A KÖTŐSZÖVETES RENDSZER SZEREPE A KOMPLEMENTER-ALTERNATÍV MEDICINA / TERMÉSZETGYÓGYÁSZAT / HAGYOMÁNYOS KÍNAI ORVOSLÁS ELMÉLETI ALAPJAINAK MEGISMERÉSÉBEN ÉS AZ EGÉSZSÉGTUDOMÁNNYAL HATÁROS TERÜLETEK INTEGRÁCIÓJÁBAN

Doktori (PhD) értekezés tézisei

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Pécs, 2013

Előszó

A természetgyógyászat (TGY), a hagyományos keleti/kínai orvoslás (HKO), a komplementer vagy alternatív medicina (KAM) kutatásának és fejlesztésének pénzügyi támogatása világszerte alulmarad a hatóanyag központú biomedicinális kutatások támogatottságához képest. Ennek ellenére, arányait tekintve, az elmúlt tíz évben növekedés tapasztalható a TGY/HKO rendszerek kutatási támogatásában az Amerikai Egyesült Államokban, Kínában, Dél-Koreában és Oroszországban egyaránt.

Mind a nyugati, mind a keleti társadalmakban a komplementer medicina módszerei elismertek, azonban terminológiája, gondolatrendszere látszólag alapjaiban eltér a modern biomedicinális értelmezéstől. Ez a tény pedig nehezíti elismertetését, oktatását. Ma már Kínán belül is ütközőpontot jelent a betegellátásban és kutatásban, nehezíti a modern „nyugati" és a hagyományos „keleti" egyetemi képzést kapott szakemberek kommunikációját.

Ezért az elmúlt években egyre erősödik az úgynevezett integratív biomedicinális szemlélet, mely alapot teremt arra, hogy akár kongresszusokon, akár szakfolyóiratokban kommunikáljanak az esetleg teljesen más hátterű, de gyakran hasonló módszereket alkalmazó, kutató szakemberek. Ennek alapfeltétele az lenne, hogy legyenek olyan fiziológiai, sejtbiológiai szintű eredmények, melyek a TGY/HKO/KAM alapmechanizmusait kutatva, de integratív szemléletből születtek és megállják a helyüket a modern tudományos társadalom publikációs, elbírálási rendszerén belül is.

Bevezetés

A természetes gyógymódok különböző eredetre, valamint eltérő jogi szabályzásra utaló elnevezéseinek sokfélesége is valószínűsíti, hogy a nyugati orvoslásban is elfogadott, modern, közös biomedicinális megközelítés nehézségekbe ütközik.

A természetgyógyászat (TGY), a hagyományos keleti/kínai orvoslás (HKO), a komplementer vagy alternatív medicina (KAM) olyan gyógymódok, kezelési eljárások összessége, melyek eredete részben függetlenül fellelhető több nép gyógyászati kultúrájában, ugyanakkor rendszert formálva az ázsiai, azon belül is a kínai történelmi-irodalmi háttéranyagban maradtak fent a legrégebbi források.

A fenti gyógymódokban közös elem az ingerpontokra és a csatornákra (akupunktúrás/akupresszúrás pontok és meridiánok) alapozott diagnózis és kezelési terv. Mivel ezek a pontok és csatornák a legtöbb esetben szinte semmilyen elfogadott anatómiai struktúrához nem vagy csak kevés tudományos igényességgel köthetők, és szerepük legkiemelkedő a HKO/TGY/KAM rendszerek gyakorlásában és oktatásában, ezért tudományos vizsgálatuk az egyik első lépcső a hagyományos keleti és a modern nyugati módszerek integrációjában vagy akár csak a kutatási kérdésfelvetések megalkotásában is.

Célkitűzés

Kutatásaim kezdeti célja az volt, hogy irodalmi forráselemzésen keresztül mutassam be, hogy vannak eredmények a keleti és a nyugati orvoslás integrációjában, és ezek az alapkutatás világában is kezdenek összeállni egy lassan átfogóvá alakuló, a TGY/HKO tudományos színvonalú integratív oktatását támogató rendszerszemléletté. Ez a kezdeti cél később személyes szakmai kapcsolatokkal bővült, mind a gyógyászati, mind a kutatói oldalon.

Mint neurobiológiában jártas kutató azt érzékeltem, hogy „vakon", a terápiás ismeretek sikerességéből adódó magabiztosságot és felbuzdulást félretéve, a biológiai alapoktól kiindulva kell megfogalmazni a kérdéseket és értékelni a kísérleteket. Az elmúlt 60 évben több látszólagos integrációt serkentő eredmény született, de az alapkutatási kérdésfeltevések precíz kidolgozásának hiánya sok esetben vakvágányokra illetve természettudományos igényességgel nem vállalható modellekhez vezetett.

Az elmúlt években végzett kutatásaim eredményeként az ingerterápia és az akupunktúra biomedicinális megközelítésében olyan új eredményeink születtek, melyek további hidat képezhetnek a keleti és a nyugati orvoslás között.

Kérdésfelvetés

1. Van-e tendencia az integrációra a stimulatív terápiák, illetve a hagyományos keleti orvoslási módszerek alapmechanizmus kutatásában?

2. A kötőszövetes mátrix rendelkezik-e olyan tulajdonságokkal, melyek anatómiai és élettani alapját képezhetik szervezet szintű kommunikációs, stabilizációs egységnek? Rendelkezik-e ez a kötőszövetes rendszer a fenti kérdéshez szükséges elektrofiziológiai és sejtélettani reaktivitási zónákkal, inhomogenitásokkal, illetve kontinuitást teremtő szövettani képletekkel?

3. Tudjuk, hogy a subdermális és az intramusculáris kötőszövet terápiás szempontból igen sokféle, látszólag eltérő inger modalitásra képes hasonlóan reagálni, azaz többféle úton képes közvetíteni a terápiás ingereket. Ez esetben léteznie kell olyan sejtélettani molekuláris kapocsnak, mely képes ezeket a különböző ingereket integrálni. Ilyen lehet az extracellulárisan regulált kináz (ERK) rendszer? Képes-e reagálni a miofasciális ERK rendszer elektromos és mechanikus ingerlésre egyaránt?

4. A statikus mágneses tereket (SMF) gyakran használják évtizedek óta a stimulatív regenerációs medicinában és a HKO/TGY rendszerekben is, de ezek neuronális vagy egyéb sejteket érintő kommunikációt szabályzó szerepét nem ismerjük. Van-e elfogadható, celluláris fiziológiai körülmények között kimutatható hatása a SMF-nek a sejthálózati kommunikációra? Ennek vizsgálatára, egy nagy elemszámban, kis költségvetéssel és gyorsan reprodukálható idegsejtmodellt választottunk (drosophila interneuronok) és olyan statikus mágneses tér impulzust, mely a diagnosztikában és a terápiában is elfogadottan használt évtizedek óta.

Módszerek

1.Kettős-rendszer (two-system therory) munka hipotézis bemutatása.

2. Virtuális test meridiánok (virtual human bodies ,VCH) három dimenziós digitális elemzése.

3. Az emberi test kötőszöveteinek elemzése computer tomographias (CT) és mágneses rezonanciás (MRI) módszerekkel.

4. A szöveti elektromos vezetőképesség mérése „konduktancia válasz" mérési módszerével.

5. In vitro elektrofiziológiai vizsgálatok (HEKA Elektronik, Lambrecht/Pfalz, Germany)

6. Immunszerologiai vizsgálatok, radioimmun módszerrel.

7. A kötőszövet és az izomszövet immunhisztológiai és elektronmikroszkópos vizsgálatai.

(A módszerek részletes ismertetése a közelményekben található.)

Eredmények és megbeszélés

A fascia hálózatról feltételezik, hogy az egy információ-átvivő rendszer mely sejtek, rostok és extracellularis mátrix elemek változataiból tevődik össze és amely rendszer felelős a test differenciált funkcionális sejtjeinek támogatásáért és regenerációjáért, a belső környezet

szabályozásáért. A „fasciológia" elmélet világít rá az olyan stimulatív terápiáknak mechanizmusaira, mint az akupunktúra és a kínai masszázs. Ezek mechanikus stimulációt fejtenek ki a szubkután fasciákra és kötőszövetre, hogy azok választ váltsanak ki a funkcionális sejtekben és ezáltal okozzanak érzékelhető terápiás hatásokat.

A jelen munkában bemutatott modell, mint kötőszövetes mátrix modell, igen ígéretes integratív terület a keleti és nyugati típusú stimulatív terápiák alapmechanizmus kutatásában. Feltétlen előnye a korábbi modellekkel szemben és azokat természetesen kiegészítve, hogy a HKO/TGY rendszerek oktathatóságát már jelenleg is, több országban könnyíti a felsőoktatásban és tanfolyami képzésekben egyaránt, ezen túlmenően további olyan biológiai jelenségekre hívja fel a figyelmet, melyet sem a keleti, sem a nyugati medicina nem ismer még eléggé, pedig úgy tűnik, ezek a szöveti regenerációs jelenségek képezhetik a modern regenerációs gyógyászat alapjait a jövőben.

Válaszok a feltett kérdésekre

1. A kettős-rendszer hipotézis magyarázattal szolgálhat a HKO és TGY klinikai eredményességének magyarázatára, mely rendszerek anatomiai és funkcionális létezését számos biológiai kutatási eredmény támogatja. Ez a keleti orvoslás és a modern nyugati medicina egyértelmű integrációs tendenciáját igazolja. (1, 2 és 3. közlemények).

2. A virtuális meridiánok digitális elemzése, és ezek összevetése a test kötőszövetes rendszerének CT, MRI elemzésével, valamint elektrofiziológiai vizsgálatokkal, egyértelműen támogatja egy anatomiailag és funkcionálisan is létező kötőszövetes matrix létezését (4. közelmény).

3. Az extracellulárisan regulált kináz (ERK 1/2), valamint a mitogén-aktivált protein kináz (p38) egyértelműen aktiválhatók voltak akupunktúrás manupilációkkal, mely bizonyítja, hogy az akpunktúra hatással van neurohormonális rendszerekre (5. közelmény).

4. A statikus mágneses tér (SMF) megváltoztatta a drosophila sejttenyészet lokális nagy interneuron hálózatának ritmikus, spontán aktivitását, és a szöveti nátrium és kálium koncentrációt. Ezen vizsgálatok az egyik első, tudományos igényű in vitro vizsgálatoknak tekinthetők, melyek igazolják a mágneses tér idegi működésekre gyakorolt hatását (6. közlemény).

Ismertek idegrendszeri kórképek, melyekben a harántcsíkolt izom fokozott aktivitását észleljük, a kálium membrán transzport zavara igazolható, ugyanakkor mégsem találunk kielégítő neurofiziológiai magyarázatot az izom hyperaktivitására. Ezen esetekben joggal gondolhatunk a myofascialis rendszer szerepére, és megfontolandó az alternatív gyógyítási módszerek kezelésbe történő bevonása (7. közlemény).

A bevezetőben feltett kérdésekre, a tételes válaszok mellett összegzésként elmondható, hogy a fasciologia vagy kötőszövetes mátrix modell képes lehet egyesíteni magában a keleti meridiánológia és a nyugati típusú stimulációs ingerterápiák alapkutatási módszereit, így előmozdítva egy közös terminológia kialakulását, tehát az alapkutatási szintből táplálkozó integrációt.

A hagyományos kínai és a modern nyugati orvoslás közötti terminológiai integrációt nehezítő tényezők:

1.Tudományos-kulturális különbségek, melyek jelentősen nehezítik a valódi integrációt: létezik egy óriási és értékes tudásanyag a TGY/HKO biomechanikai és bio-elektromágneses alapmechanizmusait és klinikai alkalmazásait tekintve, mely orosz és kínai nyelven hozzáférhető csak. Az orosz és kínai kutatók és klinikusok nagy része csak saját nyelven publikál és nem érdekeltek a Medline jegyzett, angol nyelvű publikációs kutatói társadalomban részt venni. Itt még ma is az egyetlen járható út a személyes szakmai kapcsolatokon keresztül nyílik meg és rengeteg közös fordítási és értelmezési munkaórát igényel a gyakori nevezéktani eltérések miatt.

2. Sok az esettanulmány, kevés az alapkutatás.

3. Alapkutatásban is kevés az integratív kérdésfeltevés, ezért az eredmények esetenként még nagyobb szakadékot képeznek, mert nem könnyítik a keleti orvoslás terminológiáján alapuló HKO és TGY oktatást, mivel a nem integratív kérdésfeltevésekből származó eredmények nem képeznek terminológiai hidat.

4. Nagyon kevés az egészséges humán alanyon végzett meridiánológiai kutatás. A meridiánológiai kutatások nagy része mind keleten, mind nyugaton még mindig tünet, illetve betegség központú, nem fókuszálnak az alapmechanizmusokra vonatkozó kérdésfelvetésekre

5. Az elektroakupunktúrából és a magnetoterápiás eljárásokból kifejlődött elektromágneses stimulációs terápiák jelentős térhódítáa ellenére, a mai napig nagyon kevés az olyan alapkutatási közlemény, mely a nyugati tudományos világ impakt faktoros publikációs rendszerében nyugati tudományos módszerek bevonásával tárgyalja az elektromágneses stimulációk sejtbiológiai alapjait. Ezért annak ellenére, hogy ezek a módszerek az orvoslásban keleten és nyugaton is egyre nagyobb teret hódítanak, a természet tudományos kutatói

társadalom felé továbbra is megvan a szakadék, az egyre növekvő számú esettanulmány ellenére

6. Az elektrofiziológiai szemléletű meridiánológiai munkák nagy részben klinikai szemléletűek, abból a prekoncepcióból indulnak ki, hogy a kötőszövetes rendszerben léteznek elektrofiziológia inhomogenitások, melyek átfednek az akupunktúrás meridiánok rendszerével, ezért nem végeznek megfelelő és elegendő kontrollpont mérést a vélhetően nem akupunktúrás pontokon

7. A neuronhálózati és neuroanatómiai modellek részlegesen magyarázzák a szegmentálisan, dermatómák és miotómák mentén működő fájdalomcsillapító hatásokat. A gerincvelő substantia gelatinosa állományában elhelyezkedő enkphalinerg interneuronokon keresztül megvalósuló kollaterális gátlási mechanizmus (kapukontroll modell) és a raphe magvak, illetve a periaquaeduktális középagyi szürkeállomány leszálló szerotonerg, noradrenerg és peptiderg pályái mint a perifériás és központi endogén fájdalomcsillapítás rendszerei, sokszor használt modellek az ingerterápiák hatásmechanizmusainak bemutatásában. Számos hátrányuk mellett egyik előnyük, hogy a prefrontális kérgi összeköttetéseket is tárgyalják, így részben útmutatást ad a páciens belső szándéka, motivációja és a terápia sikeressége közötti összefüggés értelmezésére is. Ezek a modellek nem tudnak mit kezdeni a meridián rendszerrel, mint a HKO hátterét adó funkcionális egységgel.

A hagyományos kínai és a modern nyugati orvoslás közötti terminológiai integrációt serkentő alapkutatási folyamatok:

1. Az elmúlt években egyre nagyobb lett az igény a humán és emlős felnőtt szervezet nem csontvelői eredetű endogén szöveti őssejt tartalékainak megismerésére, mobilizálhatóságára, sejtregenerációban betöltött szerepének vizsgálatára.

2. Az elektroakupunktúrásan serkentett exogén őssejtek szövetspecifikus beépülésének vizsgálata egy olyan integratív terület, melyben a szöveti regenerációs terápiák és az akupunktúrás módszerek kutatása közös találkozópontra ért. A kötőszövetes rendszer szerepe felértékelődött az endogén szövet specifikus őssejteket vagy legalábbis pluripotencia faktorokat mutató sejteket mobilizálni képes módszerek kutatásában is. Több, egymástól független kutatócsoport kezdi a kötőszövetes rendszert mint funkcionális és biomechanikai egységet kezelni a kísérleti és terápiás kérdésfeltevések és tervek megalkotásában is. Ez a kutatási és terápiás szemlélet túlmutat a HKO/TGY rendszerek hatásmechanizmus kutatásán, a szöveti regenerációs folyamatok mechanikai és elektromágneses úton történő stimulálhatóságának mélyebb megismeréséhez vezet.

A 2011-ben az Egészségakadémia folyóiratban megjelentetett közleményünk záró ábrájára hivatkozva a következő mondattal zárnám a dolgozatom:
Meg kell értenünk a különbséget a traumatikus sebgyógyulás és a gyógyító mikrotrauma között.

A tézis alapjául szolgáló közlemények

1. Integrative approaches in the research of fascial network for a better understanding of traditional Chinese medicine mechanisms..**Janos Palhalmi**, Yu Bai, Lin Yuan
Journal of Chinese Integrative Medicine, February 2010, Vol.8, No.2

2. Progress in fascial network research. **Janos Palhalmi**, Lin Yuan, József Bódis
Health-Academy Journal, University of Sciences Pécs. 2011, Vol 2.

3. Possible applications for fascial anatomy and fasciaology in traditional Chinese medicine.

Yu Bai, Lin Yuan, Kwang-Sup Soh, Byung-Cheon Lee, Yong Huang, Chun-lei Wang, Jun Wang, Jin-peng Wu, Jing-xing Dai, **Janos Palhalmi**, Ou Sha, David Tai Wai Yew

J Acupunct Meridian Stud 2010;3(2):125−132

4. Research methods in fasciaology: implications for acupuncture meridianology.

Yu Bai , **Janos Palhalmi**, Yong Huang , Chun Yang, Lin Yuan. **Fasciaology Journal**, Vol.1, July 30, 2011

5. Modulatory effect of acupuncture on the extracellular signal-related kinase 1/2 (ERK1/2) and p38 mitogen-activated protein kinase (p38 MAPK) signaling pathways in the subcutaneous fascia. Jiang Xue-mei, Bai Yu, **Palhalmi Janos**, Yuan Lin, Hunag Yong, Dai Jing-xing, Wang Sheng-xu, David Tai Wai Yew **Fasciaology Journal**, Vol.1, July 30, 2011

6. Static magnetic field modulates rhythmic activities of a cluster of large local interneurons in drosophila antennal lobe. Ying Yang,Ying Yan, Xiaolu Zou, Chuchu Zhang, Heng Zhang, Ye Xu, Xutian Wang, **Palhalmi Janos**, Zhiyun Yang, Huaiyu Gu

Journal of Neurophysiology, 106: 2127-2135, 2011.

7. Szerzett multifokális myoclonus pathomechanizmusa – Beteg bemutatás. Gáti István, Olof Danielsson és Pálhalmi János, **Health-Academy Journal**, University of Sciences Pécs. 2013.Vol.4,No.2.

Szerző további közleményei

1. Abrahám I, **Pálhalmi J**, Szilágyi N, Juhász G.Glucocorticoids alter recovery processes in the rat retina. Neuroreport. 1998 May 11;9(7):1465-8.

2. Bíró K, **Pálhalmi J**, Tóth AJ, Kukorelli T, Juhász G. Bimoclomol improves early electrophysiological signs of retinopathy in diabetic rats. Neuroreport. 1998 Jun 22;9(9):2029-33.

3. Galambos R, Szabó-Salfay O, Barabás P, **Pálhalmi J**, Szilágyi N, Juhász G. Temporal distribution of the ganglion cell volleys in the normal rat optic nerve. Proc Natl Acad Sci U S A. 2000 Nov 21;97(24):13454-9.

4. Pálhalmi J, Szikra T, Kékesi KA, Papp A, Juhász G. An in vivo eyecup preparation for the rat. J Neurosci Methods. 2001 Feb 15;105(2):167-74.

5. Szabó-Salfay O, **Pálhalmi J**, Szatmári E, Barabás P, Szilágyi N, Juhász G. The electroretinogram and visual evoked potential of freely moving rats. Brain Res Bull. 2001 Sep 1;56(1):7-14.

6. Barabás P, Kovács I, Kovács R, **Pálhalmi J**, Kardos J, Schousboe A. Light-induced changes in glutamate release from isolated rat retina is regulated by cyclic guanosine monophosphate. J Neurosci Res. 2002 Jan 15;67(2):149-55.

7. Hájos N, **Pálhalmi J**, Mann EO, Németh B, Paulsen O, Freund TF. Spike timing of distinct types of GABAergic interneuron during hippocampal gamma oscillations in vitro. J Neurosci. 2004 Oct 13;24(41):9127-37.

8. Pálhalmi J, Paulsen O, Freund TF, Hájos N.Distinct properties of carbachol- and DHPG-induced network oscillations in hippocampal slices. Neuropharmacology. 2004 Sep;47(3):381-9.

9. Palhalmi J: Functional regulation of acupoint electrodynamics. Does it exist? Fascia Congress 2009, Amsterdam. Peer-reviewed Presentation.

10. Gati I, Danielsson O, **Pálhalmi J**, Leijon G, Dizdar N, Fredriksson B-A,Vrethem M and Lindehammar H, Acquired multifocal myoclonus - Case histories. Submitted to **Pathology Oncology Research**

Köszönetnyilvánítás

Köszönettel tartozom a PTE ETK Doktori Iskolának, Prof. Dr. Bódis Józsefnek, és témavezetőmnek, Dr. Gáti Istvánnak, hogy lehetőséget teremtett egy ilyen nem-konvencionális téma bemutatására és hogy az előadásaik alkalmával megmutatták, hogy a biomedicinális szemléletet modern, integratív módon lehet képviselni az egészségtudományok oldaláról. Ez azért különösen fontos számomra, mert megerősítette bennem, hogy a hagyományos gyógyászati rendszerekből ismert egészségkép-énkép kapcsolat preventív alkalmazásának a nyugati/modern tudományban is lehet relevanciája.

Köszönettel tartozom Dr. Juhász Gábornak és Dr. Ábrahám Istvánnak akiktől a szakdolgozati, illetve neurobiológiai PhD munkám során olyan gondolatrendszereket kaptam, melyeket időtállóságuk miatt bármely más tudományterületen is lehet kamatoztatni.

Szeretném megköszönni Dr. Kiss-Szolingen Attilának és Dr. Eöry Ajándoknak, hogy az elmúlt 10 évben rendszeresen lehetővé tették munkám egyes állomásainak bemutatását különböző orvos-természetgyógyász kongresszusokon és egyetemi előadásokon, ezzel érdemi szakmai visszajelzéseket nyújtva.

Köszönöm Dr Kulcsár Gábornak, hogy egy új, de valójában a régi hagyományokban gyökerező muszkulo-szkeletális biomechanikai modellt ismertetett meg velem.

Köszönöm Paul Völgyesinek, egyik akupunktúra tanáromnak, hogy egy józan, tiszta gondolati modellt és gyakorlati tudást közvetített felénk.

Köszönöm Dr. Doc-Fai Wongnak, Kung Fu és Tai Chi tanáromnak, akupunktúrás szakorvosnak, hogy olyan virtuóz aku-tű technikákat mutatott, melyeket máshol nem láttam és megerősítette bennem, hogy a HKO és a mozgásművészet gyökerei közösek.

Köszönöm Dr. Németh Józsefnek, állatorvosnak, hogy rávilágított, az ingerterápiák állatokon is működnek rutinszerűen.

Köszönöm Sass István és Németh István villamosmérnök kollégáknak -akik az orosz bioelektromágneses kutatások felé irányították a figyelmemet- hogy rámutattak arra, amit a mai nyugati tudományon felnőtt „Medline társadalom" kezd felfedezni és műszaki fejlesztés alá vonni, az már több esetben, keleten évtizedek óta rutin használatban van.

Köszönettel tartozom Prof. Dr. Yuan Linnek és Dr. Bai Yunak a Dél-Kínai Orvosi Egyetem Fasciology kutatócsoprt vezetőinek, hogy a 2009-es Amsterdami Fascia Kongresszuson bemutatott munkám miatt meghívtak kutatócsoportjukba és lehetővé tették a közös kérdésfelvetéseink akadémiai szintű kidolgozásának első legfontosabb lépéseit.

Feleségemnek, Pálhalminé Mező Annának külön köszönöm, hogy mint fordító és szervező szakember támogatta az elmúlt évek munkáját és szervesen részt vett a publikációk végleges formájának kialakításában.

Szüleimnek külön szeretném megköszönni, hogy biztos háttértámogatásukkal közvetlenül és közvetetten is segítették ennek a munkának a létrejöttét.

Végezetül köszönettel tartozom az East-West Biomedicne Kft. támogatói körének, hogy a kutatások és a szervezőmunka pénzügyi oldalát megteremtették.

Conference Summary 会议纪要

Integrative approaches in the research of fascial network for a better understanding of traditional Chinese medicine mechanisms
— Summary of the Fascia Congress 2009

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人体筋膜网络的综合研究能更好地了解中医治疗机制
——2009 年筋膜大会会议纪要

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The second Fascia Congress was an interdisciplinary event, organized for promoting the connections between the societies of researchers and clinicians working in the field of alternative medicine and traditional Oriental medicine. The organizers and participants introduced several aspects of the connective tissue matrix or so called fascial network of the human and mammalian body related to integrative biomedical sciences. During the five days long schedule, high-quality presentations from the cellular and molecular levels to the clinical applications were performed to integrate the views and models of modern and traditional clinical and biomedical sciences. It was also a great opportunity to study each others' healing methods at the workshops and to build up research collaborations. The program took place in Amsterdam at Vrije University, which has a main auditorium capable of seating 900 people and at numerous smaller rooms for concurrent sessions.

During the last decades, the important function of the fascia has been neglected by anatomists and surgeons. Their viewpoints on fascia were limited by the traditional dissection process, in which connective tissues are removed to display underlying tissues. Connective tissues are usually named based on the surrounding structures, suggesting minor role for this tissue. From the structural viewpoint, connective tissues have two distinct functions: to separate or allow gliding, or to connect and transfer forces.

The scope of definition and interest in fascia at this time was extended to all fibrous connective tissues, including aponeuroses, ligaments, tendons, retinaculae, joint capsules, organ and vessel tunics, the epineurium, the meninges, the periostea, and all the endomysial and intermuscular fibers of the myofasciae. This broad definition offers several important advantages. Rather than drawing most often arbitrary demarcation lines between joint capsules and their intimately involved ligaments and tendons (as well as interconnected aponeuroses, retinacula, and intramuscular fasciae), fascial tissue is seen as one interconnected tensional network that adapts its fiber arrangement, length, density and viscoelastic properties according to local tensional demands. This terminology fits the Latin root of the term "fascia" (bundle, bandage, strap, unification and binding together) and is also synonymous to the well known term: connective tissue.

Fascia or connective tissue research has become a hot point in the last ten years because of its role in mediation of the effects of acupuncture and because of its high content of multipotent, undifferentiated cell types. The reactive properties of fascia during acupuncture needling and other mechanical stimulation methods were published and presented by Helene M. Langevin during the congress. The work of Professor Yuan Lin fundamentally integrated the Western and Oriental approach in meridianology research and put forward a new research area, called fasciaology.

The detailed description and systematic research of this body-wide network started in the last 10 years. Modern Western scientific results have lead to the point which is also known from modern traditional Chinese medicine (TCM) models that fascial network has to be considered an interconnected organ, since it has its own function and regulatory processes.

Oral presentations covered the following topics: histology, anatomy and biomechanics of fascia, surgical considerations in plastic and musculoskeletal interventions, clinical applications and different methods in alternative medicine and acupuncture.

Poster presentations mostly introduced treatment methods and several working hypotheses in clinical and basic researches based on the interconnected continuity of fascial network.

