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Original Article

Corticospinal tract alterations after ankle sprain in adolescence: Insights from the mouse model

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ABSTRACT

Purpose: Lateral ankle sprains (LAS) are associated with corticospinal pathway deficits. Existing evidence is primarily based on cross-sectional investigations and noncausal speculations. This study aims to determine whether maladaptive corticospinal pathway alterations occur pre- and postligament transection in LAS mouse models. Additionally, this study explores whether the alterations are more pronounced in adolescent mice than adults.
Methods: Twenty-four 8-week-old adolescent and twenty-four 24-week-old adult mice were randomly assigned to lateral ankle ligament transection or sham surgery. Diffusion-weighted imaging of the corticospinal pathway was performed presurgery and 8 weeks postsurgery. Fractional anisotropy (FA) values, reflecting fiber integrity within the corticospinal subregions of the medulla, pons, midbrain, and cerebrum, were extracted.
Results: Overall, 41 mice completed repeated image acquisition. Before surgery, no significant group effects on FA within the four corticospinal subregions were detected in either adolescent or adult mice. Two months after surgery, the adolescent cohort displayed a significant reduction in FA in the medulla subregion following ankle ligament transection (β-baseline-adjusted = −0.083, 95% *CI*, −0.145 to −0.021, p-corrected = 0.048). Conversely, no significant effects of ankle ligament transection on corticospinal FA were observed in the adult cohort.
Conclusion: The maladaptive alterations in the corticospinal tract could be observed in the adolescent LAS mouse model, characterized by reduced fiber integrity in the medulla subregion. While these results are derived from an animal model, they provide a foundation for future investigations into the mechanisms underlying neurological deficits following musculoskeletal injuries.

1. Introduction

Lateral ankle sprain (LAS) ranks among the most common sports-related musculoskeletal injuries, with a prevalence as high as 7 events per 1 000 person-years.[1,2] Despite the availability of a diverse range of physiotherapeutic and surgical treatments, nearly half of the individuals experiencing LAS progress from an acute ligament injury to a chronic syndrome marked by persistent dysfunction and recurrent sensations of ankle instability.[1,2] This phenomenon underscores not only biomechanical implications but also hints at potential neurophysiological disturbances affecting postinjury motor control.[3] Initial theories proposed the existence of arthrogenic muscular deficits that restrict the movement of the injured ankle as a natural protection mechanism. Moreover, maladaptive neuroplasticity associated with these deficits is common and persistent after LAS, such as lower corticospinal excitability and smaller representation of the ankle within the primary motor cortex.[3–5] Moving beyond the conventional focus on ligaments, an in-depth exploration of these neural underpinnings could reveal innovative therapeutic strategies, fostering a more comprehensive approach to the clinical outcomes of ankle sprains.

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Abbreviations

CAI	chronic ankle instability
CFL	calcaneofibular ligament
CST	corticospinal tract
DWI	diffusion-weighted imaging
FA	fractional anisotropy
FDR	false discovery rate
LAS	lateral ankle sprain
MD	mean diffusivity
MRI	magnetic resonance imaging
RD	radial diffusivity
ROI	region of interest
TMBTA	Turone Mouse Brain Atlas and Template

The execution of fine muscular movements depends on the intact corticospinal pathway, originating in the primary motor cortex and ultimately connecting to contralateral spinal motor neurons.[6] Robust neurophysiological evidence postulates that damage to the corticospinal pathway may underlie the neural origins of arthrogenic muscular deficits observed after ankle sprains.[7–9] Recently, diffusion-weighted imaging (DWI) has been employed to investigate microstructural alterations within the white neural fibers, providing a structural basis for understanding corticospinal pathway dysfunction after musculoskeletal injuries.[10,11] In their trajectory from the central cortex to peripheral motor neurons, corticospinal fibers traverse the internal capsule of the cerebrum, proceed to the cerebral peduncle of the midbrain, pass through the pons, and enter the medulla oblongata, constituting the corticospinal tract (CST).[12] In their pioneering study, Terada et al. did not observe discernible differences in the condition of CST between middle-aged and older adults with and without LAS history.[13] In contrast, Xue et al. subsequently observed maladaptively reduced CST fiber integrity in young participants with symptomatic ankle instability, quantitatively marked by reduced fiber fractional anisotropy (FA).[14] From these findings, researchers inferred that experiencing ankle sprains at a younger age might predispose individuals to more pronounced maladaptive neuroplastic changes in CST compared to their older counterparts.[11,14]

However, all these speculations on CST structure and LAS were based on cross-sectional investigations and noncausal speculations, restricting the clinical application of these neurophysiological insights.[15] Prospective studies that involve repeated measurements before and after an initial ankle sprain have been proposed to address this limitation. However, human trials of this nature are daunting due to their high costs and prolonged timelines.[15] Nevertheless, with the emergence of experimental animal models, researchers can now employ methodologies akin to those used in human studies to conduct animal research.

