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Research article

Astrocyte reactivity in spinal cord and functional impairment after tendon injury in rats

Áurea Gabriela Rodrigues Mendes [a], Gabriel Gomes Vilar de Sousa [a], Martha de Souza França [b], Carlos Alberto Marques de Carvalho [c], Evander de Jesus Oliveira Batista [d], Adelaide da Conceição Fonseca Passos [b], Karen Renata Herculano Matos Oliveira [b], Anderson Manoel Herculano [b], Suellen Alessandra Soares de Moraes [a,*]

[a] Instituto de Ciências da Saúde, Universidade Federal do Pará, Belém, Pará, Brazil
[b] Instituto de Ciências Biológicas, Universidade Federal do Pará, Belém, Pará, Brazil
[c] Departamento de Patologia, Universidade do Estado do Pará, Belém, Pará, Brazil
[d] Núcleo de Medicina Tropical, Universidade Federal do Pará, Belém, Pará, Brazil

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ABSTRACT

Astrocyte reactivity in the spinal cord may occur after peripheral neural damage. However, there is no data to report such reactivity after Achilles tendon injury. We investigate whether changes occur in the spinal cord, mechanical sensitivity and gait in two phases of repair after Achilles tendon injury. Wistar rats were divided into groups: control (CTRL, without rupture), 2 days post-injury (RUP2) and 21 days post-injury (RUP21). Functional and mechanical sensitivity tests were performed at 2 and 21 days post-injury (dpi). The spinal cords were processed, cryosectioned and activated astrocytes were immunostained by GFAP at 21 dpi. Astrocyte reactivity was observed in the L5 segment of the spinal cord with predominance in the white matter regions and decrease in the mechanical threshold of the ipsilateral paw only in RUP2. However, there was gait impairment in both RUP2 and RUP21. We conclude that during the acute phase of Achilles tendon repairment, there was astrocyte reactivity in the spinal cord and impairment of mechanical sensitivity and gait, whereas in the chronic phase only gait remains compromised.

1. Introduction

Achilles tendon injury restrains the limbs impaired due to pain, swelling and functional limitation, impacting negatively on locomotor activity (de Cesar Netto et al., 2018; Li and Hua, 2016). Much of the knowledge about Achilles tendon physiopathology comes from studies in animal models, characterizing its relevance to understanding physiological and motor behavioral changes (Murrell et al., 1992).

Among the motor behavioral changes, impairment on gait pattern is observed especially in the acute phase of tendinous injury and is considered indicative of functional limitation (Coulthard et al., 2003). Concerning quadrupeds, rat gait shows similarities to the pattern of human gait. Alternance and dissociation between the anterior and posterior limbs are important for balance and coordination during locomotion (Liang et al., 2012; Yu et al., 2001).

Previous studies showed that models of peripheral neural injury result in the intensification of responses to painful stimuli and greater activity in the spinal cord glial cells (Clark et al., 2013; Li et al., 2019; Samad et al., 2001). Glial reactivity occurs in response to central or peripheral injuries and is characterized by morphological, physiological and molecular changes in glial cells (Hansen and Malcangio, 2013; McMahon and Malcangio, 2009).

It is not yet clear whether this activation is beneficial or harmful. Beneficial changes include formation of glial scars and their contribution in tissue reorganization, as well as the release of many neuroprotective and neurotrophic factors (Pekny et al., 2014; Pekny and Pekna, 2014). However, records in the literature indicate that glial reactivity is also associated with neuron neuroplasticity and with increased synthesis and release of substances involved in hyperalgesia (Ghilardi et al., 2004; Larsson and Broman, 2011; Longo et al., 2009; Wang et al., 2009).

* Corresponding author.
E-mail address: sualessandra@yahoo.com.br (S.A.S. de Moraes).

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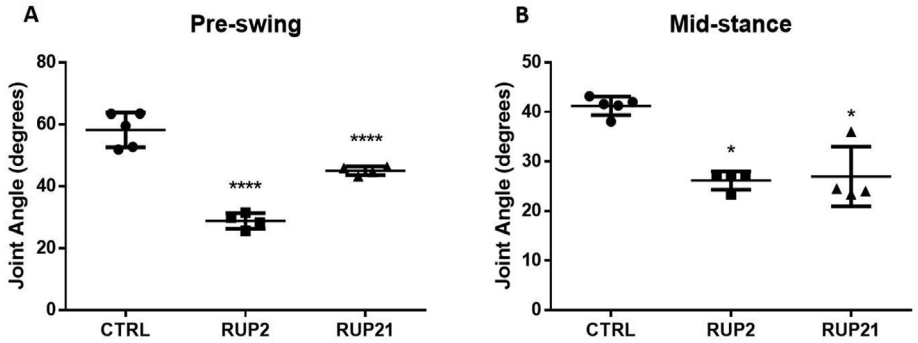

Figure 1. Analysis of ankle articular angle on acute (RUP2) and chronic (RUP21) phase of tendon repair in rats submitted to surgical tenotomy of right Achilles tendon. (A) Articular angle on pre-swing phase. (B) Articular angle on mid-stance phase. The animals were analyzed on day 0, 2, and 21 after rupture. N ≥ 4, Mean ± SD. One-way ANOVA: (A) ****$p < 0.0001$, F = 63.78 RUP2 and RUP21 vs. CTRL; RUP2 vs. RUP21. (B) *$p = 0.0001$, F = 24.68 RUP2 and RUP21 vs. CTRL.