The most prominent impact of the congress is having proved the role of fascial planes and networks in physiological and pathological biomechanics. High-quality scientific and tutorial presentations provided evidence that fascia plays a fundamental role in the mediation of force transmission between the muscle-tendon units. Authors presented several mathematical models and also clinical and basic science studies concluding that the "musculoskeletal system" name is a preconception in itself. Movement and biomechanical disorders can be basically understood only by integration of the mechanical, biophysical and physiological properties of the fascial system. Promising new approaches were presented to investigate the continuity of fascial force transmission lines and the viscoelastic properties of the connective tissue. On the basis of these above mentioned models even the macroscopic anatomical and physiological and some of the clinical hypotheses can be reconsidered in terms of fascial interplay in biomechanics.

Viscoelastic and proprioceptive properties of fascia were also presented in system level biomechanical movement regulation processes, including implications for different clinical treatment methods, like musculofascial and connective tissue stimulation therapies.

Despite the fully detailed and high-quality biomechanical studies, less was spoken about the role of fascial continuity in the regulation of interstitial fluid circulation and reactive or regenerative ability of connective tissue cell types under alternative or traditional medical stimulation circumstances.

Unfortunately the electrophysiological properties and the regenerative or remodeling potential of connective tissue system were absolutely lacking from the major congress topics, but were discussed during poster sessions.

Connective tissue has an extremely strong regenerative and tissue remodeling potential. It is well known not only from the principles of developmental biology and molecular histology but also from the modern models of traditional Oriental medicine. Fascial network is host for several multipotent and undifferentiated cell types in the adult mammalian organism. Acupuncture is the most sophisticated and oldest method for stimulating the connective tissue as an interconnected system. For example, mesenchymal stem cells can be activated by mechanical and electromagnetic stimulation too. According to the modern functional biological models of TCM, the connective tissue matrix is storing the regenerative potential of the whole body. This regenerative or remodeling ability of fascia unfortunately was not highlighted during the main frame of the congress, but was referred to in poster and parallel session presentations.

The approach of fasciaology was introduced by Professor Yuan Lin on the congress. According to his viewpoint, the fascial network is the basis of the TCM network of acupoints and meridians. The histological structure where an acupuncture needle acts upon is fascial connective tissues containing nerve endings, capillary vessels, fibroblasts, undifferentiated mesenchymal cells, lymphocytes, etc. This connective tissue matrix can be considered as a supporting storing system containing several types of multipotent cells, which play a fundamental role in tissue regeneration and remodeling and can be the biological basis of growth control system.

It is interesting to note that most of the Western scientists and even clinicians do not pay precise attention to every interconnected component of the acupuncture channels, so called meridian system. They apply the concept of the surface pathway of the main channels, because the acupoints are located there, but do not really deal with the following phenomena: internal pathways of the main channels, the channel divergences, the connecting ("luo") channels, the sinews represented by muscle-tendon units, the functional interconnection between acupuncture channels, and the overlapping between certain parts of channels.

These above mentioned systems almost fully overlap with the system of fascial lines and interconnections and functionally explain even biomechanical aspects of the fascial network. For example, force transmission line as a function is represented by the sinews (muscle-tendon meridians) and myofascial techniques are part of Chinese massage and manual therapy.

It became obvious that in order to address a relevant and conclusive question both in clinical and basic research of TCM or integrative biomedicine, it is essentially important to be fully aware of not only the different aspects of the fascial network but also the TCM meridianology and the meaning of its terminology. And it is even better if someone is able to successfully apply the system (for example acupuncture, acupressure) in healing, or at least to work in strong relation with successful people within healthcare. In this way, it is easy to avoid building up misleading experimental protocols for example in the area of placebo versus acupuncture research where many scientists try to compare oversimplified treatment protocols with sham treatments. In these cases the oversimplification of real acupuncture makes it useless in itself and obviously will generate similar result like sham treatment does.

Abstracts of the congress articles can be found on the congress website (http://www.fasciacongress.org/2009/abstracts.htm). The abstracts are divided into different categories and within each category into being oral or poster presentation.

Progress in Fascial Network Research

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Summary

The fascia framework is supposed to be an information transmission channel and is composed of variety of cells, fibres and extracellular matrix components. This system is responsible for the support and regeneration of the functional, differentiated cells of the body and for regulating the internal environment. The theory of fasciology sheds light on the mechanisms of the stimulative therapies, like acupuncture and Chinese massage, which deliver mechanical stimulation of the subcutaneous fascia and connective tissues to cause responses of specific functional cells and hence result in observable therapeutic effects. The newest findings based on the molecular and electrophysiological reactivity of the connective tissue system will be discussed in the article.

Keywords: fascia, connective tissue, acupuncture, meridians, cell signalling.

Fejlődés a kötőszövetes Mátrix kutatásban

Összefoglalás

A fascia hálózatról feltételezik, hogy az egy információ-átvivő rendszer, mely sejtek, rostok és extracellularis mátrix elemek változataiból tevődik össze és amely rendszer felelős a test differenciált funkcionális sejtjeinek támogatásáért és regenerációjáért, a belső környezet szabályozásáért. A „fasciológia" elmélet világít rá az olyan stimulatív terápiáknak mechanizmusaira, mint az akupunktúra és a kínai masszázs. Ezek mechanikus stimulációt fejtenek ki a szubkután fasciákra és kötőszövetre, hogy azok választ váltsanak ki a funkcionális sejtekben és ezáltal okozzanak érzékelhető terápiás hatásokat.
A közlemény a kötőszöveti rendszer molekuláris és elektrofiziológiai reaktivitásával kapcsolatos legújabb kutatási eredményeken alapszik.

Introduction

Fascia is the soft tissue component of the connective tissue system that permeates the human body forming a whole body continuous three-dimensional matrix of structural support. It interpenetrates and surrounds all organs, muscles, bones and nerve fibres, creating a unique microenvironment for body systems functioning (1, 2). Definition of fascia extends to all fibrous connective tissues, including aponeuroses, ligaments, tendons, retinacula, joint capsules, organ and vessel tunics, the epineurium, the meninges, the periostea, and all the endomysial and intermuscular fibres of the myofasciae. There is a substantial body of research on connective tissue generally focused on specialized genetic and molecular aspects of extracellular matrix. However, the study of fascia and its function as an organ of support has been largely neglected and overlooked for several decades. Since fascia serves both global, generalized functions and local, specialized functions, it is a substrate that crosses several scientific, medical, and therapeutic disciplines, both in conventional and complementary / alternative modalities (3).

Hypotheses which accord myofascia a central role in the mechanisms of therapies have been advanced for some time in the fields of acupuncture, massage, structural integration, chiropractic and osteopathy. Among the different kinds of tissues that are involved in musculoskeletal dynamics, fascia has received comparatively little scientific attention. Fascia, or dense fibrous connective tissue, nevertheless potentially plays a major and still poorly understood role in joint stability, in general movement coordination, as well as in back pain and many other pathologies. One reason why fascia has not received adequate scientific attention in the past decades is that this tissue is so pervasive and interconnected that it easily frustrates the common ambition of researchers to divide it into a discrete number of subunits which can be classified and separately described. In anatomic displays the fascia is generally removed, so the viewer can see the organ nerves and vessels but fails to appreciate the fascia which connects, and separates, these structures.

In particular there is increasing interest in certain therapeutic communities in the role that fascia plays in musculoskeletal strain disorders such as low-back instability and postural strain patterns of all types, fibromyalgia, pelvic pain, and respiratory dysfunction, chronic stress injuries, as well as in wound healing, trauma recovery and repair. Recent findings that advance knowledge of biomechanical and adaptive properties of fascia may account for clinical observations in health and dysfunction.

Potential influence of fasciology on modern medicine and TCM

Traditional Chinese Medicine (TCM), with a history of thousands of years, has accumulated a wealth of experience during the struggle against diseases, and has made tremendous contributions to the survival of the Chinese nation. It formed a set of independent theoretical system based on the understanding of diseases of that time. In the wake of the research of life origin and biological evolution in modern biology, this empirically based theoretical system began to face challenges by the Western medicine. However, even with the rapid development of the modern medical science, quite a number of diseases still remain clinically difficult without effective treatments; indeed, the pathogenesis of some diseases cannot find sound explanations by modern biological theories. Some of these diseases, interestingly, can be treated with TCM therapy with an unexpected effect. This is why TCM is so popular and well recognized by patients even nowadays. TCM seems to have gradually won worldwide acceptance, as more and more national legislations of developed countries have recognized the status of acupuncture in the health care system. But currently TCM is classified into Complementary and Alternative Medicine (CAM); in other words, it is not the mainstream medicine. Although modern biology research has evolved into a molecular era, some of the mysteries of the human body still wait to be uncovered. For TCM, the integration of modern biomedical science seems the only option for its development in the context of the prevailing influence of modern medical science. For this purpose, a research platform integrating both TCM and Western medicine is essential, based on the theories accepted by each other.

At present, the worldwide study of TCM theory focuses primarily on the meridians in the hope of finding a breakthrough in the identification of the material basis of the TCM theory. Great efforts have been made in such studies, and different hypotheses have been put forward, but currently no consensus has been reached over the physically tangible structures of the meridians and acupoints (4-11). While using Virtual Human Technique to mark the areas rich in connective tissue and construct a three-dimensional model of their distribution, a close

association between the traditional descriptions of the meridians and acupuncture points and the distribution of the connective tissue in the reconstructed model can be found. Such points were located mainly in the area with rich connective tissues, such as the muscular septum of the limbs, somatic motor nerve endings, internal organs with rich sensory nerve distribution, and the septum of organs. By lowering the marking threshold, more connective tissues were displayed and a complete framework distributing all over the body was presented. This finding was verified in cadavers and found that the connective tissue framework was indeed associated with the meridians (7, 12). After a comprehensive analysis of our finding and the documented descriptions in the literature concerning the meridians and acupoints, their diversity and potential clinical application, we propose the following views on the meridians and acupoints in TCM:

1. The basis of the meridian is the connective tissue network of the body. Manipulation (as insertion and rotation) of the connective tissue-rich area can generate strong biological information, such as sensory information, stretching and injury stimuli of the local tissue. Acupoints and non-points of the whole body can generate different amount of biological information, but all of a common qualitative nature. In other words, the acupoints are actually located everywhere of the body.

2. There is an anatomical relevance between the acupoints and disease sites at different anatomical levels, such as the local structures, spinal cord segments, nerve pathways and central nerve distributions.

3. The mechanism of acupuncture involve the generation of a biological effect in response to the mechanical stimulation of the connective tissue, which can regulate the body functions (the activity of tissue cells) and life activities (the repair and regeneration of tissue cells).

Two-system theory

The connective tissue framework was derived from the residual mesoderm mesenchyme after its differentiation into multiple organs and systems in earlier developmental stages. The extracellular matrix of an organism with a single germ layer, the mesogloea of a diploblastic organism, and the mesenchyme of a triploblastic organism, and the non-specific connective tissue in human were all homologous structures (11,13). Human non-specific connective tissue framework provides a scaffold for supporting the functional cells, and also serves as an undifferentiated cell reservoir and maintains the internal environment for the repair and regeneration of the functional cells. In the sense of the dynamic metabolic balance of an organism, we propose a new anatomical approach that views the human body as having two major systems, supporting-storing system and functional system which is the essence of the two system theory. The supporting-storing system consists of undifferentiated non-specific connective tissues, and the functional system is composed of various differentiated functional cells. Based on this theory, we further propose a new research field, fasciology, which studies the correlations and interactions between the two systems (12).

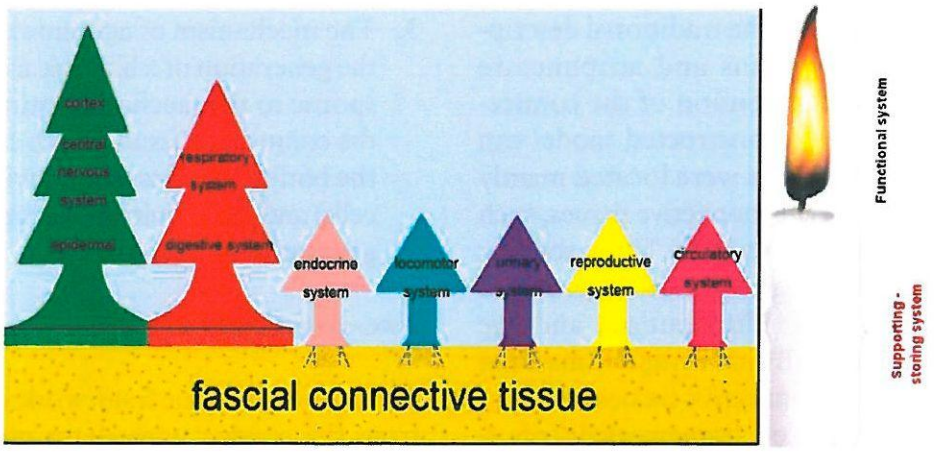

Fig. 1.
The two-system model of human structure.

From the perspective of fasciology, these features of TCM and Western medicine can be conveniently summarized: TCM emphasizes on the supporting-storing system, while Western medicine on the functional system. (Fig. 1.) The study of fasciology not only theoretically supports the biological research of TCM, but also sheds light on a new approach to the research in modern biomedical sciences. By fasciology, the mechanisms of various traditional therapies can be better explained, and better insights can be obtained into some difficult and complicated diseases. Consequently, fasciology serves as a bridge between TCM and Western medicine by combining the wisdom of the ancient Chinese and the achievements in modern science, thus promoting the development of the healthcare service (14-16).

Continuity and electrophysiology of the Connective tissue matrix

An extensive, global physiological role for connective tissue was suggested over 2000 years ago by the ancient traditional Chinese medicine. Traditional acupuncture is based on the phenomena that a network of meridians exist within the "fat greasy membranes" extending throughout the body and that this network functionally connects all parts of the body with one another. Recent evidences suggest that a correspondence may exist between the traditional meridians and the body-wide network of connective tissue matrix (16).

Connective tissue may transmit electrical, cellular, and tissue remodelling signals throughout the body, each in response to mechanical forces but on different time scales. Many tissues, including collagen, display immediate local electrical gradients in response to mechanical stress. Mechanical contacts between fibroblast cells are actively altered within minutes. Finally, tissue remodelling has been shown in tendons, ligaments and joint capsules, and if this process is also present in loose connective tissue it would provide a body wide pattern to remodel connective tissue based on movement and local tissue stress. Interactions among these three systems could provide both short term and long term responses.

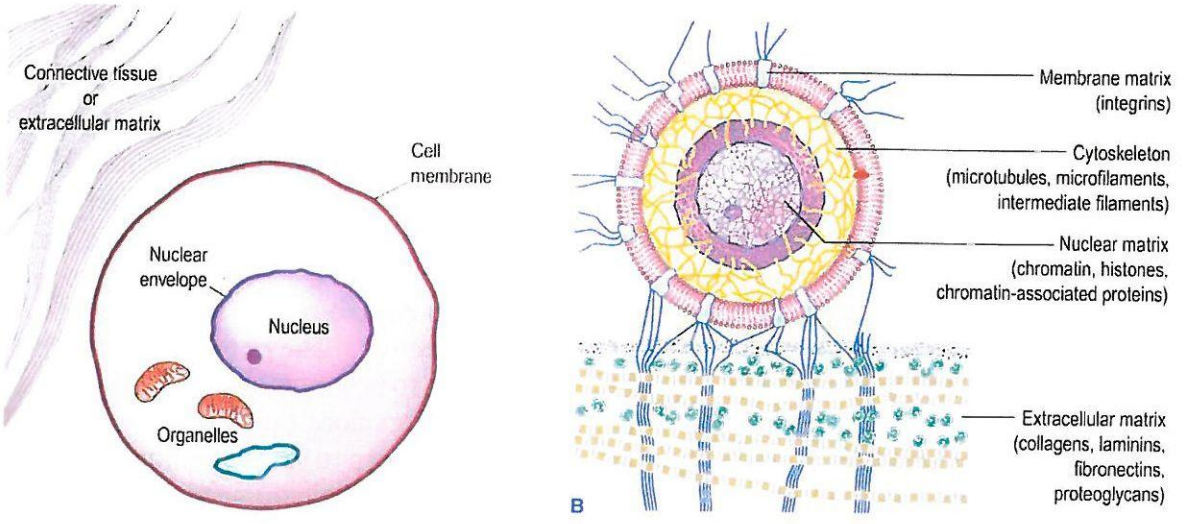

Fig. 2.
Two views of the relationship between the cell and the surrounding extracellular matrix (ECM). The traditional view is on the left, in which each element has its autonomy. On the right, a more current view, in which the nuclear matrix, the cytoskeleton and the ECM are all mechanically and functionally linked via the membrane integrated and associated protein complexes (Ref. 17).

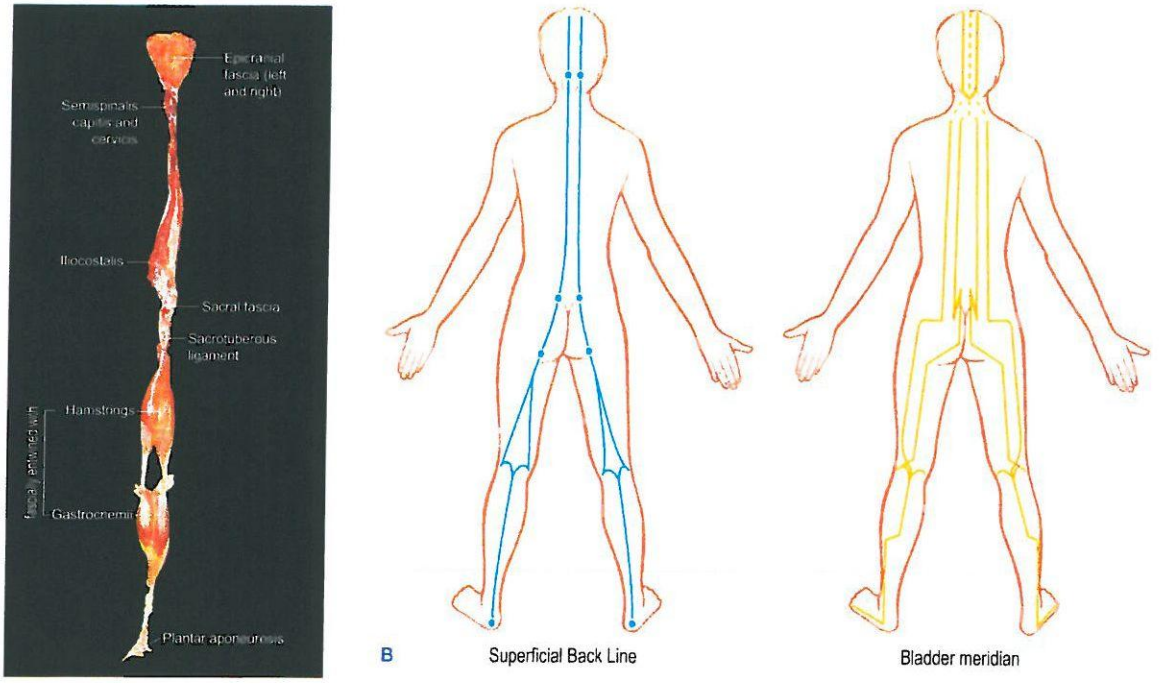

Fig. 3.
Myofascial meridian, called superficial back line dissected intact from a cadaver. The anatomical continuity between the different body regions is demonstrated. Schematic drawings show the overlapping between the traditional ("bladder") and the myofascial meridian (Ref. 17).

The idea that electronic mobility and charge transfer across biological polymeric molecules may be a fundamental mechanism in living organisms was first proposed by Szent-Gyorgyi in 1941 (18). During the next decades, some evidence was accumulated showing that a number of proteins including collagen can display semi conductive, piezoelectric and photoconductive properties in vitro (19). Whether these types of electronic phenomena occur, and have biological significance, in vivo, however, remains unknown. One of the main obstacles to studying solid state physical properties of proteins in tissues is the necessity to test these properties in a wet, ionically-filled environment. Ionic charge separation occurring locally in response to mechanical stresses (e.g. stretch, compression) is well established in specialized connective tissues and can be measured as stress-induced potentials (or "streaming potentials") (20, 21). Ionically derived potentials are known to have important local downstream effects on extracellular matrix biosynthesis (22) but usually decay over short distances. Electronic currents, on the other hand, could potentially flow over larger distances, but would require either a gradient of one type of charge carrier (leading to diffusion current) or a sustained potential difference across some region (leading to drift current). If such electronic currents do occur within connective tissue, the tissue's electrical conductance would be expected to be affected by various external influences (e.g. mechanical stress, illumination, heating).

If such an electro conductive behaviour of the connective tissue network exists, then there have to be inhomogenities in biophysical parameters along the meridian line related areas comparing to the surrounding regions. To explore this phenomenon we put forward a new approach for combining the structural and functional (electrophysiological) methods in the investigation of human fascial network.

The Conductance Density Mapping Method© was developed to carry out meridian screening in healthy and in diseased human subjects. The electrodynamic responsiveness of the sub dermal connective tissue in the meridian line and in the non-meridian line was analysed comparatively, namely, the peak of electrical conductance and resistance response and the dynamics of the response attenuation (also known as the decay time).

Region	Peak Conductance Voll value	Peak Resistance kΩ	Half Peak Decay Time (sec)
Meridian	37.8±11.7	255±66	3.2±1.5
Non Meridian	19.8±3.7	497.6±113.2	1.8±0.8

Table 1.
Electric conductance profile in the meridian and non-meridian regions recorded from 7 healthy volunteers (Mean±SE)

We demonstrated that the meridian- and non-meridian-related subdermal connective tissues had significantly different electrophysiological properties. (Table 1.) Similar results were documented in earlier reports (23, 24) but comparative and clearly reproducible results on the decay time property of responses have not been reported. Analysis of the decay or attenuation properties of the connective tissue resistance responses reveals a fundamental difference in the tissue electrodynamics between the fascial network that builds up the acupuncture meridians and the non-meridian areas. This aspect of fascial physiology can be a new approach to meridianology research and also to the research of fascial responsiveness properties. (Fig. 4.)

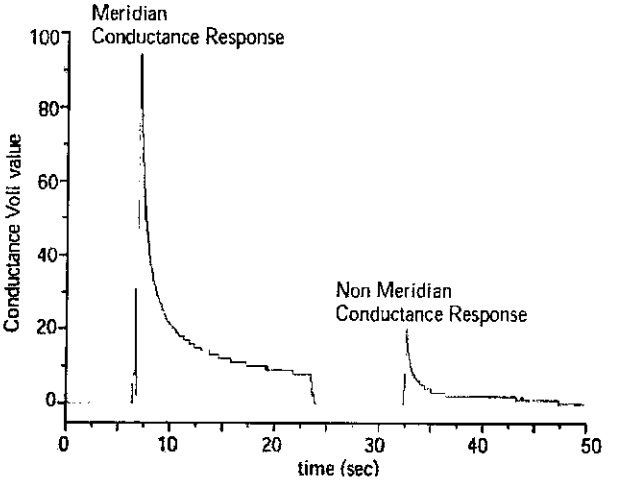

Fig. 4.
Typical DC conductance response of Sanjiao meridian compared to the nearby non-meridian connective tissue area. The peak of the conductance response is significantly higher at the Sanjiao meridian compared to the non-meridian area. (Ref. 42)

tThe higher peak amplitudes and longer decay time of meridian recordings suggest that the functional responsiveness of sub dermal connective tissue is more prominent in the meridians than in the non-meridian regions. (Fig. 5.)

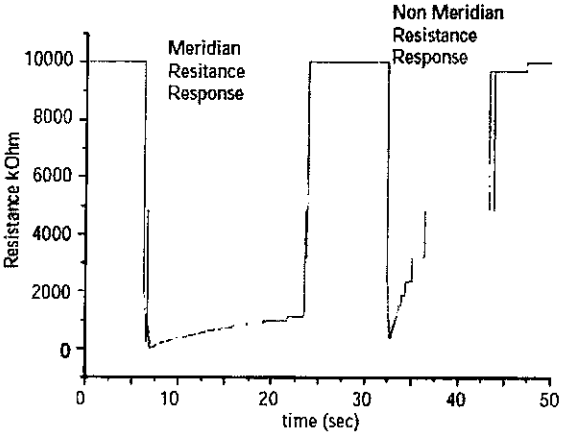

Fig. 5.
Typical DC resistance response and decay time characteristic of the Sanjiao meridian, compared to the nearby non-meridian connective tissue area. The peak amplitude and the decay time are significantly different between the Sanjiao meridian and the non-meridian area. (Ref. 42)

Mechanical stress and fascial tonus regulation

Mechanical stress is an important extracellular stimulus to maintain the cells survival and growth, and can regulate the cell metabolism and gene expression profiles. Studies have shown that regular mechanical stretch inhibits the expression of MyoD and MNF in the myoblasts (25). Stretching stimulation at a certain frequency could promote the proliferation of C2C12 type myoblast cells and these mechanical signals were thought to generate alterations in some signalling molecules, such as FAK, Rac-1, GTPase, and NF-?B (25-27). Fibroblasts are the major cells responsible for damage repair during wound healing, and they can differentiate into myofibroblasts, quite similar to smooth muscle cells with also a contractile function.

Myofibroblasts are connective tissue cells which contain dense stress fibre bundles that are mostly composed of alpha smooth muscle actin. First discovered by Majno and Gabbiani in the early 1970's, they have been shown to play a major role in wound healing and to be also involved in many other normal as well as pathological contractile tissue processes. Most of these cells develop out of normal fibroblasts stimulated by the influence of mechanical tension as well as specific cytokines. (Fig. 6.) Their smooth muscle-like contractility enables these cells to maintain a contractile force over long duration times with little energetic costs. An increased presence of myofibroblasts is a driving factor behind chronic fascial contractures, such as in plantar fibromatosis, in excessive scar formation or in frozen shoulder. Recently, the presence of myofibroblasts (or myofibroblast-like contractile cells) has also been demonstrated for normal dense connective tissues such as joint ligaments, menisci, tendons, organ capsules and others.

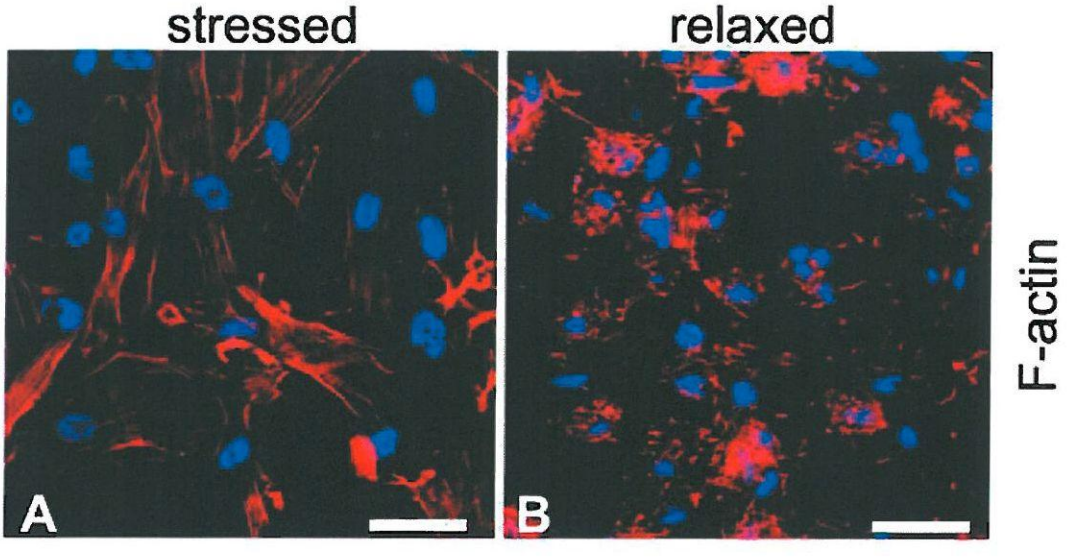

Fig. 6.
Immunocytochemical staining of fibroblast stress fibres composed of smooth muscle actin, after experimental mechanical stimulation (Ref. 28).

