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Immobilization stress sensitizes rat dorsal horn neurons having input from the low back

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Abstract

Background: Stress is known to promote several forms of muscle pain including non-specific low back pain. However, the question if stress alone activates nociceptive central neurons has not been studied systematically. Here, we investigated the influence of repeated immobilization stress on dorsal horn neurons and behaviour in the rat.
Methods: The stress consisted of immobilization in a narrow tube for 1 h on 12 days. Single dorsal horn neurons were recorded with microelectrodes introduced into the spinal segment L2. In this segment, about 14% of the neurons responded to mechanical stimulation of the subcutaneous soft tissues of the low back in naïve rats. The neurons often behaved like wide dynamic range cells in that they had a low mechanical threshold and showed graded responses to noxious stimuli.
Results: The stress-induced changes in neuronal response behaviour were (1) appearance of new receptive fields in the deep tissues of the hindlimb, (2) increased input from deep soft tissues, but unchanged input from the skin and (3) significant increase in resting activity. Surprisingly, the pressure-pain threshold of the low back remained unchanged, although dorsal horn neurons were sensitized. In the open field test, the rats showed signs of increased anxiety.
Conclusions: This study shows that stress alone is sufficient to sensitize dorsal horn neurons. The data may explain the enhanced pain low back patients report when they are under stress. The increased resting discharge may lead to spontaneous pain.

1. Introduction

Situations perceived as stressful by humans are known to induce or enhance several forms of muscle pain. Examples are non-specific low back pain (Mendelek et al., 2013), fibromyalgia syndrome (Bansevicius et al., 2001) and pain at the workplace (Sembajwe et al., 2013). The enhanced pain under stress is assumed to be mainly due to descending influences from higher central nervous centres. In contrast, other types of muscle pain, such as pain due to trauma or ischaemia as well as delayed onset muscle soreness, are due to nociceptive input from the periphery.

Possible descending influences are changes in neuronal systems such as the descending pain-modulating pathways and increased activity in the sympathetic system (Imbe et al., 2014) or a dysfunction of the hypothalamic–pituitary–adrenocortical (HPA) axis. These changes have particularly strong effects, when stress is repeated or chronic (Imbe et al., 2014). The stress-induced effects on pain and other disorders were investigated in various animal

What's already known about this topic?
- Stress is known to promote several forms of muscle pain including non-specific low back pain. However, whether stress activates central neurons processing nociceptive information from soft tissues of the low back has not been studied systematically.

What does this study add?
- The study shows that immobilization stress alone is sufficient to alter the responsiveness of spinal dorsal horn neurons processing input from soft tissues of the low back.

models. In rats, immobilization stress has been shown to lead to the formation of gastric ulcers (Goldman and Rosoff, 1968) and activation of the HPA axis (Pacák, 2000). Recent results point to the amygdala as a key structure in stress-induced pain. Under stress, neurons of this nucleus show a reduction in synaptic inhibition leading to stronger fear responses in animals (Suvrathan et al., 2013).

Although several pathological sequelae of stress are known to occur in patients and experimental animals, an important piece of knowledge is missing, namely the effect of stress on neurons in the spinal dorsal horn. Part of these neurons form the first synaptic station in the nociceptive pathway and are under the influence of both descending and afferent inputs. How the descending factors lead to subjective pain, and if stress alone is capable of changing the responsiveness of nociceptive central nervous neurons, have not been studied systematically.

The present study aimed at answering the questions, if and how immobilization stress influences the responsiveness and resting discharges of dorsal horn neurons. Moreover, stress-related changes in animal behaviour have been tested. As a method of stress induction, repeated immobilization in a restraint device was used. The immobilization stress is a common method to study acute or chronic stress and stress-related phenomena in rats (e.g. Gamaro et al., 1998; Balk Rde et al., 2011; Porterfield et al., 2011).

2. Methods

2.1. Experimental animals

Experiments were performed on 26 adult male Sprague–Dawley rats. The experimental procedure was approved by the local ethics authority responsible for animal experimentation and carried out in accordance with the German law on the protection of animals and with the ethical proposals of the International Association for the Study of Pain (Zimmermann, 1983).

Eighteen animals were divided into three groups.

Group 1 (stress, n = 6): Stress was induced on 12 consecutive days by repeated immobilization (see 2.2).

Group 2 (control, n = 6): The rats were handled daily (transport to laboratory, taken up by hand) like the stress animals in group 1 but were not repeatedly immobilized.

In groups 1 and 2, the pressure-pain threshold of the low back and exploratory behaviour in the open field test were determined 1 day before recording (Fig. 1A).

