Customized Neurotization for Hindlimb Hemiplegia After a Hemorrhagic Stroke

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Research

Keywords: Neurotization, L4 nerve root, Central hemiplegia, Skilled restoration

Posted Date: July 24th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-47611/v1>

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Abstract

Background Hemiplegia after hypertensive intracerebral hemorrhage(HICH) is prevalent,devasta-ting,and currently unable to intervene satisfactorily.Established success in neurotization spurs our interest in restoring skilled lower-limb movement.Here,we explored whether contralesional L4 transfer to ipsilesional L4 root,featuring simultaneous invigoration of flexors and extensors,reanimated dexterous locomotion for the hemiplegic hindlimb after HICH.

Methods We manufactured eligible rat models by injecting autologous vein blood into the left posterior limb of internal capsule (PLIC) and randomized thirty rats into three groups. We did not transect but expose bilateral L4 in group A (sham operation) after injection of saline,and infused equal blood followed by bilateral L4 transection and contralateral L4 transfer,in group B and group C,respectively.Behavioral,ultrastru-ctural,immunofluorescent,and electromyographic assessments were applied to efficacy analysis.MRI localized the PLIC impact.

Results We could see marked reduction in movements and capture a denervated potential in the right hindlimb of model rats ($P \leq 0.05$).In the walking tests,group C exhibited an initially high slip,but,later, a larger increment in accuracy on the paretic side, compared to group B and group A($P \leq 0.001$).Also,at Week 17,rats gained $\sim 58.2\%$ accuracy and actuated ankle motions in group C($P \leq 0.05$). In the retrograde tracing,at Week 9,fluoro-gold labeled motoneurons had been numerously present in the left anterior horn of the spinal cord from the L4-to-L4 root.Histological and ultra-microstructural assessments certified efficient axons regeneration.Electrom-yography and pawprint analysis implied the denervated muscles were reliably reinnervated,and myodynamia improvement($p > 0.05$).

Conclusions Contralateral L4 transfer could render favorable outcomes for major joints,particularly for the ankle,therefore efficaciously repaired the hemiplegic hindlimb after HICH.

Background

Hypertensive intracerebral hemorrhage(HICH) generally contributes to a catastrophic, life-changing damage with an incidence of approximately 150 in 100,000 worldwide annually,wherein $>81.3\%$ of these damages includes severe hemiplegia in the contr-alateral limbs[1–4].The victims with a limb motor dysfunction significantly lose self-help ability, placing a burden on family or forfeiting social engagement to some extent[5–10]. Traditionally,fundamental interventions to hemiplegia after HICH revolve around subsequent physical rehabilitation or physiotherapy[11–14].Nevertheless,res-ultant outcomes usually are discouraging or dissatisfactory,rousing uninterrupted quest for patient-oriented therapies.Many studies conducted in both humans and rodents have demonstrated that an injured brain cortex can re-control the contralateral limbs through a neural network remodeling across perilesional regions and contralesional hemisphere after rewiring an afferent circuit[15–18].Recently,peripheral nerve transfer,which can ignite compensational cerebral plasticity,has been exploited to rescue partial knee extension or hand prehension in patients with acute flaccid myelitis or central neurological diseases[19–22].Noticeably,though current neurotization stays in early stage and yet need to be polished[23, 24],its highlights have activated our

aspiration for its extrapolation to a lower-limb paralysis after HICH, and further interrogation of maneuvers for a dexterous motion during neurotization.

Currently, there are few data on neurotization for lower extremities hemiplegia after HICH, only several types of nerve transfer, such as L6-to-L6, L4-to-L5, and L3-to-L4 in animals, as well as obturator nerve to femoral nerve in humans, are reported for repairing the paralyzed lower-limb after central neurological diseases [25–28]. Disappointingly, only weak and rough motions were observed in the proximal major joints (i.e., hip and knee), without activities in the distal joints (i.e., ankle). One of main reasons consists in inadequate option of a source nerve. Anatomically, fine motor activities are principally accredited to a synergy between flexors and extensors chiefly composed of antero-posterior muscle groups (quadriceps femoris, semimembranosus, gastrocnemius, and tibialis anterior, etc.) in the lower extremity. Simultaneously, those muscles, coincidentally from the L4 root, drive distal joints activities. In brief, L4 root mainly bifurcates to compose both femoral and sciatic nerves and is theoretically understood as an optimal donor nerve, but need to be qualified experimentally in humans or rodents even if the initial role of the L4 could be undertaken by other lumbar roots following transection.

