UVB irradiation induces contralateral changes in galanin, substance P and c-fos immunoreactivity in rat dorsal root ganglia, dorsal horn and lateral spinal nucleus

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ABSTRACT

The selection of control group is crucial, as the use of an inadequate group may strongly affect the results. In this study we examine the effect on contralateral tissue protein levels, in a model of unilateral UVB irradiation, as the contralateral side is commonly used as a control. Previous studies have shown that UVB irradiation increases immunoreactivity for inflammatory regulated neuropeptides.

Unilateral UVB irradiation of rat hind paw was performed and corresponding contralateral spinal cord and dorsal root ganglia (DRG) were collected 2–96 h after and investigated for changes in galanin, substance P and c-fos immunoreactivity. Control tissue was collected from naïve rats. Measurement of skin blood flow from contralateral heel hind paws (Doppler), revealed no change compared to naïve rats. However, UVB irradiation caused a significant reduction in the contralateral proportion of galanin immunopositive DRG neurons, at all-time points, as well as an increase in the contralateral spinal cord dorsal horn, around the central canal and in the lateral spinal nucleus (2–48 h). The contralateral proportion of SP positive DRG neurons and dorsal horn immunoreactivity was unchanged, whereas the lateral spinal nucleus area showed increased immunoreactivity (48 h). UVB irradiation also induced a slight contralateral upregulation of c-fos in the dorsal horn/central canal area (24 and 48 h). In summary, unilateral UVB irradiation induced contralateral changes in inflammatory/nociceptive neuropeptides in spinal cord and afferent pathways involved in pain signaling already within 24 h, a time point when also ipsilateral neurochemical/physiological changes have been reported for rats and humans.

1. Introduction

Experimental unilateral inflammatory models including inflammatory pain models often use the contralateral side as control. Usually, one hind limb in rats or mice is exposed to an experimental procedure and the induced changes in the ipsilateral spinal cord and dorsal root ganglia (DRG) are compared to those on the contralateral side. This is a practical and convenient choice of control group, as long as contralateral changes do not occur as well.

Both biochemical and neurological changes have been described on the contralateral side after unilateral inflammatory lesions [1]. Anatomical and functional studies on decerebrated laboratory animals such as rats, mice and ferrets have shown that cross-communication, between ipsi- and contralateral sides, created contralateral responses at different levels of the spinal cord [1,2]. Due to the nature of these responses, the use of the contralateral side as control can be questioned, e.g. when evaluating various types of inflammatory responses. It is likely that ipsilateral changes have been underestimated when the contralateral side has been used as the control [1]. Moreover, the presence of neurons with bilateral receptive fields in the rat dorsal horn has been reported. It has been suggested that the number of detectable neurons with bilateral receptive fields increase after unilateral inflammatory stimulation as compared to the normal intact situation [3–6]. In a study conducted by Petkó and Antal (2000) [7], neurons in the superficial dorsal horn were found to project to superficial layers in the dorsal horn on the contralateral side. Furthermore, expression of some immediate-early genes and release of neurochemicals in response to inflammation have been reported in different laminae of the ipsilateral, as well as the contralateral, spinal cord after unilateral inflammatory stimulation [8–11]. The main laminae of the spinal cord receiving primary afferent projections, are ipsi- and contralateral areas of lamina I-II, and III-IV of the dorsal horn [12]. Furthermore, it has been reported that

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Denmark) were used [24]. Animals were divided into 5 experimental groups with UVB under anesthesia, and blood flow was examined using laser Doppler in awake animals immediately before and 24 h after irradiation with UVB to detect changes in response to contralateral UVB exposure.