Group 3 (naïve, n = 6): These rats were neither handled nor immobilized.

The rats of all three groups were used for electrophysiological recording. In groups 1 and 2, the electrophysiological recordings took place on day 14, 2 days after the last immobilization or control treatment (Fig. 1A).

In eight additional animals, concentrations of faecal corticosterone metabolites were determined. Four animals were treated with repeated immobilization as in group 1, and 4 animals were handled like group 2.

2.2. Stress by repeated immobilization

All rats were acclimatized for 1 week under controlled laboratory conditions. Behavioural experiments were always performed at the same time of day during the light cycle. Compared to control animals, the stress animals exhibited a significantly lower bodyweight on days 4 and 12 ($p < 0.05$), i.e. the stressed rats gained less weight than the control rats (data not shown).

Rats of group 1 were immobilized for 1 h daily on 12 consecutive days in narrow plastic tubes (inner length 16 cm, inner diameter 7 cm, Fig. 1A).

2.3. Recording of spinal dorsal horn neurons

The animals were deeply anaesthetized with thiopental sodium (Trapanal®; Altana Pharma, Germany), 100 mg/kg i.p. initially, followed by an i.v. infusion (external jugular vein) of the same anaesthetic at a constant rate of 10–20 mg/kg/h using an infusion pump to maintain a deep and constant level of anaesthesia (no flexor reflexes or blood pressure

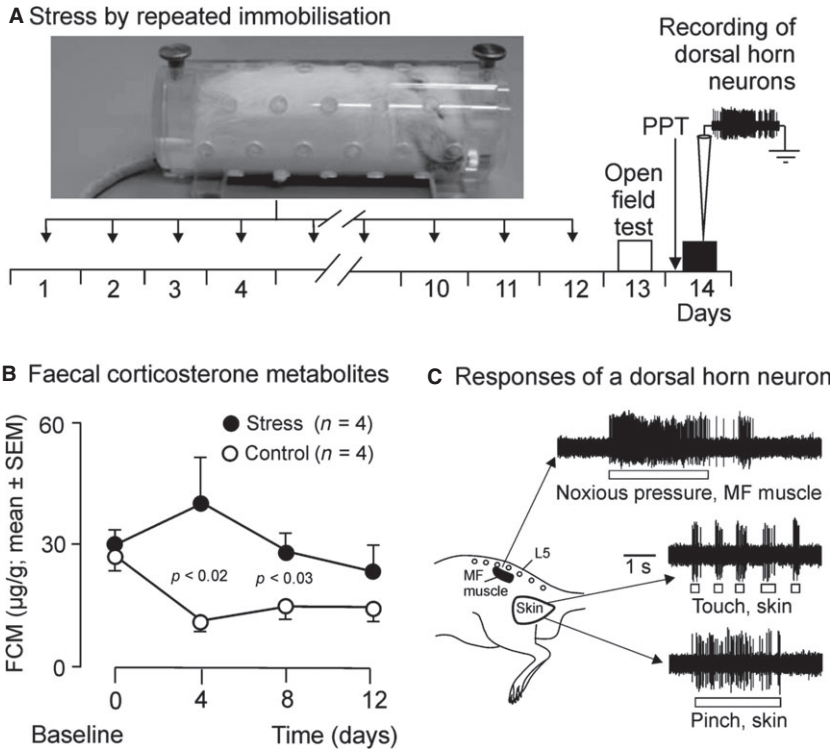

Figure 1 Experimental procedure. (A) On 12 consecutive days, the animals were immobilized in a narrow tube (inset) for 1 h every day (downwards arrows). To test locomotor activity, the animals were placed in an open field arena on day 13 (open square). The PPT was measured on day 14 just before the electrophysiological recordings. Recordings of dorsal horn neurons were performed 2 days after the last stress exposure on day 14 (filled square). (B) Mean levels of faecal corticosterone metabolites (FCM). Faeces were collected before (baseline) and at days 4, 8 and 12 after initiation of the stress experiment. Black circles: FCM levels for stressed animals (stress), open circles: control group (control). A two-way ANOVA test with the factors time and stress revealed a significant interaction of time × stress ($p = 0.007$). *p*-values between circles indicate significant differences in the *U*-test. (C) Responses of a single dorsal horn neuron recorded in a control animal. The scheme shows the approximate location and size of the receptive fields (RFs) from which the neuron could be activated. Black area: multifidus (MF) muscle; black outline: RF in the skin. The registrations show responses of the neuron to noxious pressure applied to the MF muscle and to touching and pinching the skin. Open bars indicate time and duration of stimulation. L5: spinous process L5.