Regarding LAS, Wikstrom and Hubbard et al. devised a robust LAS model by surgically transecting lateral ankle ligaments in mice.[16–18] This model effectively emulated the ankle dysfunctions observed in clinical patients through various behavioral tests, allowing repeated pre- and post-LAS measurements. Furthermore, by leveraging recent advancements in ultra-high-field magnetic resonance imaging (MRI) technologies and corresponding DWI techniques, in *vivo* analysis of the corticospinal pathway in LAS mice of different ages has become achievable. This preclinical endeavor aims to validate and provide new insights into the prevailing theoretical models of ankle injuries, which, to date, have been grounded in speculative rather than causal considerations.[15]

Therefore, the primary objective of our study was twofold. First, we aimed to determine whether the fiber integrity of the CST changes before and after ligament transection in LAS mouse models, as compared to sham surgery controls. Second, we sought to determine whether the effects of LAS on CST alterations would be more pronounced in adolescent mice than in their adult counterparts. Our hypothesis, based on prior clinical observations, posits that ligament transection leads to decreased FA values of CST in adolescent mice but not in adult specimens.

2. Methods

2.1. Animals

An initial cohort of twenty-four 6-week-old male C57BL/6 mice, each weighing 20–25 g, were obtained from Shanghai SlacLaboratory Animal Co. Ltd. After an interim of 16 weeks, a second cohort of mice with the same characteristics was procured. All mice were housed individually in plastic cages containing sawdust bedding, each accommodating six animals. These cages were situated within the vivarium of Zhangjiang Fudan International Neuroimaging Research Centre. The environment was regulated to maintain a 12-hour (h) light/dark cycle and a constant

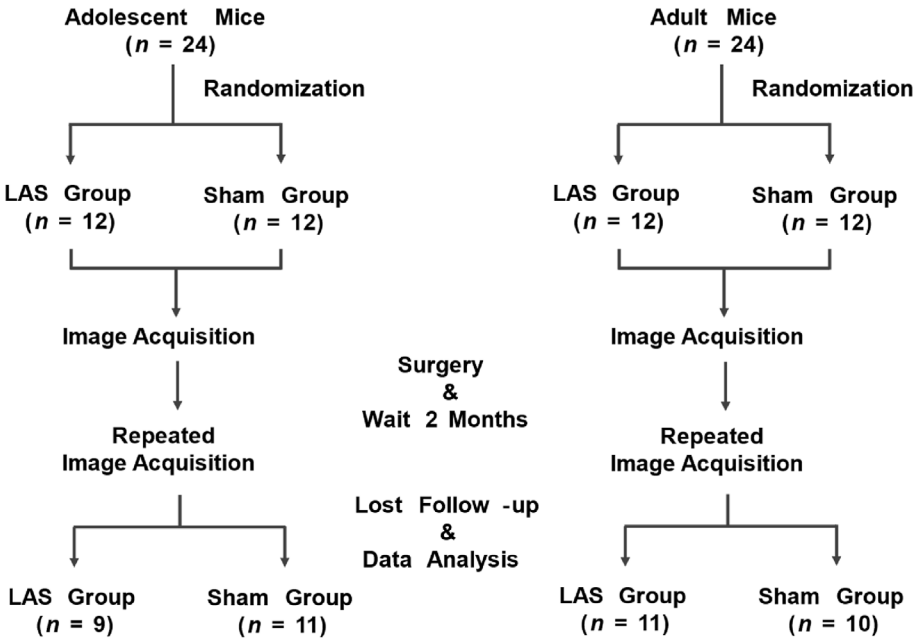

Fig. 1. Study flow diagram for both age cohorts. LAS, lateral ankle sprain.

ambient temperature ranging from 18 °C to 22 °C. Mice were allowed unrestricted mobility within their cages and had ad libitum access to water and food. A blinded member of the research team conducted daily visual inspections every 24 h.