The regulation of cell proliferation and gene expression by mechanical tension may be an important factor that affects the outcome of myofibroblast, although the mechanism of the mechanical signals and the information transmission channels remain unknown (29).

Myofibroblasts are proposed to be present in normal fascia and increase their number after mechanical stimulation. The contractive behaviour of musculoskeletal connective tissue plays a role in mediating the force transmission between different body regions and can be modified by different complementary and alternative medical modalities. Force transmission lines, so called "myofascial meridians" are regulated by the contractile properties of myofibroblast. This mechanism can be in the background of different stimulative therapies like acupuncture, massage therapy, movement and mobilization therapies.

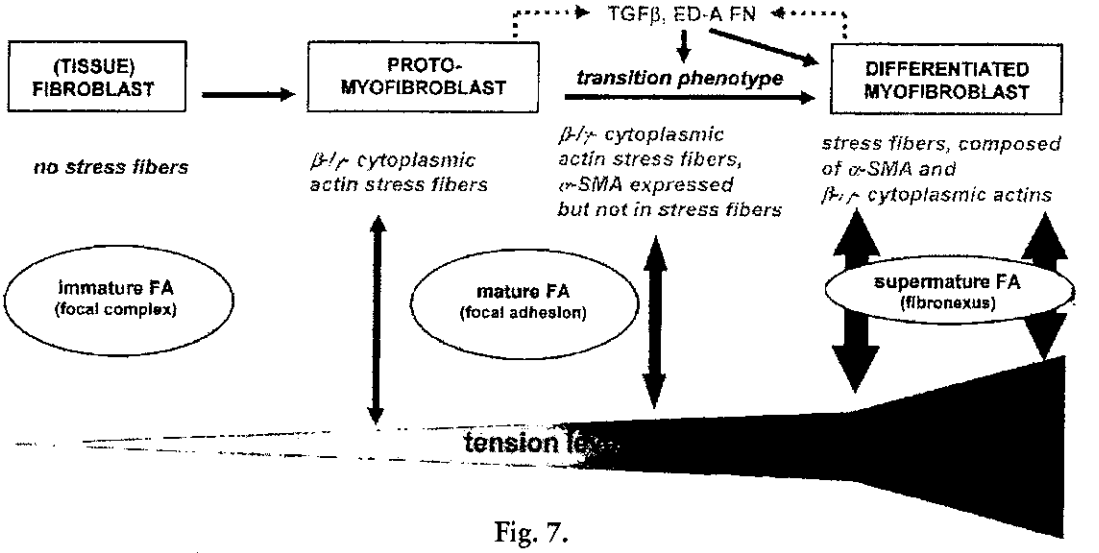

Fig. 7.
The feedback relations between matrix stiffness, adhesion, maturation and myofibroboast differentiation.
In most normal tissues and on compliant culture substrates, fibroblasts exhibit only immature if any cell-matrix contacts and do not develop stress fibres. However, these small complexes are sufficiently strong to promote initial matrix remodelling and tension development. Increasing matrix tension lead to the formation of the proto-myofibroblast, characterized by mature adhesions and contractile stress fibres that are composed of cytoplasmic actins. The contractile force exerted by stress fibres further augments tension in the matrix, promoting gradual supermaturation of focal adhesion molecules. The concerted action of tension, TGFB and fibronectin induces de novo expression of smooth muscle actin, which organizes transitorily in the cytoplasm. (Ref. 41)

Molecular reactivity of the subcutaneous fascia for mechanical and electrical stimulation

Extracellularly regulated kinases (ERK) play a major role in transferring both mechanical and biophysical signals between the extracellular matrix and the intracellular microcompartment (30). Electroacupuncture can promote ERK signal transduction in rat subcutaneous fascia, ERK1/2 and p38 mitogen-activated protein kinase (p38 MAPK) are also activated in the fibroblasts during collagen matrix contraction under isometric tension (30). Activation of ERK1/2 is indispensable in cell proliferation and survival

(31-33). It has been reported that mechanical stimulation results in the activation of ERK 1/2 and p38 MAPK in various cells, including osteoblasts (34-36). The members of the MAPK family, including ERK1/2, N-terminal Jun kinase (JNK1/2) and p38 MAPK, have been proposed as the fundamental signalling components linking extracellular stimuli to intracellular responses. ERK family proteins play a key role in transforming the mechanical stretch signal to the level of ligand-activated transcription factors in osteocytes and osteoblasts (37, 38). As a mechanical therapy, acupuncture produces shear stress or mechanical stretch at the cellular level in the fascia and muscle tissues, suggesting the possible roles of ERK1/2, p-ERK1/2, p38 and p-p38 in the effect of acupuncture on the fascia and muscle. It has been previously found that the activity of ERK1/ 2, JNK and P38 MAPK increased following low shear stress treatment; inhibition of ERK1/2, JNK1/2 and p38 MAPK with PD98059, SP600125 and SB203580, respectively, led to the suppression of the shear stress-induced IL-8 gene expression (39). It is interesting to notice that in our experiments, directed ion movement evoked by electric acupuncture caused more intensive ERK signal elevation than simple mechanic acupuncture (40). (Fig. 8.)

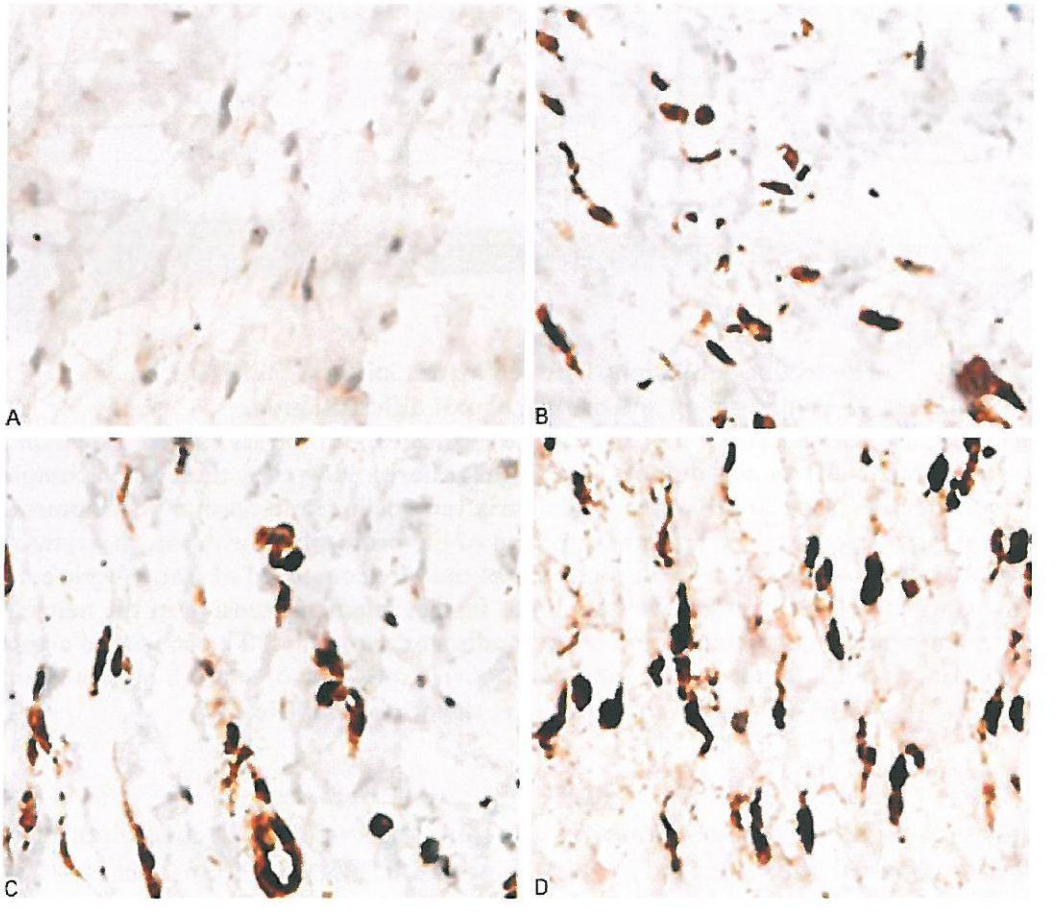

Fig. 8.
Immunohistochemical staining of ERK1/2 in the fascia in different groups (×400). A: Control group; B: Manual acupuncture at ST36 group; C: Electro-acupuncture at ST36 group; D: Manual acupuncture at non-acupoint. The number of positive cells increased after acupuncture in B, C and D groups (arrowhead) (Ref. 40).

Langevin and his colleagues suggested (7) that fibroblasts of the connective tissue form an extensive interconnected cellular network, and communicate with each other through close apposition. It is reasonable to hypothesize that the effect of fascia on the immunity, blood vessels and internal organs is different from that of the nervous system. Furthermore, MAPK cell signalling pathway is present in almost all the eukaryotic cells, and serves as the primal intercellular communication pathway. We hypothesize that acupuncture affects the cellular morphology of the fascia connective tissue to cause alterations in the expressions of MAPKs signal proteins such as ERK1/2 and P38, hence affecting the biochemical profiles and finally the functional status of the organism. We found that acupuncture can promote the expression of ERK1/2 and P38, which suggests a new perspective in the understanding how mechanical acupuncture signals in the fascia are transferred. Understanding of the regulatory role of fascia connective tissue in the physiologic and pathologic states of the body and the mechanism of signal transduction of MAPK from a biological viewpoint can be of vital importance. Questions, including how the changes of the signal proteins are conducted in the fascia connective tissue, and whether this paracrine communication within the fascia can spread, remain to be answered.

Fibroblast secretes matrix metalloproteinases and tissue specific matalloproteinase inhibitors (MMP and TIMP), which play an important role in reconstruction and matrix remodelling and basement membrane permeability. TIMP is directly related to the malignant cell phenotype, tumour cell metastasis and collagen fibre content within the tissue, and participates in the injury repair process. The undifferentiated mesenchymal cells in the connective cells possess the ability of multipotent differentiation and transdifferentiation. It is indicated that increased local number of mesenchymal stem cells, or their transdifferentiation, promotes tissue damage repair. Studies using gene transfer technique have shown the possibility of reversing the fibroblasts to the original state of embryonic stem cells which possesses multipotent differentiation ability across all the three germ layers (43-45).

Connective tissue specific transforming growth factor (CTGF) plays an important role in the various pathophysiological processes such as matrix formation and degradation, organ fibrosis, tumorgenesis, and distant metastasis of cancer cells (45). The stimulation by tensile stress (twisting and insertion of the needle) causes lowered expression of CTGF (46). The experimental tensile stimulations are very similar to the traditional Chinese acupuncture stimulation, in which the needle inserted in the connective tissue interacts and tightly intertwines with the collagen fibres by electrostatic force, and twisting the needle causes increased fibre tension, which is translated into intracellular signals to cause cytoskeletal remodelling induced by activation of the Rho pathway and alterations in the target gene transcription (47-49). The effect of stretch stimulation on CTGF expression shows a correlation to the stimulation frequency, amplitude and time, and this effect was also affected by such cytokines as IL-6 and PG (50). The CTGF mRNA encodes a distinct mitogen with immunogenic and chemotactic capacities, which can induce fibroblast proliferation and secretion of extracellular matrix to regulate cell proliferation, differentiation, embryonic development and wound healing – this might be the biological significance of fascia. TGF is constituted by two subunits, α and β. Three isoforms of TGF have been identified in mammals, namely TGF-β1, TGF-β2, and TGF-β3. TGF plays important roles in the outcomes of many diseases. It was reported that mechanical stress can also regulate its transcription (51).

Tensile stimulation down-regulated the expression of TGF-β1, similar to the results of other researches (52). Many types of cells

can synthesize TGF. Four groups of the main downstream effectors of TGF have been found, namely the cell cycle-related factors (including cyclins, CDKs, and CDIs), transcription factors (c-myc, RB, c-fos, c-jun, myb, and E2F), apoptosis-related factors (Bcl-2 and Bax), and mesenchymal elements. The transcription level of TGF-β1 also plays an important role in the modulation of the immune function, cell growth and differentiation, extracellular matrix synthesis and storage, embryonic development, and wound healing (53). From another prospective, the alterations of TGF-β1 mRNA expression in the connective tissue in response to mechanical stimulation demonstrate the important biological functions of fascial tissues that may related to tissue remodelling, regeneration and self-renewal.

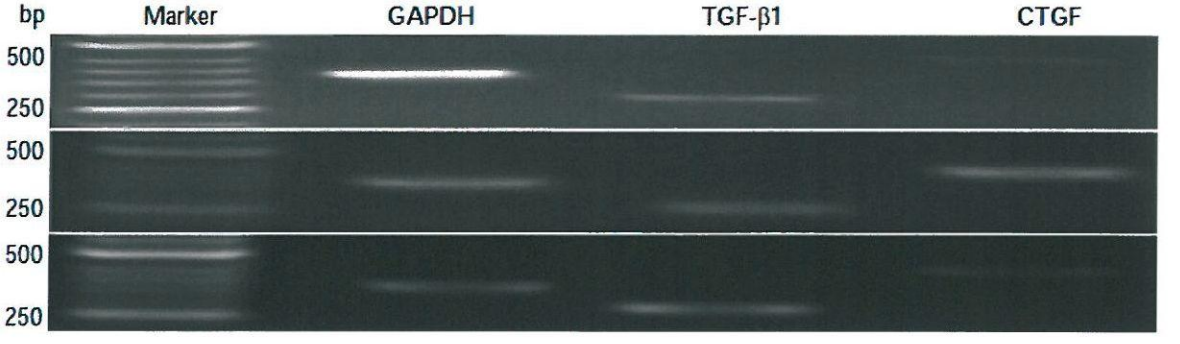

Fig. 9.
Loose connective tissue is involved in the response to tensile load, which causes changes in the cell proliferation and CTGF and TGF-β1 gene transcription. CTGF, TGF-β and GAPDH mRNA expression in different groups. From top to bottom: twisting group, insertion group, and blank control group. The size of the PCR products of CTGF, TGF-β and GAPDH are 499 bp, 329 bp, and 426 bp, respectively. (Ref. 52)

Summary

The above findings led many researchers to the following facts, those generated a working hypotheses which we call microtrauma model of the stimulative therapies.
Different modalities of stimulations used in Chinese and oriental or natural medicine - so called microtraumatic stimulation of the subcutaneous connective tissue- evokes the following local reactions:

→ Local neuroimmunomodulatory reaction: neuropeptiderg modulation of connective tissue microenvironemnt via C-fiber efferent activity is pivotal in the initiation of changes of the communication between fibroblasts, T-helper cells, macrophags, adipocytes and free nerve endings
→ tissue remodelling and proliferation of multipotent connective tissue cells
→ changes of electrophysiological, biomechanical, molecular properties of fascial meridians
→ cell migration through the myofascial meridians(?)
→ epitehlial-mesenchimal transition initiated by a substance P mediated mechanism
→ better connective tissue circulation and activation of regenerative cell potential
→ enhancement of collagen synthesis and MMP (matrix metalloprotease) activation
→ ERK activation and increase of local, paracrine hormone synthesis (IGF-1, IGF-2, TGFbeta1, TNFalpha, TNF beta, FGF-1, FGF-2).

The investigation of the above micro tissue remodelling process thought to be a key to the understanding and integrating of the natural stimulative therapies and some aspects of the oriental medical interventions.

Fasciology is a new method for putting forward the appropriate integrative research questions and for integrating the education of the traditional/natural oriental medicine on the basis of modern biomedical aspects.

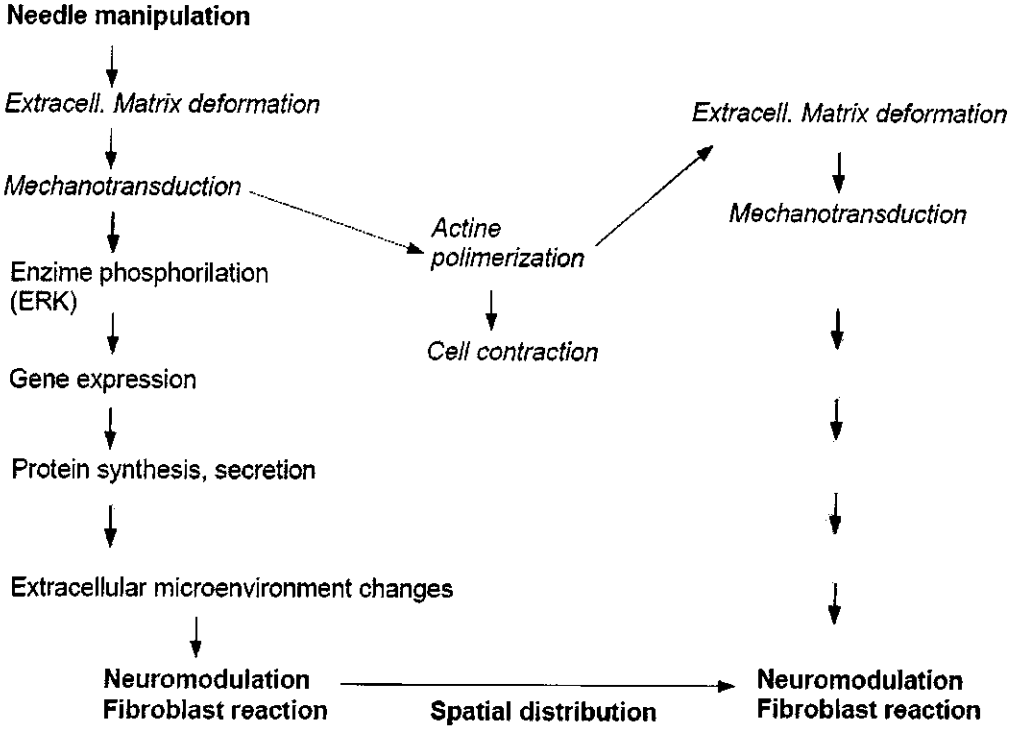

Fig. 10.
Mechano- or electric stimulation evoked changes of the molecular microenvironment spreads away from the locus by the contribution of extracellular matrix mediated mechanotransduction. (Ref. 54)

References

1. Fei L, Cheng HS, Cai DH, et al. The meridian material basis and functional characteristics of the experimental exploration and research prospect Chin Sci Bull, **1998**, 43 (06): 658-72.
2. Langevin HM, Yandow JA. Relationship of acupuncture points and meridians to connective tissue planes. Anat Rec, **2002**, 269(6): 257-65.
3. Palhalmi J, Bai Y, Yuan L. Integrative approaches in the research of fascial network for a better understanding of traditional Chinese medicine mechanisms. Journal of Chinese Integrative Medicine (Zhong Xi Yi Jie He Xue Bao) February **2010**, Vol.8, No.2.
4. Dang RS, Chen EY, Shen XY, et al. The relationship between Lung meridian acupoints and connective tissue structure Shanghai Acupunct, **1997**, 16 (04): 28-9.
5. Ding GH, Yang J, Chen EY, et al. Human tissue fluid directional flow and meridian. Prog Nat Sci, **2001**, 8: 29-36.
6. Langevin HM, Churchill DL, Wu J, et al. Evidence of connective tissue involvement in acupuncture. FASEB J, **2002**, 16(8): 872-4.
7. Langevin HM, Cornbrooks CJ, Taatjes DJ. Fibroblasts form a body-wide cellular network. Histochem Cell Biol, **2004**, 122(1): 7-15.

8. Langevin HM, Rizzo DM, Fox JR, et al. Dynamic morphometric characterization of local connective tissue network structure in humans using ultrasound. BMC Syst Biol, 2007, 1: 25.
9. Konofagou EE, Langevin HM. Using ultrasound to understand acupuncture. Acupuncture needle manipulation and its effect on connective tissue. IEEE Eng Med Biol Mag, 2005, 24(2): 41-6.
10. Langevin HM. Connective tissue: a body-wide signaling network? Med Hypotheses, 2006, 66(6): 1074-7.
11. Wang J, Dong WR, Wang CL, et al. From meridians and acupoints to self-supervision and control system: a hypothesis of the 10th functional system based on anatomical studies of digitized virtual human. South Med Univ, 2007, 27(5): 573-9.
12. Bai Y, Yuan L, Soh KS, Lee BC, Huang Y, Wang CL, Wang J, Wu JP, Dai JX, Palhalmi J, Sha O, Yew DT. Possible applications for fascial anatomy and fasciaology in traditional Chinese medicine. Journal of Acupuncture and Meridian Studies. 2010 Jun; 3(2):125-32.
13. Yuan Lin The theory of fasciology. Fasciology Journal, Guangzhou University. Vol.1. July 30, 2011.
14. Langevin HM, Churchill DL, Cipolla MJ. Mechanical signaling through connective tissue: a mechanism for the therapeutic effect of acupuncture. Faseb J, 2001, 15(12): 2275-82.
15. Wang J, Wang CL, Shen BL, et al. Explanation of essence and substance basis of channels and collaterals with fasciology. Zhongguo Zhen Jiu, 2007, 27(8): 583-5.
16. Huang Y, Yuan L, He ZQ, et al. Study on the meridians and acupoints based on fasciaology: an elicitation of the study on digital human being. Zhongguo Zhen Jiu, 2006, 26 (11): 785-8.
17. H. M. Langevin Connective tissue: A body-wide signaling network? Fascia Congress 2009, Amsterdam. Pre reviewed Presentation.
18. T. W. Myers Anatomy trains of myofascial meridians. Elsevier Limited. 2009.
19. A. Szent-Györgyi The study of energy-levels in biochemistry. Nature 148 (1941) pp. 157-159
20. F. W. Cope. A review of the applications of solid state physics concepts to biological systems. Jornal of Biol Phys. 3 (1975) pp. 1-41.
21. C.T. Chen, R.P. McCabe, A.J. Grodzinsky, R. Vanderby Transient and cyclic responses of strain-generated potential in rabbit patellar tendon are frequency and pH dependent. Journal of Biomech. Engineering. 122 (2000) (5) pp. 465-470
22. W.M.Lai, V.C.Mow, D.D. Sun, G.A. Ateshian On the electric potentials inside a charged soft hydrated biological tissue: streaming potentials versus diffusion potential. Journal of Biomech. Engineering. 122 (2000) (4) pp. 336-346
23. Y.J. Kim, L.J. Bonassar, A.J. Grodzinsky The role of cartilage streaming potential, fluid flow and pressure in the stimulation of chondrocyte biosynthesis during dynamic compression. J Biomech. 28 (1995) (9) pp. 1055-1066
24. Ahn AC, Wu J, Badger GJ, et al. Electrical impedance along connective tissue planes associated with acupuncture meridians. BMC Complement Altern Med, 2005, 5: 10.
25. Lee MS, Jeong SY, Lee YH, et al. Differences in electrical conduction properties between meridians and non-meridians. Am J Chin Med, 2005, 33(5): 723-8.
26. Kumar A, Murphy R, Robinson P, et al. Cyclic mechanical strain inhibits skeletal myogenesis through activation of focal adhesion kinase, Rac-1 GTPase, and NF-kappaB transcription factor. FASEB J, 2004, 18(13): 1524-35.
27. Qiu XZ, Li XN, Chen WY, et al. Effect of cycling stretch on the proliferation of C2 C12 myoblasts. Chin J Clin Anat, 2006, 24(2): 183-5.
28. Clark CB, McKnight NL, Frangos JA. Stretch activation of GTP-binding proteins in C2C12 myoblasts. Exp Cell Res, 2004, 292(2): 265-73.
29. Lin Yuan Acupuncture stimulation and the fascial tonus regulation. Fasciology Congress 2010, Guangzhou, China.
30. Rockwell WB, Cohen IK, Ehrlich HP. Keloids and hypertrophic scars: a comprehensive review. Plast Reconstr Surg, 1989, 84(5): 827-37.
31. Jiang XM, Yang C, Yuan L, et al. Expression of extracellular signal-regulated protein kinases in the subcutaneous fascia of rats and their changes after acupuncture. J South Med Univ, 2009, 29(4): 623-6.
32. Werry TD, Christopoulos A, Sexton PM. Mechanisms of ERK1/2 regulation by seven-transmembrane-domain receptors. Curr Pharm Des, 2006, 12(14): 1683-702.
33. Boulton TG, Nye SH, Robbins DJ, et al. ERKs: A family of protein-serine/threonine kinases that are activated and tyrosine phosphorylated in response to insulin and NGF. Cell, 1991, 65(4): 663-75.
34. Guyton KZ, Liu Y, Gorospe M, et al. Activation of mitogen-activated protein kinase by H2O2. Role in cell survival following oxidant injury. J Biol Chem, 1996, 271 (8): 4138-42.
35. Whelchel A, Evans J, Posada J. Inhibition of ERK activation attenuates endothelin-stimulated airway smooth muscle cell proliferation. Am J Respir Cell Mol Biol, 1997, 16(5): 589-96.
36. Inoue D, Kido S, Matsumoto T. Transcriptional induction of FosB/DeltaFosB gene by mechanical stress in osteoblasts. J Biol Chem, 2004, 279(48): 49795-49795803.

37. Kumar A, Knox AJ, Boriek AM. CCAAT/ enhancer-binding protein and activator protein-1 transcription factors regulate the expression of interleukin-8 through the mitogen- activated protein kinase pathways in response to mechanical stretch of human airway smooth muscle cells. J Biol Chem, 2003, 278(21): 18868-76.
38. Yuge L, Okubo A, Miyashita T, et al. Physical stress by magnetic force accelerates differentiation of human osteoblasts. Biochem Biophys Res Commun, 2003, 311(1): 32-8.
39. J. Ignacio Aguirre, Lilian I. Plotkin, Arancha R, et al. A novel ligand-independent function of the estrogen receptor is essential for osteocyte and osteoblast mechanotransduction. J Biol Chem, 2007, 282(35): 25501-8.
40. Cheng M, Wu J, Li Y, et al. Activation of MAPK participates in low shear stress-induced IL-8 gene expression in endothelial cells. Clin Biomech, 2008, 23 (Suppl 1): S96-S103.
41. JIANG Xue-mei, BAI Yu, Palhalmi Janos, YUAN Lin, HUNAG Yong, DAI Jing-xing, WANG Sheng-xu, David Tai Wai Yew: Modulatory effect of acupuncture on the extracellular signal-related kinase 1/2 (ERK1/2) and p38 mitogen-activated protein kinase (p38 MAPK) signaling pathways in the subcutaneous fascia. Fasciology Journal, Guangzhou University. Vol.1. July 30, 2011.
42. Boris Hinz: Masters and servants of the force: The role of matrix adhesions in myofibroblasts force perception and transmission. Fascia Congress 2009, Amsterdam. Pre reviewed Presentation.
43. Bai Yu, Janos Palhalmi, Huang Yong, Yang Chun, Lin Yuan: Research methods in fasciaology: implications for acupuncture meridianology. Fasciology Journal, Guangzhou University. Vol.1. July 30, 2011.
44. Li H, Han Y, Guo Q, et al. Cancer-expanded myeloid derived suppressor cells induce anergy of NK cells through membrane-bound TGF-beta 1. J Immunol, 2009, 182(1): 240-9.
45. Malays Mok PL, Cheong SK, Leong CF. In vitro differentiation study on isolated human mesenchymal stem cells. J Pathol, 2008, 30(1): 11-9.
46. Chu CY, Chang CC, Prakash E, et al. Connective tissue growth factor (CTGF) and cancer progression. J Biomed Sci, 2008, 15(6): 675-85.
47. Nonaka TS, Fujita T, Takahashi T, et al. TGF-beta1 and CTGF mRNAs are correlated with urinary protein level in IgA nephropathy. J Nephrol, 2008, 21(1): 53-63.
48. Iatridis JC, Wu J, Yandow JA, et al. Subcutaneous tissue mechanical behavior is linear and viscoelastic under uniaxial tension. Connect Tissue Res, 2003, 44(5): 208-17.
49. Langevin HM, Churchill DL, Wu J, et al. Evidence of connective tissue involvement in acupuncture. FASEB J, 2002, 16(8): 872-4.
50. Langevin HM, Vaillancourt PD. Acupuncture: does it work and, if so, how? Semin Clin Neuropsychiatry, 1999, 4(3): 167-75.
51. Leask A, Parapuram SK, Shi-Wen X, et al. Connective tissue growth factor (CTGF, CCN2) gene regulation: a potent clinical bio-marker of fibroproliferative disease? J Cell Commun Signal, 2009, 3(2): 89-94.
52. Wang Y, Maciejewski BS, Soto-Reyes D, et al. Mechanical stretch promotes fetal type II epithelial cell differentiation via shedding of HB-EGF and TGF-alpha. J Physiol, 2009, 587(8): 1739-53.
53. ZHANG Xue-quan, YANG Chun, DAI Jing-xing, QU Rong-mei, YUAN Lin: Effect of tensile load on cell proliferative activity in rat loose connective tissue. Fasciology, Vol.1, July 30, 2011
54. Dehnavi E, Soheili ZS, Samiei S, et al. The effect of TGF-beta2 on MMP-2 production and activity in highly metastatic human bladder carcinoma cell line 5637. Cancer Invest? 2009, 27(5): 568-74
55. Janos Palhalmi: The scientific basis of acupuncture. International congress of the Integrated biological regulation system (IBR Sys.) 2007 and 2008. Budapest.

