	−7.47	Sham>SNI
Retrosplenial	Bilateral	2.3, −7.2, 7.8 −2.6, −7.1, 7.1	RSGa RSGa	−6.51 −5.75	Sham>SNI
Entorhinal	Bilateral	6.5, −6.9, 2.1 −6.2, −6.5, 1.5	DIEnt DIEnt	−7.33 −7.40	Sham>SNI
S1J/Fr3/AID	Right	3.7, 3.6, 5.6	Fr3/AID	−4.44	Sham>SNI
S1FL	Left	−3.7, 3.6, 5.6	S1FL	−3.68	Sham>SNI

[¥]according to Paxinos and Watson, 2005, given in mm x (0 = centre, left is negative), y (relative to Bregma), and z (ventral to dorsal); two peaks shown for bilateral regions. PFC, prefrontal cortex, includes secondary motor cortex (M2) and ACC areas 24 and 32. Retrosplenial includes retrosplenial dysgranular (RSD (area 30)) and granular (RSGa/b) cortices. Entorhinal includes dorsolateral (DLEnt), intermediolateral (DIEnt), and ventral intermediate (VIEnt) entorhinal cortex. S1J/Fr3/AID, S1 jaw area, Frontal cortex area 3, dorsal agranular insula cortex. S1FL, S1 forelimb area.

Table 2

Results from von Frey linear model.

Cluster or ROI name	Side	Coordinates of peak(s) voxel[¥]	Peak region	Peak t-value	VF partial correlation corrected for age[†]	
					SNI (df=45)	Sham (df=27)
S1HL/M1	Right	1.8, −1.1, 8.8	M1	3.92	0.400 **	−0.013
S1HL/M1	Left	−2.2, −1.1, 8.8	S1HL	3.35	0.334 *	−0.067
ACC (24)	Left	−0.5, 3.0, 8.4	ACC (24)	3.57	0.342 *	−0.202
ACC (24)/M2	Right	0.9, 3.0, 8.4	ACC (24)/M2	3.19	0.408 **	0.162
Insula	Left	−5.5, −1.0, 3.1	DI/GI	2.84	0.375 **	−0.057
ACC (32)	Midline	−0.2, 4.0, 6.0	ACC (32)	3.19	0.365 *	0.172

[¥] according to Paxinos and Watson, 2005, given in mm x (0 = centre, left is negative), y (relative to Bregma), and z (ventral to dorsal); two peaks shown for bilateral regions.

[†] Correlation coefficients between log-(50% VF thresholds) and the mean log-Jacobian determinant values for the cluster at post-surgery time points.

* $p < 0.05$,

** $p < 0.01$, two-tailed.

S1HL/M1, S1 hindlimb area/primary motor cortex, ACC (24), anterior cingulate cortex area 24, M2, secondary motor area, ACC (32), anterior cingulate cortex area 32. Insula includes granular (GI) and dysgranular (DI) insula cortex.