As well-known, any practical technique will entail technological evolution in animal models preceding its extrapolation to patients.

Thus, in the study, we proposed the L4-to-L4 transfer prototype in a rat model, in which robust motions for the hip and knee joints and whole activities for the ankle were observed, and yet projected reliable data to researchers for positive reference. Besides, its feasibility and availability were confirmed by the behavioral, histological, and electromyographic evidence, as well as anatomic interpretations, also predicted a profound prospect.

Methods And Materials

Standard protocol approvals and registrations.

We conducted animal experiments by the principles and procedures outlined in the NIH Guide for the Care and Use of Laboratory Animals (National Academies Press, 2011), enforced procedures concerning animals by the Institutional Animal Care and Use Committee at Nanjing Medical University (Nanjing, Jiangsu, China; approval no. IACUC-1906009), and performed all surgical procedures under chloral hydrate anesthesia. All attempts were made to minimize animals suffering.

Animals

Thirty Sprague-Dawley male rats (250~300 g) were used in this study, purchased from the Laboratory Animal Center at Nanjing Medical University, and housed three per cage in the controlled animal facility of Nanjing Medical University at $22 \pm 1^\circ\text{C}$, humidity $60 \pm 10\%$ with water and food *ad libitum*. The animal care unit was sustained at a 12 h light–dark cycle, with lights on at 7:00 am. Animals were randomly

assigned into three groups, each 10 rats. Group A was subjected to sham operation. Group B received bi-L4 transection, and Group C underwent L4-to-L4 root transfer following a hematoma-driven lesion in PLIC. Noiseless room was kept for rat subjects.

Creation of an internal capsule insult

Based on the published neural tracing results[29, 30], we created a hematoma-caused lesioning in the posteromedial area of PLIC. Rats were anesthetized with 10% chloral hydrate (400 mg/Kg body weight) intraperitoneally with body temperature at $37^{\circ}\text{C} \pm 0.5$ using a heating pad. Briefly, after restrained in a stereotactic frame (RWD-68025, Shengzheng Biotechnology Co., Ltd.), a rat was made a scalp incision along the midline followed by hemostasis using ophthalmobipolar, drilled a small hole in the skull. We gradually inserted a hydraulic microinjector, perpendicularly, into the target area (AP -3.1 from bregma; ML ± 3.3 from the midline; DV -7.8; $n = 30$) (Fig. 1A-B), slowly injected $\sim 180 \mu\text{l}$ blood or saline into the target site for 15 min at a rate of $12 \mu\text{l}/\text{min}$, with a 30G Hamilton syringe connected to an UltraMicroPump (WPI, Sarasota, FL, USA). The sham group received saline injection following by only exposure of bi-L4. Post-surgery, rats were transported to a recovery chamber with ketoprofen (2 mg/kg, i.m.) for analgesia for three consecutive days. Seven days post-injection, we used a T2-weighted MRI image to indicate location of the investigated area (Fig. 1C). Both behavioral and electromyographic tests supported an adjudication of a rat model.

Choice of a donor nerve

In a preliminary experiment, twenty-four naive rats (weighed 250–300 g) were classified into two groups, each 12 rats. Unilateral L4 root was excised sharply in one group, with-out transection of all other lumbar roots, while in another group, the counterpart was retained with other lumbar roots excised completely (i.e., L2, L3, L5, and L6). Postoperatively, rats received an intramuscular injection of penicillin (80,000U per day) for three days, application of lidocaine ointment to the skin incision for five days, underwent observations and measurements in a blinded fashion. Seven days later, behavioral and electromyographic tests confirmed eligibility of L4 root as a donor target.