As far as we know, there is no data about occurrence of such reactivity in the spinal cord after tendon rupture nor in which repair period this event could occur, although it has already been described that the consequences of rupture involve pain associated with loss of function, neuroinflammatory events and proliferation of local nerve branches (Danielson, 2009).

Thus, the aim of this study is to investigate, in two different repair phases, whether total Achilles tendon injury leads to astrocyte activation in the spinal cord and its relation to changes on gait pattern and mechanical sensitivity.

2. Material and methods

2.1. Animals

Fifteen Male Wistar rats (200–300g) were obtained from the bioterium of the Universidade Federal do Pará. Animals were housed in polypropylene cages under a 12/12-h light-dark cycle at 23 °C, with free access to standard rat food and water. Use and care of animals was approved by the ethics committee in research with experimental animals (CEUA-UFPA/114-13) and performed following the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health.

2.2. Experimental groups

The study included two experimental groups: a control and a rupture group. The control group (CTRL, n = 5) was constituted by uninjured animals, while rupture group was subdivided in animals submitted to experimental tenotomy and euthanized after 2 dpi (RUP2, n = 5) or 21 dpi (RUP21, n = 5) as representative times of acute and chronic phases of tendon repair, respectively.

2.3. Tendon injury model

Rats were intraperitoneally anesthetized with 10% ketamine hydrochloride (80 mg/kg) and 2% xylazine (12 mg/kg). Experimental tenotomy was performed in the right hind limb under aseptic conditions as previously described (Moraes et al., 2013). Tendon was exposed through a posterior midline skin incision at the ankle. Afterwards, the tendon was totally transected from 0.5 cm above the calcaneal insertion followed by tendon suture in accordance to the Kessler method and simple skin suture (Barmakian et al., 1994). No immobilization, movement restriction or analgesy was utilized. After 2 or 21 days post-injury, all animals were euthanized by transcardiac perfusion with 4% PFA and the spinal cord was dissected, carefully removed and immediately post fixed in 4% PFA. The spinal cords segment L5 was used to histological analysis due they relation with motor and sensorial control of ankle.

2.4. Gait analysis

For gait analysis, the articular angle test was used (Liang et al., 2012). The animals had the right ankle, knee and hip joints identified and marked with a permanent ink to facilitate the articular angle analysis. The test consisted of filming (Sony, Japan) the animal gait in straight line on a rectangular apparatus (10 × 60 × 20 cm) followed by analysis of the articular ankle on pre-swing and mid-stance gait phases by the software Image J (National Institute of Mental Health, USA) utilizing the tool "angle".

2.5. Mechanical sensitivity

Paw withdrawal threshold (PWT) was determined before surgery (baseline) and once daily on days 2 and 21 after surgery using von Frey

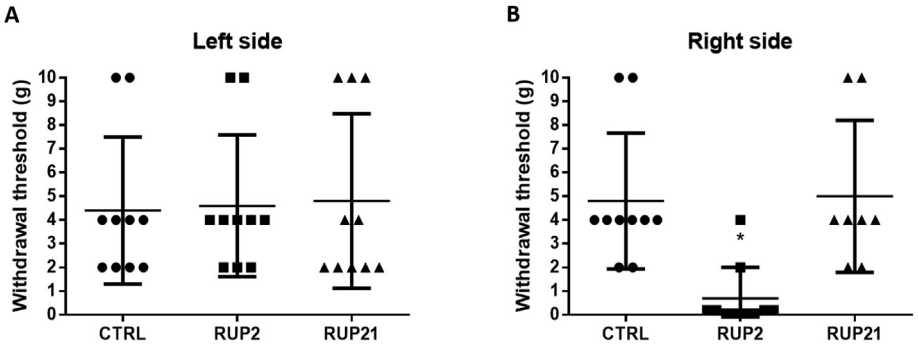

Figure 2. Analysis of mechanical sensitivity before tendon injury and on acute (RUP2) and chronic phase (RUP21) of tendon repair in rats submitted to surgical tenotomy of right Achilles tendon. Mechanical sensitivity was measured in the left (A) and right (B) hind paws. N ≥ 4, Mean ± SD. Kruskal-Wallis, *$p = 0.0003$ RUP2 vs. CTRL and RUP21.

filaments (SORRI-BAURU, Brazil) applied in ascending order of bending force to the plantar surface. Each rat was placed for 30 min to habituation in a transparent plastic dome with a plastic mesh floor, allowing access to the plantar surface of the hind paw. The time between tests was of 5 min and each trial was repeated 3 times. A von Frey filament was pressed perpendicular to the plantar surface of each left hind paw with sufficient force to cause slight bending, and was held for approximately 3 s. The PWT was the lowest weight of monofilament that elicited a withdrawal reflex, characterized as licking or shaking of the paw (Abaei et al., 2016). All behavioral tests were performed by an examiner blinded to the treatment groups.