J Acupunct Meridian Stud 2010;3(2):125–132

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PERSPECTIVES

Possible Applications for Fascial Anatomy and Fasciaology in Traditional Chinese Medicine

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Received: March 9, 2010
Accepted: April 13, 2010

KEY WORDS:
acupoints;
anatomy;
fasciaology;
meridians;
primo-vessel;
traditional Chinese medicine

Abstract
Research using medical imaging instruments such as computed tomography and magnetic resonance imaging has led to the proposal that the fascial network distributed over the human body is the anatomical basis for the acupoints and meridians of traditional Chinese medicine. Therefore, we put forward a new theory of anatomy called fascial anatomy. In fascial anatomy, a human body is divided into two major systems. One is the supporting-storing system of unspecialized connective tissues. The other is a functional system. An undifferentiated non-specific connective tissue network, with the participation of the nervous and the immune systems, constitutes the supporting-storing system of the human body. The various differentiated functional cells in the body that are supported and surrounded by the supporting-storing system constitute the functional system. The discipline that studies the supporting-storing system and the mutual relationship between this system and the functional system in a living human body is called *fasciaology*. The establishment of fascial anatomy and fasciaology opens a new research field in anatomy; consequently, fasciaology will play a significant role in biological medicine and traditional Chinese medical research, as well as future clinical practice.

1. Introduction

Meridian and collateral theory is the basis of traditional Chinese medicine (TCM). Much work has been carried out to find the anatomical component of meridians and collaterals; however, their existence is still disputed [1]. In the visible Chinese human project, a national basic research program of China, the computer constructed areas rich in fascial connective tissues were found to be very close to the locations of meridians and collaterals [1–3]. Based on the visible Chinese human digital datasets, virtual three-dimensional (3-D) structures of the areas rich in fascial connective tissues along meridians in the body's trunk and limbs were constructed [4]. They revealed a line-like structure similar to that

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of acupoints and meridians or collaterals [5]. Furthermore, these fascial strings were also close to virtual meridians in distance. More fascial connective tissue areas were constructed into 3-D structures, and more line-like structures were found. After the 3-D structures of all fascial connective tissues throughout the body had been constructed, a body-shaped connective tissue network appeared. Subsequently, based on computed tomography and magnetic resonance images of living human bodies, the 3-D structures of fascial connective tissue were constructed [6]. Similarly, fascial connective tissue also appeared in a line-like pattern and was co-localized with traditional Chinese meridians. Furthermore, using dynamic ultrasound, "*Deqi*" (a sore and numb feeling at acupoints) was found to occur only when a needle penetrated or stimulated the connective tissue of the fascia [7]. The histological composition of meridians has been regarded as non-specific connective tissue, including loose connective tissue and fat tissue [8–11]. The effective sites of acupuncture are therefore thought to be fascial connective tissue, including, amongst others, cells and tissues inside sites such as nerve endings, capillaries, fibroblasts, undifferentiated mesenchymal cells and lymphocytes.

The fascia of the human body is homologous in structure to the extracellular matrix of a single germ layer organism, the mesoglea of a diploblastic organism, and the mesenchyme of a triploblastic organism. Their common function is to sustain stability of the internal environment of an organism [12]. During evolution, the constitutive elements of organisms can be summarized into two types of systems (Figure 1), which are (1) the supporting and storing system containing adipose and loose connective tissues and (2) the functional systems composed of specialized cells. In other words, the fascial network, the mesenchyme, and the extracellular fluid are homologous, and their common function is to sustain stability of the internal environment of an organism. The mesoderm further evolves into organs and systems with specific functions, including the locomotor, urinary, reproductive and circulatory systems [13]. Mesenchyme remnants then differentiate into connective tissues that are distributed throughout the body. The network of fascial connective tissues provides support for the functional systems, which are composed of specialized cell [14]. The functions of the connective tissues play an important role in maintaining a longer life span for the organism. Organisms whose fascia systems are not well evolved have shorter life spans; conversely, those organisms with well evolved fascia systems have longer life spans.

Consequently, two new terms have been defined. First, fascial anatomy is a new anatomical theory

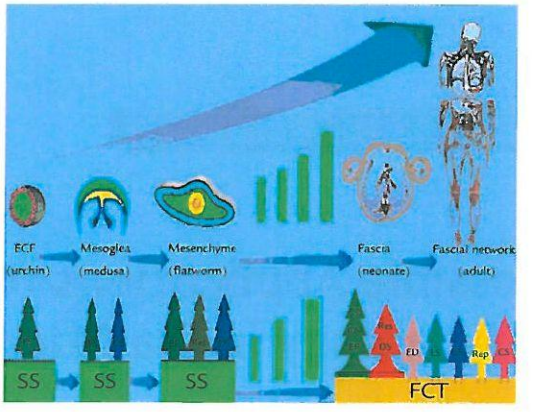

Figure 1 Schematic diagram showing that the fascia of the human body is homologous in structure to the extracellular matrix of a single germ layer organism, the mesoglea of a diploblastic organism, and the mesenchyme of a triploblastic organism. SS=supporting-storing system; FS=functional system; CC=cerebral cortex; CN=central nerve; EP=epidermis; Res=respiratory system; DS=digestive system; ES=endocrine system; LS=locomotor system; US=urinary system; Rep=reproductive system; CS=cardiovascular system; FCT=fascial connective tissue; ECF=extracellular fluid.

[4,6,8]. According to fascial anatomy, each living organism is composed of two major systems. One is the supporting-storing system, which is composed of a network of unspecialized connective tissues. The other is the functional system, which contains organs and tissues surrounded by the supporting-storing system. Second, fasciaology is the research field that studies the supporting-storing system and the mutual relationships between the above two major systems in a living organism. The theories of fascial anatomy and fasciaology highlight the significance of fascial research, which is important not only in the field of TCM but also in other fields of biomedical research and clinical therapy. TCM theories may be scientifically interpreted through the use of fasciaology.

2. Anatomy of Fascia

2.1. The origin of fascia

The fascia network is homologous with the extracellular matrix of a single germ layer organism, the middle lamella of a two germ layer organism, and the mesenchyme of a three embryonic layer organism. When the structures are retrospectively displayed and modeled, the components of an organism during each period can be summarized into two major systems (Figure 1). Functional systems are composed of cells with specific functions. They

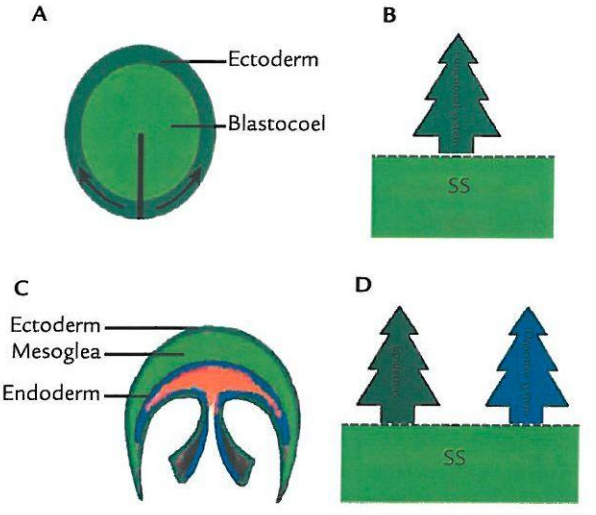

Figure 2 (A,B) Schematic diagram of a sea urchin embryo, the ectoderm is its functional system, and the blastocoel is its supporting-storing system (SS). (C,D) Diagram of a jellyfish, the ectoderm and the endoderm are its functional system while the mesoglea is its SS.

derive from twists and folds of the ectoderm and the endoblast (Figure 2). In contrast, the network fascia is composed of undifferentiated mesoderm tissue. The major histological compositions of fascia are loose connective tissue and adipose tissue. These tissues provide a stable internal environment for the cells of the functional system [15,16]. The above theory is the basis of fascial anatomy.

2.2. Fascial anatomy

Fascial anatomy is a new perspective on anatomy. It classifies body structures into the supporting-storing system and the traditional functional system. This perspective is also applicable to all living organisms, from a primitive unicellular organism to a higher mammal. It studies the morphological transformation during organic evolution from simple to complex. It also investigates how an organism maintains a longer life span through the evolution of the supporting-storing system.

During evolution, the constitutive elements of each organism can be divided into two major systems, the supporting-storing system composed of undifferentiated cells from the network of unspecialized connective tissues, including adipose tissue and loose connective tissue, and the functional systems composed of specialized cells (Figure 3). The boundary of the two major systems is the basement membrane originating from the endoderm and ectoderm and the basement-like membrane originating from the mesoderm. Undifferentiated stem

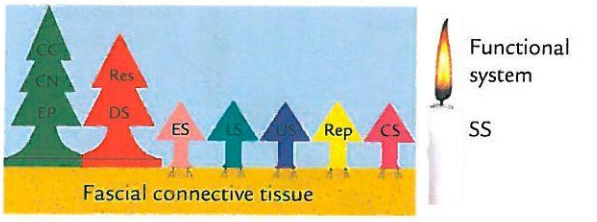

Figure 3 Schematic diagram showing that the living condition of an organism is like that of a lit candle. The fascial system composed of the network of unspecialized connective tissues is equivalent to the wax of the candle, and the functional system is equivalent to the flame. CC=cerebral cortex; CN=central nerve; EP=epidermis; Res=respiratory system; DS=digestive system; ES=endocrine system; LS=locomotor system; US=urinary system; Rep=reproductive system; CS=cardiovascular system, FCT=fascial connective tissue; SS=supporting-storing system.

cells in the supporting-storing system incessantly migrate over the above membranes, differentiate into committed-stem cells, and further differentiate into functional cells. The structures and functions of an organism are maintained by the incessant supplementation and refreshment from the supporting-storing system to the functional system. Meanwhile, under the regulation of the nervous and immune systems, the fascia network throughout the body regulates the functional and living status of cells and provides a stable environment for the survival of functional cells [17,18].

The functional system does not refer to the traditional nine major systems in systemic anatomy, which include the musculoskeletal, nervous, endocrine, cardiovascular, immune, respiratory, urinary, digestive and reproductive systems; rather, it refers to all the cells, tissues and organs that are wrapped and segmented by the supporting-storing system. The functional system is composed of various committed stem cells and functional cells. These cells differentiated from the mesoderm and the folded endoderm and/or from ectodermal cells [9]. Functional cells work together to fulfill living activities.

Fascial anatomy studies the structure of an organism based on the two-system theory. Fascial anatomy is different from traditional regional anatomy and systematic anatomy. Regional anatomy usually only studies human structures according to different regions of the body, whereas systematic anatomy studies the human body according to both the morphologies and functions of organs. Fascial anatomy encloses a third parameter, time (Figure 4), studying not only the structures and functions of the body, but also the morphological transformation during organic evolution and embryonic development. It investigates how an organism, such as an

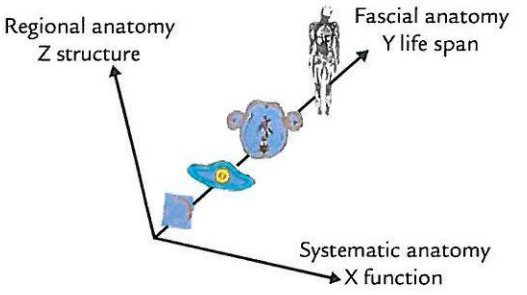

Figure 4 The relationship between fascial anatomy, systematic anatomy and regional anatomy.

advanced primate, can maintain a longer life span through evolution of the supporting-storing system from the mesoderm. Therefore, fascial anatomy helps scientists better understand the biological essence of an organism by reminding them to study anatomy in a dynamic perspective, that is, all cells and organs maintain their normal structures and functions through the interaction between the supporting-storing system and the functional system. In other words, fascial anatomy switches anatomical study from the "dead" to the "living". When the supporting-storing system wears out, the body will die. If the living condition of an organism is regarded as a lit candle, then the fascial system, the network of unspecialized connective tissues containing adipocytes and stem cells, corresponds to the wax of the candle, and the functional system corresponds to the flame of the candle (Figure 3). The supporting-storing system provides energy and cell reserve for the functional system, in the same way that the wax of the candle provides fuel to the flame. When the wax is depleted, the flame will extinguish, as with the human body. When the supporting-storing system wears out, the body will die.

3. Physiology of Fascia

Fasciaology studies the supporting-storing system, looking, for example, at how this system influences the functional system after medical treatments, and how its mesenchymal stem cells differentiate into functional cells and supplement the functional system. Fasciaology also studies the role of the supporting-storing system in the living human body and the interactions between the two major systems involved in the mechanisms of acupuncture and TCM, evolutionary biology, holistic therapies, integrated medicine and complementary medicine. The research methods of fasciaology include developmental biology analysis, non-specific connective tissue function research, stem cell research, cytobiology, molecular biology, and TCM theory research. Fasciaology also studies the curative property of mesenchymal stem cells for certain diseases, such as senile dementia, cancer, osteoporosis, degenerative disease, etc. Fasciaology helps us to study the mechanism of some therapies, such as the acupuncture, herbal medicine, holistic therapies, integrated medicine and complementary medicine of TCM. Therefore, fasciaology promotes the discovery of new therapies.

Stem cells in the supporting-storing system are undifferentiated cells. These cells have potential to differentiate into committed stem cells. Committed stem cells are the intermediate types of cells between stems cells and functional cells. They may also have certain proliferating ability to increase in number. Functional cells are those that possess strong specialized physiological functions in the human body. However, they have already lost proliferating ability. All functional cells have a short lifespan because the maintenance of normal morphology and physiological functions of an organism requires an incessant renewal of functional cells. This corrects the erroneous idea that the number of certain functional cells is unchangeable in the human body. For example, it had previously been thought that the number of neurons in the central nervous system could only decrease rather than increase after birth and that myocardial cells could only increase in volume, but not in number. Fasciaology corrects these mistakes and provides clear explanations [7].

Cells can migrate and differentiate over the germ layers. According to traditional developmental biology, cells can only differentiate in their own blastoderm rather than migrating into another germ layer. Recently, differentiation over blastoderms has been found to occur in special conditions, for example, during recovery from a trauma and the growth of a carcinoma. The theory of fasciaology addresses the above scientific question of cell differentiation. In fasciaology, all cells originate from the stem cells of the supporting-storing system. As previously mentioned, stem cells are undifferentiated cells from the mesoderm. Undifferentiated stem cells differentiate into various committed stem cells and finally into functional cells during migration over basement and basement-like membranes [6].

4. Biomedical Significance of Fascial Anatomy and Fasciaology

Fascial anatomy studies the morphological changes from growing to aging and offers a new perspective on the study anatomy. Therefore, it is different from both regional anatomy and systematic anatomy. In developmental biology, the mesoderm-derived mesenchyme differentiates into multiple organ systems during individual development, while

the endoderm and ectoderm form a network of non-specific fascial connective tissues. This network is an independent system composed of stem cells and constitutes the supporting-storing system regulated by the nervous and the immune systems [19]. Stem cells in non-specific connective tissue constantly differentiate into committed stem cells and functional cells. Therefore, human cells can be classified into three categories: stem cells, committed stem cells and functional cells. To maintain a longer biological life span and a stable interior environment in the body, body cells constantly renew themselves. In fascial anatomy, human life involves the continuous consumption of the supporting-storing system.

Fasciaology is a combination of developmental biology, embryology, cytobiology, anatomy, TCM, and holistic therapies. With the theory of fasciaology, scientists may perceive the human body in a simpler, easier and more understandable way because the human body and all other organisms are simplified into two major systems. In this way, we can understand how important the supporting-storing system is, and determine the key effect of non-specific connective tissue upon the human body. The mechanisms of some therapies can also be easily understood in fasciaology and inscrutable TCM therapies can be explained in a scientific way [8].

The simple generalization that each organism is composed of two major systems may explain the mechanism of TCM and may free TCM from the shackles of its traditional theories and ancient records, promoting the modernization of Chinese medicine. The proposal of fasciaology also frees classical acupuncture therapy from the fetters of ancient meridian records by looking at the human body from a new perspective. The simple theory explains the mechanism of TCM and ushers it into a new frontier of medical science. This theory will guide people to find new and more effective treatments.

5. Possible Applications of Fasciaology Research in TCM

The theory of fasciaology may scientifically explain the mechanism of TCM which is an empirical subject. The foundation of TCM theories originates from many subjective assumptions rather than scientific experiments [20]. There may be some placebo effects and other unknown mechanisms existing in TCM therapy. Different ancient TCM books have different records on TCM theories [21], creating the impression that TCM is a pseudo-science. In fasciaology, stimulated stem cells in the supporting-storing system differentiate to functional cells in the functional system, and therefore, provide a cell supplement for this system. This may be one of the mechanisms for TCM therapy. The result is a mobilization of the body's reserves of stem cells and a regulation of the endocrine system. Electrolyte balance and the water balance in the body can also occur via this system allowing disease cure. Although TCM treatment regimes differ from person to person, a common principle and mechanism for treatment has been addressed above [22,23].

In TCM, there are many kinds of physical treatments, including acupuncture, *Gua Sha*, plum blossom needle, and so on. They all stimulate the fascia network so as to regulate the metabolism and functions of the functional cells [11,24]. The essence of TCM meridians may be explained in the view of fascial anatomy. The anatomical basis of meridians is the fascia network throughout the body, and the histological composition of meridians is non-specific connective tissues, including loose connective tissue and fat tissue. The histological structure where an acupuncture needle produces an action is fascial connective tissue containing nerve endings, capillary vessels, fibroblasts, undifferentiated mesenchymal cells, lymphocytes, etc. [25–27]. Acupoints are those sites that produce strong biological reactions when stimulated. Since fascial connective tissue is distributed throughout body, acupoints may exist in every part of the body [28–30]. The difference between clinically so-called acupoints and non-acupoints and between main acupoints and supplementary acupoints is the intensity of the biological reactions rather than the structural components.

Since non-specific connective tissue is the anatomical basis of meridians and acupuncture and the connective tissue network is distributed throughout the body, the targets of acupuncture therapy may exist all over the body [22,31,32]. Acupoints in the human body are sites that can generate significant response when stimulated. Non-meridian extra acupoints are areas rich with fascial connective tissues, and they are located outside meridians. The locations and depths of these areas differ among individuals, as do their sensitivity, causing therapeutic effects to also differ [33–35]. The ancient records of meridians and acupoints, as well as those of herbal medicine, have no scientific basis. These books, therefore, can only serve as references and should not be strictly followed as textbooks [11,23,24,36]. Fascial research may provide evidence for the accuracy of acupoints.

6. Primo-vascular System in the Fascia

Several authors have suggested that an acupuncture meridian corresponds to connective tissue, as histological features can distinguish the acupuncture

points from surrounding tissues [37,38]. Heine found that acupuncture points perforated the dermal fascia that separated subcutaneous tissue from muscle tissue [39–41]. He demonstrated the lung meridian as a fascia-myo-tendon chain [42,43], a finding which has been supported by others [44–48]. Some others consider acupuncture points as neurovascular bundles [49–51] or veins perforating the dermal fascia [52]. Langevin observed that most acupoints appeared to coincide with intermuscular or intramuscular connective tissue planes [11].

Although many investigators have observed a close connection between acupuncture meridians and fascia, separate anatomical structures corresponding to acupuncture points or meridians that are not known in Western biology or medicine have also been suggested. Bong-Han Kim is unique in the sense that he found a novel circulatory system that formed a weblike network throughout an animal's body [53,54]. His claims, however, remained unconfirmed by other groups, except for a Japanese anatomist Fujiwara [55] who reproduced some parts of Kim's claims. Only recently has intensive reinvestigation revealed the primo-vessels (Bonghan ducts) that correspond to the meridian-like structures in the animal body [56]. It turns out that almost all the primo-vessels lie in the fascia. For example, the primo-vessels on the surfaces of internal organs were sometimes attached to the peritoneal fascia surrounding abdominal organs or abdominal walls. The primo-vessels were well visualized by using Trypan blue and were observed to form a network in the omentum and the peritoneum, which are representative fascia [57]. A surprising discovery was made by using this Trypan-blue-staining method; that is, the primo-vascular system existed on the fascia surrounding tumor tissues [58], which, in turn, raised two possibilities. One is control or treatment of a tumor by acupuncture through the primo-vascular system. Another is the metastasis of cancer through the primo-vessels. These two opposing possibilities may offer novel approaches to cancer biology and medicine. Further observation of primo-vascular systems on the arachnoid mater of the brain and perineurium of nerves have been reported very recently [57].

The close relationship between the primo-vascular system and the fascia is natural considering recent developments in anatomical understanding and in applications to manual and movement therapies, including acupuncture, of the fascia system [59]. The significance of fascia anatomy as a supporting-storing system is augmented by the presence of the novel primo-vascular system in various fascia. It also explains why acupuncture meridians (or the primo-vessels) are difficult to identify by using ordinary histological techniques involving hematoxylin and eosin. The primo-vessels are made of the same material, collagen fibers, as the fascia, and their distinction requires specific techniques, which need to be fully developed in the future.

7. Conclusions

The anatomical basis of acupoints and meridians is the fascial network distributed throughout the body. Meridians consist of non-specific connective tissues, including loose connective tissue and fat tissue. Acupoints are sites that produce strong biological reactions when stimulated. The non-specific connective tissue network has been proposed as the supporting-storing system in the hypotheses of fascial anatomy and fasciaology. Recent observation of the primo-vascular system in various fascia surrounding internal organs and tumor tissues allows more detail and evidence for these hypotheses. These hypotheses are being investigated through research into the mechanism of acupuncture and TCM, evolutionary biology, holistic therapies, integrated medicine, and complementary medicine. Research methods include developmental biology analysis, aging mechanism research, non-specific connective tissue function research, stem cell research, cytobiology, molecular biology, and TCM theory research.

Acknowledgments

The work described in this paper was supported by the National Basic Research Program (973 Program, grant no. 2007CB512705) and by the National Natural Science Foundation of China (grant no. 30801464).

References

1. Yuan L, Tang L, Huang WH. Construction of dataset for virtual Chinese male. *Di Yi Jun Yi Da Xue Xue Bao* 2003; 23:520–3. [In Chinese]
2. Wang CL, Yuan L, Wang J, Jiao PF. Contrast study on the line course of fascia meridians made by three dimensional reconstruction and classical meridians in human body. *Chin J Anat* 2007;30:340–3.
3. Wang JW, Dong W, Wang C, Yao D, Zhao B, Shen B, et al. From meridians and acupoints to self-supervision and control system: a hypothesis of the 10th functional system based on anatomical studies of digitized virtual human. *J South Med Univ* 2007;27:573–9.
4. Wang CL, Wu JP, Wang J. An interpretation on the essence of meridians and acupuncture mechanism from fasciaology view. *Chin J Basic Med Tradit Chin Med* 2008;14:312–4.
5. Zhong SZ, Yuan L, Tang L, Huang WH, Dai JX, Li JY, et al. Research report of experimental database establishment of digitized virtual Chinese No.1 female. *Di Yi Jun Yi Da Xue Xue Bao* 2003;23:196–200. [In Chinese]