Behavioral tests

We conducted the beam and ladder rung walking tests as well as footprint analysis as per the established protocols at baseline, 9, 13, and 17 weeks, respectively[31]. Fifteen days before the baseline evaluations, rats were trained on a per-procedure basis. A schematic illustration of the corresponding protocol is exhibited in Fig. 1D.

In the walking-beam test, animals ($n = 10/\text{group}$) were trained to cross a horizontal beam prior to surgery. A single run was deemed as satisfaction if the animal walked through the beam incessantly at a constant

gait. Nine satisfying runs in each animal were used to calculate their performance in a blinded manner by an experienced reviewer. The number of times that an animal slipped off the ledge with its affected hindlimb was recorded and then normalized for the sum of steps taken. Slips onto the ledge were rated as a full slip (given a score of 1) and a half slip (given a score of 0.5) was scored if the hindlimb touched a side of beam. A slipping ratio was designated as the times of slip per the sum of right hindlimb steps.

Likely, in the walking-ladder test, we trained rats ($n = 10/\text{group}$) to travel across a horizontally-placed ladder from a neutral cage to their home cage in advance. Traveling across the ladder at a uniform velocity was described as a favorable status, and nine qualified runs per animal were collected for their performance calculations. A slipping rate was evaluated as the number of slip per the sum of right hindlimb steps.

In the footprint analysis, we enticed rats to walk across the gangway straightly for three times for acclimatization. Subsequently, rats were tested in a gangway coated by aligned white papers, with a black cage at the end, following the right forepaw and handsaw were rendered blue and red ink respectively. The eligible data were gathered for efficacy analysis of intervention.

We calculated open field walking in conformity with the Basso, Beattie and Bresnahan (BBB) rating scale. We left individual rat subjects in an open field ($90 \times 150 \text{ cm}$), observed for five minutes, and rated hindlimb activities from 0 to 21.

L4 Nerve Roots Surgery Procedures

Two weeks after creation of rat hemiplegia models, we performed nerve root transfer in group C, conducted bi-L4 transection in group B, and carried out the only exposure of bi-L4 in group A. After anesthetized with 10% chloral hydrate intraperitoneally ($0.4 \text{ ml} / 100 \text{ g}$), laid supine, shaved, and fixed on the miniature operation table, animals were made a median incision 3~4 cm long longitudinally in the abdomen, which centered on the L4 and paralleled the anterior superior iliac spine. Subsequently, bi-L4 nerve roots were seen under the operating microscope (SZ61, Olympus). In Group C, the left L4 root (intact side) was traced and transected as distally to intervertebral foramen as possible, while the right L4 root was severed as proximally as possible. The proximal stump of the left L4 nerve root was transferred to the distal stump of the right L4 root using 10 – 0 Prolene sutures, with the right proximal stump secured to the ambient psoas major. In Group B, the stumps of bi-L4 roots were fixed with the muscle to avoid neural reconnection (Fig. 1E-F). An absorbable hemostatic sponge was carefully stuffed around the operative field before strict skin closure.

T2-Weighted MRI for localization

Seven days after blood injection, T2-weighted MRI showed the PLIC lesion on a Bruker Biospec 7-T MRI system. Animals were anesthetized with 5% and maintained with 2% halothane (in 30% O₂:70% N₂O),

vol/vol), and were then intubated and mechanically ventilated at 65 beats/min. A second T2-weighted image set was gained throughout the lesion.