Following a two-week acclimation period, formal assessments began for the two mice cohorts. Cohort 1 included adolescent mice selected from the pool of twenty-four 8-week-old mice, whereas Cohort 2 utilized adult mice drawn from the twenty-four 24-week-old mice. The CST evaluation was performed before and 8 weeks after the surgery to correspond to the ages representing adolescent (sprain at 8 weeks and follow-up at 16 weeks old) and adult patients (sprain at 24 weeks and follow-up at 32 weeks old) at the repeated evaluation.[19,20] Given that mice attain sexual maturity at 10 weeks of age and senescent changes in mice begin at 40 weeks, these mice age groups align with the adolescent and adult stages of human development.[19,20] Each cohort involved randomly assigning mice cages to two distinct groups: the LAS group (n = 12) and the sham surgery group (n = 12). Pre- and post-surgical MRI acquisitions were conducted, with the postsurgical scan scheduled two months following the surgical intervention. The selected sample size and time points were informed by previous research that identified motor function deficits and ankle degeneration in LAS mouse models, as illustrated in Fig. 1.[16–18,20] The Animal Welfare and Ethics Group of the Department of Experimental Animal Sciences granted the necessary approvals for all animal experimentation procedures involved in the study (Approval ID 2020JS-645).

2.2. Image acquisition

Brain MRI scans were performed with an 11.7 T preclinical MRI scanner equipped with a 4-channel phased array torso coil (Bruker BioSpec, Billerica, MA). The operator conducting the scans was blinded to the group assignments. Anesthesia was administered to the mice using 2%–3% isoflurane complemented by oxygen and an MR-compatible respiratory gating system. Subsequently, mouse heads were stabilized using bite and ear bars to minimize movement. Core body temperature was monitored using a rectal probe and maintained at approximately 36 °C using a heating pad supplemented by a warm-water circuit (Thermo Scientific, Waltham, MA). The imaging protocols included: (a) structural imaging via T2-weighted Rapid Acquisition with Refocused Echoes sequence: image size = 256 × 256, number of slices = 45, voxel size = 0.07 × 0.07 × 0.30 mm^3, echo time = 30 ms, and repetition time = 2 500 ms; (b) DWI using Echo-planar Imaging sequence: image size = 150 × 150, number of slices = 45, voxel size = 0.12 × 0.12 × 0.3 mm^3, echo time = 20 ms, repetition time = 3 500 ms, with five baseline imaging with b value of 0 $s{\cdot}mm^{-2}$ (i.e., b0 images) and 30 diffusion gradient directions with b value of 1 000 $s{\cdot}mm^{-2}$. After the scanning procedure, the mice were placed beneath a warming lamp until they regained normal mobility.

2.3. Surgical instrumentation

Surgical procedures were initiated one day after the baseline MRI scans on 8-week-old mice, following the established methods described in previous references.[16–18,21–24] The procedure included the following steps: (a) Each mouse was anesthetized with 2%–3% isoflurane mixed with supplemental oxygen and then placed on a clean surgical surface; (b) The right ankle was shaved and cleaned with alcohol, followed by disinfection using chlorhexidine; (c) A 2 mm incision was made behind the lateral malleolus; (d) In the LAS group, the calcaneofibular ligament (CFL) was cut, whereas in the sham group, no ligaments were altered; (e) The incision was then sealed using surgical adhesive. After the procedure, pain relief was provided by subcutaneous injection of carprofen at 5.0 $mg{\cdot}kg^{-1}$. Mice were subsequently positioned beneath a warm light until they regained their usual locomotor activity (e.g., moving around the cage). In this study, a "Blank" group, which would not undergo any interventions, was not established. Prior research has shown that the effect caused by the "sham" surgery group does not significantly differ from that of the "blank" group.[24]

2.4. DWI analysis

Diffusion data preprocessing and region of interest (ROI) analysis adhered to established protocols, prominently utilizing the Functional MRI of the Brain software library version 6.0 (University of Oxford, UK).[13] The workflow is illustrated in Fig. 2. Initially, the voxel dimensions of all primary images, atlases, and templates were amplified by a consistent factor of 10 to enhance their functionality within the widely-used neuroimaging software.[25] Diffusion images were corrected for eddy currents and head motion. An affine technique was used to align them to the associated averaged b0 image via the Linear Image