Experimental exposure of the skin to ultraviolet B (UVB) irradiation results in inflammation and mechanical hyperalgesia in humans, rats and mice [16–20] as well as induction of neurophysiological changes in rats [22] which correlate with the neurophysiological changes observed in humans [23] after UVB exposure. Thus, the UVB-model can be considered a translational pain model [16–21]. The peak for cortical activity in rats, 24 h after UVB irradiation, correlates with the time point reported from human studies, 24–32 h [17,23]. Furthermore, UVB irradiation of the rat hind paw will induce rapid increase in galanin, substance P and c-fos immunoreactivity in the ipsilateral spinal cord, and also reduce the proportion of galanin positive neurons in the corresponding dorsal root ganglia. The induced changes peak within a similar time frame (24–48 h) [24] as neurophysiological changes observed in a previous rat study [22]. Thus, it seems that UVB irradiation will rapidly induce both neurophysiological and biochemical changes, in a similar time frame, within the nervous system. However, whether unilateral UVB irradiation will also induce contralateral changes in the spinal cord and dorsal root ganglia remains unknown. In this follow up study, we investigated the modulation of the inflammatory regulated neuropeptides (SP and galanin), as well as a transcription factor commonly used as an activity marker (c-fos), on the contralateral side. The present study focuses on the investigation of associated contralateral changes as it is of crucial importance to clarify whether the contralateral side is an appropriate control in various neurophysiological studies.

2. Materials and methods

In the present study we have utilized tissues from the contralateral side of the spinal cord and DRG from animals in one of our previously published studies [24], to evaluate if there are any contralateral changes in galanin, substance P and c-fos immunoreactivity after UVB irradiation of the rat hind paw. The rationale for using this material (not previously analyzed), rather than generating identical new contralateral tissues, is that it will allow for a direct comparison with the changes observed at the ipsilateral side since all histology/analysis is performed under the exact same conditions, and also minimize the number of rats subjected to painful stimulation, namely UVB irradiation.

2.1. Experimental animals

In short, a total of 44 female Sprague-Dawley rats (Tacorian, Denmark) were used [24]. Animals were divided into 5 experimental groups (n = 8 per group), and a control group (naive, untreated rats; n = 4 rats, however both left and right sides were examined). Animals in the experimental groups were perfused and tissues harvested 2, 12, 24, 48, or 96 h after UVB irradiation, as previously described [24]. In all experimental groups, the heel area of the right hind paw was irradiated with UVB under anesthesia, and blood flow was examined using laser Doppler in awake animals immediately before and 24 h after irradiation to evaluate the degree of inflammation, for details see [24]. The increase in blood flow in the heel area ipsilateral to the UVB irradiation was almost doubled compared to pre-irradiation values or values from the contralateral side which were not affected [24].

2.2. Tissue preparation and immunohistochemistry

Animals were anesthetized using fentanyl/midazolam (0.1 mg/kg, and 10 mg/kg respectively) administration as described by Etemadi et al. 2017 [24]. The animals were subsequently perfused with ~150 mL room tempered saline (0.9 % NaCl in distilled water), followed by ~300 mL of ice-cold 4 % paraformaldehyde (PFA), in 0.1 M phosphate buffer (pH 7.4). Dissected tissues were postfixed for 1½–2 h in 4 % PFA, cryoprotected in 25 % sucrose solution, frozen on dry ice and stored at −80 °C until sectioned. Horizontal, 16 μm sections of spinal cord lumbar enlargement (spinal cord: L5), and corresponding dorsal root ganglia (DRG: L5) were obtained in the same manner as previously described in Etemadi et al. 2017 [24]. All sections were stored at −25 °C until immunostaining. The following antibodies were used: i) galanin (1:1000, rabbit anti-galanin, Cat. Nr. 7100T(4-326), Peninsula), ii) substance P (1:1000, rabbit anti-SP, Cat. Nr. 20064, Immunostar), iii) c-fos (1:10,000, rabbit anti-c-fos, Cat. Nr. PC38, Calbiochem) and iv) HuC/HuD (1:600, mouse anti-HuC/HuD, Cat. Nr. A-21271, Life technology). On the first day of staining, tissues were hydrated in phosphate buffered saline (PBS) (0.01 M, pH = 7.4), and then incubated in blocking solution (5% normal goat serum in 0.25 % Triton X-100 in PBS) for 1 h. In the next step, primary antibody incubation was performed over night at room temperature. The following day, tissues were washed repeatedly in PBS, and incubated in light sealed boxes for 2 h with the nuclear marker (DAPI) (4′,6-diamidino-2-phenylindole), (1:1000, Cat. Nr. D3571, Invitrogen), and secondary antibodies (goat anti-rabbit IgG Alexa 594 and goat anti-mouse IgG Alexa 488 (1:500), anti-rabbit, Cat. Nr. A11005 and anti-mouse Cat. Nr. A11001, Invitrogen), diluted in the blocking solution. After this, slides were rinsed in PBS, and mounted using DABCO (Fluka/Sigma-Aldrich, Switzerland) mounting media. Previous studies have already confirmed the specificity of the primary antibodies used in the current study, galanin [24–26] substance P [27–29] and c-fos [30,31]. Moreover, to test the specificity of the secondary antibodies, incubation with omission of the primary antibodies was performed.