reactions exceeding 10 mmHg to noxious stimuli). Muscular relaxation was induced with pancuronium bromide (Inresa, Germany, 0.6 mg/kg/h i.v.). Mean arterial blood pressure measured in the right common carotid artery and body core temperature were continuously monitored and kept at physiological levels (above 80 mmHg, 37–38 °C). The animals were artificially ventilated with a gas mixture of 47.5% O_2, 2.5% CO_2 and 50% N_2 (Hoheisel et al., 2013). A laminectomy was performed to expose the spinal segments L1 to L5. The laminectomy did not affect the caudal back muscles and the overlying fascia (Taguchi et al., 2008).

Extracellular recordings from dorsal horn neurons were made in the spinal segment L2 that receives strong input from deep low back tissues (Hoheisel et al., 2013). Recordings were made with glass-microelectrodes filled with 5% NaCl (10–38 MΩ). Microelectrode penetrations were made to a depth of 1000 µm. As a search stimulus, the dorsal roots L3 to L5 were electrically stimulated together with a single electrode (intensity 5 V, width 0.3 ms, repetition rate 0.33 Hz). All dorsal horn neurons giving stable responses to this stimulus were accepted for the study. The latencies of the neurones varied between 1.5 and 3.8 ms (stress: mean 1.93 ± 0.06 SEM; control: 1.93 ± 0.05; naïve: 2.10 ± 0.07) indicating a synaptic input from myelinated afferents. No significant difference was found between the treatment groups.

2.4. Identification of receptive fields and neuron classification

The receptive fields (RFs) of the neurons were identified with mechanical stimulation of low back

structures bilaterally, both hindlimbs, hip, lateral abdominal wall and tail. As innocuous stimuli, touch with an artist's brush and moderate pressure with a blunt probe were used; as noxious stimuli, pinching with a sharpened watchmaker's forceps (skin, thoracolumbar fascia, TLF) or noxious pressure with a blunt probe (muscle). In some cases, as a noxious chemical stimulus, 5% NaCl (50 μL) was injected into muscles underlying a mechanosensitive cutaneous or fascial RF.

In the quantitative evaluation, the input of a given neuron was the criterion. When a certain input is mentioned (e.g. skin), the input could exist with or without input from another source. When a neuron responded to touching or pinching of the skin, it was considered a neuron having skin input. In neurons that responded to moderate or noxious pressure applied to a muscle or other deep somatic structures but did not respond to brushing and pinching of the overlying skin, the RF was considered to be in the muscle or other deep structures. The deep somatic tissues of the low back could be tested directly, because the skin of that area was opened. Since recent data emphasized the importance of the TLF as an input source for lumbar dorsal horn neurons (Taguchi et al., 2008; Hoheisel et al., 2013), some cells with or without input from the fascia were evaluated separately. To identify a RF in the TLF, it was pinched with a watchmaker's forceps. Intramuscular injections of 5% NaCl were given to identify RFs in a muscle underneath a mechanosensitive RF in the fascia or skin. Size and location of the RFs (in case of muscular RFs, their projection on the surface) were recorded on a standard outline of the rat body. The search for RFs was carried out following a strict protocol in all experimental groups: The responsiveness of each neuron was tested by stimulating the body regions in a fixed order (see sketch in Fig. 2C) starting with the toes and followed by metatarsus, heel, lower leg, knee, thigh, base of the tail, low back and lateral abdomen.

The resting (ongoing) discharge of each neuron was determined before it was tested with experimental stimuli. A neuron was defined as having resting activity if it fired ≥1 impulse per min.