2.6. Immunofluorescence and nuclei cell staining

The spinal cord segment L5 was first cryoprotected by immersion in 10%, 20%, and 30% sucrose batteries for three days. The specimens were then sectioned at 20 μm by a cryostat (Leica CM3050 S; Leica, Germany) and collected on glass slides. Indirect immunofluorescence staining was performed as described previously (Previtali et al., 2003) to glial fibrillary acidic protein (GFAP) using a rabbit anti-GFAP polyclonal antibody (1:500; Santa Cruz, USA). After overnight incubation with primary antibodies, samples were incubated with Alexa Fluor 488-conjugated goat anti-rabbit IgG (1:1000; Molecular Probes, USA). Nuclei were stained with 1:10,000 DAPI (Sigma, USA) for 3 min and briefly mounted on glass slides. The section was analyzed with the confocal laser scanning microscope LSM 510 META (Carl Zeiss, Germany) using a 10x objective with numerical aperture of 0.30 controlled by the software ZEN 2011 (Carl Zeiss, Germany). Intensity of GFAP staining was quantified from the photomicrographs as published elsewhere (Magni et al., 2019), with some modifications. Briefly, the mean values of pixel intensity for GFAP staining were automatically evaluated using the software ImageJ 1.53e (National Institutes of Health, USA) and expressed as ratios to the mean values of pixel intensity for DAPI staining to normalize as to the number of cells in each spinal cord quadrant. Finally, variations between conditions were referred to as percentages relative to the mean of all four quadrants.

2.7. Statistical analysis

Data are reported as mean ± SD. Comparison between two means of different groups was performed by the Student t test. Multiple comparisons were made using Kruskal–Wallis or one-way ANOVA, and p values less than 0.05 were considered statistically significant. All statistical analyses were performed using the software Prism 6 (GraphPad, USA).

3. Results

Our group investigated the impact of Achilles tendon injury on gait pattern in acute or chronic repair phases in rats. In Figure 1, the kinematic of pre-swing and mid-stance phase of ankle articular angle are shown. On the pre-swing phase, we observed a significant decrease of articular angle in both RUP2 and RUP21 when compared to CTRL and in RUP2 when compared to RUP21 ($F = 63.78$, $p < 0.0001$). In this way, at both time points the ankle angle was impaired and led to a worsening of function.

Similar to pre-swing, in the mid-stance phase the differences between the CTRL and RUP2 and RUP21 were highly significant ($F = 24.68$, $p = 0.0001$). However, there was no difference between RUP2 and RUP21, suggesting that both were equally impaired. Since we inferred that the decrease of the joint angle could be related to pain, we next investigate whether Achilles tendon injury led to mechanical sensory impairment at the time points analyzed.

In Figure 2, the result of hind paw withdrawal threshold is presented. In the CTRL group, the von Frey hair stimulus used to evoke withdrawal at day 0 (baseline) was 4.8 ± 2.86 and 4.4 ± 3.1 on right and left paws, respectively. However, in the RUP2 group, these values were 0.7 ± 1.3 in the right and 4.6 ± 2.9 in the left side, and, in the RUP21 group, the paw withdrawal threshold obtained was 5.0 ± 3.2 on the right and 4.8 ± 3.67 on the left side. Only the right side of RUP2 was significantly different compared to paw withdrawal value obtained from the CTRL and RUP21 groups ($p = 0.0003$). The right hind paw withdrawal threshold increased