2.3. Quantitative and qualitative analysis of immunoreactivity in DRG and spinal cord

2.3.1. Quantification of galanin, SP and c-fos immunoreactivity in DRG

The proportion of galanin and SP-immunopositive neurons in experimental/control DRG tissue was analyzed first by qualitative screening of all sections (6 sections/experimental condition and animal) and then quantitatively from montages of 10X photomicrographs from DRG: L5 sections captured using a fluorescence microscope (Nikon Eclipse 80i, Japan) connected to a DS-Ri1 digital camera (Nikon Instruments, Japan). Representative sections, i.e. sections reflecting the overall labeling for the DRGs exposed to the same experimental condition and without tears or folds were defined and in these at least 250 HuC/HuD positive neuronal profiles were evaluated per experimental condition. All sections were processed under the same conditions during the entire immunohistochemical and quantitative analysis. The examined neuronal profiles were evaluated, with regards to presence of galanin-, SP-, or c-fos-immunoreactivity, and compared with control tissues to detect changes in response to contralateral UVB exposure.

2.3.2. Quantification of galanin and SP immunoreactivity in spinal cord

To evaluate the density of galanin and SP immunoreactivity in the contralateral spinal cord we first examined six SC: L5 sections/experimental group and animal (n = 8 animals for each experimental condition) and n = 4 for the control animals (both left and right sides) qualitatively. In the subsequent quantification process, we analyzed photomicrographs obtained from one representative section per experimental condition and animal (sections reflecting the overall labeling of spinal cords exposed to the same experimental conditions, without tears or folds). The photomicrographs of the spinal cord sections immunostained for galanin or SP were captured under a 10X objective, using a DS-2 MV digital camera (Nikon, Japan) connected to a Nikon eclipse 80i microscope (Nikon, Japan). Measurements of the density of labeling (immunoreactive area for galanin or SP respectively) is reported as the proportion of labeled area in relation to the total area and was analyzed using an image analysis system, NIS-Elements 3.1 software (Nikon, Japan).
Instruments, Japan). For quantification, two rectangular regions of interest (ROIs) with different sizes were defined: i) a larger ROI encompassing the dorsal spinal cord area (ROI: 1200 × 1800 μm) (Fig. 1a), and ii) a smaller ROI encompassing the area around the central canal (ROI: 1000 × 700 μm) (Fig. 1b). Quantifications of these ROIs were performed in spinal cord tissue contralateral to UVB irradiation, and from the control group consisting of naïve animals. In a first step, the threshold for each marker was set at a fixed ratio of the mean background intensity (this value was calculated individually by two researchers) for each image, to ensure that specific antigen labelling and not unspecific background staining was evaluated. As the value for this threshold is related to the binding properties of the antibody to its cognate antigen, the threshold for signal to background ratio will differ between different markers and for galanin immunofluorescence it was set at 4.5 times above background and for SP immunofluorescence to 9 times above background intensity. For determination of the fraction of galanin or SP immunofluorescence in the dorsal horn and LSN area, immunoreactivity of the tissue delineated by the spinal cord border in the larger ROI was quantified. These results are presented as the dorsal horn + LSN area (see Results). In a second step, after exclusion of the immunopositive dorsal horn area, the LSN immunopositive area was obtained (see Results). The smaller ROI was used to quantify the immunoreactivity in the area around the central canal.