6. Yuan L, Wang J, Wang CL, Wang CL, Dai JX, Huang Y. Fasciaology: a new theory on the human self-supervision and control system. *Sci Tech Rev* 2006;24:85–9.
7. Yuan L, Zhong SZ. Human auto-testing and modulating system-anatomic basis involved in meridianal system. *Tianjin J Tradit Chin Med* 2004;21:356–9.
8. Huang Y, Yuan L, He ZQ, Wang CL. Study on the meridians and acupoints based on fasciaology: an elicitation of the study on digital human being. *Zhongguo Zhen Jiu* 2006; 26:785–8. [In Chinese]
9. Wang XY, Zhong C. The morphological basis of mesotherapy. *Chin J Aesth Plast Surg* 2007;18:166–8.
10. Langevin HM, Churchill DL, Cipolla MJ. Mechanical signaling through connective tissue: a mechanism for the therapeutic effect of acupuncture. *FASEB J* 2001;15:2275–82.
11. Langevin HM, Yandow JA. Relationship of acupuncture points and meridians to connective tissue planes. *Anat Rec* 2002;269:257–65.
12. Marieb EN, Mallatt J, Wilhelm PB. Basic Embryology. In: *Human Anatomy*. San Francisco: Benjamin Cummins, 2008: 51–60.
13. Ross MH, Pawlina W. Connective Tissue. In: *Histology, A Textbook and Atlas*. Baltimore: Lippincott Williams & Wilkins, 2005:146–8.
14. Thomas WM. Structural integration-developments in Ida Rolf's 'Recipe'-I. *J Bodywork Mov Ther* 2004;8:131–42.
15. Robert S. Fascial plasticity–a new neurobiological explanation: Part 1. *J Bodywork Mov Ther* 2003;7:11–19.
16. Robert S. Fascial plasticity–a new neurobiological explanation Part 2. *J Bodywork Move Ther* 2003;7:104–16.
17. Ciczek LSW, Szopinski J, Skrzypulec V. Investigations of morphological structures of acupuncture points and meridians. *J Tradit Chin Med* 1985;5:289–92.
18. Moore KL, Agur AMR. Introduction to clinical anatomy. In: *Essential Clinical Anatomy*. New York: Lippincott Williams & Wilkins, 1995:8–9.
19. Hashimoto PH. The perineurial vessel: a possible candidate for the structural basis of the meridian (Jing-Luo) in Chinese medicine. *Anat Sci Int* 2005;80:177–80.
20. Board of Science and Education, British Medical Association. The evidence base of acupuncture. In *Acupuncture: Efficacy, Safety and Practice*. Amsterdam: Harwood Academia Publishers, 2000:11–21.
21. Birch SJ, Felt RL. The theoretical basis of acupuncture: fundamental concepts and explanatory models. In: *Understanding Acupuncture*. Edinburgh and New York: Churchill Livingstone, 1999:110–13.
22. Sutherland JA. Meridian therapy: current research and implications for critical care. *AACN Clin Issues* 2000;11:97–104.
23. Ho MW, Knight DP. The acupuncture system and the liquid crystalline collagen fibers of the connective tissues. *Am J Chin Med* 1998;26:251–63.
24. Omura Y, Takeshige C, Shimotsuura Y, Suzuki M. Imaging of the stomach, and localization of the stomach meridian & its acupuncture points in a human cadaver by the use of the indirect "Bi-Digital O-Ring Test Imaging Technique". *Acupunct Electrother Res* 1988;13:153–64.
25. Ma W, Tong H, Xu W, Hu J, Liu N, Li H, et al. Perivascular space: possible anatomical substrate for the meridian. *J Altern Complement Med* 2003;9:851–9.
26. Yung KT. Birdcage model for the Chinese meridian system: part VI. Meridians as the primary regulatory system. *Am J Chin Med* 2005;33:759–66.
27. Konofagou EE, Langevin HM. Using ultrasound to understand acupuncture. Acupuncture needle manipulation and its effect on connective tissue. *IEEE Eng Med Biol Mag* 2005;24:41–6.
28. Ahn AC, Wu J, Badger GJ, Hammerschlag R, Langevin HM. Electrical impedance along connective tissue planes associated with acupuncture meridians. *BMC Complement Altern Med* 2005;5:10.
29. Robb RA. 3-D visualization in biomedical applications. *Annu Rev Biomed Eng* 1999;1:377–99.
30. Swartz MA, Tschumperlin DJ, Kamm RD. Mechanical stress is communicated between different cell types to elicit matrix remodeling. *Proc Natl Acad Sci USA* 2001;98:6180–5.
31. Dung HC. Anatomical features contributing to the formation of acupuncture points. *Am J Acupunct* 1984;12:139–43.
32. Bensoussan A. The nature of the meridians. In: *The Vital Meridian: A Modern Exploration of Acupuncture*. Melbourne and New York: Churchill Livingstone, 1991:51–3.
33. Langevin HM, Churchill DL, Wu J, Badger GJ, Yandow JA, Fox JR, et al. Evidence of connective tissue involvement in acupuncture. *FASEB J* 2002;16:872–4.
34. Bossy J. Morphological data concerning the acupuncture points and channel network. *Acupunct Electrother Res* 1984;9:79–106.
35. Scheip R, Klinger W, Lehmann-Horn F. Active fascial contractility: fascia may be able to contract in a smooth muscle-like manner and thereby influence musculoskeletal dynamics. *Med Hypotheses* 2005;65:273–7.
36. Langevin HM, Churchill DL, Fox JR, Garra BS, Krag MH. Biomechanical response to acupuncture needling in humans. *J Appl Physiol* 2001;91:2471–8.
37. Matsumoto K, Birch S. *Hara Diagnosis: Reflections on the Sea*. MA: Paradigm Publications, 1988.
38. Ho MW, Knight DP. The acupuncture system and the liquid crystalline collagen fibers of the connective tissues. *Am J Chin Med* 1998;26:251–63.
39. Heine H. Anatomical structure of acupoints. *J Tradit Chin Med* 1988;8:207–12.
40. Heine H. Morphologie der Ohrakupunkturpunkte. *Dtsch Zschr Akup* 1993;36:99–103. [In German]
41. Heine H. Der Akupunkturpunkt–ein Meridianorgan. *Dtsch Zschr Akup* 1996;39:75–80. [In German]
42. Heine H. Zur Morphologie der Akupunkturpunkte. *Dtsch Zschr Akup* 1987;30:75–9. [In German]
43. Heine H. Functional anatomy of traditional Chinese acupuncture points. *Acta Anat* 1995;152:293–7.
44. Zerlauth B, Böheim C, Moriggl B. Histologie der akupunkturpunkte. *Dtsch Ztschr Akup* 1992;35:34–8. [In German]
45. Egerbacher M. Veterinaeakupunktur. Anatomische und histologische Struktur ausgewaehlter Akupunkturpunkte bei Rind und Hund. *Dtsch Ztschr Akup* 1993;36:75–80. [In German]
46. Draempehl D, Ottensmeier A, Kleinpeter A, Kiupel M. Morphologische Untersuchungen an den Akupunkturpunkten und Meridianen bei Katzen und Hunden. *Dtsch Ztschr Akup* 1993;36:104–9. [In German]
47. Zhang B. Studies on the morphology and function of meridian lines with reference to neurogenic inflammation by ICR mouse. *Dtsch Zschr Akup* 1996;39:29–38.
48. Stecco L. *La Manipalazione Neuroconnettivale. Disfunzioni segmentarie dell'apparato locomotore*. Roma: Marrapese, 1996. [In Italian]
49. Rabischong P, Niboyet JEH, Terral C, Senelar R, Casez R. Bases experimentales de l'analgesie acupuncturale. *Nouv Presse Med* 1975;4:2021–6. [In French]
50. Senelar R. Les characteristiques morphologiques des points Chinois. In: Niboyet JEH ed. *Nouveau traite d'acupuncture*. Paris: Maisonneuve, 1979. [In French]
51. Bossy J. Morphological data concerning the acupuncture points and channel network. *Acupuncture Electrother Res* 1984;9:79–106.
52. Plummer JP. Anatomical findings at acupuncture loci. *Am J Clin Med* 1980;8:170–80.
53. Kim BH. On the Kyungrak system. *J Acad Med Sci DPR Korea* 1963;90:1–41. [In Korean]

54. Kim BH. Sanal Theory. *J Jo Sun Med* 1965;108:39–62. [In Korean]
55. Fujiwara S, Yu SB. 'Bonghan Theory' Morphological Studies. *Igaku no Ayumi* 1967;60:567–77.
56. Soh KS. Bonghan circulatory system as an extension of acupuncture meridians. *J Acupunct Meridian Stud* 2009;2: 93–106.
57. Lee BC, Soh KS. A novel model for meridian: Bonghan systems combined with fascia (Bonghan-Fascia Model). In: *Proceedings, Fascia Research II: Basic Science and Implications for Conventional and Complementary Health Care*. Amsterdam: Elsevier, 2009:144.
58. Yoo JS, Kim HB, Ogay V, Lee BC, Ahn S, Soh KS. Bonghan ducts as possible pathways for cancer metastasis. *J Acupunct Meridian Stud* 2009;2:118–23.
59. Myers TW. *Anatomy Trains: Myofascial Meridians for Manual and Movement Therapists*, 2nd ed. Oxford: Churchill Livingstone, 2008.

Original Article

Research methods in fasciology: implications for acupuncture meridianology

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ABSTRACT

Objective The theory of meridians and collaterals is the basis of acupuncture in the traditional Chinese medicine; however, their anatomical existence is still unknown. The aim of this study is to introduce new and combined research approaches to the investigation of the anatomical basis and physiological properties of the fascial network and the acupoints and meridians. ***Methods*** Based on the digital datasets of Virtual Chinese human (VCH) bodies, 3-dimensional (3-D) structures of the virtual meridians and fascia connective tissue-rich areas were constructed. The shortest distances between the virtual acupoints and the constructed fascia connective tissues were measured. The 3-D structures of the fascial connective tissue-rich areas were also constructed based on CT and MRI images of living human subjects, and also compared with meridians. The electrophysiological properties of a sample meridian were analyzed on the basis of Conductance Density Mapping© method to investigate the physiological inhomogenities of the human connective tissue overlapping the above mentioned anatomical structures. ***Results*** The 3-D structures of fascial connective tissue-rich areas in the VCH bodies showed a special beads-on-strings pattern of distribution. The reconstructed fascial lines co-localized precisely with the traditional meridian lines. The more fascial connective tissue areas were constructed, the more string-like structures were found. When the 3-D structures of all the fascial connective tissues throughout the body were constructed, a body-shaped connective tissue network appeared. The 3-D structures of the fascial connective tissue-rich areas constructed from both CT and MRI images also showed beads-on-string patterns, and co-localized with traditional Chinese meridians. In physiological experiments, the Sanjiao (triple warmer) channel was the chosen sample meridian. The peak amplitudes of conductance, resistance responses and decay time of the resistance response showed statistically significant difference (P=0.00392, 0.00454, and 0.000377, respectively) between the subdermal connective tissue overlapping the meridian and non-meridian areas. ***Conclusions*** The fascia network throughout the body is the anatomical basis of acupoints and meridians in traditional Chinese medicine. The histological composition of the meridian is the non-specific connective tissue (including loose connective tissue and fat tissue). Connective tissue related to the meridian areas expresses intensified functional responsiveness compared to non-meridian fascia lines. We put forward a new approach for combining the structural and functional (electrophysiological) methods in the investigation of human fascial network. According to this view, the human body consists of two major systems: one is the supporting and storing system and the other is the functional system. The anatomical discipline based on this division method is named fascial anatomy. The discipline which studies the supporting and storing system and the mutual relationship between this system and the functional system is named fasciology.

Key words: fasciology; methodology; acupuncture; meridianology; virtual human technique

INTRODUCTION

The theory of meridian system is the fundamental basis of acupuncture, moxibustion and massage therapy in Traditional Chinese Medicine (TCM) [1]. The meridian system is composed of 12 principal meridians and 8 extra meridians. The principal meridian systems,

Received: 2011-02-15 Accepted: 2011-04-08
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including their deep and surface pathways and also divergent and connecting channels, provide the linking between different organs, and the extra meridians communicate with the principal meridians [2-3]. This meridian map has been well established by the ancient Chinese, and is still the basis of the treatment methods like traditional Chinese acupuncture, moxibustion and Chinese massage therapy. Efficiency of these methods is not questionable [4], but the meridians are still "invisible" to modern scientists.

Many efforts have been made to prove the existence of acupoints and meridians, which have been described as different substances, such as neurovascular bundles, neuromuscular attachments, sensory nerve endings, perivascular space and perineurial vessels [5-10]. Recently, more and more researchers have suggested that a correspondence may exist between acupuncture meridians and connective tissues [11-14]. However, there is an argument about the types of connective tissues. Omura et al [15] linked the acupuncture points mainly to dense connective tissue, while Langevin and Yandow [12] viewed the meridians as the network formed by interstitial connective tissue. These studies examined only small parts of the human body and only a few meridians.

Since the meridian concept is more functional than structural, scientists have made a huge effort to describe several electro-physiological properties of acupoints and meridians in relation to their surrounding areas. A tremendous amount of studies proves the special electrical features of acupoints and meridians, but surprisingly only a few of them are able to meet modern scientific criteria [13]. Conductance Density Mapping©, developed by East-West BioMedicine Ltd., is a systematic method to explore the electrical properties of acupoints and meridians based on the multidimensional analysis of electric resistance parameters recorded through the puncturing needles. In this way the electric properties of connective tissue planes related to meridian and non-meridian areas can be compared very precisely. In this study, the basis of the method is demonstrated on a sample meridian part.

The virtual human technique is a new methodology: visualization of 3-D human renderings allows an accurate anatomical and functional mapping and a quick analysis of structure-to-function relationships [16-20]. The data of Virtual Chinese Human (VCH) bodies was employed in this project. The first aim of this study was to investigate the anatomical structures of acupoints and meridians 3-dimensionally in VCH bodies. The second aim of this study is to demonstrate how the Conductance Density Mapping© method can be combined with the anatomical approaches to differentiate the meridian-related connective tissue planes from their surroundings on the basis of the functional properties.

METHODS

Anatomical studies

VCH image datasets

Digital image datasets, including a set of VCH Male 1 (VCH-M1) and a set VCH Female 1 (VCH-F1) were collected and prepared [21]. A 176-cm-tall male cadaver and a 155-cm-tall female cadaver were perfused with a red filling material through the femoral artery before freezing and embedding. The red filling material was a mixture composed of gelatin, cinnabar and starch. The whole cadavers were sectioned by a JZ1500A vertical milling machine at intervals of 0.2 mm. Images of all the sequential sections were captured with a FujiFinePixS2Pro camera and saved as Tagged Image File Format (TIFF). The digitized VCH male and female image datasets were established [22].

Construction of 3-D structures of connective tissue

From the image database of VCH-M1, 100

images of the upper extremity and 100 images of the lower extremity, all at 5 mm intervals, were sequentially selected [23]. Using 4 marker points on the original images, image registration was performed followed by confirmation with a standard edge detection algorithm (Sobel). During the localization, the parameters were adjusted according to the actual images. After removal of the backgrounds in the images, 3 groups of images were obtained (Fig.1). The images were then compared with the standard human body tomograms to identify the connective tissues. Green circles were inscribed in the center of thick connective tissue areas, which were on the border of the subdermal and intramuscular connective tissues and usually showed triangular or polygonal shape (Fig.2A, C). The diameter of a green circle was half of the maximum diameter of the thick connective tissue area (Fig.4A). The maximum diameter of this thick connective tissue area is the vertical distance between the intermuscular septum and the subcutaneous thick connective tissue under the dermis. Subsequent image segmentation and diminution by bicubic interpolation resulted in 3 groups of images, each with a size of 810 × 390 pixels. The images were converted into grey color. Finally, a 3-D model was reconstructed.

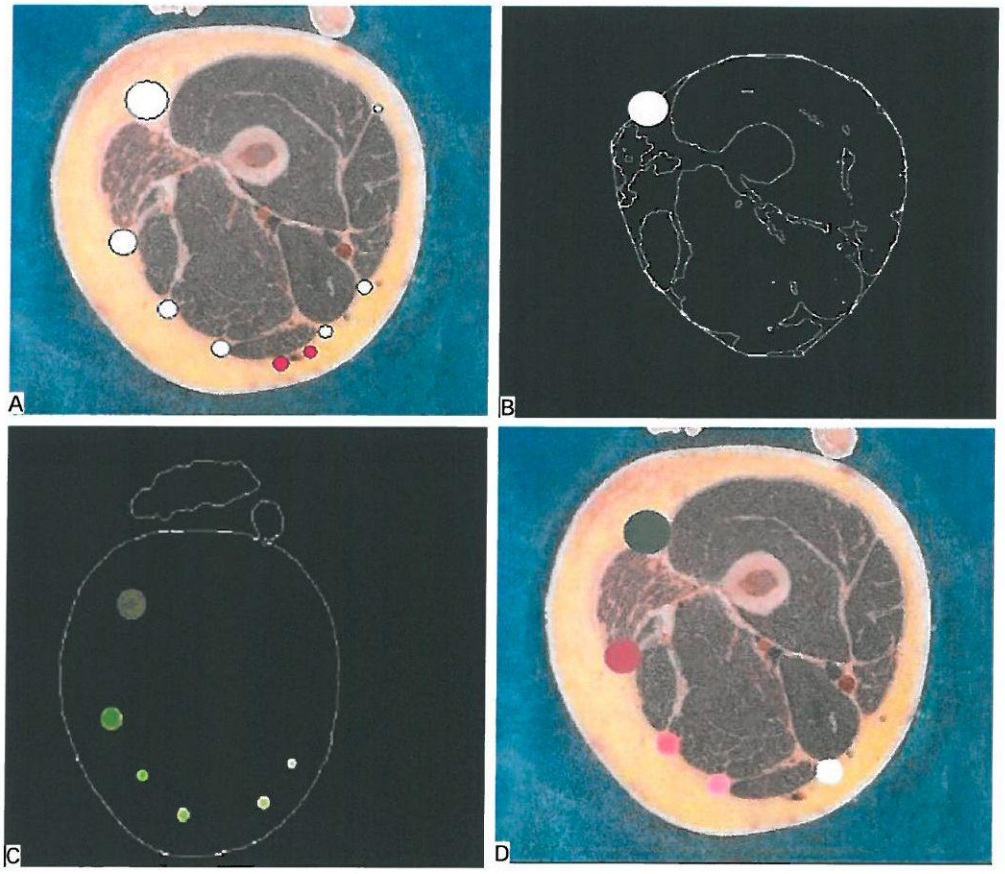

Fig.1 Marking procedure. The areas rich in connective tissues in a VCH image are marked in A. The muscular tissue is extracted B, and the connective tissue areas are auto-marked by the software C. D shows the marked VCH image.

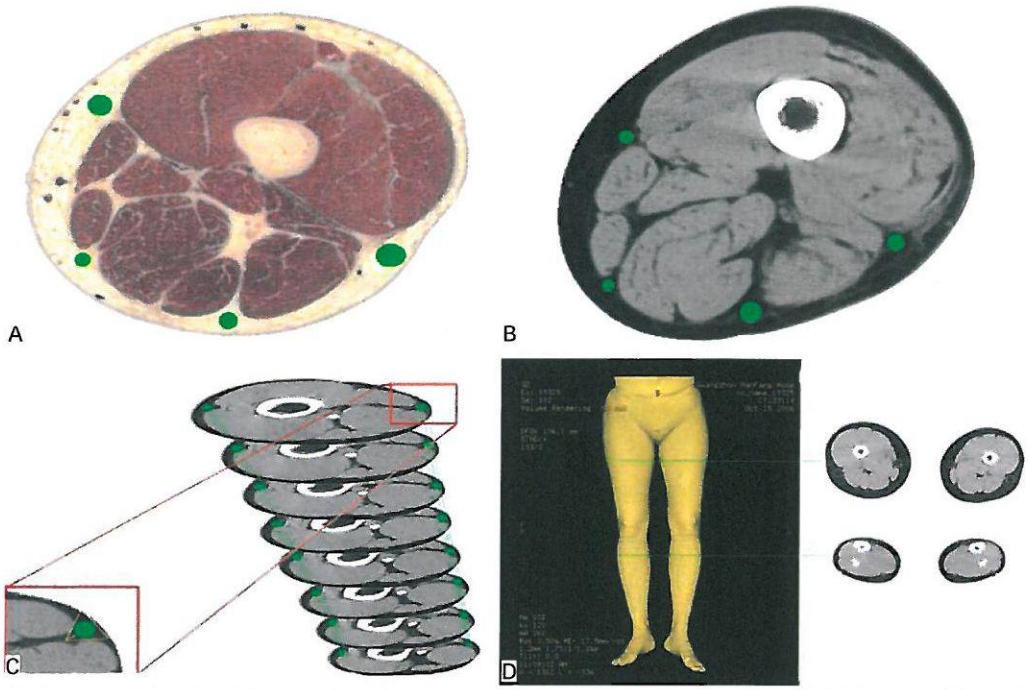

Fig.2 3-D reconstruction. Circles are inscribed in the connective tissues in VCH (A) and CT (B) images. The 3-D structures are then reconstructed (C and D).

Methodological approach

The distances between the traditional (virtual) meridian lines and the fascial lines were analyzed bidirectionally in the following two ways: (1) 10 points were randomly selected on each examined fascial line. The distances between these points and the traditional meridian lines were measured. (2) 10 acupoints were randomly selected on each examined traditional meridian. The distances between these acupoints and the fascial lines (indicated by areas containing rich connective tissue, showing special "beeds on string" structure) were measured.

These 2 datasets (derived from the distances, measured by mm) were compared with the results of the visual observers, who were experts in anatomy and meridianology. Overlapping (colocalization) between the traditional meridians and fascial lines was accepted when the result of visual observation was identical with the result of the bidirectional procedure described above.

Comparison of 3-D connective tissue strings with traditional meridians in VCH image datasets

The 3-D connective tissue structures were rendered from the green circles inscribed in the VCH images close to the anterior and posterior midlines of the trunk, as well as close to the medial and the lateral lines of the upper and lower extremities. For this introductory methodological study the courses of six major constructed connective tissue strings were compared with those of meridians in TCM, including Ren and Du meridians in the trunk, Large Intestine meridian of Hand-Yangming (LI) and Triple-warmer meridian of Hand-shaoyang in the upper limbs, as well as Kidney meridian

of Foot-shaoyin (KI) and Galdbladder meridian of Foot-shaoyang (GB) in the lower limbs. The vertical distances were measured between the traditional meridians and the reconstructed fascial lines (the so-called connective tissue strings) at 10 different, randomly selected points on respective lines. Results are summarized in Tab.1.

Study of CT images of living human bodies

The lower limbs of 12 volunteers were scanned by GE Lightspeed 16 row spiral CT. Circles were added into the connective tissue-rich areas in the CT images in a fashion similar to that in VCH images (Fig.2B, C). The 3-D structures were then reconstructed through 3-D volume rendering. The output data were transformed from DICOM to JPEG format. The correlation between the acupoints and fascia converging areas was analyzed. We measured the distance between the traditional meridians and the reconstructed fascia converging lines. First, 10 acupoints were selected along every 6 standard meridian lines, then the vertical distance from the 10 acupoints was measured in every traditional meridian to the reconstructed fascia converging lines in the vicinity [24].

Study of MRI images of living human bodies

Magnetic resonance (MR) images of the volunteers were randomly selected for 3-D connective tissue reconstruction. The traditional Chinese meridians and acupoints were marked on one upper limb of the volunteers, and a plastic tracer-filled tube was placed along the marked meridians. GE 3.0 MRI was used to scan the limbs. The fascia converging areas in the MRI images were marked and their 3-D structures were reconstructed using MIMICS11.02 software. The distances between the tracer-filled traditional (virtual) meridians and the fascial lines were analyzed.

Physiological study

The measurement was carried out with a tissue conductance recording electroacupuncture device produced by Minel Ltd. During the recording, a maximum of 10 μA direct current was applied to test the conductance between the electrode tips inserted into the acupoints. In Voll scaling system, the conductance values are expressed as a percentage of the maximal conductance on a 1/x scale (zero resistance: 100% or 100 Voll value), and 50 Voll value equals 100 kΩ. When DC resistance recording occurs, for the therapists, it is easier to use a so-called Voll scale which is proportional to the conductance and magnified to the specific range of human tissues, so that the nonlinear nature (1/x scale) of the recording method is not so disturbing. For research purposes, it is important to record both the Voll values and resistance in kΩ in parallel to render the results comparable to earlier clinical observations for accurate analysis.

With this measurement technique it is not the sweet gland activity parameters of the vegetative tone that are reflected, but those of the deep tissue electrodynamics. Stainless steel (0.3 mm diameter) acupuncture needles were used for recording to generate comparable results with ongoing clinical observations.

The Conductance Density Mapping Method© was developed to carry out meridian screening in healthy and in diseased human subjects. In this study the most elemental part of the method was applied, only in DC recording mode to look for functional inhomogenities overlapping the anatomical inhomogenities of connective tissue.

The negative electrode was connected to the needle inserted to Sanjiao 6 acupoint and the positive electrode was connected to the Sanjiao 9 acupoint and to the control measuring nonacupoint (CMP) approximately 3 cm from Sanjiao 9. The electrodynamic responsiveness of the subdermal connective tissue in the meridian line (Sanjiao 6-9) and in the non-meridian line (Sanjiao

6-CMP) was analyzed comparatively, namely, the peak of electrical conductance and resistance response and the dynamics of the response attenuation (also known as the decay time). The decay time was calculated at the half attenuation point compared to the peak response.

The results from the 7 healthy volunteers were analyzed by one-way ANOVA.

RESULTS

Anatomical study

Datasets of VCH bodies

A total of 9 232 and 8 556 TIFF images were obtained from the male and female cadavers, respectively. The database size was 161.56 GB for VCH-M1 and 149.7 GB for VCH-F1.

3-D constructions of VCH connective tissues The reconstructed fascia strings (green) overlapped the traditional meridians (red) (Fig.3). The reconstructed fascia strings showed beads-on-string structures in certain parts of the limbs and the body trunk. For instance, the reconstructed connective tissue line in the back and in the abdomen showed a beads-on-string pattern and overlapped the traditional Du and Ren meridians (Fig.3A). This is partly due to the regional anatomical structure, the vertebral column. The green connective tissue strings in upper limbs were colocalized with the classical Large Intestine meridian of Hand-yangming and Triple Energizer meridian of Hand-shaoyang that were indicated in red (Fig.8B). The connective tissue strings in lower limbs were also colocalized with the Kidney meridian of Foot-shaoyin and Gallbladder meridian of Foot-shaoyang (Fig.3C). The results showed a strong consistency between the intermuscular connective tissues and distributions of meridians. Data are shown in Tab.1.

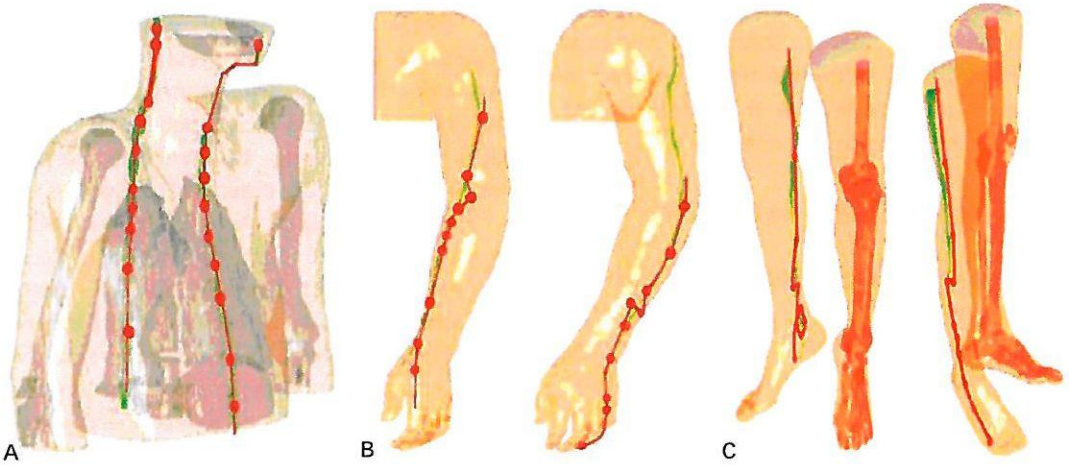

Fig.3 Reconstructed fascial lines (green) and classical meridians (red) in the trunk (A), upper limbs (B) and lower limbs (C) in the VCH body.

The data show an extremely strong colocalization between traditional meridians and fascial lines reconstructed from the connective tissue converging areas. Even in the case of

Tab.1 Vertical distances between cognominal meridians pairs at 10 randomly selected points (mm)

Meridians	Distances between TCM meridians and fascial lines										*Mean±SD*
Large Intestine Meridian (LI)	14.1	1.7	-1.0	2.1	-3.2	1.8	1.2	3.8	0.4	3.6	3.3±3.9
Sanjiao Meridian (SJ)	0.1	0.2	0.2	-0.8	-2.9	-8.1	9.5	0.2	0.6	3.0	2.5±3.5
Kidney Meridian (KI)	18.8	10.2	-7.9	-5.7	2.5	-6.7	6.5	-5.6	1.1	9.0	7.4±4.8
Gallbladder Meridian (GB)	-17.0	-11.5	-9.6	-4.5	1.4	-1.1	0.5	2.5	-1.6	4.1	5.4±5.5
Ren Meridian (RN)	0	0	0	0	0	0	0	0	0	0	0
Du Meridian (DU)	0	0	0	0	0	0	0	0	0	0	0

Kidney meridian, the 7.4±4.8 mm difference is within the error margin of the imaging and marking procedure.

When more connective tissue 3-D structures were reconstructed, more fascia strings, or meridians, were found. When the fascia connective tissues of the whole body were reconstructed and their 3-D structure was rebuilt, the complete fascia network was presented (Fig.4). The connective tissues were extensively distributed over various parts of the human body and formed a complete connective tissue framework. All the human organs were coated with connective tissues, which also extended into the organs to form septa within the organs.

Study of CT images

The distribution of the rebuilt fascia meridians overlapped with that of the traditional ones. When the classical meridians were compared with connective tissue string reconstructed from the CT images, the distribution of the reconstructed fascia meridians overlapped with the traditional ones (Fig.5).

Study of MRI images

When the classical meridians were compared with the reconstructed connective tissue string from MRI images, the distribution of the reconstructed fascia meridians colocalized with that of the traditional ones. The results were similar to those from VCH study (Fig.6).

Physiological study

All the 3 calculated parameters of DC resistance properties showed statistically significant differences between the meridian and non-meridian datasets. The conductance profile expressed on Voll scale (Fig.7) was sharp and quickly attenuated, while the resistance decay profile (Fig.8) was more elongated as expected on the basis of the literature. The higher peak amplitudes and longer decay time of meridian recordings suggest that the functional responsiveness of subdermal connective tissue is more prominent in the meridians than in the non-meridian regions (Tab.2).