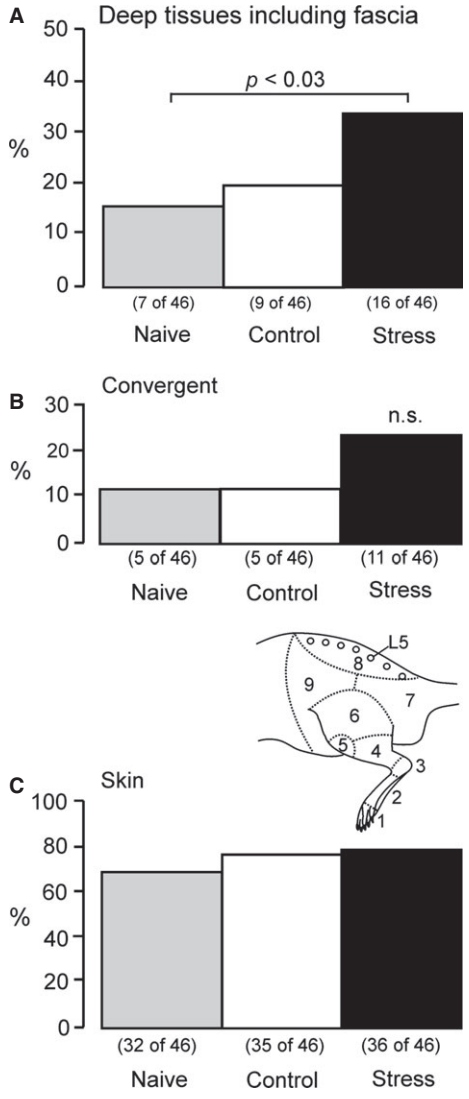

Figure 2 Proportion of neurons with deep or cutaneous input. (A) Neurons with input from deep tissues (muscles, thoracolumbar fascia and other deep tissues), (B) proportion of convergent neurons with receptive fields in at least two types of tissue including skin), (C) neurons with input from the skin. Stress: handled and immobilized animals; control: handled animals, not immobilized; naïve: animals not handled and not immobilized. Each panel was constructed from the same population of neurons (46 neurons in total; see numbers underneath the bars). n.s. = not significant compared to naïve or stressed rats. The scheme in (C) shows the order of body regions used for searching for receptive fields: 1, toes; 2, metatarsus; 3, heel; 4, lower leg; 5, knee; 6, thigh; 7, base of tail; 8, low back; 9, lateral abdomen.

2.5. Behavioural experiments

To test for mechanical hyper- or hypoalgesia, the pressure-pain threshold (PPT) of deep tissues in the low back was measured at the vertebral level L5. Previous experiments had shown that input from this level was mainly processed in the segment L2. An electronic von Frey anaesthesiometer (Life Science Instruments, Woodland Hills, CA, USA) with a blunt tip area of 3.46 mm^2 was pressed at increasing intensity to the intact skin. With the blunt tip, mainly nociceptors in deep structures are excited (Takahashi et al., 2005). The PPT was defined as the

minimum pressure that elicited a pain-related reaction (withdrawal, escape movements, vocalization). The PPT was measured directly before anaesthetizing the animals for the neuronal recordings.

Spontaneous motor behaviour was tested in an open field arena that consisted of four identical compartments (50 cm × 50 cm × 45 cm, Chourbaji et al., 2008). One day before recording, the distance travelled, speed of the animals and time spent in the central zone (distance >12.5 cm to the nearest wall) were recorded for 30 min with a digital camera at a light intensity of 50 lx. For data evaluation, the program Viewer^2 (Biobserve GmbH, St. Augustin, Germany) was used. The number of rearings (standing on the hindlimbs) was counted manually.

2.6. Faecal corticosterone metabolites

Faeces were collected 1 day before the immobilization stress started (baseline) and at day 4, 8 and 12 during the stress sessions or at the equivalent days for the unstressed animals. All faeces were collected at the same time of day. Twelve hours after the stress session, the animals were placed in a new cage and all faeces were collected after 2 h.

The fresh faeces were stored at −20 °C until all sampling was terminated. Then, the faeces were dried for 3 h at 60 °C and homogenized. A total of 0.1 g was weighted into new vials. For extraction, 2 mL methanol (80%) were added, shaken for 30 min and centrifuged (Palme et al., 2013). Portions of the 0.5 mL supernatant were stored at −20 °C until analysis. Corticosterone metabolites were measured with a 5α-pregnane-3β,1β,21-triol-20-one enzyme immunoassay (Touma et al., 2003 and Lepschy et al., 2007).

2.7. Data analysis

Comparison between treatment groups was made using the *U*-test of Mann and Whitney. Proportions of neurons were compared with Fisher's exact probability test and levels of corticosterone metabolites by two-way ANOVA and *U*-test. A probability level of less than 5% was regarded significant.

3. Results

3.1. Faecal corticosterone metabolites

Compared to control animals, the concentration of the faecal corticosterone metabolites was higher in animals stressed by repeated immobilization (two-way ANOVA factor stress: $F(1, 6) = 4.972$; $p = 0.067$). The highest concentration in stressed animals was found at day 4 of the stress period, thereafter it decreased slowly to baseline value (two-way ANOVA factor time $F(3, 18) = 3.374$; $p = 0.041$; interaction time × stress: $F(3, 18) = 5.559$; $p = 0.007$). Using the *U*-test, differences between stress and control animals were significant at days 4 and 8 of the stress period (Fig. 1B); at day 12, levels of corticosterone metabolites were also higher in stressed animals but the difference was not significant.