2.3.3. Qualitative evaluation of c-fos immunoreactivity in spinal cord

For qualitative evaluation of c-fos immunopositive neuronal profiles, we examined 6 sections per animal/experimental group (n = 8 for each experimental condition and n = 4 for the control group) for each area of evaluation. One representative section (i.e. of good technical quality/devoid of tears and folds, processed under same conditions, and reflecting the overall labeling of spinal cord exposed to the same experimental conditions), from each individual animal and experimental group was selected. Based on our initial examination, we chose two different areas for evaluation of the distribution of c-fos immunoreactivity: i) the dorsal part of the spinal cord and ii) the area around the central canal. Spinal cord sections stained for c-fos were imaged under a 20X objective, using a DS-2 MV digital camera (Nikon, Japan) connected to a Nikon eclipse 80i microscope. A blinded qualitative analysis of presence of c-fos immunoreactivity was performed for all photomicrographs, by an experienced histologist who was unaware of the section identity, using a modified method for qualitative scoring [24,32-34]: i) + none or very few c-fos immunopositive neuronal profiles; ii) ++ moderate number of c-fos immunoreactive neurons, and iii) +++ more frequent immunofluorescent c-fos staining of neuronal profiles. As we could not detect any obvious changes in c-fos expression in the LSN area, no further analysis of this area was performed.

2.4. Statistical analysis

The Kruskal-Wallis test was used for statistical analysis, followed by Dunn’s post hoc test when adjustment for multiple comparisons was needed. A p-value of ≤ 0.05 was considered statistically significant. The data is presented as box and whiskers diagrams with an indication of the median values. The box represents the 25th and 75th percentile and the whiskers represent the minimum and maximum values. Graphpad Prism® 6.0 (Graphpad Software Inc., USA) was used to analyze the data.

3. Results

3.1. DRG

3.1.1. Galanin

In the control (naïve) group, approximately 10 % of the neuronal profiles in the DRG showed immunoreactivity for galanin (Figs. 2 a, b and 3 a). After UVB irradiation the proportion of galanin immunopositive neuronal profiles on the contralateral side was reduced to approximately 5%. This reduction was statistically significant (Figs. 2 c, d and 3 a), for all time points.

3.1.2. SP

Our analysis showed that UVB irradiation had no significant effect on the proportion of SP immunopositive profiles in contralateral DRG neurons compared to neuronal profiles in control animal DRG. The proportion was 16 % in the naïve, control group and 15 % in contralateral DRGs (Fig. 3b).
3.1.3. C-fos

No c-fos activity could be observed in DRG sections from the control group (data not shown). UVB irradiation did not induce detectable c-fos expression in DRG neuronal profiles on the contralateral side.

3.2. Spinal cord

Analysis of the L5 spinal cord, was conducted for three different areas: the dorsal horn, the LSN, and around the central canal, for control animals and spinal cord tissue contralateral to UVB irradiation. The
immunoreactivity was evaluated for all three markers: galanin, SP and c-fos.

3.2.1. Galanin distribution

In the control group, spinal cord sections showed galanin immunoreactivity, typically in fibers, in the superficial layers of the spinal cord dorsal horn (Fig. 4a). In the UVB treated animals, contralateral staining for galanin was increased after UVB radiation compared to the control group in both the dorsal horn and LSN area (Fig. 4b-d, Supplementary Fig. S1, and 6a-b). The increased immunoreactivity for galanin in the dorsal part of the spinal cord (dorsal horn + LSN area), was significant 24 h after irradiation (Fig. 6a). When the dorsal horn was analyzed separately no significant change was observed, however when the LSN area only was analyzed the immunoreactivity for galanin was increased at 24 h compared to the control group (Fig.6b). The area around the central canal showed significant induction of contralateral galanin positive immunostaining at all selected time points, except at 96 h after UVB irradiation (Fig. 6c).