Electrophysiological evaluation at a set period

Electromyographic examination

Five days following the left PLIC insult, we conducted surface electromyograms for the identification of a rat model. Mildly anesthetized animals with 10% chloral hydrate (0.15 ml/100 g), we shaved the bilateral lower-limbs and placed a reference electrode and recording electrode on the lateral or medial thigh of the hindlimb detected, respectively. Rats were stimulated at an increasing intensity of current from 0.5 mA to 1 mA, with self-adhesive electrodes in place. Based on neuroanatomic association in lower extremities, we performed serial electromyography with a concentric needle in the anterior-posterior muscle groups 10 days following the severance of a lumbar root, recorded myokymic potentials and positive sharp waves to locate muscles from the L4, and achieved according data under the condition of the only excision of L4 or only retention of L4, respectively. At 9, 13, and 17 weeks post-transfer, by acupuncture electromyogram, we tested the L4-supplied muscles in the case of early neurogenic or MUAP (compound muscular action potential, MUAP) changes, besides the denervated potentials. Additionally, we excluded positive sharp waves without myokymic potentials as a confounding variable, assessed neurogenic or myogenic alterations of MUAPs, and finally detected a reinnervated potential in the investigated muscles at a scheduled time.

H-reflex recording in the gastrocnemius

As such, after the aforementioned preparations, we directed a pair of receiving electrodes into the gastrocnemius in the right hindlimb and placed a stimulating electrode transcutaneously behind the medial malleolus. All the electrodes linked with a four-channel electrophysiology instrument (Galileo NT LineKey, Italy). We stimulated the reinnervated nerve using a single pulse (2 ms, 5 Hz) at an initial current of 0.1 mA, with 0.1 mA increment until reaching a maximum current, and determined latencies for H-wave and M-wave.

Immunofluorescence for regenerated nerves

Rats were injected an overdose of 10% chloral hydrate intraperitoneally and transcardially perfused with 600 ml saline and 400 ml 4% paraformaldehyde solution. The regrowing section of a regenerative nerve, 5 mm away from the coaptation site, was resected rostrally and caudally, immersed to 4% paraformaldehyde overnight at 4°C, followed by 30% sucrose in 0.1 M phosphate buffer overnight at 4°C, and were sliced longitudinally at a thickness of 20 µm with a manual rotary microtome (LEICA CM1905). Every fourth section was chosen for immunofluorescence. Three chosen sections per segment

were retained and processed on a per-procedure basis. Next, we incubated the specimens with microtubule-associated protein-2 (MAP-2, Abcam) diluted at 1:250 in primary antibody dilution buffer, laid them on a reciprocal shaker running overnight at 4°C. The primary antibody was skipped in the negative control. Following MAP-2 incubation, carefully washed in 0.01 M PBS, and incubated with Alexa Fluor-693 (1:250, Sigma) at 37°C for 30 min, tissue sections were added an anti-quenching reagent and quickly photographed under a fluorescence microscope (LEICA DM2500).

Retrograde tracing of the motoneurons to the quadriceps femoris

At week 9, 13 and 17 post-transfer, we surgically exposed the quadriceps femoris on the paretic side in parallel with severance of other lumbar roots (i.e., L2, L3, L5 and L6), infused 1 μ l 4% fluoro-gold (UE-F4040) into the muscle at 3 points in the treated rats, and finally closed the incision after saline wash. In a naïve rat, we infused the equal dose into the counterpart in the intact hindlimb for a control. Seven days later, we sectioned the lumbar spinal cord from the L4-to-L4 root at 20 μ m thickness, taking pictures under confocal microscopy (Zeiss LSM880 with NLO & Airyscan). Soma and partial dendritic arbors tagged by fluoro-gold were enrolled in this study. Under 10 objective lens, at 50 μ m separation photograph, we captured the labeled motoneurons at 480 nm wave-length. z-stack was used for reconstruction of optical sections.

Ultrastructural assessment for the regrowing nerve

To illustrate the reinnervated dynamics, the regrowing roots, 3 mm away from the coaptation site, were transected rostrally and caudally for ultrastructural evaluation at a prescribed timepoint. Generally, nerve sections obtained were fixed in 2.5% glutaraldehyde, dehydrated in both graded ethanol and propanone, permeated with resin. Next, the specimens examined were subjected to longitudinal and transverse ultrathin slices, then stained with 3% uranyl acetate–lead citrate. Ultrastructures for the regenerative axons were observed under FEI (Tecnai Spirit Biotwin, USA), and captured at a various resolution. *G-ratio* is described as an axon diameter divided by a fiber one, directly representing the size of a regenerative axon. We repetitively calculated it and deemed it as a convective parameter reflecting axons remyelination.