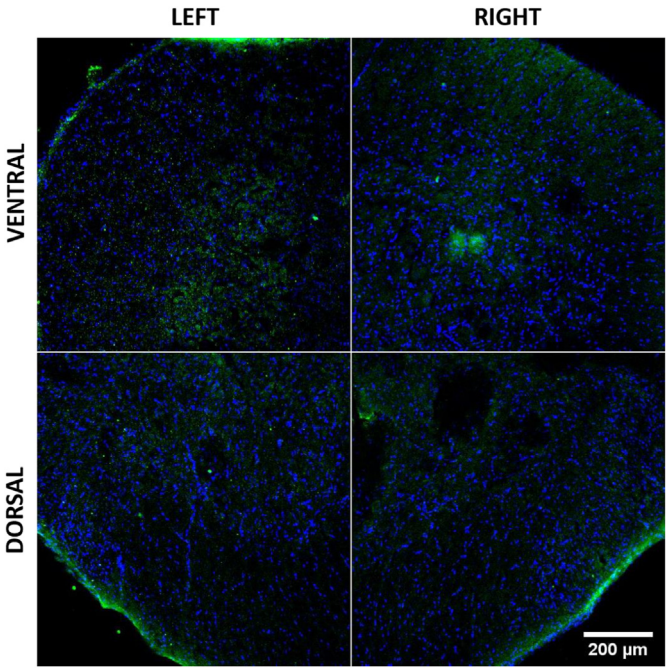

Figure 3. Immunofluorescent staining to GFAP (green) and nuclear staining with DAPI (blue) in transversal sections of spinal cord at the L5 segment of control (CTRL) group, which was comprised by uninjured rats. Immunofluorescence detection was performed on a confocal microscope using a 10x objective lens.

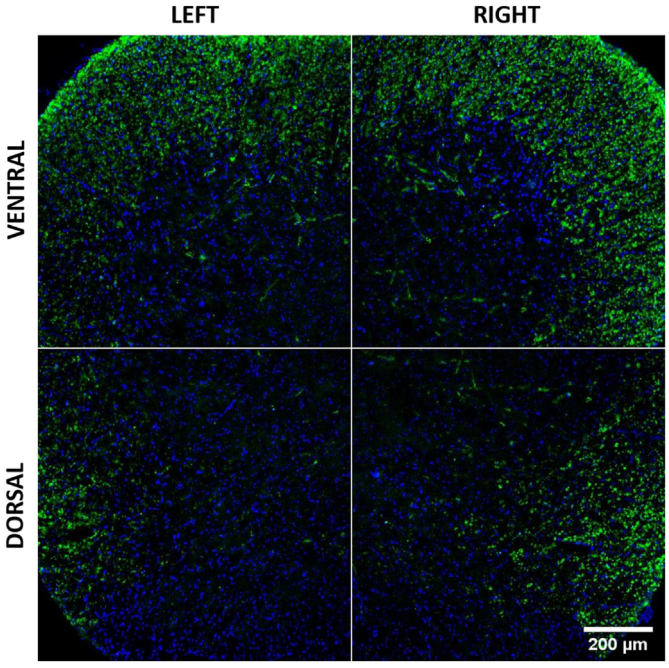

Figure 4. Immunofluorescent staining to GFAP (green) and nuclear staining with DAPI (blue) in transversal sections of spinal cord at the L5 segment of acute (RUP2) group, which was comprised by rats submitted to experimental tenotomy of right Achilles tendon and euthanized after 2 dpi. Immunofluorescence detection was performed on a confocal microscope using a 10x objective lens.

on day 21 to CTRL values, with no significant difference between CTRL and RUP21. There was also no difference between groups for hind paw withdrawal threshold on the left side ($p = 0.9211$).

As we observed divergences on gait and sensorial responses between acute and chronic phases of Achilles tendon injury, we decided to investigate whether during these repair phases there would be changes in astrocyte reactivity on the spinal cord segment related to Achilles tendon. Representative photomicrographs of the L5 spinal cord segment obtained from CTRL, RUP2 and RUP21 groups are shown in Figures 3, 4, and 5, respectively. After 2 days, Achilles tendon injury led to a ~70% overall increase in GFAP labeling in this segment, markedly in its right ventral quadrant, in comparison to CTRL group. In contrast, 21 days after tendon injury, a sharp overall decrease of ~96% in GFAP labeling was observed in comparison to RUP2 group (Figure 6).

4. Discussion

In this work, we have shown for the first time that the occurrence of impairment of locomotion and mechanical sensitivity due to acute Achilles tendon injury may be related with astrocyte reactivity in the L5 segment of spinal cord. This phenomenon was not observed in the chronic phase, when only gait pattern was still impaired.

The increase in GFAP expression in the spinal cord is well reported in models of peripheral nerve injury. In a model of spinal root insults, the increase in ipsilateral reactivity occurred at 1 dpi (Rothman and Winkelstein, 2007), while, in a similar model, the increase of GFAP in the ipsilateral spinal cord occurred after 3 dpi (Wang et al., 2009). Another study with spinal nerve injury assessed GFAP expression in spinal cord at both 28 and 42 dpi and their results suggest that, by these time points, glial activation is no longer occurring (Tawfik et al., 2007). These data are consistent with our study, as we also found remarkable reactivity in the acute phase and little expression at the long term, even in a different lesion model.

Concerning the effect of damage on more distal nerve structures, several studies showed that, after the induction of sciatic nerve injury, there is an increase in the reactivity of ipsilateral astrocytes to injury in 7 (Cirillo et al., 2010), 10 (Cirillo et al., 2011; Gallo et al., 2015) and 14 dpi (Taccola et al., 2016). Our group showed the increase of astrocyte reactivity in the acute phase in both sides of the spinal cord at the L5 segment. Concerning this finding, previous studies induced a unilateral monoarthritis in rats and observed a robust increase in glial reactivity in both sides of the spinal dorsal horn in 1, 3 and 10 dpi (Shan et al., 2007; Sun et al., 2008). The bilateral increase of astrocytes reactivity in the dorsal horn was also found after 6 h of injury in a monoarthritis model, which suggests that, in cases of peripheral inflammation, glial reactivity occurs since the acute phase, but does not follow a pattern of ipsilateral expression due to the possibility of activation of multiple ascending sensory pathways, and it is may explain our findings (Bressan et al., 2010).