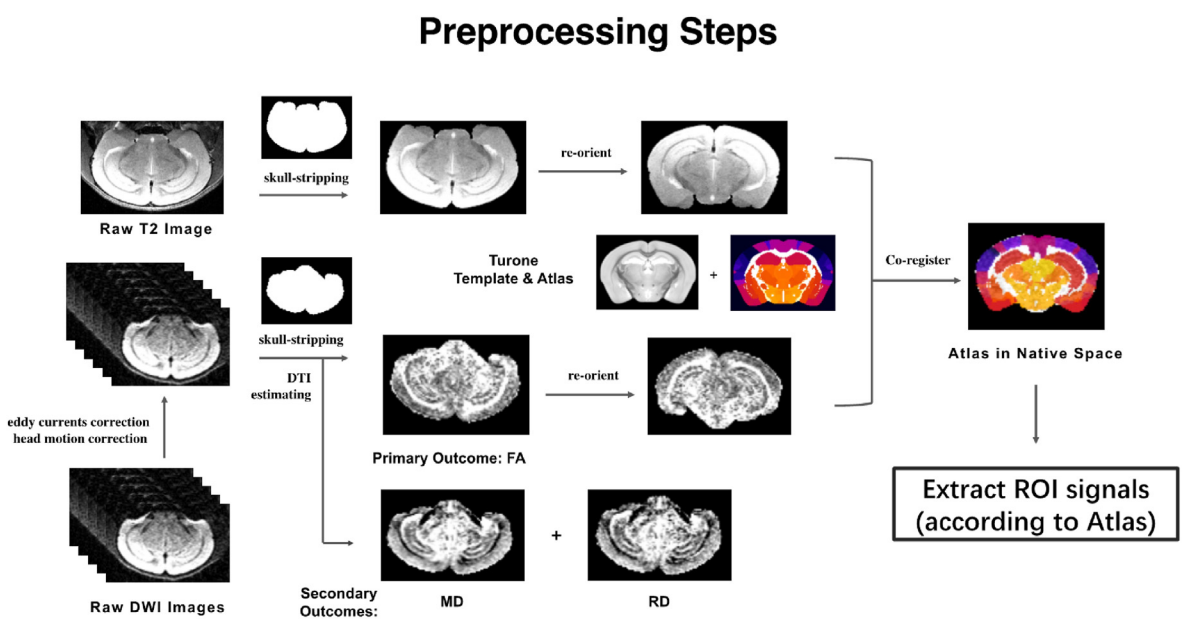

Fig. 2. Schematic representation of the imaging data processing and analysis pipeline. DWI: diffusion-weighted imaging; ROI, region of interest; FA, fractional anisotropy; MD, mean diffusivity; RD, radial diffusivity.