Statistical Analysis

Differences among groups were calculated using repeated-measures ANOVA, with post-hoc LSD pairwise comparisons applied as appropriate. All data were expressed as the mean $\pm$ S.E.M., with SPSS 21.0 (SPSS Inc.) and image J (1.52p) used for data analysis. The statistical significance value was set at a *p* value of 0.05 or lower.

Data availability

Results

Identification of a right hindlimb paralysis

Seven days after injury to the dorsomedial area of PLIC, animals had an onset of pronouncedly reduced activities in the right lower extremity, anterogradely galloped in circles centering on the right hindlimb. In the walking tasks, there was > 85% slip rate in the right hindlimb, as opposed to in the left lower-limb ($P < 0.05$). In footprint test, the fore-hindpaw prints were non-overlapping for at least 6 months, with a certain spacing (19.2 ± 0.7 mm) in between ($P > 0.05$). Additionally, denervated MUAPs with a lengthened duration and shortened amplitude, were detected in the hemiplegic hindlimb, by contrast to in the left hindlimb, further corroborating a truth that fore-hindpaw print was separated. The results implied an establishment of a rat model (Fig. 2).

Availability of L4 nerve root as a donor nerve

Ten days after the only transection of L4, with acupuncture electromyography, rats were markedly detected myokymic potentials and positive sharp waves in the quadriceps femoris, moderately in both semimembranosus and gastrocnemius, mildly in the tibialis anterior, and hardly in the biceps femoris, maximally scored 20 in BBB score scale. Instead, in the context of retention of L4 root but excision of other lumbar roots, rats were captured normal potentials in the corresponding muscles, particularly in the quadriceps femoris, semimembranosus, gastrocnemius, and tibialis anterior, maximally rated 13 of BBB score. We could conclude from BBB score that under the only retention of the L4 nerve root, the hindlimb function might recover to ~70% of a native state within 2 weeks, as compared to under the only excision of the L4 nerve root, and from the electromyography that the L4-dominating muscles could actuate flexion and extension for major joints in the hindlimb, especially for the ankle. These results demonstrated eligibility of L4 as a source nerve (Fig. 3).

Behavioral assessment among three groups

In the footprint test, the right fore-hindpaw interval is summarized in Supplementary Table 1. Also, in-between space in Group C significantly diminished over time till an overlapping status emerged at 17 weeks post-surgery, in contrast to group B ($P < 0.001$). In the walking-beam and -ladder tests, the overall slip and error rate for the bilateral hindlimbs were not statistically significant between group B and C, and attained 90% at baseline ($P > 0.05$). At 1 week, the slip and error ratio in group B drastically declined to 44% ~56% as compared to group C. At 3 weeks, animals in Group A achieved ~95% accuracy, compared with in group B and C ($P \approx 0.05$). At week 5, in group B and C, growing behavioral improvement nevertheless started to emerge, but higher increment in group B. Nonetheless, from 9th week onwards, animals in group C displayed larger reduction in the slip and error rate than did in group B ($P < 0.05$). Although increase in

accuracy after 9 weeks, animals in these two groups failed to improve to the level in group A at all time points. The BBB score was significantly higher at 17 weeks than at 9 and 13 weeks ($P < 0.001$). The data exhibited effective motor function regeneration for the hemiplegic hindlimb through L4 transfer (Fig. 4).

Electrophysiological evaluation for contralateral L4 transfer.

In group C, the reinnervated MUAPs could be elicited with concentric needles in the quadriceps femoris, semimembranosus, lateral gastrocnemius and tibialis anterior of the right hindlimb in 4 out of 10 rats (40%) at 9 weeks, in 7 rats (70%) at 13 weeks, and in 9 rats (90%) at 17 weeks, post-intervention. Also, the denervated MUAPs detected in the target muscles markedly decreased temporally. In H-reflex recording, the M/H-wave latency for the right gastrocnemius was remarkably longer at week 9 than at week 17 ($P < 0.05$) (Supplementary Table 2). The latency shortened over time till approximating to a normal level ($P > 0.05$). Together, the results suggested that the L4-to-L4 root efficiently reinnervated the targeted end-organs (Fig. 5).