3.2. Stress-induced sensitization of dorsal horn neurons

All in all, 138 neurons were evaluated in the 18 animals of groups 1–3. Of the 138 neurons, 119 responded to at least one of the mechanical or chemical stimuli used (stress: 43; control: 39; naïve: 37). Of these 119 neurons, 15.8% received input from deep tissues (muscles, fascia and other deep soft tissues) and 74.8% input from the skin. Many neurons had a highly convergent input in that they responded to stimulation of receptors in skin, muscles and/or fascia (e.g. Fig. 1C). In our sample, 10.9% of all neurons recorded responded exclusively to stimulation of deep soft tissues. Neurons having input from two and more types of tissue were called convergent (Fig. 2B).

The recording depth of the dorsal horn neurons studied ranged from 80 to 900 μm; there were no significant differences between the treatment groups. Of the neurons, 55.1% was recorded at a depth of 400–800 μm which corresponds to laminae IV, V and VI of the dorsal horn. 75.5% of the neurons having input from deep somatic tissues were located in these laminae.

The proportion of neurons having input from the various tissues of the low back, hip and hindlimb is shown in Fig 2. Stressed animals exhibited an increase in the proportion of neurons responding to stimulation of at least one of the deep somatic tissues tested, namely muscles, fascia and non-identified soft tissues (Fig. 2A). Compared to naïve animals ($p < 0.03$), but not to control animals ($p = 0.08$), the increase was statistically significant.

In those neurons that had convergent input from various sources (deep tissues including fascia, skin), there was just a trend towards an increase under stress ($p = 0.08$, Fig. 2B).

Fig. 2C shows that most of the neurons had input from the skin (many exclusively). In contrast to the results obtained with stimulation of deep tissues, the skin input did not change under stress.

A prominent effect of the immobilization stress was the appearance of new RFs in deep tissues other than the TLF (Fig. 3A). These new RFs appeared outside the low back in the hip and in the entire hindlimb (grey areas in Fig. 3A). In stressed animals, 17.4% of the RFs were located in the hip and hindlimb, whereas in control rats, the proportion was 8.7% and in naïve ones 4.3%. The difference between naïve and stressed animals was statistically significant ($p < 0.045$). Interestingly, in the same population of neurons, the number of RFs in the low back, close to the spinal column, did not increase significantly ($p = 0.38$; outlined areas in Fig. 3A).

When only those neurons were evaluated that received input from the TLF (among other sources), no significant differences were found. However, in stressed animals, there was a trend towards an increased number of neurons with TLF input (Fig. 3B).

For each neuron, also the responsiveness to mechanical stimulation of the contralateral hip and hindlimb was tested. None of the recorded neurons possessed or acquired RFs on the contralateral side.

Taken together, these results indicate that in the stressed animals, the dorsal horn neurons reacted more strongly to stimulation of deep soft tissues in the low back, hip and hindlimb.

The most striking effect of the immobilization stress did not concern the response behaviour of the neurons but their resting activity. Both the proportion of neurons having resting activity ($p < 0.007$; Fig. 4A) as well as the mean impulse activity of all – discharging and silent – neurons increased significantly ($p < 0.007$; Fig. 4B). The stress-induced increase in mean resting activity was due to the high discharge rate in a considerable subpopulation of neurons. Some neurons reached very high frequencies. Only in stressed animals, three neurons were found that had a resting activity of more than 1000 imp/min (Fig. 4D).

3.3. Behavioural experiments

The stressed animals did not exhibit a lowered PPT in the deep low back tissues, although the dorsal horn neurons were sensitized. The PPT of the deep tissues was determined close to the spinal process L5. As mentioned earlier, the input from this body region was mainly processed in the segment L2.

The PPT did not differ significantly between the three treatment groups (stress: mean 589.0 g ± 60.1 SEM; control: 591.8 g ± 17.1; naive: 560.8 g ± 26.9). The data clearly show that there was no stress-induced mechanical hyperalgesia in deep soft tissues of the low back.