Astrocytes activation can be initiated by CNS injury, microbial invasion, pain states, and by the products released by activated microglia (Sun et al., 2008; Zhang et al., 2012). Previous studies have shown that glial modifications after peripheral nerve and spinal cord injury (Cirillo et al., 2010; Pekny and Nilsson, 2005; Wang et al., 2009; Zhang et al., 2012) are associated with increased stimuli by primary afferents as C and A-δ fibers (Colangelo et al., 2008), morphological changes of the nerves

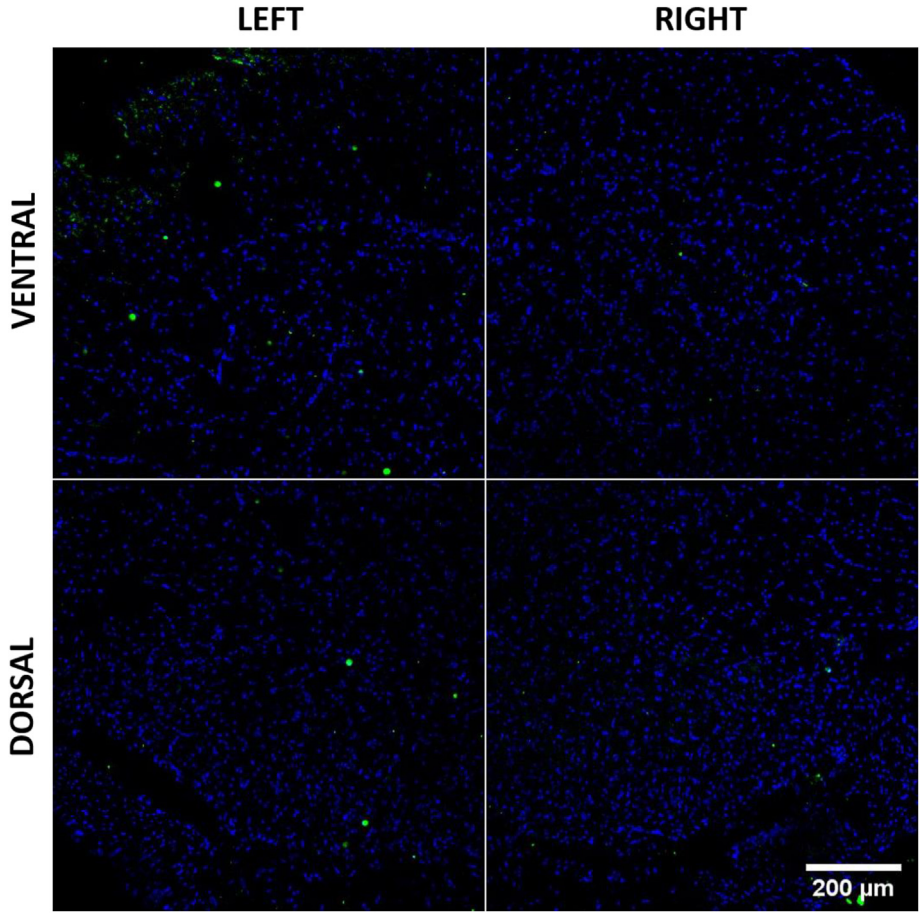

Figure 5. Immunofluorescent staining to GFAP (green) and nuclear staining with DAPI (blue) in transversal sections of spinal cord at the L5 segment of chronic (RUP21) group, which was comprised by rats submitted to experimental tenotomy of right Achilles tendon and euthanized after 21 dpi. Immunofluorescence detection was performed on a confocal microscope using a 10x objective lens.

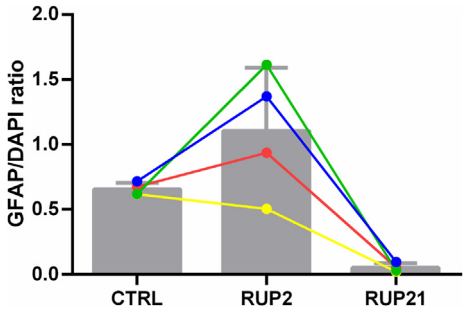

Figure 6. Quantification of astrocyte activation in response to tendon injury. The intensity of GFAP staining in transversal sections of spinal cord at the L5 segment of control (CTRL), acute (RUP2) and chronic (RUP21) groups was measured as a ratio to the intensity of DAPI staining for their left ventral (blue), right ventral (green), left dorsal (yellow) and right dorsal (red) quadrants. Gray columns represent mean ± SD of all four quadrants of the spinal cord for each condition.

myelination, and alterations in the architecture of the dorsal root ganglia (Cirillo et al., 2010), thus establishing correlation between glial plasticity and sensory alterations.