DISCUSSION

Disputes still remain in the existence of the anatomical basis of the meridians and collaterals in TCM. In the Virtual Chinese Human (VCH) project, the computer-constructed areas rich in

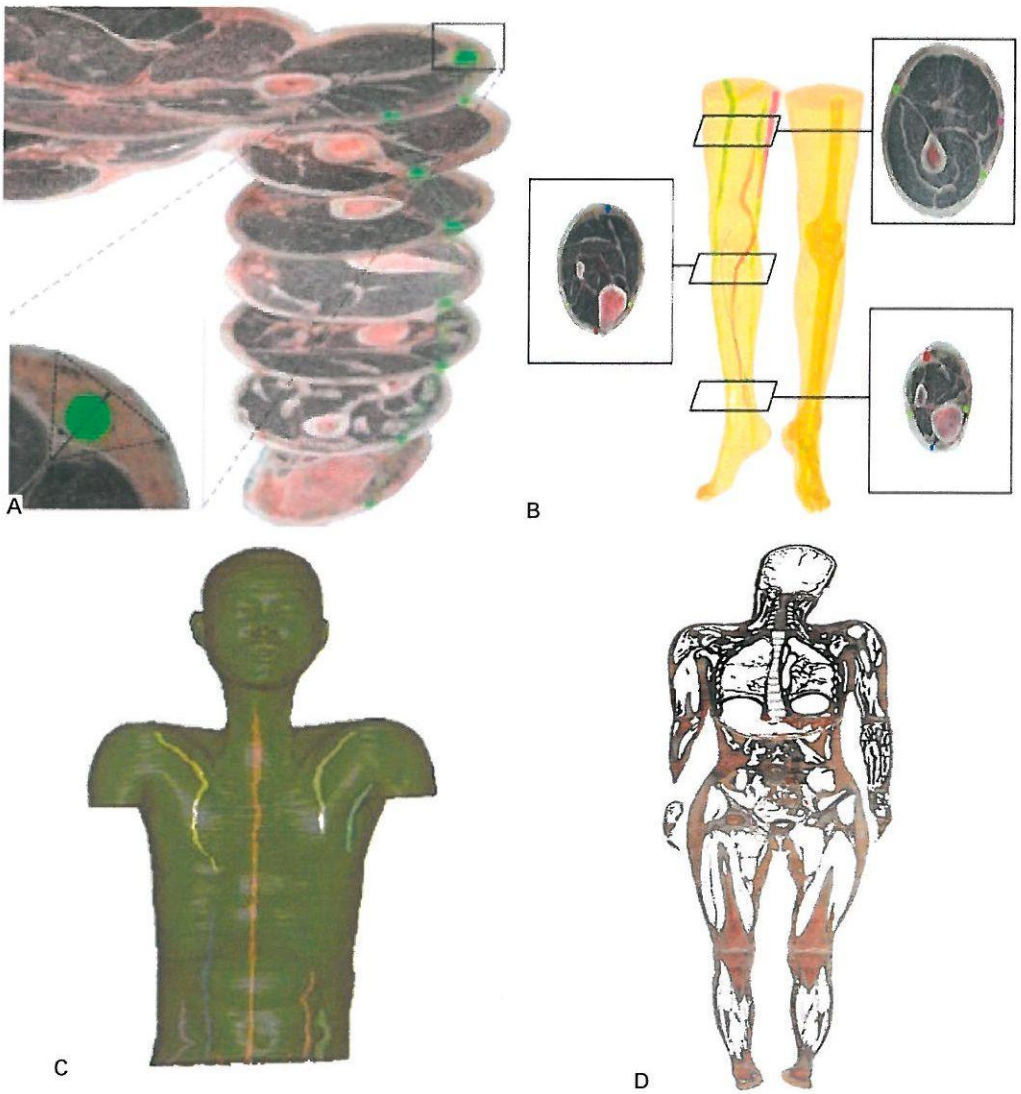

Fig.4 The connective tissues in VCH images are marked and their 3-D structures are rendered (A to C). When fascia connective tissues of the whole body are marked and their 3-D structures are rebuilt, the complete fascia network is generated (D).

fascial connective tissues were found to distribute in a pattern very close to that of meridians and collaterals. These areas containing rich fascial connective tissues constitute a linear structure, and colocalized with the meridians in TCM. On the other hand, "Deqi" (a sore and numb feeling at acupoints) was found, by means of dynamic ultrasound, to occur only when a needle penetrated or stimulated the connective tissue of the fascia [7]. Histologically, the meridians are thought to be composed of non-specific connective tissues, including loose connective tissues and fat tissues [8, 11]. Therefore we propose that the effective sites of acupuncture should be the fascial connective tissues, including the cells and tissues inside these sites, such as the nerve

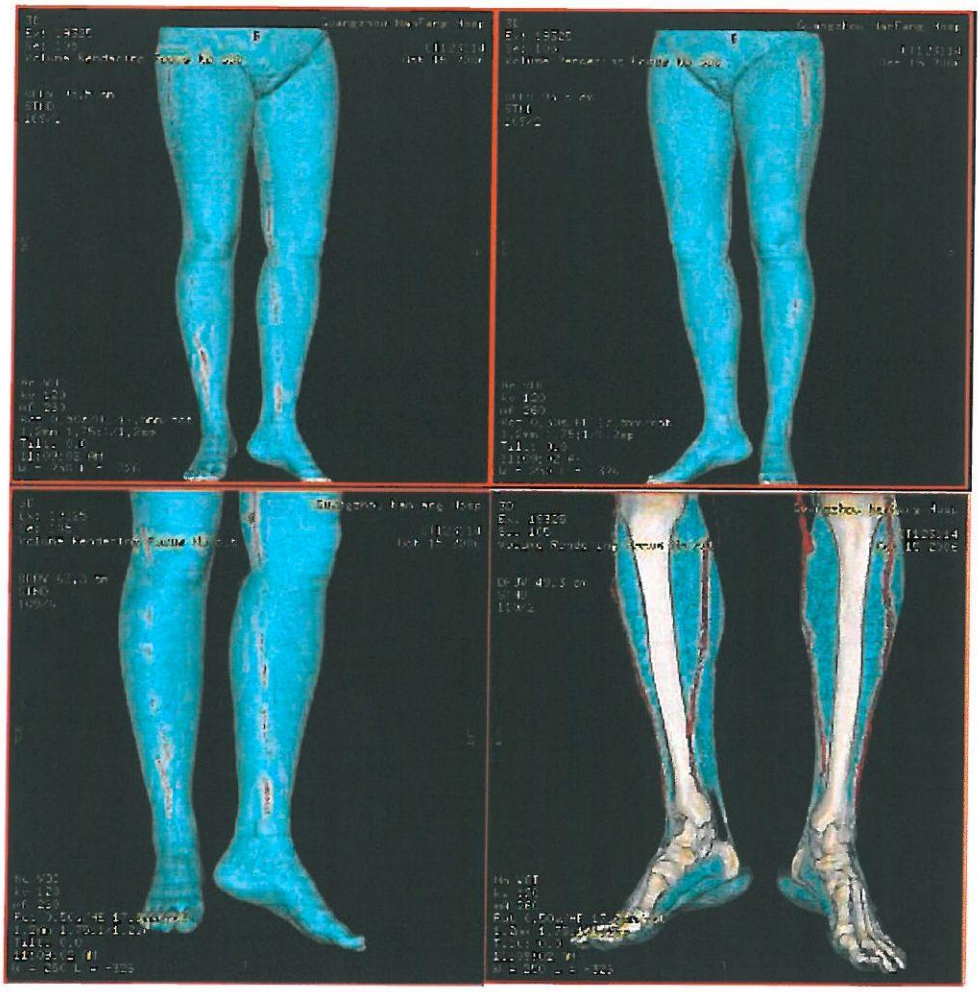

Fig.5 Reconstruction of the 3-D structures of the areas containing rich connective tissues based on the CT images of the lower limbs.

endings, capillaries, fibroblasts, undifferentiated mesenchymal cells, lymphocytes, etc.

In this work we combined the anatomical approach of fasciology with an electrophysiological method for mapping the connective tissue areas related to the meridians. Conductance Density Mapping© method can be conveniently combined with the VCH research and also with the CT- and MRI-based research methods for investigating the physiological nature of meridians and acupoints. We demonstrated that the meridian- and non-meridian-related subdermal connective tissues had significantly different electrophysiological properties. Similar results were documented in earlier reports [13,25], but comparative and clearly reproducible results on the decay time property of responses have not been reported. Analysis of the decay or attenuation properties of the connective tissue resistance responses reveals a fundamental difference in the tissue electrodynamics between the fascial network that builds up the

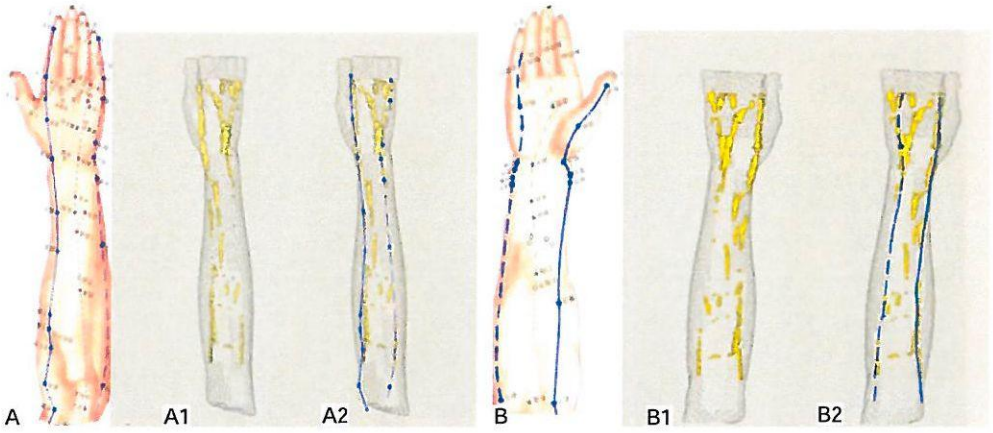

Fig.6 Based on MRI images of upper limbs, the constructed connective tissue strings (A1, B1) are compared with blue Large Intestine meridian of Hand-yangming (A, A2) and Lung meridian of Hand-taiyin (B, B2). The constructed connective tissue strings are rendered yellow and the traditional meridians are shown in blue.

Tab.2 Electric conductance profile in the meridian and non-meridian regions recorded from the 7 healthy volunteers (Mean±SE)

Region	Peak Conductance Voll value	Peak Resistance kΩ	Half Peak Decay Time (sec)
Meridian	37.8±11.7	255±66	3.2±1.5
Non Meridian	19.8±3.7	497.6±113.2	1.8±0.8

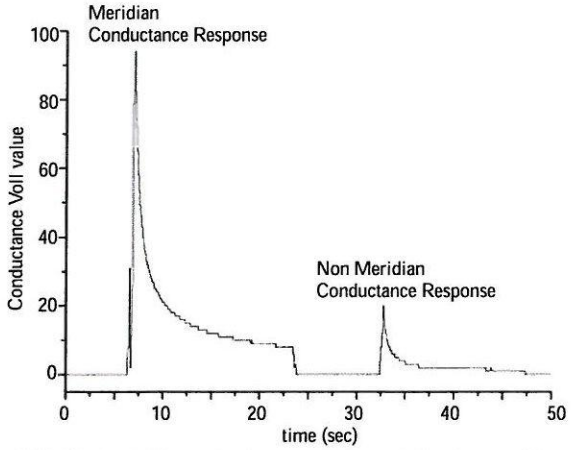

Fig.7 Typical DC conductance response of Sanjiao meridian compared to the nearby non-meridian connective tissue area. The peak of the conductance response is significantly higher at the Sanjiao meridian compared to the non-meridian area.

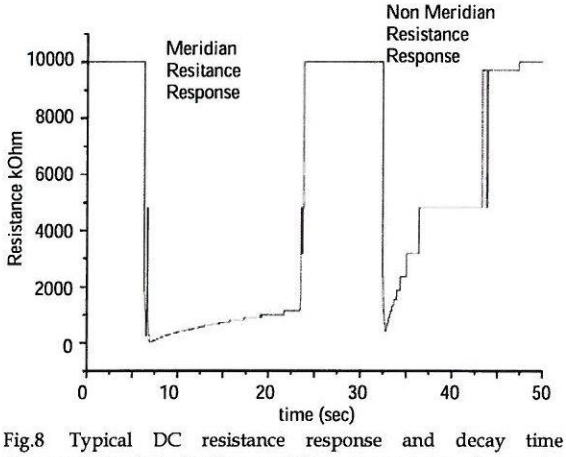

Fig.8 Typical DC resistance response and decay time characteristic of the Sanjiao meridian, compared to the nearby non-meridian connective tissue area. The peak amplitude and the decay time are significantly different between the Sanjiao meridian and the non-meridian area.

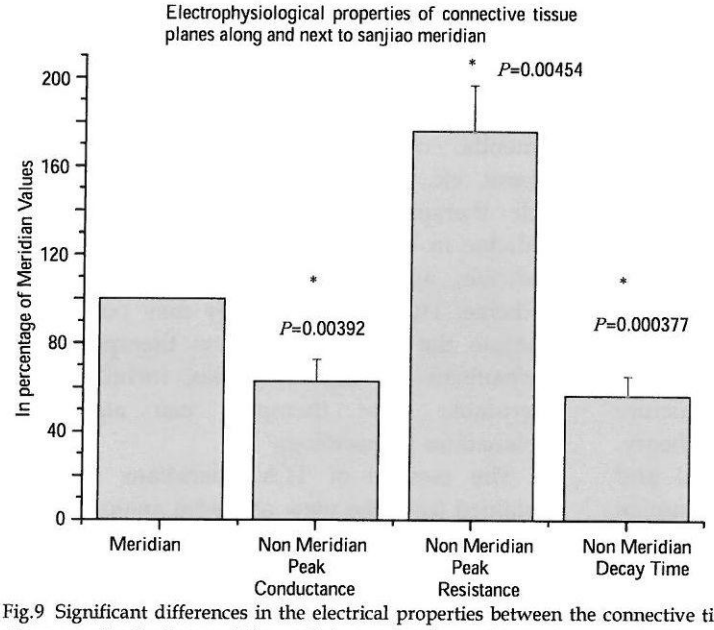

Fig.9 Significant differences in the electrical properties between the connective tissue plane near the Sanjiao meridian and the surrounding subdermal connective tissue (n= 7, ANOVA).

acupuncture meridians and the non-meridian areas. This aspect of fascial physiology can be a new approach to meridianology research and also to the research of fascial responsiveness properties.

Our results show that connective tissues permeate the whole body and form a network. The acupoints are located in the connective tissues either between the muscles or between the muscle and bones [26-27]. The fascia has a self-regulating function to adapt to the body pressure, and massage therapy can affect its tension, viscoelasticity and structure [28-30]. It is also responsible for maintaining the structural integrity of the body. The fascia connective tissue is rich in blood vessels, nerves, lymph, and provides the matrix that allows for intercellular communication. It may play an important role in surveillance and regulation of the body [31-32].

From an evolutionary point of view, the fascia network is homologous with the extracellular matrix of a single-germ-layer organism, the middle lamella of a two-germ-layer organism, and the mesenchyma of a three-germ-layer organism. The components of an organism during each evolutionary period can be summarized into two major systems (Fig.1), namely the supporting and storing system containing adipose and loose connective tissues, and the functional system constituted by specialized cells. In other words, the fascial network, mesenchyme and extracellular fluid (ECF) are homologous, and their common function is to maintain the stability of the internal environment of an organism. The mesoderm further evolves into organs and systems with specific functions, including the locomotor system, urinary system, reproductive system, circulatory system, and so forth [33]. The residues of the mesenchyme then differentiate into connective tissues which are distributed throughout the body. This theory is the basis of fascial anatomy.

Here two new terms must be defined: (i) Fascial anatomy. According to fascial anatomy, each living organism is composed of two major systems, one is the supporting-storing system constituted by the network of unspecialized connective tissues, and the other is the functional system containing the organs and tissues that are surrounded by the supporting-storing system. (ii) Fasciology. This term refers to the study of the supporting-storing system and the relationships between the supporting-storing system and the functional system in a living organism [34-35]. The theory of fascial anatomy and fasciology highlight the significance of fascial research, which is important not only in the field of TCM, but also in other biomedical research and clinical therapies. Some TCM theories may

also find scientific interpretations in fasciology.

In the theory of Fasciology, the functional system does not refer to the conventionally established nine major systems in systemic anatomy; rather, it refers to a system consisting of all the cells, tissues and organs encapsulated and segmented by the supporting-storing system. The functional system contains a variety of committed stem cells and functional cells. These cells are different from the mesoderm and folded endoderm and/or ectoderm cells [25]. The functional cells work together to fulfill living activities. Fascial anatomy studies the structure of an organism based on the two-system theory. Different from the conventional regional and systematic anatomy, which studies human structures in terms of their morphologies and functions, fascial anatomy integrates a third dimension, time (Fig.4). It studies not only the structures and functions of the body, but also the morphological transformation during evolution and embryonic development. Therefore, fascial anatomy helps scientists better understand the biological essence of an organism, because it reminds scientists to study anatomy from a "living" perspective, in which all cells and organs maintain their structures and functions. In other words, fascial anatomy switches anatomical study from "dead" to "living". When the supporting-storing system wears out, the body will die.

Fasciology studies the supporting-storing system in light of its influences on the functional system, differentiation of mesenchymal stem cells into functional cells, and cell supplementation of the functional system. Fasciology also studies the role of the supporting-storing system in the living human body and the interactions between the two major systems, which form the basis of the mechanisms of acupuncture and TCM, holistic therapies, integrative medicine and alternative or complementary medicine. The research methods of fasciology include developmental biology analysis, non-specific connective tissue function research, stem cell research, cytobiology, molecular biology, electrophysiology and TCM theory research. Fasciology also studies the curative property of mesenchymal stem cells on certain diseases, such as senile dementia, cancer, osteoporosis, degenerative disease, etc., and explores the mechanisms of such therapies as acupuncture and herbal medicine in TCM, holistic therapies, integrative medicine, and alternative or complementary medicine. Therefore, fasciology may potentially promote the discovery of new therapies. The mechanisms of some therapies, including the inscrutable TCM therapies, can also find explanations in fasciology [18].

The essence of TCM meridians may be explained from the view of fascial anatomy. The anatomical basis of meridians is the fascia network throughout the body, and the histological composition of meridians is non-specific connective tissues, including loose connective tissue and fat tissue. The histological structure where an acupuncture needle stimulates is the fascial connective tissue [7]. Acupuncture points are the sites that produce strong biological reactions when stimulated. Since fascia connective tissue is distributed throughout body, acupoints may exist in every part of the body [12, 30]. Moreover, we believe that Gua Sha therapy is also targeted at the dermal dense connective tissue all over the body. The difference between the acupoints and non-acuppoints, as well as between main acupoints and supplementary acupoints, is more of the intensity of biological reactions than of structural components. This is confirmed by our electrophysiological results that showed a prominently different electrodynamical nature of the subdermal connective tissue under acupoints and non acupoints. Early studies by R. Voll and Nakatani (Nakatani 1958) have demonstrated that tissue conductance recorded from or above the degenerative tissues is not sustainable and quickly decays. In modern electrophysiology, it can be explained by the changes in ion channel composition and time-constant characteristic and also by the changes

in the molecular composition and quality of interstitial matrix under degenerative conditions. Our research shows that meridian and acupoint-related connectives tissue show stronger responsiveness and more sustainable resistance characteristics than their surrounding tissues. This electrodynamical nature of acupoints and meridians is consistent with the concept of fasciology, according to which the connective tissue under acupoints and meridians possess stronger regenerative potentials.

Since the non-specific connective tissue is the anatomical basis of meridians and acupuncture, and the connective tissue network is distributed throughout the body, the targets of acupuncture therapy may exist all over the body [2-3, 5]. Acupoints in the human body are the sites that can generate significant response when stimulated. Non-meridian extra acupoints are the areas rich in fascial connective tissues, and they are located outside the meridians. The histological composition of the target areas of acupuncture can be classified into 5 types, namely the dermal dense connective tissue, subcutaneous loose connective tissue, loose connective tissue in the intermuscular septum, and loose connective tissue around the neurovascular tract, and loose connective tissue in the hilus and tunica of the visceral organs.

The location and depth of these areas are different in all individuals, as is sensitivity, hence the therapeutic effects vary [4, 27, 36]. The ancient records of meridians and acupoints as well as herbal medicine of TCM lack a scientific basis. Fascial research may provide the evidence for the accuracy of acupoints.

CONCLUSIONS

The anatomical basis of acupoints and meridians is the fascial network throughout the body. The histological composition of meridians is non-specific connective tissues, including loose connective tissue and fat tissue. Acupoints are the sites that produce strong biological reactions when stimulated. The non-specific connective tissue network is proposed as the supporting-storing system in the hypotheses of fascial anatomy and fasciology. These hypotheses involve researches of the mechanisms of acupuncture and TCM, evolutionary biology, holistic therapies, integrative medicine, and alternative/complementary medicine.

ACKNOWLEDGEMENTS

This work was supported by the National 973 Program for Basic Research Development (2007CB512705), National Natural Science Foundation of China (30801464). We are grateful to Anna Palhalmine Mezo for editing the manuscript and support in the physiological experiments.

REFERENCES

[1] Birch SJ, Felt RL. The theoretical basis of acupuncture: fundamental concepts and explanatory models. In: Understanding Acupuncture [M]. Edinburgh and New York: Churchill Livingstone, 1999.

[2] Bensoussan A The nature of the meridians. In: The vital meridian: a modern exploration of acupuncture [M]. Melbourne and New York: Churchill Livingstone, 1991.

[3] Sutherland JA. Meridian therapy: current research and implications for critical care [J]. AACN Clin Issues, 2000, 11: 97-104.

[4] Board of Science and Education, British Medical Association. The evidence base of acupuncture. In: Acupuncture: efficacy, safety and practice [M]. Amsterdam: Harwood Academia Publishers, 2000.

[5] Bossy J. Morphological data concerning the acupuncture points and channel network [J]. Acupunct Electrother Res, 1984, 9: 79-106.

[6] Dung HC. Anatomical features contributing to the formation of acupuncture points [J]. Am J Acupunct, 1984, 12: 139-43.

[7] Ciczek LSW, Szopinski J, Skrzypulec V. Investigations of morphological structures of acupuncture points and meridians [J]. J Trad Chin Med,1985, 5: 289-92.

[8] Ma W, Tong H, Xu W, et al. Perivascular space: possible anatomical substrate for the meridian [J]. J Altern Complement Med, 2003, 9: 851-9.

[9] Hashimoto PH. The perineurial vessel: a possible candidate for the structural basis of the meridian (Jing-Luo) in Chinese medicine [J]. Anat Sci Int, 2005, 80: 177-80.
[10] Yung KT. Birdcage model for the Chinese meridian system: part VI. Meridians as the primary regulatory system [J]. Am J Chin Med, 2005, 33: 759-66.
[11] Ho MW, Knight DP. The acupuncture system and the liquid crystalline collagen fibers of the connective tissues [J]. Am J Chin Med, 1998, 26: 251-63.
[12] Langevin HM, Churchill DL, Wu J, et al. Evidence of connective tissue involvement in acupuncture [J]. FASEB, 2002, 16: 872-4.
[13] Ahn AC, Wu J, Badger GJ, et al. Electrical impedance along connective tissue planes associated with acupuncture meridians [J]. BMC Complement Altern Med, 2005, 5: 10.
[14] Konofagou EE, Langevin HM. Using ultrasound to understand acupuncture. Acupuncture needle manipulation and its effect on connective tissue [J]. IEEE Eng Med Biol Mag, 2005, 24: 41-6.
[15] Omura Y, Takeshige C, Shimotsuura Y, et al. Imaging of the stomach, and localization of the stomach meridian & its acupuncture points in a human cadaver by the use of the indirect "Bi-Digital O-Ring Test Imaging Techuique" [J]. Acupunct Electrother Res, 1988, 13: 153-64.
[16] Ackerman MJ, Spitzer VM, Scherzinger AL. The Visible Human data set: an image resource for anatomical visualization [J]. Medinfo, 1995, 8: 1195-8.
[17] Moore KL, Agur AMR. Introduction to clinical anatomy. In: Essential clinical anatomy. New York: Lippincott Williams & Wilkinsm, 1995.
[18] Caon M. Voxel-based computational models of real human anatomy: a review [J]. Radiat Environ Biophys, 2004, 42: 229-35.
[19] Huang Y, Yuan L, He ZQ, et al. Study on the meridians and acupoints based on fasciology: an elicitation of the study on digital human being [J]. Zhongguo Zhen Jiu, 2006, 26: 785-8.
[20] Robb RA. 3-D visualization in biomedical applications[J]. Annu Rev Biomed Eng, 1999, 1: 377-99.
[21] Zhong SZ, Yuan L, Tang L, et al. Research report of experimental database establishment of digitized virtual Chinese No.1 female [J]. J First Mil Med Univ, 2003, 23: 196-200.
[22] Yuan L, Tang L, Huang WH. Construction of dataset for virtual chinese male No.1 [J]. J First Mil Med Univ, 2003, 23: 520-3.
[23] Wang XY, Zhong C. The morphological basis of mesotherapy [J]. Chin J Aesth Plast Surg, 2007, 18: 166-8.
[24] Wang CL, Yuan L, Wang J, et al. Contrast study on the line course of fascia meridians made by three dimensional reconstruction and classical meridians in human body [J]. Chin J Anat, 2007, 30: 340-3.
[25] Lee MS, Jeong SY, Lee YH, et al. Differences in Electrical Conduction Properties Between Meridians and Non-meridians[J]. Am J Chin Med, 2005, 33(5): 723-8.
[26] Wang CL, Wu JP, Wang J. An interpretation on the essence of meridians and acupuncture mechanism from fasciology view [J]. Chin J Basic Med Trad Chin Med, 2008, 14: 312-4.
[27] Langevin HM, Churchill DL, Fox JR, et al. Biomechanical response to acupuncture needling in humans [J]. J Appl Physiol, 2001, 91: 2471-8.
[28] Scheip R. Fascial: plasticity -a new neurobiological explanation Part 2 [J]. J Bodyw Mov Ther, 2003, 7: 104-16.
[29] Myers Thomas W. Structural integration-developments in Ida Rolf's 'Recipe'-I [J]. J Bodyw Mov Ther, 2004, 8: 131-42.
[30] Swartz MA, Tschumperlin DJ, Kamm RD. Mechanical stress is communicated between different cell types to elicit matrix remodeling [J]. Proc Natl Acad Sci USA, 2001, 98: 6180-5.
[31] Scheip R, Klinger W, Lehmann-Horn F. Active fascial contractility: fascia may be able to contract in a smooth muscle-like manner and thereby influence musculoskeletal dynamics [J]. Med Hypotheses, 2005, 65: 273-7.
[32] Langevin HM, Churchill DL, Cipolla MJ. Mechanical signaling through connective tissue: A mechanism for the therapeutic effect of acupuncture [J]. FASEB J, 2001, 15: 2275-82.
[33] Ross MH, Pawlina W. Connective Tissue. In: Histology. A textbook and atlas [M]. Baltimore: Lippincott Williams & Wilkins, 2005.
[34] Yuan L, Wang J, Wang CL, et al. Fasciology: a new theory on the human self-supervision and control system [J]. Sci Tech Rev, 2006, 24: 85-9.
[35] Yuan L, Zhong SZ. Human auto-testing and modulating system—anatomic basis involved in meridianal system [J]. Tianjin J Trad Chin Med, 2004, 21: 356-9.
[36] Langevin HM, Yandow JA. Relationship of acupuncture points and meridians to connective tissue planes [J]. Anat Rec (New Anat), 2002, 269: 257-65.