3.2.2. SP distribution

Immunostaining of spinal cords from control animals showed SP immunoreactivity mainly in nerve fibers, which were typically detected in the superficial layers of the dorsal horn and LSN area (Fig. 5a). We could not detect any significant changes with regards to SP immunoreactivity when comparing the dorsal spinal cord area (dorsal horn + LSN area), from the contralateral side with control animals. However, a quantitative analysis of SP immunoreactivity in the LSN area alone, revealed a significant increase in SP immunoreactivity in the contralateral LSN 48 h after UVB irradiation (Fig. 5b-d, Supplementary Fig. S2, and 6d). When the dorsal horn area was analyzed separately, no significant changes were detected. There were no detectable changes in SP immunoreactivity within the central canal area.

3.2.3. C-fos distribution

In the control group we could detect sparse immunostaining for the c-fos marker in the dorsal horn area and the area around the central canal (Figs. 7 and 8). In comparison with the control group, contralateral spinal cord dorsal horn area, received a higher scoring for c-fos immunoreactivity 24 and 48 h after UVB irradiation (Figs. 7b and 8 a). A similar, but less obvious pattern could be observed in the area around central canal (Figs. 7b and 8 b).

4. Discussion

In this study we have investigated induction of contralateral biochemical changes in the rat DRG and spinal cord after UVB irradiation of the rat hind paw. Our findings show a reduction in the proportion of galanin positive neurons (i.e. neuronal profiles) in the DRG contralateral to UVB irradiation at all selected time-points (2–96 h) as compared to the control group (naïve). As we have reported previously [24], the proportion of galanin immunoreactive DRG neurons ipsilateral to UVB irradiation decreased compared to controls, from approximately 10 % in the control group (naïve) to 3.5 % ipsilateral to UVB treatment [24]. In the present study of contralateral changes, we also found a reduction, and the proportion of galanin immunopositive DRG neurons was approximately 5%. Compared to the ipsilateral side, UVB irradiation had a milder effect on galanin expression on the contralateral side. The proportion of galanin positive DRG neurons during normal conditions has been estimated to be about 5–10 % [24,35–37]. Note however that galanin synthesis is considered to be a dynamic process that may involve up to about 40 % of the DRG neuronal population during normal conditions [38]. The proportion of SP positive neurons in the contralateral DRGs was unchanged when compared to the control group. The control levels of SP in DRGs neurons found in this study, 16 %, are well in line with previous findings of about 20 % [39]. As suggested previously [39] peptides may be divided in two groups, the first (type I) being peptides which are detected in rather large amounts during normal conditions such as SP. The second group (type II) containing peptides expressed in low amounts during normal conditions. In some cases, a peptide may be placed in both groups and galanin is an example of such a peptide. In DRG, galanin may be categorized as type II whereas in hypothalamus it can be categorized as type I. No induction of c-fos immunoreactivity in DRGs was found after UVB irradiation, and thus no changes were reported since c-fos is not detectable in DRG neurons in naïve animals [24,40].