In our study, the astrocyte reactivity was associated with a decrease in the withdrawal threshold of the injured paw at 2 dpi, therefore with a change in mechanical sensitivity, while the reduction of reactivity was related to sensory normalization. Several studies have also found that ipsilateral sensory impairment was associated with increased glial reactivity in various lesion models and for different time periods (Cirillo et al., 2010; Gallo et al., 2015; Goff et al., 1997; Sun et al., 2008; Wang et al., 2009). A study subjected animals to an adapted model of spared nerve injury and compared those who developed painful hypersensitivity due to injury with those who did not develop it (Taccola et al., 2016). It was observed that animals whose increase in glial reactivity was noted were those with hypersensitivity, suggesting that reactivity is associated with pain.

There is evidence that increased glial reactivity induces the expression of various amino acid transporters, neurotransmitters, proinflammatory mediators, and receptors involved in nociceptive signaling. In addition, close structural relationships with neurons allow astrocytes to directly influence neuronal changes (Cirillo et al., 2011; Ghilardi et al., 2004; Goff et al., 1997; Hansen and Malcangio, 2013).

Increase in synthesis and expression of type I interleukin (IL-1β) stimulates the expression of cyclooxygenase type 2 (COX-2) in the CNS, implying in decrease of the threshold of mechanical pain, intensified by other proinflammatory cytokines (Samad et al., 2001). Other studies showed that, after a few weeks of injury induced by injection of an irritant agent in the paw, there is a complete recovery of the mechanical sensitivity. Both studies corroborate our findings, associating inflammatory conditions in CNS with pain and showing total sensory recovery in the later phase of a paw lesion (Goff et al., 1997; Huang et al., 2015).

Although our group verified normalization of astrocyte reactivity and sensitivity after 21 days of injury, locomotor activity remained impaired, which was evidenced by the reduction in the ankle joint angle, leading to gait impairment in the middle-stance and pre-swing phases. The functional analysis of gait is a very sensible method to assessing the recovery condition of an Achilles tendon and should be realized by video-based gait system with measures of ankle articular angles. Achilles tendon injury with and without surgical repair impairs mid-stance and pre-swing gait phases leading to decrease of articular angle even in 21 dpi, which confirms our findings (Liang et al., 2012).

After tendon injury there is an increase in the duration of the double support phase, characterizing a functional worsening associated with an increase in the synthesis of neuropeptides involved in nociception and leading to a change in gait pattern (Lui et al., 2010). On the other hand, in studies whose locomotion was evaluated by footprints in animals with lesions, the worsening in the parameter analyzed occurred in the first days after the injury, with tendency to normalize between 14 and 21 dpi. This supports the hypothesis that the joint angle is the more sensitive parameter to evaluate functional tendon recovery (Aro et al., 2013; Moraes et al., 2013).

After frequent pain stimulus, compensatory changes may occur in healthy joint movement in rats with the injured paw to maintain gait stability. All gait pattern is automatically controlled by spinal cord, and motor control may remain altered for a long time after changes in muscle recruitment pattern due to injury and pain (Liang et al., 2012). Significant functional deficits are still present after 2 years of ruptured tendon in patients regardless of whether there was surgical repair or not (Olsson et al., 2011).

In conclusion, our study suggests that Achilles tendon injury by total rupture followed by repairment induces astrocyte reactivity on the spinal cord, which is associated with the development of sensorial impairment. Furthermore, the gait impairment occurs together with this event but persists on the long term.

Declarations

Author contribution statement

Áurea Gabriela Rodrigues Mendes, Gabriel Gomes Vilar de Sousa, Adelaide da Conceição Fonseca Passos: Performed the experiments; Wrote the paper.

Martha de Souza França: Performed the experiments; Analyzed and interpreted the data.

Carlos Alberto Marques de Carvalho: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Evander de Jesus Oliveira Batista: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Karen Renata Herculano Matos Oliveira: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Anderson Manoel Herculano: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Suellen Alessandra Soares de Moraes: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

Abaei, M., Sagar, D.R., Stockley, E.G., Spicer, C.H., Prior, M., Chapman, V., Auer, D.P., 2016. Neural correlates of hyperalgesia in the monosodium iodoacetate model of osteoarthritis pain. Mol. Pain 12.

Aro, A.A., Simões, G.F., Esquisatto, M.A.M., Foglio, M.A., Carvalho, J.E., Oliveira, A.L.R., Gomes, L., Pimentel, E.R., 2013. Arrabidaea chica extract improves gait recovery and changes collagen content during healing of the Achilles tendon. Injury 44 (7).