Fluorogold-labeled motoneurons in the ventral horn from the L4-to-L4 root.

In the ventral horn, we could see many fluorogold-labeled motoneurons, despite largest labeled area existed in the control ($P > 0.05$). At 9, 13, and 17 weeks, tagged moto-neurons increased temporally, in contrast with the contralateral side ($P < 0.05$). These results exhibited that a high proportion of regenerated axons efficaciously reinnervated the motor endplates (Fig. 6).

Immunofluorescence staining and ultrastructure for the reinnervated nerve root

At the coaptation site, regrowing axons were remarkably observed, with mature ones tagged by MAP-2 accounting for $25.61 \pm 5.18\%$ at week 9, $38.52 \pm 3.64\%$ at week 13, and $57.89 \pm 6.25\%$ at week 17. Additionally, increasing reinnervation between the stumps occurred temporally till reaching nearly utter reinnervation at week 17 ($P > 0.05$) (Supplementary Fig. 1). Simultaneously, ultrastructure for the regenerative nerves exhibited a coexistence of myelinated and unmyelinated axons, further suggesting an uneven axon regeneration at the three timepoints ($1,081 \pm 469$, $12,572 \pm 953$, and $15,928 \pm 1147$, $P < 0.05$). Over time, *g-ratio* for a regrowing axon declined, in parallel with an elevation of myelinogenesis. Additionally, more reinnervated axons occurred in a realigned and rearranged manner. The results demonstrated effective regeneration of nerve fibers (Supplementary Fig. 2).

Discussion

Given currently dissatisfied outcomes and large HICH populations, we need additional intervention to limb hemiplegia[32–35]. Rationales behind the previous C7-to-C7 transfer for the paretic upper limb are beginning to be accepted and understood as scalability[36–40]. Reinnervation of the denervated muscles, γ -circuit interruption, and establishing a new pathway remarkably contributed to a motor recovery post-transfer[41, 42]. Contralateral lumbar neurotization engaging the similar mechanisms was considered available for a lower limb paresis after central neurological injury. Thus, in this project, we would rather extend the L4-to-L4 transfer to hindlimb hemiplegia than other transfer modalities in rats.

Why the L4 root was identified as an optimal source nerve, compared to other lumbar nerves?

First, transecting the L4 root may not impair the lower extremity motion owing to compensation from neighbor roots, as shown by the BBB score. Second, biomechanically, synergies between flexors and extensors lead to a stable and refined movement. Anatomically, the L4 is a sole root that simultaneously drives anteroposterior muscle groups, theoretically actuating robust flexion and extension in a hindlimb. Finally, given the muscles innervated by the L4, including the quadriceps femoris, semimembranosus, gastrocnemius, and tibialis anterior, etc., it was inferred that the L4 may not only power reliable proximal joints activities but also distal joints motions in the lower limb. The L4 as a donor nerve might represent a priority in functional recovery following nerve transfer.

In our study, we broke off the efferent pathway via damage to the posteromedial area of PLIC, to create a qualified model rat, as indicated by electromyography and pawprint tests. Yet, only slight or no spasm was observed in a model rat. Besides, we presented the availability of the L4-to-L4 transfer for motor recovery. As well-known, a denervated muscle may be atrophic if a nerve fiber fails to reinnervate at a certain time, i.e., 3 to 6 months. On the one hand, the results from H-reflex and retrograde tracing implied that the regenerative axons had strongly reinnervated the target muscles. On the other hand, based on a shortened latency for M/H-wave and g-ratio in ultrastructure temporally, we strongly speculated that nearly all regrowing fibers could be myelinated and well-functioned. Finally, depending on an increasing regeneration between stumps, we suggested that larger numbers of regenerated fibers reached the motor end-organ to generate better functional outcomes. Collectively, post-surgery, the axons could regenerate into the target muscles to undertake due responsibility before their atrophy.