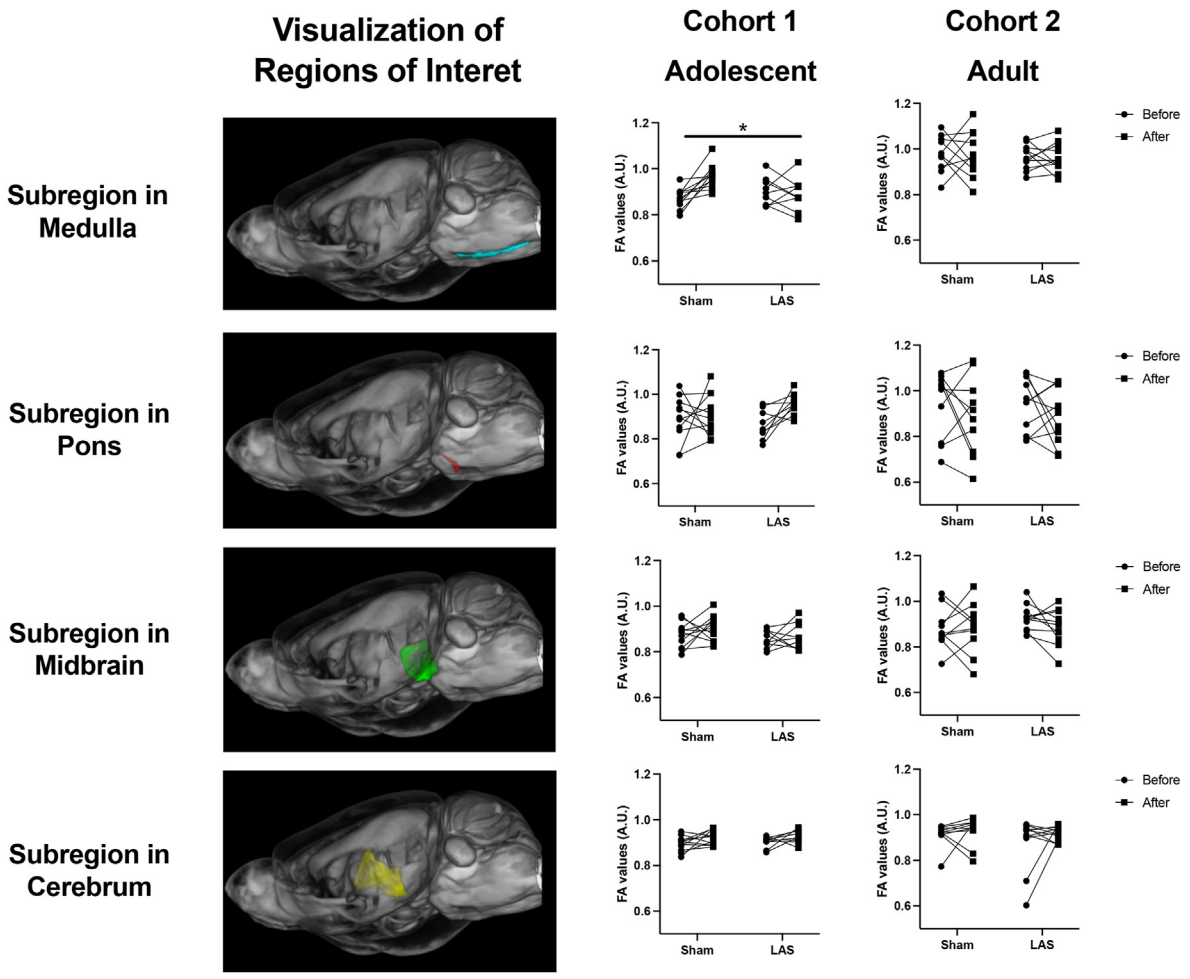

Fig. 3. Regions of interest (ROI) depiction and paired data results. Statistically significant effects of the LAS/Sham groups are highlighted with an asterisk (*). LAS, lateral ankle sprain.

Registration Tool. Individual brain masks for skull-stripping were devised from the b0 and T2 images with the Brain Extraction Tool to eliminate non-neural tissues. Subsequently, the Diffusion Toolbox was employed to compute FA, mean diffusivity (MD), and radial diffusivity (RD) maps: FA indicates the degree of anisotropy of water diffusion within a voxel, with higher values reflecting highly directional diffusion as seen in intact fibers; MD represents the average diffusion within a voxel, where higher values suggest increased extracellular space, associated with pathological conditions like edema or necrosis; RD measures diffusion perpendicular to the principal direction of fiber tracts, with higher values indicating potential demyelination. Following reorientation, the Turone Mouse Brain Atlas and Template (TMBTA) transitioned from the standardized space to the native domain through the T2 images and the FA maps using linear/nonlinear Image Registration Tool. Finally, averaged DWI values within the binarized masks of the CST (from lower to higher subregions, including the ones within the medulla, pons, midbrain, and cerebrum) were extracted for further estimation (Fig. 3). Given that the surgical intervention was applied to the right ankle—governed by the contralateral CST—the left side's DWI outcome was designated as the ROI. The ipsilateral hemisphere was also examined for supplementary analysis.

2.5. Statistical analysis

Statistical analyses were performed using R software, version 4.2.2 (R Foundation for Statistical Computing). Linear regression analyses were conducted separately for each age cohort to assess the impact of group assignment (LAS vs. sham control) on post-surgical DWI outcomes of the corticospinal subregion sections. To account for potential baseline differences and study longitudinal changes, presurgical DWI outcomes were included as covariates, with postsurgical DWI outcomes serving as the dependent variables. Based on our hypothesis that adolescents might exhibit higher neuroplasticity changes in FA, whereas adults might show weaker changes of FA (as mentioned in the introduction), we applied a false discovery rate (FDR) correction to the results of the group variables within the adolescent group for the four subregions after adjusting for baseline data to minimize errors from multiple comparisons. The MD and RD, which did not differ in the previous clinical research, were presented as supplementary analyses. The alpha level was set at $p < 0.05$ for all statistical tests.

3. Results

Repeated measurements of image acquisition were conducted on 41 mice. Among them, 4 mice succumbed, and 3 exhibited imaging abnormalities (Fig. 1). Before surgery, no significant group effects on the FA of the four corticospinal subregions were detected in the adolescent and adult experiments ($p > 0.05$). Two months after surgery, the FA of the medulla subregion of mice in the adolescent cohort increased from (0.873 ± 0.044) to (0.967 ± 0.053) in the control group. In contrast, the LAS group demonstrated a decline from (0.901 ± 0.061) to (0.809 ± 0.072), revealing a statistically significant effect once the baseline was adjusted ($\beta = -0.083$, 95% *CI*: -0.145 to -0.021, P-FDR = 0.048) (Fig. 3 and Table 1). No significant LAS surgery effects on the FA of corticospinal outcomes were noted in the adult experiments, other DWI outcomes, or ipsilateral outcomes (Supplementary appendix).

Table 1
Changes in CST integrity across different brain subregions following LAS in adolescent and adult mice cohorts.