Barmakian, J.T., Lin, H., Green, S.M., Posner, M.A., Casar, R.S., 1994. Comparison of a suture technique with the modified Kessler method: resistance to gap formation. J. Hand Surg. 19 (5).

Bressan, E., Mitkovski, M., Tonussi, C.R., 2010. LPS-induced knee-joint reactive arthritis and spinal cord glial activation were reduced after intrathecal thalidomide injection in rats. Life Sci. 87 (15–16).

Cirillo, G., Bianco, M.R., Colangelo, A.M., Cavaliere, C., Daniele, D.L., Zaccaro, L., Alberghina, L., Papa, M., 2011. Reactive astrocytosis-induced perturbation of synaptic homeostasis is restored by nerve growth factor. Neurobiol. Dis. 41 (3).

Cirillo, G., Cavaliere, C., Bianco, M.R., De Simone, A., Colangelo, A.M., Sellitti, S., Alberghina, L., Papa, M., 2010. Intrathecal NGF administration reduces reactive astrocytosis and changes neurotrophin receptors expression pattern in a rat model of neuropathic pain. Cell. Mol. Neurobiol. 30 (1).

Clark, A.K., Old, E.A., Malcangio, M., 2013. Neuropathic pain and cytokines: current perspectives. J. Pain Res. 6.

Colangelo, A.M., Bianco, M.R., Vitagliano, L., Cavaliere, C., Cirillo, G., De Gioia, L., Diana, D., Colombo, D., Redaelli, C., Zaccaro, L., Morelli, G., Papa, M., Sarmientos, P., Alberghina, L., Martegani, E., 2008. A new nerve growth factor-mimetic peptide active on neuropathic pain in rats. J. Neurosci. 28 (11).

Coulthard, P., Simjee, S.U., Pleuvry, B.J., 2003. Gait analysis as a correlate of pain induced by carrageenan intraplantar injection. J. Neurosci. Methods 128 (1–2).

Danielson, P., 2009. Reviving the "biochemical" hypothesis for tendinopathy: new findings suggest the involvement of locally produced signal substances. Br. J. Sports Med. 43 (4).

de Cesar Netto, C., Godoy-Santos, A.L., Pontin, P.A., Natalino, R.J.M., Pereira, C.A.M., de Oliveira Lima, F.D., da Fonseca, L.F., Staggers, J.R., Cavinatto, L.M., Schon, L.C., de Camargo, O.P., Fernandes, T.D., 2018. Novel animal model for Achilles tendinopathy: controlled experimental study of serial injections of collagenase in rabbits. PloS One 13 (2).

Gallo, A., Dimiziani, A., Damblon, J., Michot, B., Rieux, A., Des De Kock, M., Hermans, E., Deumens, R., 2015. Modulation of spinal glial reactivity by intrathecal PPF is not sufficient to inhibit mechanical allodynia induced by nerve crush. Neurosci. Res. 95.

Ghilardi, J.R., Svensson, C.I., Rogers, S.D., Yaksh, T.L., Mantyh, P.W., 2004. Constitutive spinal cyclooxygenase-2 participates in the initiation of tissue injury-induced hyperalgesia. J. Neurosci. 24 (11).

Goff, J.R., Burkey, A.R., Goff, D.J., Jasmin, L., 1997. Reorganization of the spinal dorsal horn in models of chronic pain: correlation with behaviour. Neuroscience 82 (2).

Hansen, R.R., Malcangio, M., 2013. Astrocytes - multitaskers in chronic pain. Eur. J. Pharmacol. 716 (1–3).

Huang, W.Y., Dai, S.P., Chang, Y.C., Sun, W.H., 2015. Acidosis mediates the switching of Gs-PKA and Gi-PKCε dependence in prolonged hyperalgesia induced by inflammation. PloS One 10 (5).

Larsson, M., Broman, J., 2011. Synaptic plasticity and pain: role of ionotropic glutamate receptors. Neuroscientist: Rev. J. Bringing Neurobiol. Neurol. Psychiatr. 17 (3), 256–273.

Li, H.Y., Hua, Y.H., 2016. Achilles tendinopathy: current concepts about the basic science and clinical treatments. BioMed Res. Int. 2016.

Li, L., Li, Y., Fan, Z., Wang, X., Li, Z., Wen, J., Deng, J., Tan, D., Pan, M., Hu, X., Zhang, H., Lai, M., Guo, J., 2019. Ascorbic acid facilitates neural regeneration after sciatic nerve crush injury. Front. Cell. Neurosci. 13.

Liang, J.I., Chen, M.Y., Hsieh, T.H., Liu, C.Y., Lam, C.F., Chen, J.J.J., Yeh, M.L., 2012. Video-based gait analysis for functional evaluation of healing achilles tendon in rats. Ann. Biomed. Eng. 40 (12).

Longo, U.G., Ronga, M., Maffulli, N., Orth, F., 2009. Achilles tendinopathy. Sports Med. Arthrosc. Rev. 17 (2), 112–126.