The general impression when evaluating galanin immunoreactivity in the dorsal part (dorsal horn + LSN) of the spinal cord was that there was an increased reactivity on the contralateral side. However, the only significant change was found 24 h after UVB irradiation, which corresponds to the data reported from the ipsilateral side [24]. The increase found on the contralateral side is similar to what was found ipsilaterally, except for 24 and 48 h after irradiation when ipsilateral changes were even greater (roughly twice as large). An increase in galanin immunoreactivity in the ipsilateral dorsal horn and LSN may be explained by an increased release/export of galanin from the DRG neurons, which are
Fig. 6. Diagrams showing the proportion of galanin and SP positive area in the L5 spinal cord dorsal horn area, the LSN area and the area around the central canal, in control animals and contralateral to UVB irradiation. UVB irradiation induced increased galanin immunoreactivity in the L5 dorsal spinal cord area (a), in the LSN area (b), and also in the area around the central canal (c) on the side contralateral to UVB exposure. The quantitative analysis of the contralateral dorsal part of the spinal cord revealed that the immunoreactivity for galanin was upregulated 24 h after UVB irradiation (a). This increase was statistically significant compared to control animals (p < 0.05). Quantification of the immunoreactivity in the LSN area alone (b) showed a statistical difference 24 h after UVB irradiation, with an increased galanin immunoreactivity (p < 0.05). In the area around the central canal an increased immunoreactivity for galanin, could be detected, mainly in fibers, at all time points except 96 h after exposure to UVB radiation (p < 0.05). UVB irradiation induced a significantly increased SP immunoreactivity (d) in the LSN area (48 h after UVB irradiation), but not in the area around the central canal, compared with control animals (p < 0.01).

Fig. 7. Immunohistochemical staining of c-fos activity (red) in the contralateral L5 spinal cord dorsal horn and around the central canal 24 h after UVB irradiation. The immunoreactivity for the c-fos marker was increased in the dorsal horn, as well as in the area around the central canal (24 h) after UVB irradiation (b) compared to the control group (a). The blue arrowheads indicate the dorsal horn area, and green arrowheads indicate the central canal area. The white arrows point out some of the c-fos positive neurons. Scale bar: a and b = 100 μm.

Fig. 8. Diagrams visualizing the qualitative scoring of the proportion of c-fos immunopositive staining of the L5 spinal cord dorsal horn area and the area around the central canal in control animals, and contralateral to UVB irradiation. The spinal cord contralateral to UVB exposure shows an enhanced c-fos immunoreactivity 24 and 48 h after UVB irradiation, both in the dorsal horn area (a) and the area around the central canal (b).
showing a rapid decrease in galanin immunoreactivity after UVB irradiation [24]. Another option is that increased galanin immunoreactivity in the dorsal horn may come from spinal interneurons, since Lamina I-II contain interneurons which produce galanin [38]. The observed increase in galanin immunoreactivity on the contralateral side may also be caused by release/export from DRG neurons on the same side. When analyzing the galanin immunoreactivity in the contralateral LSN area, we could observe significant changes after 24 h. In our previous paper, we also reported significant changes in the ipsilateral dorsal part (dorsal horn + LSN) of the spinal cord at 24 and 48 h after UVB irradiation, and when the ipsilateral LSN area was analyzed separately significant changes could be observed at 12 and 24 h after UVB irradiation. As discussed above (for DRG), the contralateral findings for the dorsal part of the spinal cord also demonstrate a similar but less pronounced change in galanin immunoreactivity after UVB irradiation, when compared to our previous ipsilateral findings. In the area around the central canal, we could detect a significantly increased galanin immunoreactivity at 2–48 h after UVB exposure. UVB irradiation will increase galanin immunoreactivity in the area around the central canal on both the ipsilateral and contralateral side after UVB irradiation when compared to control (naïve) rats. For SP, our immunohistochemical analyses revealed that in general the immunoreactivity was unaffected in the dorsal part of the spinal cord (dorsal horn + LSN area), but a significant increase could be detected at 48 h in the LSN area. This finding is slightly different from our previous report, where we could detect increased SP immunoreactivity from the spinal cord (dorsal horn + LSN), 48 h after UVB irradiation [24]. Our analysis of c-fos, showed a significant increase in the induction on the contralateral side 24 and 48 h after irradiation when compared to the control group, both for the spinal cord dorsal horn area and around the central canal. We have reported similar changes in c-fos in these areas for the ipsilateral spinal cord after UVB irradiation of the rat hind paw [24].