Cohort 1	Subregion	Sham Group ($n = 11$)		LAS Group ($n = 9$)		Baseline not corrected		Baseline corrected		
		Before (mean ± *SD*)	After (mean ± *SD*)	Before (mean ± *SD*)	After (mean ± *SD*)	estimate (95%*CI*)	*p* value	estimate (95%*CI*)	*p* value	FDR corrected *p*
Adolescent	**Medulla**	0.873 ± 0.044	0.967 ± 0.053	0.901 ± 0.061	0.809 ± 0.072	−0.077 (−0.135 to −0.018)	0.013	−0.083 (−0.145 to −0.021)	0.012	0.048*
	Pons	0.891 ± 0.100	0.906 ± 0.094	0.862 ± 0.065	0.956 ± 0.048	0.05 (−0.023 to 0.122)	0.165	0.045 (−0.030 to 0.119)	0.220	0.293
	Midbrain	0.875 ± 0.055	0.905 ± 0.055	0.855 ± 0.038	0.869 ± 0.059	−0.036 (−0.089 to 0.018)	0.176	−0.039 (−0.095 to 0.017)	0.157	0.293
	Cerebrum	0.895 ± 0.033	0.922 ± 0.032	0.902 ± 0.024	0.928 ± 0.029	0.006 (−0.023 to 0.035)	0.655	0.008 (−0.022 to 0.037)	0.592	0.592

Cohort 2	Subregion	Sham Group ($n = 10$)		LAS Group ($n = 11$)		Baseline not corrected		Baseline corrected		
		Before (mean ± *SD*)	After (mean ± *SD*)	Before (mean ± *SD*)	After (mean ± *SD*)	estimate (95%*CI*)	*p* value	estimate (95%*CI*)	*p* value	–
Adult	**Medulla**	0.980 ± 0.080	0.979 ± 0.103	0.965 ± 0.055	0.961 ± 0.064	−0.018 (−0.096 to 0.059)	0.629	−0.016 (−0.096 to 0.064)	0.674	–
	Pons	0.939 ± 0.145	0.888 ± 0.172	0.939 ± 0.118	0.887 ± 0.120	−0.001 (−0.135 to 0.133)	0.984	−0.001 (−0.135 to 0.132)	0.983	–
	Midbrain	0.881 ± 0.089	0.882 ± 0.111	0.928 ± 0.055	0.886 ± 0.078	0.004 (−0.083 to 0.091)	0.928	−0.015 (−0.105 to 0.074)	0.722	–
	Cerebrum	0.913 ± 0.051	0.928 ± 0.063	0.880 ± 0.115	0.918 ± 0.031	−0.010 (−0.054 to 0.035)	0.655	−0.010 (−0.057 to 0.037)	0.661	–

CST, Corticospinal Tract; LAS, Lateral Ankle Sprain; *CI*, confidence intervals; *SD*, standard deviation; FDR, False Discovery Rates. FDR corrected *p*-values are included for the adolescent cohort based on our hypothesis that alterations would be more pronounced in adolescent mice than in adults. Significant effects are marked with an asterisk (*).

4. Discussion

The relationship between musculoskeletal injuries, particularly ankle sprains, and the subsequent neural changes in the corticospinal pathway has been a longstanding subject of scientific intrigue.[3] The central finding of this study is the presence of maladaptive neuroplasticity in the CST following LAS, with a more pronounced impact observed in adolescent mice than in adults. Notably, this study marks the inaugural application of the LAS mouse model in conjunction with ultrahigh-field DWI to investigate corticospinal alterations triggered by ankle ligament injuries. Furthermore, it provides the first longitudinal evidence of this phenomenon.

While conventional cross-sectional studies offer static insight into changes in fiber integrity within the CST, our longitudinal animal study adds a dynamic dimension by tracking these changes before and after the onset of injury. This methodological advancement is pivotal in delineating causal relationships, thereby enriching our understanding of the temporal CST adaptations following a sprain. Although our findings are grounded in preclinical data, we are optimistic that this attempt will unearth fresh insights into the prevailing theoretical models of ankle injuries. Furthermore, we anticipate that this study's findings will facilitate formal clinical investigations of the central mechanism of arthrogenic corticospinal dysfunctions.[15] And this study could be an example that has the potential to drive further research into the causality exploration behind residual defects following sports injuries, which is clearly of interest to both clinicians and patients.