Lui, P.P.Y., Chan, L.S., Fu, S.C., Chan, K.M., 2010. Expression of sensory neuropeptides in tendon is associated with failed healing and activity-related tendon pain in collagenase-induced tendon injury. Am. J. Sports Med. 38 (4).

Magni, G., Boccazzi, M., Bodini, A., Abbracchio, M.P., van den Maagdenberg, A.M., Ceruti, S., 2019. Basal astrocyte and microglia activation in the central nervous system of Familial Hemiplegic Migraine Type I mice. Cephalalgia 39 (14), 1809–1817.

McMahon, S.B., Malcangio, M., 2009. Current challenges in glia-pain biology. Neuron 64 (1).

Moraes, S.A.S., Oliveira, K.R.M., Crespo-López, M.E., Picanço-Diniz, D.L.W., Herculano, A.M., 2013. Local NO synthase inhibition produces histological and functional recovery in Achilles tendon of rats after tenotomy: tendon repair and local NOS inhibition. Cell Tissue Res. 353 (3).

Murrell, G. a, Lilly, E.G., Davies, H., Best, T.M., Goldner, R.D., Seaber, a V., 1992. The achilles functional index. J. Orthop. Res.: Off. Publ. Orthop. Res. Soc. 10 (3), 398–404.

Olsson, N., Nilsson-Helander, K., Karlsson, J., Eriksson, B.I., Thomée, R., Faxén, E., Silbernagel, K.G., 2011. Major functional deficits persist 2 years after acute Achilles tendon rupture. Knee Surg. Sports Traumatol. Arthrosc. 19 (8).

Pekny, M., Nilsson, M., 2005. Astrocyte activation and reactive gliosis. Glia 50 (4).

Pekny, M., Pekna, M., 2014. Astrocyte reactivity and reactive astrogliosis: costs and benefits. Physiol. Rev. 94 (4).

Pekny, M., Wilhelmsson, U., Pekna, M., 2014. The dual role of astrocyte activation and reactive gliosis. Neurosci. Lett. 565.

Previtali, S.C., Nodari, A., Taveggia, C., Pardini, C., Dina, G., Villa, A., Wrabetz, L., Quattrini, A., Feltri, M.L., 2003. Expression of laminin receptors in Schwann cell differentiation: evidence for distinct roles. J. Neurosci. 23 (13).

Rothman, S.M., Winkelstein, B.A., 2007. Chemical and mechanical nerve root insults induce differential behavioral sensitivity and glial activation that are enhanced in combination. Brain Res. 1181 (1).

Samad, T.A., Moore, K.A., Sapirstein, A., Billet, S., Allchorne, A., Poole, S., Bonventre, J.V., Woolf, C.J., 2001. Interleukin-1 β-mediated induction of Cox-2 in the CNS contributes to inflammatory pain hypersensitivity. Nature 410 (6827).

Shan, S., Qi-Liang, M.Y., Hong, C., Tingting, L., Mei, H., Haili, P., Yan-Qing, W., Zhi-Qi, Z., Yu-Qiu, Z., 2007. Is functional state of spinal microglia involved in the anti-allodynic and anti-hyperalgesic effects of electroacupuncture in rat model of monoarthritis? Neurobiol. Dis. 26 (3).

Sun, S., Cao, H., Han, M., Li, T.T., Zhao, Z.Q., Zhang, Y.Q., 2008. Evidence for suppression of electroacupuncture on spinal glial activation and behavioral hypersensitivity in a rat model of monoarthritis. Brain Res. Bull. 75 (1).

Taccola, G., Doyen, P.J., Damblon, J., Dingu, N., Ballarin, B., Steyaert, A., Rieux, A. des, Forget, P., Hermans, E., Bosier, B., Deumens, R., 2016. A new model of nerve injury in the rat reveals a role of Regulator of G protein Signaling 4 in tactile hypersensitivity. Exp. Neurol. 286.

Tawfik, V.L., Nutile-McMenemy, N., LaCroix-Fralish, M.L., DeLeo, J.A., 2007. Efficacy of propentofylline, a glial modulating agent, on existing mechanical allodynia following peripheral nerve injury. Brain Behav. Immun. 21 (2).

Wang, W., Wang, W., Mei, X., Huang, J., Wei, Y., Wang, Y., Wu, S., Li, Y., 2009. Crosstalk between spinal astrocytes and neurons in nerve injury-injuced neuropathic pain. PloS One 4 (9).

Yu, P., Matloub, H.S., Sanger, J.R., Narini, P., 2001. Gait analysis in rats with peripheral nerve injury. Muscle Nerve 24 (2).

Zhang, H., Yoon, S.Y., Zhang, H., Dougherty, P.M., 2012. Evidence that spinal astrocytes but not microglia contribute to the pathogenesis of paclitaxel-induced painful neuropathy. J. Pain 13 (3).