Original Article

Modulatory effect of acupuncture on extracellular signal-related kinase 1/2 and p38 mitogen-activated protein kinase signaling pathways in rat subcutaneous fascia

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ABSTRACT

Objective To study the changes of expressions of ERK1/2 and p38 in the subcutaneous connective tissue after acupuncture treatments and investigate the molecular biological reactivity of subcutaneous fascia in different body regions overlapping the meridian and non-meridian lines. ***Methods*** Twenty Sprague-Dawley rats were randomly divided into 4 groups. The rats in the control group received no treatment. The rats in the other 3 groups received manual acupuncture at acupoint ST36, electro-acupuncture at acupoint ST36, or manual acupuncture at a non-acupoint (inguinal fat pad). Ten days after the treatments, the rats were sacrificed and the tissues, including the fascial and muscular tissues at the acupuncture sites, were collected for immunohistochemical (IHC) staining and Western blotting to evaluate the expression of ERK1/2, phosphorylated ERK1/2 (p-ERK1/2), p38 and phosphorylated p38 (p-p38). ***Results*** IHC staining showed an increase in the positive ERK1/2 signals in all the 3 acupuncture groups, among which the non-acupoint acupuncture group showed the strongest signals. p38-positive signals increased significantly only in the non-acupoint group. Western blotting revealed significantly increased expressions of ERK1/2, p-ERK1/2 and p-p38 in the all 3 acupuncture groups, and the expression intensity was the strongest in the non-acupoint acupuncture group and the least in the ST36 acupoint manual acupuncture group. ***Conclusions*** Acupuncture results in up-regulation of ERK1/2 and p38 in the subcutaneous fascia. Subcutaneous connective tissues in different regions show variations in the intensity of reactivity to mechanical or electrical needling stimulation. This indicates that needling manipulation of the fascial/connective tissue network evokes a local, extracellular matrix-initiated response, besides the common neurohormonal regulating effect of acupuncture.

Key words: acupuncture; extracellular signal-related kinase 1/2; p38 mitogen-activated protein kinase; subcutaneous fascia

INTRODUCTION

It has been suggested that the distribution of acupuncture points and meridians of the human body is closely related to the connective tissue network [1-3]. Subcutaneous fascia, a part of the loose connective tissue, is the site directly stimulated by the acupuncture needle. During the therapy, the needle closely contacts the collagen fibers of the connective tissue, and the latter transfer the

Received: 2011-05-09 Accepted: 2011-06-30
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mechanical signals to cause various biochemical and functional responses [4]. However, it is still unknown how the needling-induced extracellular signals are translated into intracellular responses.

Extracellularly regulated kinases (ERK) play a major role in transferring both mechanical and biophysical signals between the extracellular matrix and the intracellular microcompartment. According to our previous study [5], electro-acupuncture can promote ERK signal transduction in rat subcutaneous fascia. ERK1/2 and p38 mitogen-activated protein kinase (p38 MAPK) are also activated in the fibroblasts during collagen matrix contraction under isometric tension [6]. Activation of ERK1/2 is indispensable in cell proliferation and survival [7-9]. It has been reported that mechanical stimulation results in the activation of ERK 1/2 and p38 MAPK in various cells, including osteoblasts [10-12].

In this study, we aim to examine the biological effects of acupuncture on ERK1/2 and p38 MAPK signaling pathways in the subcutaneous fascia, and compare the effects of different acupuncture methods on the two signal pathways in different body regions related to different meridian lines.

MATERIALS AND METHODS

Materials

KeyGen protein extraction kit and KeyGen protein assay kit were purchased from Guangzhou Jetway Biotechnology, China. Mouse anti-p-ERK1/2 and anti-p-p38 monoclonal antibodies (mAbs), rabbit anti-ERK1/2 and anti-p38 polyclonal mAbs, Immobilon®-P polyvinylidene difluoride (PVDF) membranes, and enhanced chemiluminescence (ECL) detection kit were purchased from Santa Cruz Biotechnology, USA. Goat anti-rabbit and anti-mouse peroxidase-conjugated secondary mAbs were from Zhoushan Goldenbridge Biotechnology, China. Alkaline phosphatase-conjugated streptavidin- biotin complex (SABC-AP, rabbit IgG) and SABC- AP (mouse IgG) kits were provided by Wuhan Boster, China. Tissue homogenizer was from Fluko Company, Germany. Trans-blot semi-dry electrophoretic transfer cell was from BIO-RAD Laboratories, Inc., USA. All the other reagents used were of analytical grade.

Animals grouping and treatments

Twenty female Sprague-Dawley rats (SPF grade, Certificate No.24301050) weighing 180-220 g were provided by the Experimental Animal Center of Southern Medical University. The rats were randomized into 4 groups (n=5), namely the control group without treatment, acu-ST36 (Zusanli, the 36th acupoint of the Stomach Meridian of Foot-Yangming) group receiving mechanical acupuncture at the acupoint ST36 with twisting manipulation for 4 min (120 times per minute), ele-acu-ST36 group treated with electro-acupuncture at the acupoint ST36 at the frequency of 2 Hz (current intensity of 48 mA) for 20 min, and non-acupoint group receiving mechanical acupuncture at a non-acupoint (midpoint of the inguinal groove) with twisting manipulation for 4 min (120 times per minute). The treatments in every acupuncture group were performed once daily for a total of 10 days.

Paraffin sectioning

After anesthesia with 10% $CCl_3CH(OH)_2$, the rats were sacrificed by perfusion with 0.86% saline followed by 4% paraformadehyde (PFA) in 0.1 mol/L phosphate buffer (pH 7.4). The subcutaneous tissues and muscles surrounding the needling point (with an area 1 cm in diameter) were removed and fixed in 4% PFA overnight at 4 ℃. The tissues were dehydrated and embedded in paraffin for sectioning at a 4 mm thickness.

Immunohistochemical staining

The paraffin sections were rehydrated and treated with 3% H_2O_2 for 2 min in an icebox,

followed by microwave treatment. After rinsed in 0.01 mol/L phosphate buffered saline (PBS, pH 7.4), the sections were treated with citrate buffer for 15 min, followed again by microwave treatment. The sections were then allowed to cool down at room temperature and blocked with 1% bovine serum albumin (BSA) for 30 min. Then sections were incubated with either rabbit anti-ERK1/2 and rabbit anti-p38 mAbs (both at 1: 50), with microwave treatment for 2 min. After washing thoroughly in PBS, the sections were incubated with 1: 400 anti-rabbit secondary mAb for 2 h. Finally freshly prepared DAB solution was added to the sections for observation under microscope. The presence of yellow or brown granules in the cells indicated a positive staining.

Western blotting

After anesthesia, the rats were sacrificed and the subcutaneous tissues and muscles surrounding the needling point were removed and separated. The fresh fascia and muscle tissues were separately lysed in a buffer solution containing 4% phenylmethylsulfonyl fluoride. The lysates were incubated on ice for 10 min, vortexed for 45 s and kept on ice for another 10 min. After centrifugation, the supernatant was collected and the proteins were quantified using bicinchoninic acid method. Briefly, the lysate proteins diluted in 6× Laemmli sample buffer were loaded at 30 μg per lane, separated by sodium dodecyl sulfate- polyacrylamide gels containing 10% acrylamide, and electrotransferred to PVDF membranes. After blocking with 5% non-fat milk in TBST containing 50 mmol/L Tris, 200 mmol/L NaCl and 0.1% Tween-20 (pH 7.2-7.4), the membranes were incubated with anti-ERK1/2, anti-p38, anti-p- ERK1/2, and anti-p-p38 mAbs separately (all diluted at 1: 400 in TBST containing 5% BSA) at 4 ℃ overnight. After washing, the membranes were incubated separately with horse radish peroxidase- conjugated secondary mAbs (1: 400, diluted with TBST containing 5% BSA) for 2 h at room temperature. Finally, the blots were developed by the ECL kit with Kodak Image Station 2000MM System and digitalized with Image Tool 3.0 software. The expressions of ERK1/2, p-ERK1/2, p38 and p-p38 were presented as the ratios of their gray scales to that of β-actin.

Statistical analysis

All the data were analyzed with SPSS 13.0 software package. The data were presented as Mean±SD and analyzed using One-way ANOVA. Least Significant Differences (LSD) method was used for multiple comparisons if ANOVA gave a significant result. When heterogeneity of variance existed between the data groups, approximate F analysis Welch Methods was used to compare differences and Dunnett's T3 method was used to perform comparisons between multiple groups. A P value less than 0.05 was considered to indicate a statistical significance.

RESULTS

Immunohistochemical staining

In all the 4 groups, positive ERK1/2 signals were located mainly in the cytoplasm surrounding the nuclei of the fibroblasts and macrophages (Fig.1). The signals increased after acupuncture, especially in the fascia of the non-acupoint group. Positive p38 signals (weaker than the ERK1/2 signals) were also detected in the fascia of all the groups (Fig.2). A significant increase of p38 signals was found in the fascia of the non-acupoint group.

Expressions of ERK1/2 and p-ERK1/2

Compared with the control group, ERK1/2 and p-ERK1/2 expressions were enhanced significantly in all the acupuncture (experimental) groups (Fig.3 and 4). Their expressions in the fasciae were also significantly stronger than those

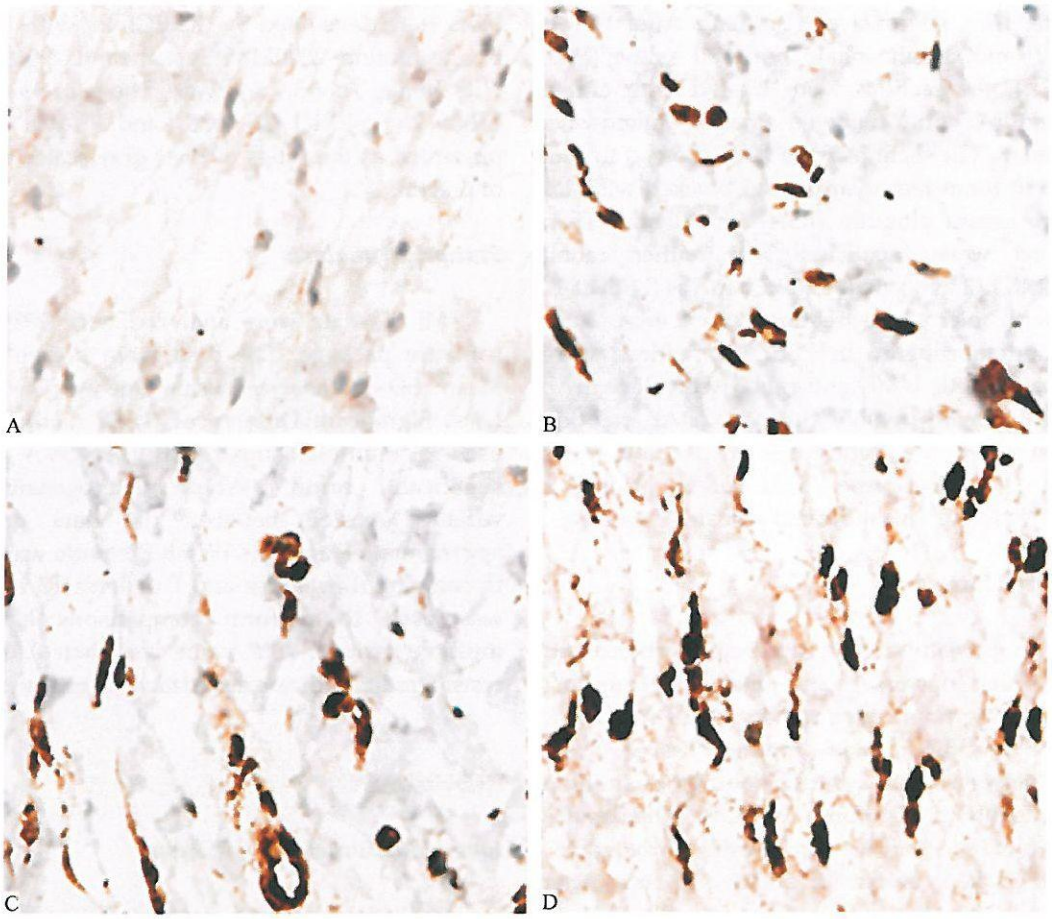


Fig.1 Immunohistochemical staining of ERK1/2 in the fascia in different groups (×400). A: Control group; B: Manual acupuncture at ST36 group; C: Electro-acupuncture at ST36 group; D: Manual acupuncture at non-acupoint. The number of positive cells increased after acupuncture in B, C and D groups (arrowhead).

in the muscular tissues ($P<0.001$). Among the acupuncture groups, the non-acupoint group showed the most obvious increase in the expression of ERK1/2 in both the fascia and muscular tissues, followed by ele-acu-ST36 group, and then by acu-ST36 group. p-ERK1/2 expressions showed a similar pattern among the groups (Fig.3 and 4).

Expressions of p38 and p-p38

The expression of p38 also increased significantly in both the fascia and muscles in all the experimental groups, when compared to the control group (Fig.5). The differences of p38 expressions between the fascia and muscles were significant in all the experimental groups ($P<0.001$). In both the fascia and muscle, p38 expressions increased the most obviously in the non-acupoint group, followed by ele-acu ST36 group, and then by acu-ST36 manual acupuncture group. The expression of p-p38 was not detected probably due to its weak expression in the fasciae and muscles.

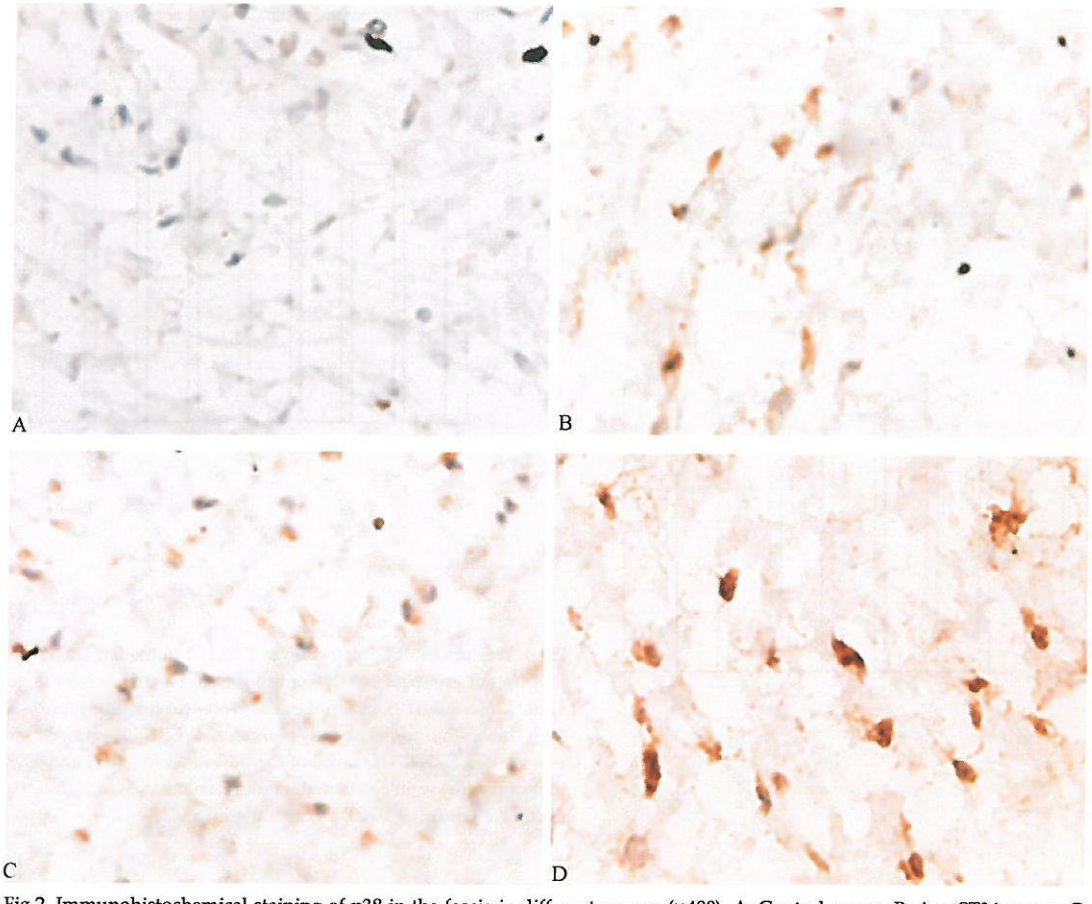


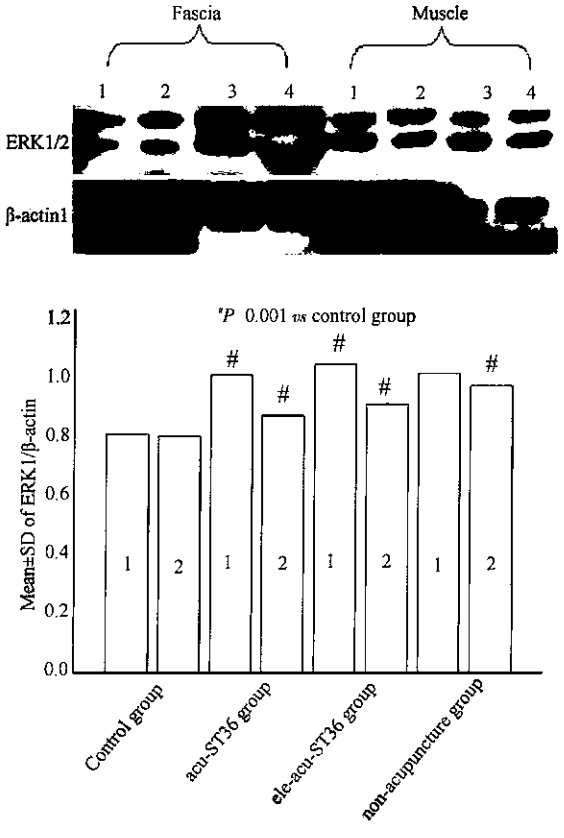
Fig.2 Immunohistochemical staining of p38 in the fascia in different groups (×400). A: Control group; B: Acu-ST36 group; C: Ele-acu-ST36 group; D: Non-acupuncture group.

DISCUSSION

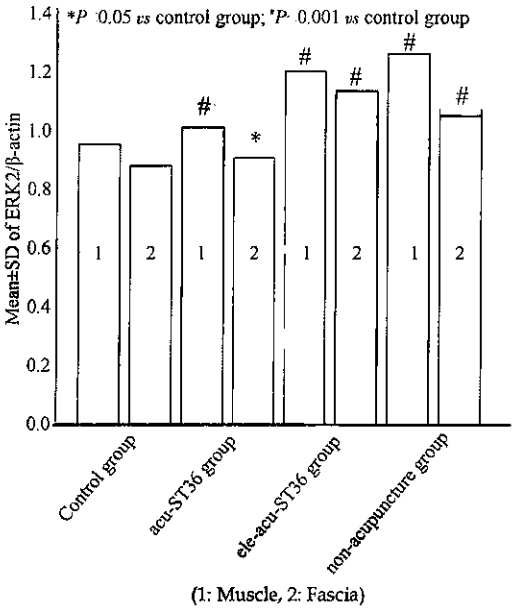
In this study, we investigated the molecular biological effects of acupuncture stimulation in the fascia and the surrounding muscular structures. This is different from the commonly used study models of acupuncture, which examine mainly the organ-specific effects of acupoint stimulation. We explored the general biological mechanism of acupuncture by observing the expressions of ERK1/2 and p38 MAPK signal pathway proteins, which represents a new approach to the study of the mechanism of acupuncture.

The members of the MAPK family, including ERK1/2, N-terminal Jun kinase (JNK1/2) and p38 MAPK, have been proposed as the fundamental signaling components linking extracellular stimuli to intracellular responses. ERK family proteins play a key role in transforming the mechanical stretch signal to the level of ligand-activated transcription factors in osteocytes and osteoblasts [13].

ERK1/2 isoforms are ubiquitously expressed and activated by dual-specific MAPK kinases (MAP or ERK kinase 1 and 2, MEK1/MEK2) in response to stimuli [6]. It has been demonstrated that fluid shear stress activates



(1: Muscle, 2: Fascia)



(1: Muscle, 2: Fascia)

Fig.3 Western blotting detection of ERK1/2 in the fascia and muscle in different groups. Lane 1: Control group; Lane 2: Acu-ST36 group; Lane 3: Ele-acu-ST36 group; Lane 4: Non-acupuncture group (A). In each lane, the upper band represented ERK 1 and the lower represented ERK2. The ratios of the intensities of ERK1 and ERK2 to that of β-actin were calculated, and presented as Mean+SD. *P<0.05 *vs* control group; *P<0.001 *vs* control group

ERK1/2 and low shear stress activates ERK1/2, JNK1/2 and p38 MAPK in human umbilical vein endothelial cells (HUVECs) [14-15]. The inhibition of ERK1/2 decreases shear stress-induced c-fos expression [16] and nuclear factor-κB (NF-κB) activity [17].

In this study, we demonstrated that in the control rats, the expression of ERK1/2 was slightly higher, whereas p-ERK1/2 expression was slightly lower in the fascia than in the muscles. Acupuncture stimulation caused a more obvious increase of p-ERK 1/2 expression in the fascia than in the muscles. This may be attributed to the fact that the acupuncture effect is produced by the mechanical coupling between the needle and the connective tissue to cause winding of the tissue fibers around the needle during needle rotation, but not by muscle contraction [4]. Both ERK1/2 and p-ERK1/2 participate in cell differentiation and proliferation, and play a role in the maintenance of normal metabolism of an organism [18]. Further study is still needed to fully understand the biological significance of the up-regulation of ERK1/2 and p-ERK1/2 in the muscles, which may provide important insights into the mechanism of the therapeutic effect of acupuncture.

We found that among all the acupuncture groups, the non-acupoint group showed the most obvious increase in ERK1/2 and p-ERK1/2 expression in both the fascia and muscle tissues. p38 MAPK is activated both by stress (such as

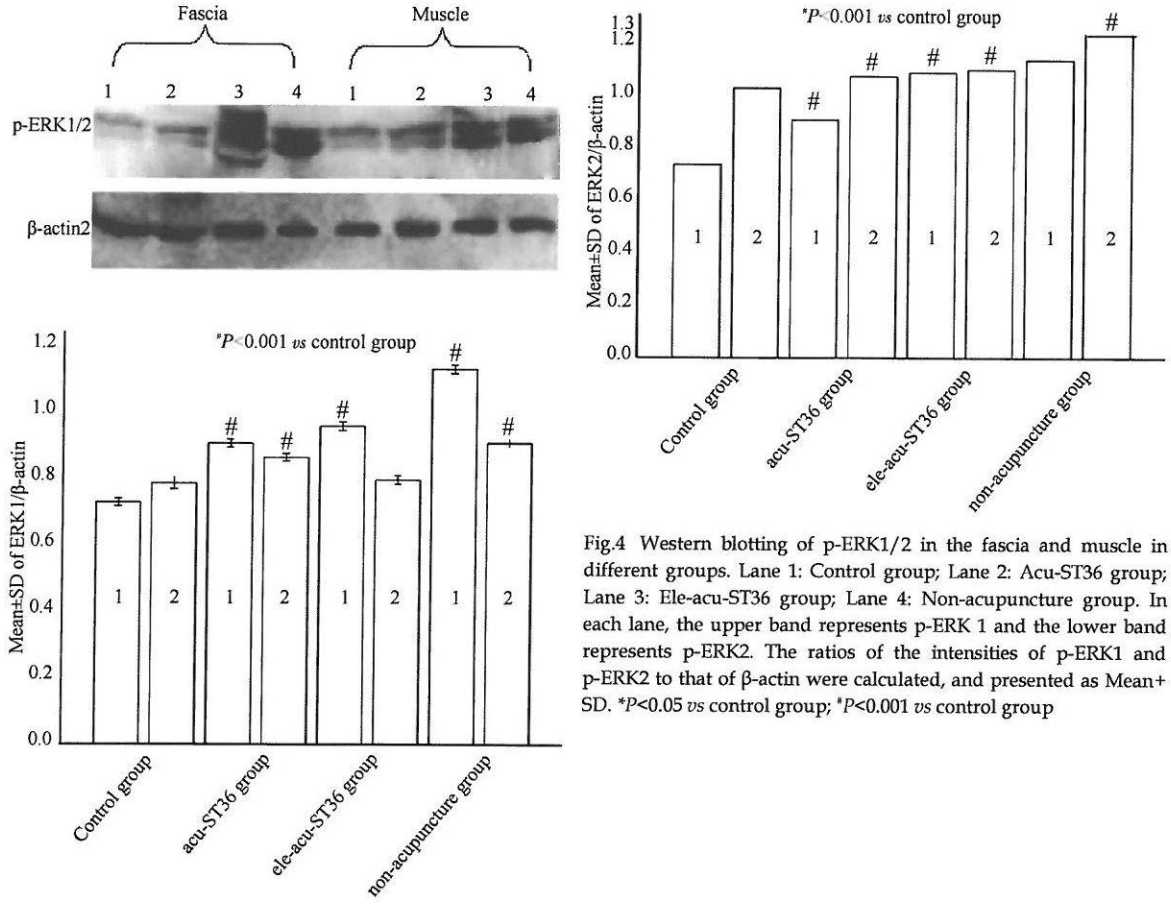


Fig.4 Western blotting of p-ERK1/2 in the fascia and muscle in different groups. Lane 1: Control group; Lane 2: Acu-ST36 group; Lane 3: Ele-acu-ST36 group; Lane 4: Non-acupuncture group. In each lane, the upper band represents p-ERK 1 and the lower band represents p-ERK2. The ratios of the intensities of p-ERK1 and p-ERK2 to that of β-actin were calculated, and presented as Mean+SD. *P<0.05 *vs* control group; #P<0.001 *vs* control group

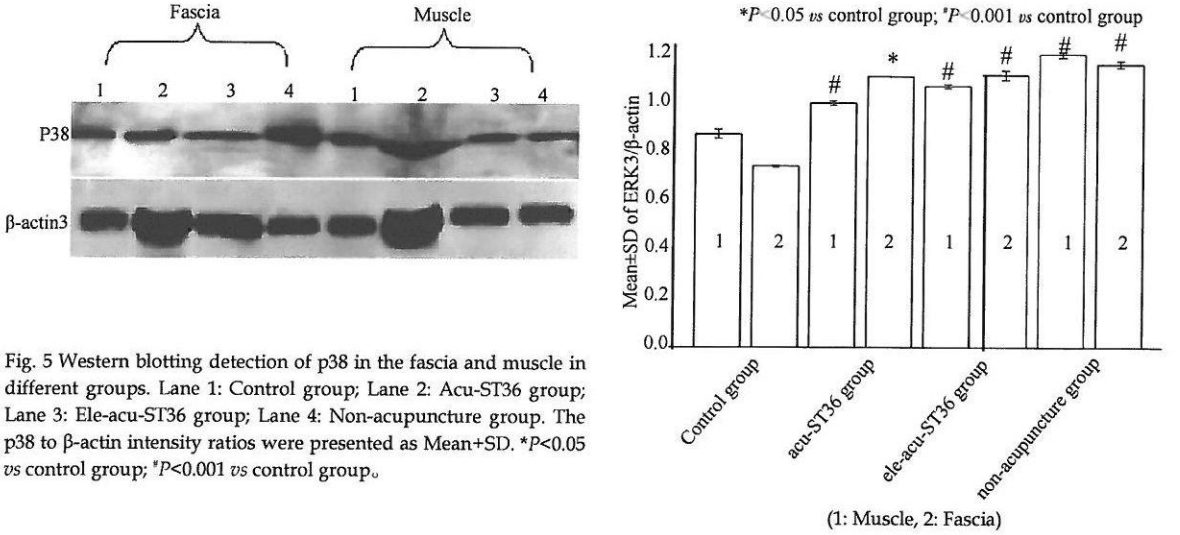


Fig. 5 Western blotting detection of p38 in the fascia and muscle in different groups. Lane 1: Control group; Lane 2: Acu-ST36 group; Lane 3: Ele-acu-ST36 group; Lane 4: Non-acupuncture group. The p38 to β-actin intensity ratios were presented as Mean+SD. *P<0.05 *vs* control group; #P<0.001 *vs* control group。

osmotic pressure, heat shock and ultraviolet radiation) and by chemical mediators (such as lipopolysacharides, tumor necrosis factor-α, and interleukin-1). Activated p38 MAPK has been shown to regulate the generation of inflammatory cytokines [19-20]. In the present work, the increase of p38 MAPK in both tissues was the most obvious in the non-acupoint. Dissection of the non-acupoint and ST36 acupoint areas in rats revealed much richer subcutaneous fascia and fat pads in the non-acupoint (inguinal groove region) than in the ST36 region. A twisting needle in the inguinal region can stimulate a greater extent of connective tissues and needs a stronger pull-out strength than that in the ST36 acupoint. This may explain the reason for the most significant upregulation of ERK1/2, p-ERK1/2 and p38 expressions in the non-acupoint group. Another possible explanation is that there are regional differences in the connective tissue reactivity to needle-induced trauma. This is why the following experiments are necessary to compare the tissue response of histologically similar body regions located at the meridian and non-meridian lines. The expression of p-p38 was not detected in the samples possibly because of limitations of the experimental conditions.