4.1. Changes in CST following ankle sprains

Our findings underscore the profound impact of ankle sprains on adaptations within the CST. The CST is a crucial neural highway that facilitates the transmission of motor commands from the cerebral cortex to the spinal motor neurons, and its integrity is vital for motor functionality.[12,26] An ankle sprain introduces not only a localized tissue injury but also an ensuing influx of sensory inputs to the central nervous system, such as regional pain and proprioceptive deafferentation.[3,4] Following the initial injury, the sprained ankle may further exhibit degenerative changes due to ankle and subtalar joint instability, which can also lead to neuromuscular deficits and exacerbate the condition through inflamed soft tissues and damaged articular cartilage.[27] The atypical sensory information following an ankle sprain might trigger modifications within the CST, akin to the brain's arthrogenic response, which is thought to restrict limb movement by altering muscle-activation patterns as a protective mechanism to aid in injury recovery.[4,5,28] Electrophysiological studies on corticospinal excitability have shown that patients with LAS encounter difficulties in activating muscles around the injured ankle via CST fibers, and there is evidence of atrophy in the functionally concentrated areas and volumes of the ankle-related motor cortex.[29,30] Building upon existing theoretical frameworks and corroborated by clinical findings, our preliminary preclinical study observed a significant decrease in FA within the CST, which likely indicated maladaptive neuroplasticity, reflecting disruptions in the normal anisotropic diffusion of water molecules along the tract and the potential axonal degeneration. However, definitive answers to the precise mechanisms underlying these observations still require further investigations at the cell molecular level.

In particular, our study has unveiled notable alterations within the CST after an ankle sprain, notably confined to the lower medulla segment, whereas no corresponding changes were observed in the higher pons, midbrain, and cerebrum regions. We speculate that several factors might contribute to this localized neural response. First, the medulla, as an extension of the spinal cord, has direct neural connections with the lower limbs, rendering it highly responsive to injuries in the peripheric regions.[31] In the pursuit of neuroplasticity efficiency, the brain may prioritize adaptations where proximity allows for swift adjustments.[31] This theory might explain the significant reductions in fractional anisotropy observed specifically in the medulla subregion, which is closer to the peripheral injury site, and likely undergoes more direct influence compared to the more distal regions like the pons, midbrain, and cerebrum.[31] The robustness of more distal brain regions may derive from their complex and redundant neural circuits, which facilitate rapid adaptation post-injury and confer enhanced stability to these areas.[26] Conversely, the medulla, which may lack such redundant pathways, exhibits greater susceptibility to disruptions caused by peripheral injuries.[26] However, this study does not provide definitive answers to the precise mechanisms underlying our observations, necessitating further investigations. Nonetheless, we hope that our findings and theories underscore the pivotal role of the medulla in the neural response to ankle

injuries, thereby facilitating a deeper understanding of its unique changes.

4.2. Interaction of age with CST changes post ankle sprains

Our study sheds light on the significant differences in CST adaptations following ankle sprains between adolescent and adult mice. The prevalence of initial ankle sprains during adolescence, typically between the ages of 15 and 19 years in humans, underscores the relevance of studying this age group. However, the current understanding of the impairments associated with chronic ankle instability (CAI) predominantly centers on adolescents and college-aged adults.[32] This focus has led to inconsistent findings regarding CST alternation following ankle sprains in different age groups.[13,14] Our findings suggest that these differential responses affirm the intricate interplay between neuroplasticity and aging.[33] Emerging research consistently suggests that younger brains possess enhanced neuroplastic capabilities, allowing them to adapt more readily to stimuli or injuries.[33] The heightened adaptability observed in younger organisms, such as adolescent mice, may stem from their reservoir of immature synapses, which provide a substantial capacity for neuroplastic modifications. These abundant, adaptable synapses within the CST of adolescent mice are speculated to rapidly adjust in response to altered sensory inputs, such as the increased nociceptive signaling and reduced proprioceptive feedback following LAS or ligament tears.[3] In contrast, the neural systems of mature or elderly organisms often exhibit diminished neuroplasticity, reflecting a lower density of such flexible synaptic connections.[33]

Although existing models of ankle sprains and CAI posit that older patients experience more severe dysfunction, our results challenge this notion.[34] Given that ankle deficits following sprains result from various mechanical and sensorimotor impairments, we postulate that the mechanism of injury-induced maladaptive neuroplasticity may interact with age. In line with our findings, Terada et al. observed more pronounced cerebellar deficits than corticospinal defects in middle-aged and older adult patients with ankle injuries.[13] Therefore, from a clinical perspective, understanding age-dependent CST alterations after ankle sprains is paramount. Our findings suggest that rehabilitation protocols may need to be tailored based on the patient's age.[35] For example, it would be advisable for clinicians to encourage adolescent patients experiencing their first LAS to seek rehabilitative care promptly, with a particular focus on intensive motor control training to prevent abnormal neuroplastic changes in the CST. Additionally, there is growing evidence supporting the effectiveness of neuromodulation techniques, such as transcranial direct current stimulation targeted at the corticospinal pathway, which may be also especially beneficial for adolescents with ankle sprains.[36] However, these suggestions need to be further validated by clinical trials to confirm their effectiveness and safety. While our observations are preliminary, the complex interplay we have unveiled between age, CST adaptations, and ankle sprains holds promise for uncovering new insights. We are optimistic that these insights will open up promising avenues for developing more age-specific therapeutic strategies for after-ankle injury rehabilitation.