Compared to the control group, stressed animals travelled significantly longer distances at a higher average speed ($p < 0.05$; Fig. 5A and B). Stressed

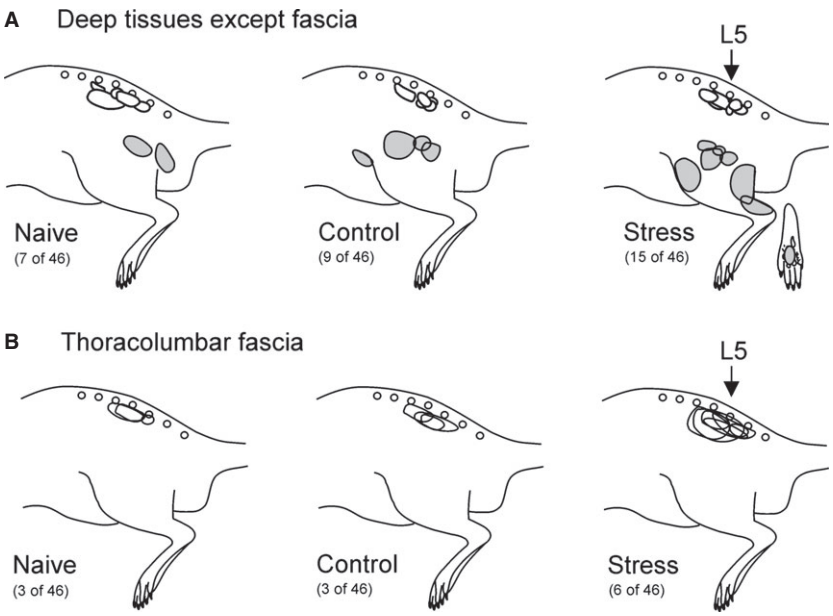

Figure 3 Location and size of the deep receptive fields (RFs). (A) RFs in deep tissues others than the thoracolumbar fascia. (B) RFs in the thoracolumbar fascia. Black outlines: RFs in fascia (B) or other soft deep tissues of the low back (A). Grey areas in (A): RFs in deep tissues outside the low back. Arrows: spinous process L5. Experimental groups and numbers in parentheses as in Fig. 2.

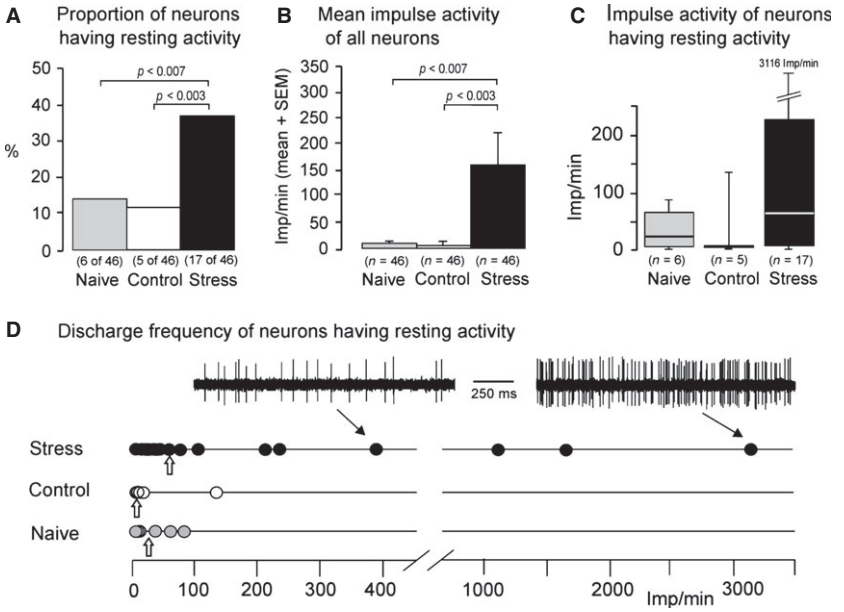

Figure 4 Resting activity of dorsal horn neurons. (A) Proportion of neurons having resting activity. (B) Mean impulse activity of neurons with and without resting activity. (C) Impulse activity of neurons with resting activity (≥1 imp/min). Shown are median, first and third quartile (box) and range (whiskers). (D) Discharge frequency of neurons having resting activity. Insets show original registrations of the resting activity from neurons marked by filled arrows and medians are indicated by open arrows. Experimental groups and numbers in parentheses as in Fig. 2.

animals also showed increased rearing behaviour ($p< 0.05$; Fig 5C). In contrast, the animals of the stress group spent significantly less time in the central zone of the open field ($p < 0.05$; Fig. 5D).