Here, we also demonstrated distinct observations from the L4-to-L4 transfer after a comparison of the other transfer modalities. Post-operation, in the distal major joints such as hip and knee joints, rats could conduct large-range, dexterous, and strong activities. Additionally, in the ankle joint, full flexion and extension were often seen at the early stage, as further implied in the walking-beam and walking-rung tests. As we know, central neurological injuries commonly cause motor control deficits in the whole leg, specifically refined ones. To achieve contiguous skilled locomotions, rats oblige to balance the weight support and true up limb placement rapidly. Hence, fine muscle, leg coordination, and ability to equilibrate weight-bearing stepping movements are required for a favorable performance in the walking tests. For that reason, movement accuracy and gait in the intact leg were provisionally negatively impacted as well. Briefly, the extent to which action accuracy was improved and deficits in gait were rescued could be revealed by the skilled walking tasks, pawprint test, as well as acupuncture

electromyography. Nevertheless, relatively long recovery time was taken after the contralateral L4 transfer. Also, on the intact hindlimb, a comparatively much loss of motor function emerged after transecting the L4, although it did not impair the locomotion. Despite these shortcomings could not eclipse the neurotization application, we still hope that they will be overcome in the future by multidisciplinary evolutions.

Several limitations to this study remained to be solved in the future. Noteworthily, post-intervention, no significant motion was observed in digits, possibly due to a short experiment duration. The exact number of L4 nerve fibers on the intact side innervating the contralateral muscles and the ratios to other lumbar nerve roots were yet not clarified. As rats are quadrupeds, a lower-limb is more frequently implicated in the skilled motion in rats than in humans. Slight motor degradation tends to be more detectable in rats. However, the present study still extrapolates the concept to clinical practice.

In conclusion, contralateral L4 neurotization enables an effective motor recovery in the distal joint, intensifies performance in the proximal joints, and may be an optimal option for the hindlimb paralysis secondary to HICH. Further researches into the mechanisms underlying axonal regeneration and cortical plasticity in hindlimb recovery are inevitably desirable.

Abbreviations

HICH: Hypertensive intracerebral hemorrhage

PLIC: Posterior limb of internal capsule

Declarations

Acknowledgements

Not applicable

Declarations of interest

None.

Authors' contributions

Q.T.D. and Z.X.F. designed the analysis. S.J., M.T. and Y.W.Y. collected and extracted the data. Q.T.D., W.J.H. and M.T. carried out the statistical analysis. Q.T.D., H.B.S. and L.L.X. drafted the manuscript. All authors reviewed and approved the final report.

Funding:

The authors disclosed receipt of the following financial support for the research, authorship, and publication of this article: this study was supported by grants from the National Natural Science Foundation of China (No. 81171147), “Key Medical Talents of Qiangwei Project” Research Foundation of Health Department of Jiangsu Province (No. ZDRCA2016010), “Xingwei Project” Key Personal Medical Research Foundation of Health Department of Jiangsu Province (No. RC201156), Jiangsu Province’s Natural Science Foundation (BK20171064), Research Foundation of Jiangsu Provincial Medical Youth Talent, the Project of Invigorating Health Care through Science, Technology and Education (QNRC2016858). Jiangsu Province’s Key Discipline of Medicine (No. XK201117) and the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD). Jiangsu Province’s Natural Science Foundation (BK20171064), Research Foundation of Jiangsu Provincial Medical Youth Talent, the Project of Invigorating Health Care through Science, Technology and Education (QNRC2016858).

Availability of data and materials

All the data mentioned in this article are available on published article.

Ethics approval and consent to participate

Not applicable

Consent for publication

All the authors have approved the manuscript.

Competing interests

All authors report no disclosures relevant to the manuscript, and claim no conflict of interest.