It is not surprising that both galanin and SP immunoreactivity increased in the LSN area on both the ipsi- and contralateral side after UVB irradiation. Ling et al. [41], has presented neuroanatomical evidence that LSN receive direct inputs from both ipsi- and contralateral primary afferents. Furthermore, other anatomical and electrophysiological studies have suggested that the LSN area receive innervation from the contralateral lamina I, and that LSN has ipsilateral projections to spinal cord laminae I, II, V and VII which influence the spinal cord activity level in response to peripheral noxious stimuli [13–15,42–44]. Petkó and Antal [7] reported that the LSN receive input from neurons located on the lateral side of the contralateral superficial dorsal horn. Therefore, it is possible that nociceptive afferents activated by UVB irradiation project to the contralateral side as well, inducing an increased immunoreactivity for galanin and SP in the contralateral LSN, which was observed in the present study. It is also noteworthy, that the LSN area is receiving input from spinal interneurons containing SP and other neuropeptides [13,45,46]. In addition, local production of SP in the LSN area has also been reported [47,48].

Previous studies have demonstrated alterations in neuropeptide/receptor expression in contralateral DRG neurons in response to unilateral inflammatory stimulation [1,49–51]. It is well established that peripheral inflammation will decrease galanin content in ipsilateral DRG neurons, while increasing the amount of galanin mRNA positive neurons in the ipsilateral dorsal horn [52,53]. Lang R. et al. [39] reported that most of the galanin produced in the DRGs will be exported to laminae II of the spinal cord via afferent terminals. The present finding of a decreased proportion of galanin positive DRG neurons contralaterally, in response to unilateral UVB stimulation of the hind paw, is an interesting finding which to our knowledge has not been observed before and the mechanism for this reduction is unclear. However, a similar phenomenon has been described by von Banchet et al. [49], where monoarticular arthritis induced bilateral upregulation of neurokinin 1 and bradykinin 2 receptors in rat DRG. Since the upregulation observed by von Banchet et al. [49] was symmetrical and segmental they suggested that these changes are due to a symmetrical afferent innervation projecting to both sides of the spinal cord. It has been demonstrated that the central nervous system is involved in inducing bilateral inflammatory pain [54]. As suggested by von Banchet et al. [49], effector CNS activity might induce receptor expression in DRG neurons. If such activity also can induce changes in galanin expression on the contralateral side is not known.

In the present study, unilateral UVB irradiation induced an increased contralateral c-fos immunoreactivity 24 and 48 h post irradiation in the dorsal part of the spinal cord, as well as in the area around the central canal. Similar observations have previously been reported, e.g. contralateral expression of c-fos in different laminae of the spinal cord in response to a peripheral painful stimuli [1,8–11,14]. Together with our study, this supports that there is an activation also of contralateral neurons during such conditions. As reviewed by Shenker et al. [1], anatomical studies have revealed that sensory fibers in the spinal cord decussate via the dorsal commissure providing a possibility for crosstalk between both sides of the spinal cord. Afferent fibers conveying information about pain and temperature, and which are likely activated during inflammation, cross the midline at the segmental level before projectingcranially on the contralateral side. The present, and our previous study [24], suggest that the translational inflammatory pain model using UVB irradiation will induce changes on both the ipsilateral and contralateral side. As pointed out by Shenker et al. [1] results from studies where the contralateral side is used as the control might underestimate the response on the ipsilateral side. The present study supports the claim made by Shenker et al. [1] that contralateral changes are detected when the contralateral side is compared to a naïve control group. Along with our previous study (Etemadi et al. 2017) [24] this also strongly emphasizes that when studying changes in the nervous system, induced by inflammation, the control group should consist of normal naïve animals and not be composed of the contralateral side.

Authors’ contributions

All authors (LE, LMEP, ND) made substantial contribution to conception, planning and design, interpretation of data and revising the manuscript critically for important intellectual content. LE (Leila Etemadi) carried out all experiments, analyzed all data aided by LMEP (Lina Pettersson) and wrote the manuscript. Manuscript revision was done by ND (Nils Danielsen) and LMEP (Lina Pettersson). All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors reported no declarations of interest.

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Appendix A. Supplementary data

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