4. Discussion

In this study, we show for the first time that repeated immobilization stress has a direct effect on the discharge behaviour of dorsal horn neurons. Other effects such as an activation of muscle nociceptors by the immobilization cannot be excluded but seem unlikely. Because repeated or chronic (as opposed to acute) stress is assumed to be particularly effective in enhancing muscle pain (Imbe et al., 2014), the rats were repeatedly immobilized for a prolonged period of time. The increase in faecal corticosterone metabolites indicated that the immobilization was an effective technique for stress induction.

4.1. Electrophysiological data

The majority of neurons with input from deep soft tissues were of the WDR type (Fig. 1C). These neurons are assumed to have a nociceptive function (Chung et al., 1979). The WDR neurons with input from deep soft tissues of the low back exhibited an

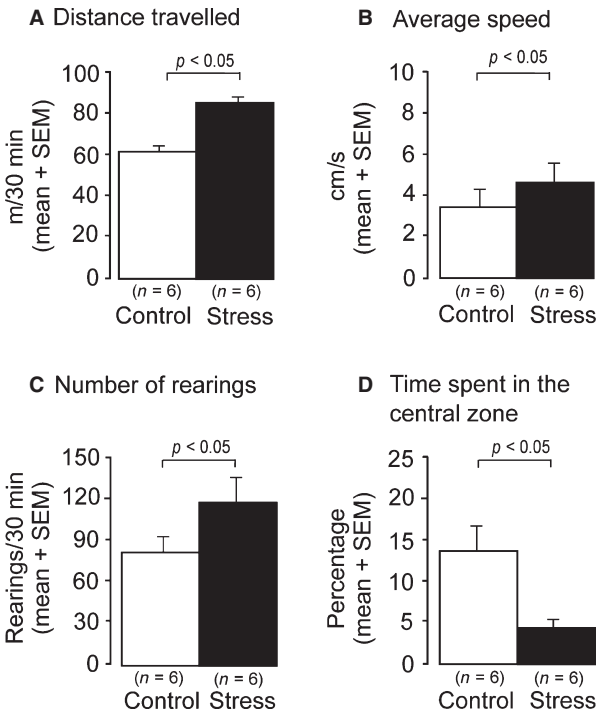

Figure 5 Locomotor behaviour in the open field. Distance travelled (A), average speed (B) and number of rearings (C) were significantly enhanced in stressed rats, while the time spent in the central zone was reduced (D). Experimental groups as in Fig. 2. *n*, numbers of animals tested.

increase in deep input under stress (Fig. 2A). Moreover, the resting discharge of many neurons had significantly higher frequencies in the stressed animals.

Neurons with input from the skin were most numerous (Fig. 2C), whereas the proportion of cells having input from deep soft tissues was much smaller (Fig. 2A). The latter showed a significant increase in number under immobilization stress, but neurons with skin input were not affected. This difference may be due to the fact that cutaneous afferents have highly effective synaptic connections on dorsal horn neurons from the beginning, which are not susceptible to plastic changes. In contrast, many of the synapses between the afferents from deep tissues and spinal neurons are ineffective initially and may become effective when the responsiveness of the neurons is increased under stress. Thus, the stress affected only the input from the deep soft tissues to dorsal horn neurons.

The functional significance of the cutaneous input in neurons also having input from the deep tissues of the low back is unknown. One possibility is that the cutaneous input facilitates the localization of deep stimuli. Probably, the neurons of our study had even more connections with other sources, e.g. joints (Schaible et al., 1987) and viscera (Akeyson and Schramm, 1994), but this possibility was not tested.

The appearance of new RFs in dorsal horn neurons is not a specific effect of stress; we have observed it also after peripheral input. For instance, new RFs appeared after injection of algesic substances into the gastrocnemius–soleus muscle (Hoheisel et al., 1993) and after injections of NGF into the multifidus muscle (Hoheisel et al., 2013). The formation of new RFs may be due to the unmasking of formerly ineffective synapses on spinal neurons. Such a mechanism has been described in rat dorsal horn neurons by Wall (1977) and Koerber et al. (2006).

In patients with non-specific low back pain, the appearance of new RFs in nociceptive spinal neurons of the low back may explain the expansion of pain to the lower leg in humans (Andersen et al., 2012). The formation of new RFs in the hindlimb as observed in our study may be interpreted as follows: The new RFs are due to the unmasking of ineffective synapses on neurons that originally had input from the low back and probably mediated low back pain. If this assumption is correct, stimulation of the RFs in the hindlimb should evoke pain in the low back and not in the hindlimb.