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Figures

Figure 1

Diagrammatic demonstration of manufacture of rat models and customized treatments in each group. (A) The employed coordinate is in accordance with an illustration by Paxinos and Watson. (B) A hole 0.8mm in diameter was obtained in rat cranium, 3.1mm posterior to the bregma, 3.3mm lateral to the midline. (C) A coronal T2-weighted MRI image indicates the hematoma inhabiting the posterior limb of the left internal capsule (pink arrow). (D) A protocol on the experiment is presented. (E) sham-operation

was conducted in Group A(bilateral L4 nerve roots were not severed intraoperatively,n=10).(F)Bilateral L4 nerve roots were sharply transected in Group B(n=10), and the stumps were secured to the psoas major muscles or capped with soft tissues.(G) The L4-to-L4 transfer was performed in Group C(n=10).Contralesional L4 was resected as distally as possible,while proximal severance occurred in the ipsilesional L4; Interrupted suture was applied to stumps anastomosis.(R=Right; L= Left).

Figure 2

Behavior and electromyographic assessments confirming eligibility of a rat model. (A) and (B) In the walking tasks, >85% slip rate was seen in the right hindlimb(affected side), compared with in the left one (intact side)($P \leq 0.05$). (C) Superposed fore-hindpaw print, generally characteristic of a naive rat, is presented as a reference. (D) A contralateral detached state was present in rats with an injury to the posteromedial area of PLIC. In this case, normal MUAPs (E) were replaced with the denervated ones (F).

Figure 3

Availability of the L4 as a source nerve. Anatomically, a nerve root dominating the flexors and extensors is usually considered as an optimal candidate. (A) Under only severance of the L4, the electromyographic test indicated that the positive waves or denervated potentials occurred in the QF, SM, TA, and GC. (B) Surprisingly, under the only retention of the L4, normal MUAPs were evidently recorded from the same target muscles. (C) With respect to the similar treatment, >12 in the BBB scale score was scored after only retaining the L4 when ≈ 20 was scored following only severing the one ($P < 0.05$). The data suggested high weight of the L4 in the lumbar plexus and an effective compensation from neighbor lumbar roots. (QF= Quadriceps femoris, SM= Semimembranosus, GC= Gastrocnemius, TA= Tibialis anterior).

Figure 4

Behavior improvement following the L4-to-L4 transfer. (A) Fore-hindpaw prints for a naive rat (A1) and model rat (A2) are presented as a reference. (B) Judging from data at week 9 (B1), week 13 (B2), and week 17 (B3), the detached status was gradually pulled back to an overlap post-procedure. (C) and (D) Also, in the walking tasks, no statistical significance in accuracy occurred at baseline between group B and group C. With time, a larger decline in slip rate for the affected side was observed in group C, as compared to in group B ($P < 0.05$). Additionally, in group C, there was also a relatively high slip rate for the intact side owing to body imbalance. Later on, there was also improvement in accuracy. (E) Eventually, at week 17, rats in group C scored ~13 in the BBB scale score. These results implied a positive role of the intervention in motor regeneration. (BL=Baseline).

Figure 5

Electromyographic assessment revealing efficacious reinnervation of the denervated muscles. (A1) At week 9, many positive sharp waves were seen post-operation ($P \leq 0.05$). (A2) MUAPs began to appear and the positive sharp waves disappeared. (A3) Numerous MUAPs were detected ($P \leq 0.05$). (B) The reinnervated potentials were prominently recorded in QF, moderately detected in SM and QC, and slightly captured in TA ($P \leq 0.05$). (C) H-reflex was evoked in a naïve rat, as a control. At 9, 13, and 17 weeks, H-reflex was markedly elicited in the gastrocnemius of an affected hindlimb, the latency for which shortened over time and approximated to a normal standard. (QF= Quadriceps femoris, SM=Semimembranosus, GC=Gastrocnemius, TA=Tibialis anterior).

Figure 6

Retrograde tracing showing increasing motoneurons tagged by fluoro-gold temporally. To mirror the ability of the L4-to-L4 root to transmit signals, we set up a control group (A) for quantitative comparison. At week 9 (B), week 13 (C), and week 17 (D), many labeled motoneurons are observed in the ventral horn, and the tagged area expands temporally.

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