4.3. Limitations

This study has several limitations that must be discussed. First, we recognize the inherent limitations of using quadrupedal animals to model bipedal human injuries. However, the mouse model remains a widely accepted method in the preliminary stages of biomedical research, and we hope it can provide valuable insights and benefit future human studies.

Second, the animal model employed in our study relies on surgical ligament transection, differing from the natural mechanism of ankle sprains. Although this model has been widely validated by robust previous studies and is proven to effectively emulate the ankle dysfunctions observed in clinical patients, including gait and balance deficits and reduced physical activity.[16–18,21–24] we still acknowledge the necessity of more ankle inversion-based animal models to further validate our findings.[37] Third, we omitted behavioral assessments, such as balance and gait tests, which were frequently employed in prior studies on ankle sprain models.[16–18,23,24] Notably, methodologies for assessing ankle corticospinal deficits in patients with LAS have not been adapted for mouse models, posing challenges for their integration into our study.[3] The lack of such behavioral assessments in our research could potentially weaken our model's applicability to human LAS. Fourth, we lost follow-up for seven mice in the two cohorts caused by the relatively long scanning time for mice. Notably, the lost-to-follow-up rate remains $<$ 20% and is consistent among groups, minimizing the risk of bias. Fifth, the intervals for postsurgery measurements and the chosen age groups were constrained by practical limitations, including the high costs associated with 11.7 T neuroimaging, obtaining older mice, and extended animal care for our research group. Nonetheless, the single postsurgical measurement at the 8-week was supported by existing literature as a suitable time point for observing significant neuroplastic and degenerative changes after LAS.[20,24,27] We anticipate that future studies might overcome this limitation by merging central evaluations with comprehensive, life-long observations, building on the seminal work of Wikstrom et al.[17] Sixth, we were unable to discern a connection between CST and the development of CAI or copers in animal models.[16] While a post hoc division through the balance beam test might be feasible, the associated costs could become prohibitive as the sample size increases. Seventh, the ROIs of the CST, as delineated by the existing atlas, did not specify segment areas governing muscles of the injured ankle. This lack of specificity potentially reduces our detection sensitivity and accuracy for identifying localized neural changes. Given that mice were anesthetized during scanning, pinpointing ankle movement centers proved challenging. Also, the regions we observed represent only segments of the full CST, and we hope that future advancements in imaging technology will allow for comprehensive visualization of the entire neural pathway. Eighth, our study refrained from an exhaustive exploration of neural differences across the entire brain, which would require a larger sample size and extensive whole-brain multiple comparisons. Instead, guided by the aforementioned robust existing evidence of corticospinal deficits post-ankle sprains, we only focused our hypothesis-driven analysis specifically on the CST. However, the authors suggested that this approach could be enough to targets this specific knowledge gaps effectively, and also adheres to ethical principles for animal research by minimizing the required sample size. Finally, despite employing an animal model combined with congruent animal DWI techniques, the evidence grade remains relatively moderate, limiting the immediate clinical applicability of our findings. Subsequent studies should aim to corroborate our conclusions within a human patient cohort.

5. Conclusions

Our research effectively bridges an important knowledge gap, illuminating the maladaptive responses within the central nervous system's corticospinal pathway following ankle injuries (evidenced by reduced fiber integrity). Notably, this study underscores the significant mediating role of age in these adaptive changes. Although these findings are based on animal models, they emphasize the need to customize motor rehabilitation for LAS according to the individual's age.

Conflict of interest

The authors have no direct or indirect interests that are in direct conflict with the conduction of this study.

Ethical approval statement

The Animal Welfare and Ethics Group of the Department of Experimental Animal Sciences granted the necessary approvals for all animal

experimentation procedures involved in the study (Approval ID: 2020-JS645).

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Data statement

The extracted MRI outcomes are available upon reasonable request corresponding to the senior authors (Y.H., hua_cosm@aliyun.com; H.W., hewang@fudan.edu.cn)

CRediT authorship contribution statement

Xiao'ao Xue: Conceptualization, Data curation, Formal analysis, Writing – original draft. **Xicheng Gu:** Conceptualization, Data curation, Formal analysis, Writing – original draft. **Yuwen Zhang:** Conceptualization, Data curation, Formal analysis, Writing – original draft. **Xuejun Wu:** Methodology, Writing – review & editing. **Tian Xia:** Investigation, Writing – review & editing. **Rong Lu:** Formal analysis, Methodology, Software, Writing – review & editing. **He Wang:** Funding acquisition, Supervision, Writing – review & editing. **Yinghui Hua:** Funding acquisition, Supervision, Writing – review & editing.

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Appendix A. Supplementary data

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