The massive increase in resting activity of the dorsal horn neurons was a prominent finding of the present study. High levels of resting discharges are assumed to be associated with spontaneous pain as opposed to pain evoked by external stimuli (Suzuki and Dickenson, 2006; Isnard et al., 2011). In behaving animals, spontaneous pain is difficult to detect, and our rats did not appear to be in persistent pain. Their eating and sleeping behaviour in the laboratory cage was normal; the stress effects became apparent only when they were tested in the open field.

4.2. Behavioural experiments

The unchanged PPT in the stressed animals can be explained by the fact that the threshold was determined over the multifidus muscle close to the spinal column, i.e. at a location where the RFs were almost not affected by stress. Only the number of neurons having RFs in the TLF exhibited a trend towards an increase. This change was not reflected in the PPT. With regard to low back pain patients, there are reports of a lowered or unchanged PPT depending on the site of testing and other factors, e.g. age, sex and chronicity of pain (LeResche et al., 2013; O'Neill et al., 2014).

Another interpretation is that the unchanged PPT is due to stress-induced analgesia, which abolished behavioural responses to external pressure. However, stress analgesia is assumed to be due to activation of descending pain inhibition and should lead to a PPT increase (Yilmaz et al., 2010), which does not fit in other results of our study (e.g. increased neuronal responsiveness and fearful behaviour).

All movement parameters in the open field test showed significant changes in the stressed animals: With the exception of the time spent in the central zone of the arena, the distance travelled, the average speed and the number of rearing were significantly increased. In contrast, the time spent in the central zone of the arena was significantly shorter, i.e. the rats moved along the walls of the test cage and avoided crossing the centre. This behaviour is an indication of increased anxiety and reliable evidence for the effectiveness of the chosen stress protocol.

4.3. Possible pathways for the stress-induced changes

In the stress-induced effects on neuronal and animal behaviour, two pathways are probably involved.

(1) *Descending pain-modulating pathways:* The main stress-induced effects on dorsal horn neurons were increased resting discharge and enhanced responsiveness. These effects may be due to either a

decreased descending pain inhibition or increased pain facilitation (Heinricher et al., 2009). In a previous study (Yu and Mense, 1990), we found that a block of descending pain-inhibiting pathways leads to an increased responsiveness and resting discharge in dorsal horn neurons similar to what we have seen in the present study. This finding points to a decreased pain inhibition as one possible explanation for the present results. The decreased pain inhibition is likely associated with increased pain facilitation (Heinricher et al., 2009) and with a stress-induced increase in the activity of the amygdala (Suvrathan et al., 2013). The movement pattern in the open field test, particularly the avoidance of the centre zone, speaks for an involvement of the amygdala.
(2) Increased sympathetic activity: Gillette et al. (1994) found that nociceptive dorsal horn neurons, including WDR cells, could be excited by stimulation of the lumbar sympathetic chain. They concluded that the dorsal horn activation is partly due to a sympathetic (efferent) activation of primary afferent neurons from the low back and viscera. This mechanism could contribute to the higher responsiveness and resting discharge of the dorsal horn neurons.

4.4. Limitations of the study

(1) Because of the location of the recording electrode close to the low back – i.e. close to the site of stimulation – a statistical comparison of the neurons' response magnitudes between naïve, control and stressed animals could not be made.
(2) Only a few neurons in the superficial dorsal horn are included in our sample.
(3) The character of the study is mainly descriptive. No statements can be made regarding the mechanisms underlying the stress-induced changes. In future studies, possible mechanisms such as a changed function of descending pain-modulating pathways or the sympathetic nervous system will be investigated.

5. Conclusions

The immobilization model differs from our previous models in that no nociceptive input from the periphery has been induced (unless a sympathetic effect on primary afferents is assumed, see above). The stress alone was capable of inducing an increase in the responsiveness and resting discharge of WDR – presumably nociceptive – dorsal horn neurons. In the inflammatory animal models, the outcome was different in that the increase in responsiveness was large and that in resting discharge small. Therefore, the present animal model could mimic a group of low back patients whose pain is mainly dependent on psychological stressors (Viniol et al., 2013), whereas the inflammatory models may represent patients whose pain is due to lesions in the soft tissues of the low back.

Author contributions

U.H. was responsible for conception of the study and design of the experiments, acquisition, analysis and interpretation of the data, drafting the manuscript. M.V. was responsible for design of the experiments, acquisition, analysis and interpretation of the data. R.P. was responsible for acquisition and analysis of faecal corticosterone metabolites. P.G. was responsible for conception of the study, interpretation of the data. S.M. was responsible for conception and design of the study, interpretation of the data, writing the manuscript. All authors discussed the results and commented on the manuscript.

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