As a mechanical therapy, acupuncture produces shear stress or mechanical stretch at the cellular level in the fascia and muscle tissues, suggesting the possible roles of ERK1/2, p-ERK1/2, p38 and p-p38 in the effect of acupuncture on the fascia and muscle. It has been previously found that the activity of ERK1/2, JNK and P38 MAPK increased following low shear stress treatment; inhibition of ERK1/2, JNK1/2 and p38 MAPK with PD98059, SP600125 and SB203580, respectively, led to the suppression of the shear stress-induced IL-8 gene expression [21]. It is interesting to notice that directed ion movement evoked by electric acupuncture caused more intensive ERK signal elevation than simple mechanic acupuncture.

The fascia and connective tissues exist throughout the human body and form a 3-dimensional intercellular matrix for support, continuity, and communication. According to Yuan Lin and his colleagues [22], after the mesoblastic mesenchyme differentiates into the functional organs and systems, the rest of the mesenchyme forms the fascia and connective network. The latter provides cellular storage and supply functions for further reproduction of the functional cells, and participates in the regulation of the functional status of the body. However, it is still unknown how the fascia network regulates the physiopathological conditions of the body [23].

Langevin and his colleagues [24] suggested that fibroblasts of the connective tissue form an extensive interconnected cellular network, and communicate with each other through close apposition. It is reasonable to hypothesize that the effect of fascia on the immunity, blood vessels and internal organs is different from that of the nervous system. Furthermore, MAPK cell signaling pathway is present in almost all the eukaryotic cells, and serves as the primal intercellular communication pathway. We hypothesize that acupuncture affects the cellular morphology of the fascia connective tissue [25] to cause alterations in the expressions of MAPKs signal proteins such as ERK1/2 and P38, hence affecting the biochemical profiles and finally the functional status of the organism. We found that acupuncture can promote the expression of ERK1/2 and P38, which suggests a new perspective in the understanding how mechanical acupuncture signals in the fascia are transferred.

Understanding of the regulatory role of fascia connective tissue in the physiologic and pathologic states of the body and the mechanism of signal transduction of MAPK from a biological viewpoint can be of vital importance. Questions, including how the changes of the signal proteins are conducted in the fascia connective tissue, and whether this paracrine communication within the fascia can spread, remain to be answered.

CONCLUSIONS

Acupuncture needling results in up- regulation

of ERK1/2 and p38 in the subcutaneous fascia, which is possibly correlated to the pathway of MAPK signal transduction. This indicates that acupuncture may rely on another regulating system, the general fascia connective tissue network, besides the common neuro- hormonal regulating network.

ACKNOWLEDGEMENTS

This work is supported by National973 Program for Basic Research development (2007CB512705) and National Natural Science Foundation of China (30801464).

REFERENCES

[1] Fei L, Cheng HS, Cai DH, et al. Study on the basis structure of Meridians and Channels and its functional characteristics [J].Chin Sci Bull, 1998, 43: 658-72.

[2] Yuan L, Jiao PF, Tang L, et al. Anatomical Basic of Traditional Chinese Medical Channels [J]. Chin Bas Sci, 2005, 3: 44-8.

[3] Langevin HM. Biomechanical response to acupuncture needling in humans [J]. J Appl Physiol, 2001, 91(6): 2471-8.

[4] Langevin HM, Churchill DL, Cipolia MJ. Mechanical signaling through connective tissue: a mechanism for the therapeutic effect of acupuncture [J]. FASEB J, 2001, 15(12): 2275-82.

[5] Jiang XM, Yang C, Yuan L, et al. Expression of extracellular signal-regulated protein kinases in the subcutaneous fascia of rats and their changes after acupuncture [J]. J South Med Univ, 2009, 29(4): 623-6.

[6] Werry TD, Christopoulos A, Sexton PM. Mechanisms of ERK1/2 regulation by seven-transmembrane-domain receptors [J]. Curr Pharm Des, 2006, 12(14): 1683-702.

[7] Boulton TG, Nye SH, Robbins DJ, et al. ERKs: A family of protein-serine/threonine kinases that are activated and tyrosine phosphorylated inresponse to insulin and NGF [J]. Cell, 1991, 65(4): 663-75.

[8] Guyton KZ, Liu Y, Gorospe M, et al. Activation of mitogen-activated protein kinase by H_2O_2. Role in cell survival following oxidant injury [J]. J Biol Chem, 1996, 271 (8): 4138-42.

[9] Whelchel A, Evans J, Posada J. Inhibition of ERK activation attenuates endothelin-stimulated airway smooth muscle cell proliferation [J]. Am J Respir Cell Mol Biol, 1997, 16(5): 589-96.

[10] Inoue D, Kido S, Matsumoto T. Transcriptional induction of FosB/DeltaFosB gene by mechanical stress in osteoblasts [J]. J Biol Chem, 2004, 279(48): 49795-49795803.

[11] Kumar A, Knox AJ, Boriek AM. CCAAT/enhancer-binding protein and activator protein-1 transcription factors regulate the expression of interleukin-8 through the mitogen- activated protein kinase pathways in response to mechanical stretch of human airway smooth muscle cells [J]. J Biol Chem, 2003, 278(21): 18868-76.

[12] Yuge L, Okubo A, Miyashita T, et al. Physical stress by magnetic force accelerates differentiation of human osteoblasts [J]. Biochem Biophys Res Commun, 2003, 311(1): 32-8.

[13] J. Ignacio Aguirre, Lilian I. Plotkin, Arancha R, et al. A novel ligand-independent function of the estrogen receptor is essential for osteocyte and osteoblast mechano-transduction [J]. J Biol Chem, 2007, 282(35): 25501-8.

[14] Jo H, Sipos K, Go YM, et al. Differential effect of shear stress on extracellular signal-regulated kinase and N-terminal Jun kinase in endothelial cells. Gi2- and Gbeta/gamma-dependent signaling pathways [J]. J Biol Chem, 1997, 272(2): 1395-401.

[15] Cheng M, Wu J, Li Y, et al. Activation of MAPK participates in low shear stress-induced IL-8 gene expression in endothelial cells [J].Clin Biomech, 2008, 23Suppl 1: S96-S103.

[16] Bao X, Clark CB, Frangos JA. Temporal gradient in shear-induced signaling pathway: involvement of MAP kinase, c-fos, and connexin43 [J]. Am J Physiol Heart Circ Physiol, 2000, 278(5): H1598-H1605.

[17] Davis ME, Grumbach IM, Fukai T, et al. Shear stress regulates endothelial nitric-oxide synthase promoter activity through nuclear factor kappaB binding [J]. J Biol Chem, 2004, 279(1): 163-8.

[18] Lee DJ, Rosenfeldt H, Grinnell F. Activation of ERK and p38 MAP kinases in human fibroblasts during collagen matrix contraction [J]. Exp Cell Res, 2000, 257(1): 190-7.

[19] Kaminska B. MAPK signalling pathways as molecular targets for anti-inflammatory therapy - from molecular mechanisms to therapeutic benefits [J]. Biochim Biophys Acta, 2005, 1754(1-2): 253-62.

[20] McCubrey JA, Lahair MM, Franklin RA. Reactive oxygen species-induced activation of the MAP kinase signaling pathways [J]. Antioxid Redox Signal, 2006, 8(9-10): 1775-89.

[21] Cheng M, Wu J, Li Y, et al. Activation of MAPK participates in low shear stress-induced IL-8 gene expression in endothelial cells [J]. Clin Biomech, 2008, 23(Suppl 1): S96-S103.

[22] Yuan L, Wang J, Wang CL, et al. Fasciology: A new theory on the human self-supervision and control system [J]. Sci Tech Rev, 2006, 216(24): 85-9.

[23] Bai Y, Yuan L, Soh KS, et al. Possible applications for fascial anatomy and fasciaology in traditional Chinese medicine [J]. J Acupuncture Meridian Stud, 2010, 3(2): 125-32.

[24] Langevin HM, Cornbrooks CJ, Taatjes DJ. Fibroblasts form a body-wide cellular network [J]. Histochem Cell Biol, 2004, 122(1): 7-12.

[25] Langevin HM, Boaffard NA, Badger GJ, et al. Dynamic fibroblast cytoskeletal response to subcutaneous tissue stretch ex-vivo and *in vivo* [J]. Am J Physiol Cell Physiol, 2005, 288(3): C747-C756.

J Neurophysiol 106: 2127–2135, 2011.
First published July 20, 2011; doi:10.1152/jn.00067.2011.

Static magnetic field modulates rhythmic activities of a cluster of large local interneurons in *Drosophila* antennal lobe

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Submitted 7 February 2011; accepted in final form 18 July 2011

Yang Y, Yan Y, Zou X, Zhang C, Zhang H, Xu Y, Wang X, Janos P, Yang Z, Gu H. Static magnetic field modulates rhythmic activities of a cluster of large local interneurons in *Drosophila* antennal lobe. *J Neurophysiol* 106: 2127–2135, 2011. First published July 20, 2011; doi:10.1152/jn.00067.2011.—With the development of superconducting magnets, the chances of exposure to intense static magnetic fields (SMFs) have increased. Therefore, safety concerns related to magnetic field exposure need to be studied, especially the effects of magnetic field exposure on the central nervous system. Only a limited number of studies prove a direct connection between magnetic fields and electrophysiological signal processing. Here we described a cluster of large local interneurons (LNs) located laterally to each antennal lobe of *Drosophila melanogaster*, which exhibit extensive arborizations throughout the whole antennal lobe. Dual recordings showed that these large LNs demonstrated rhythmic spontaneous activities that correlated with other LNs and projection neurons (PNs) in the olfactory circuit. The results suggest that 3.0-T SMF can interfere with the properties of the action potential, rhythmic spontaneous activities of large LNs, and correlated activity in pairs of ipsilateral large LN/LN in the olfactory circuit. This indicates that *Drosophila* can be an ideal intact neural circuit model and that the activities of the olfactory circuit can be used to evaluate the effects of magnetic field stimulations.

neural circuit; *Drosophila melanogaster*

ALL LIVING ORGANISMS are continuously exposed to the natural geomagnetic field, which is ~0.5 G. With the development of superconducting magnets, especially the introduction of magnetic resonance imaging (MRI) in the twentieth century, the chances of exposure to intense static magnetic fields (SMFs) around several teslas (T) have increased (Schenck 2005; Silva et al. 2006). Considering the rapid increase in the strength of magnetic fields used in medical diagnosis, various studies have investigated the effects of acute or chronic exposure to SMFs on humans and animals over the past 30–40 years. These studies indicate that SMFs are capable of influencing a number of biological systems (Gowland 2005; Saunders 2005). A significant decrease in both amplitude and variability of the evoked response in the striate cortex of adult cats was detected after exposure to a 1,200-G SMF, which is considered to be due to the action of the magnetic field at the synapse (Rosen and Lubowsky 1987). Further research on the effects of a 1,230-G SMF in principal cells in the cat lateral geniculate body suggested that the spontaneous discharge frequency was decreased and the discharge pattern was changed on account of the distortion of ion channels (Rosen and Lubowsky 1990). Evidence has shown that the action of SMFs is mainly based on their effects on the molecular structure of excitable membranes, especially imbedded ion channels, which can interfere with the mechanism of calcium and sodium channel activation (Miyakoshi 2005; Rosen 2003b). A previous study has shown that exposure to a 120-mT SMF results in a slight reduction in the peak calcium current amplitude, a shift in the current-voltage relationship, and a slowing of the channel activation rate without any change in the inactivation rate (Rosen 1996). Since ion channels play important roles in cellular excitability and neural function, the effect of SMFs on the nervous system has attracted more scholarly attention. Exposure to 2- to 3-mT SMF has been proven to modulate synaptic excitability in a mouse hippocampal slice preparation (Wieraszko 2000). However, previous studies have mainly focused on cultured neurons and brain slice preparations; the effects of SMFs on intact neural circuit function are still far from being completely understood.

The antennal lobe of *Drosophila*, serving like the vertebrate olfactory bulb, provides an ideal intact neural network model to investigate neural circuit function (Ng et al. 2002). Three classes of neurons form synapses in the antennal lobe and participate in the processing of olfactory information, including olfactory receptor neurons (ORNs), projection neurons (PNs), and local interneurons (LNs). The ORNs expressing the same olfactory receptors project to the same glomerulus and connect to the PNs in the antennal lobe, modulated by LNs. Then PNs transmit olfactory information to the mushroom body and the lateral horn (Shang et al. 2007; Wilson et al. 2004). The morphology, physiology, and development of PNs and ORNs have been widely documented, while LNs have not received much attention (Chou et al. 2010; Das et al. 2008). A previous study has shown that correlated spontaneous activity exists in the *Drosophila* antennal lobe, which is essential for information processing by neural circuits (Kazama and Wilson 2009). Thus the *Drosophila* antennal lobe could be an ideal model for investigating the effects of SMFs on the central nervous system, especially on the network of neural circuits, and may provide more information about the mechanisms of SMF action and the risk factors associated with the use of intense SMFs.

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To address these questions, we describe a cluster of large LNs in the *Drosophila* antennal lobe and report the effects of 3.0-T SMF on their extracellular and intracellular spontaneous activities by using a *Drosophila* whole brain recording system (Gu and O'Dowd 2007) in wild-type Canton-S female fly pupae 2 days before eclosion. Our results demonstrated that these large LNs generated rhythmic spontaneous activities that correlated with the activities of other LNs and PNs in the ipsilateral antennal lobe, and we found that 3.0-T SMF can modulate the rhythmic activities and correlated activity of large LNs.

METHODS

Fly strains. Drosophila melanogaster stocks were reared on standard cornmeal agar medium supplemented with dry yeast at 24°C and 60% relative humidity. All experiments were performed on wild-type Canton-S female flies 2 days before eclosion. In our lab, flies produce new adults in 14 days. To accurately record the time point, flies that are 2 days before eclosion are double-identified by red eyes and transparent wings in the puparium.

3.0-T SMF exposure. The experimental group was deposited in a box filled with a 2-cm sound-absorbing sponge to eliminate potential noise. The box was tightly stuck to the center of the magnetic resonance spectrometer (Siemens MAGNETOM Trio A Tim System 3.0T MRI machine) with a 3.0-T SMF, and there was no movement of pupae in the field. Although a electromagnetic wave shield barrier was constructed between the examination room and the operation room of the MRI machine, a mT-level magnetic field still existed in the operation room. To ensure that the control group was free from magnetic field, we deposited the control group in our own lab. The temperatures of the examination room of the MRI machine and our lab were both controlled at 24°C, and the humidity was 60%. Other influence factors of these two groups were consistent.

Electrophysiological recordings from large LNs in isolated whole brain. All brains were obtained from female flies 2 days before eclosion. The entire brain, including optic lobes, was removed and prepared for recording in standard external solution containing 20 U/ml papain with 1 mM L-cysteine as previously described (Gu and O'Dowd 2006, 2007). The standard external solution contained (in mM) 101 NaCl, 1 $CaCl_2$, 4 $MgCl_2$, 3 KCl, 5 glucose, 1.25 NaH_2PO_4, and 20.7 $NaHCO_3$, pH 7.2, 250 osmol/kgH_2O. Then the dissected brains were mounted in an RC-26 perfusion chamber (Warner Instruments, Hamden, CT) containing the recording solution bubbled with 95% O_2 and 5% CO_2 (2 ml/min) throughout the experiments with the anterior face of the brain up. Pipettes were targeted to LNs in the dorsal neuron cluster in the antennal lobe.

Cell-attached and whole cell recordings were performed with pipettes (9–12 MΩ) filled with an internal solution containing (in mM) 102 K-gluconate, 0.085 $CaCl_2$, 1.7 $MgCl_2$, 17 NaCl, 0.94 EGTA, and 8.5 HEPES, pH 7.2, 235 osmol/kgH_2O. Gigaohm seals were achieved before recording in cell-attached configuration. Recordings were made at room temperature, and only a single large local neuron or a pair of neurons was examined in each brain.

All electrophysiological recordings were carried out with a BX51WI upright microscope (Olympus, Lehigh Valley, PA). Signals were acquired with an EPC10 amplifier (HEKA Elektronik, Lambrecht/Pfalz, Germany), filtered at 5 kHz with a built-in filter, and digitized at 5 kHz.

Biocytin staining and fluorescence imaging. The morphology and identity of a single large LN were confirmed by post hoc staining with biocytin. In some cells, 0.4% biocytin was added to the internal pipette solution. To make sure that biocytin could be injected into the soma and the terminals, the recording pipette was maintained in the whole cell configurations for at least 30 min. After electrophysiological recording, the brain was fixed in phosphate buffer containing 4% formaldehyde at 4°C for 10 h and subjected to biocytin staining. Then the brain was washed in 1% PBS three times, blocked, and incubated in a blocking buffer (0.1 M PBS, 0.1% Triton X-100, 1% BSA) containing streptavidin-Cy3 (Molecular Devices) for 3 h at room temperature. After incubation, the brain was washed three times with 5-min intervals in PBS. A BX51WI microscope with a ×40 objective and an Imag-Pro plus 7.0 (Olympus) camera was used to acquire photos of dendritic arborization of the large local neurons in the antennal lobe.

Data analysis. All data were analyzed by Clampfit 10.2 (Molecular Devices). Two or more spikes with interval time <100 ms were defined as a burst, and a single spike or a burst was regarded as an event. Low-frequency oscillations <10 Hz were isolated by low-pass filtering from voltage traces acquired in whole cell current-clamp configuration. Cross-correlation function was performed by Clampfit 10.2 with 2,000-ms lag time.

Statistics. The differences between control and SMF treatment were evaluated by independent *t*-test and Mann-Whitney rank sum test where appropriate. Values of $P < 0.05$ were considered significant.

RESULTS

A cluster of large LNs in the Drosophila antennal lobe demonstrated rhythmic activities. Previous studies have shown that LNs in the *Drosophila* antennal lobe are diverse in their neurotransmitter profiles, connectivity, and physiological properties, and they play important roles in information processing by olfactory neural circuits (Chou et al. 2010; Das et al. 2008). The whole brain of female fly pupae 2 days before eclosion was dissected and observed. A cluster of three or four large LN somata was found located laterally to each antennal lobe, with an impressively larger size than other cell bodies nearby, around 9.68 ± 0.31 μm in diameter (Fig. 1, *A* and *B*). These neurons were approached with a standard glass electrode. To show detailed morphology of these neurons, biocytin was used when whole cell patch-clamp recording was applied. The cluster of large LNs was intrinsic to the ipsilateral antennal lobe, with thick processes extending from the laterally situated cell body into the center of the lobe and exhibiting extensive arborizations that almost covered the entire antennal lobe (Fig. 1, *A* and *C*). These features were consistent with some types of LNs previously reported (Das et al. 2008).

Electrophysiological properties of these large LNs were monitored by patch-clamp recording. The cell-attached recording showed that they demonstrated spontaneous rhythmic spikes (3.02 ± 0.47 Hz) 2 days before eclosion (Fig. 2*A*). Under whole cell current-clamp recording, these neurons revealed a mean resting membrane potential (RMP) of −48.22 ± 1.80 mV and fired spontaneous action potentials (APs) actively with a mean amplitude of 32.09 ± 3.40 mV and a mean firing rate of 3.39 ± 0.92 Hz, including bursting pattern with consecutive spikes (Fig. 2*B*). The spikes could be blocked by tetrodotoxin (TTX) (data not shown). Whole cell voltage-clamp recording demonstrated the presence of spontaneous oscillatory activities (Fig. 2*C*).

Large LNs showed correlated activity with other LNs and projection neurons in the antennal lobe. Correlated spontaneous activities have been widely documented in neurons in the brain, which are important in information coding and processing by neural circuits (Kashiwadani et al. 1999; Kreiter and Singer 1996; McCarthy et al. 2011). A previous study has shown that homotypic PNs produce highly correlated spikes in

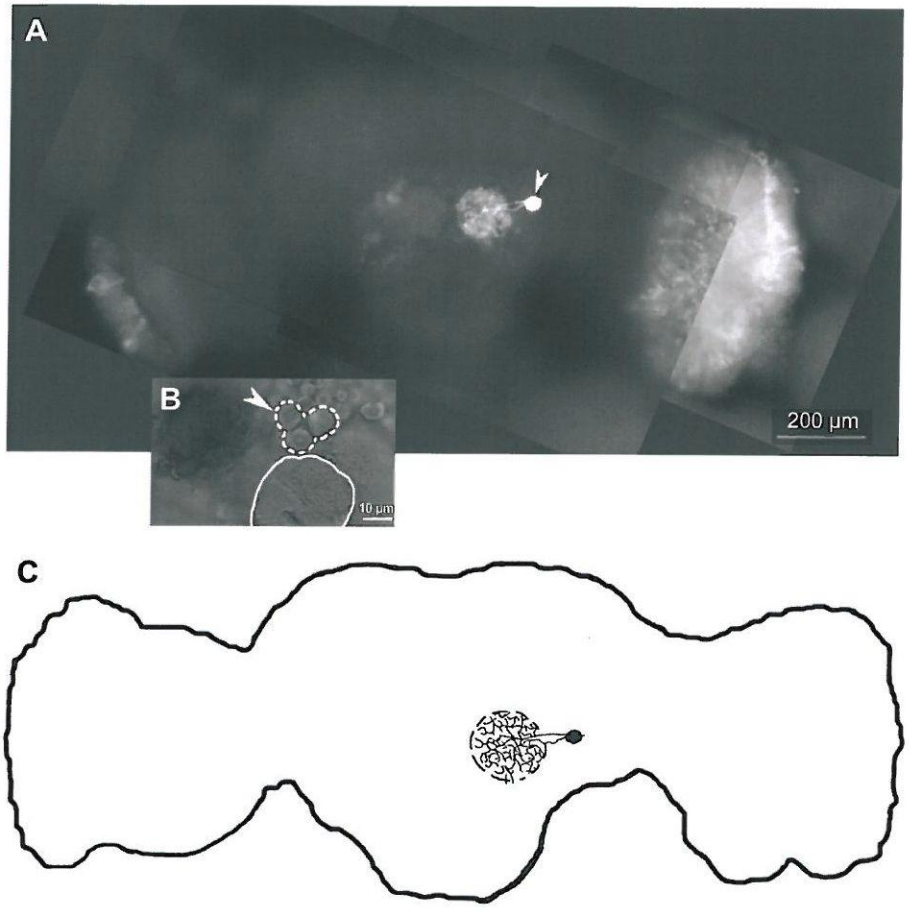


Fig. 1. A cluster of large local interneurons (LNs) in the antennal lobe of *Drosophila*. *A*: fluorescent microscopy demonstrated a large LN found in the antennal lobe. The soma of the neuron (arrowhead) was located laterally to antennal lobe, with the neural arborizations spreading over nearly the entire antennal lobe. *B*: bright-field image of the large LNs. There was a cluster of 3 or 4 large LN somata (dashed line and arrowhead) located laterally to each antennal lobe (solid line). The mean diameter of the soma was 9.68 ± 0.31 μm. *C*: camera lucida demonstrated the detailed morphology of these large LNs. There were thick neural processes spreading from the soma stalk of the recorded large LN. The neural processes then developed into small collaterals that extended anteriorly, and the arborizations innervated throughout the antennal lobe (dashed line) glomeruli.

Drosophila antennal lobe (Kazama and Wilson 2009). To determine whether these large LNs showed correlated activity with other neurons in the olfactory circuit, dual recordings from pairs of ipsilateral large LN/PN and large LN/LN in the antennal lobe were performed. In cell-attached recording, large LNs, PNs, and LNs generated spontaneous spikes (Fig. 3*A* and *B*; Fig. 4*A* and *B*). Two or more spikes with an interval time <100 ms were defined as a burst, and a single spike or a burst was regarded as an event. Then the cumulative frequency distribution of the interval time between events was detected. No significant differences in pairs of large LN/PN ($P = 0.095$, Kolmogorov-Smirnov test; Fig. 3*E*) and pairs of large LN/LN ($P = 0.12$, Kolmogorov-Smirnov test; Fig. 4*E*) were found.

In whole cell current-clamp recording, these three types of neurons revealed spontaneous APs and oscillations (Fig. 3*C*, Fig. 4*C*). Considering that oscillations are a common feature of spontaneous activity in the olfactory system and are thought to play an important role in information processing and memory in a variety of brain areas (Galan et al. 2006; Gelperin 2006; Ravel et al. 2003), low-frequency oscillations <10 Hz were isolated by low-pass filtering from voltage traces acquired in whole cell current-clamp configuration (Fig. 3*D*, Fig. 4*D*). The timing of low-frequency oscillation was found to be correlated in both pairs of ipsilateral large LN/PN and large LN/LN by computing the cross-correlation function. The cross-correlation analysis presented a clear peak with mean value at 0.62 ± 0.10 (lag time = 0 ms) ($n = 12$) in pairs of large LN/PN (Fig. 3*F*) and 0.79 ± 0.17 (lag time = 0 ms) ($n = 16$) in pairs of large LN/LN (Fig. 4*F*). Low-frequency oscillation correlation was largely decreased from 0.78 ± 0.01 to 0.20 ± 0.09 by 20 μM curare ($n = 3$, $P < 0.05$, paired *t*-test) but unaffected by 10 μM picrotoxin ($n = 3$, $P = 0.15$, paired *t*-test).

These results indicated that large LNs showed correlated activity with other ipsilateral LNs and PNs in the antennal lobe, suggesting that the activities of large LNs can partially reveal the activities of the entire local olfactory circuit.

3.0-T static magnetic field-modulated rhythmic activities of large LNs. The effects of SMFs on the central nervous system have been widely investigated in cultured neurons and brain slice preparations (Saunders 2005). Previous studies have demonstrated that weak SMF can change the properties of APs of neurons (McLean et al. 1995; Nikolic et al. 2008). As large LNs showed correlated activity with other LNs and PNs in olfactory circuit of antennal lobe, the effects of 3.0-T SMF on the rhythmic extracellular activities and APs of large LNs as well as correlated activity in large LN/LN pairs were monitored in order to investigate the alterations of the neural circuit under intense SMF exposure.

Cell-attached and whole cell recording were performed on the large LNs in isolated whole brain of female fly pupae 2 days before eclosion. Two or more spikes with an interval time <100 ms were defined as a burst. The changes of spike and burst frequency induced by exposure to 3.0-T SMF for 8 h were detected. In cell-attached configuration, the mean fre-

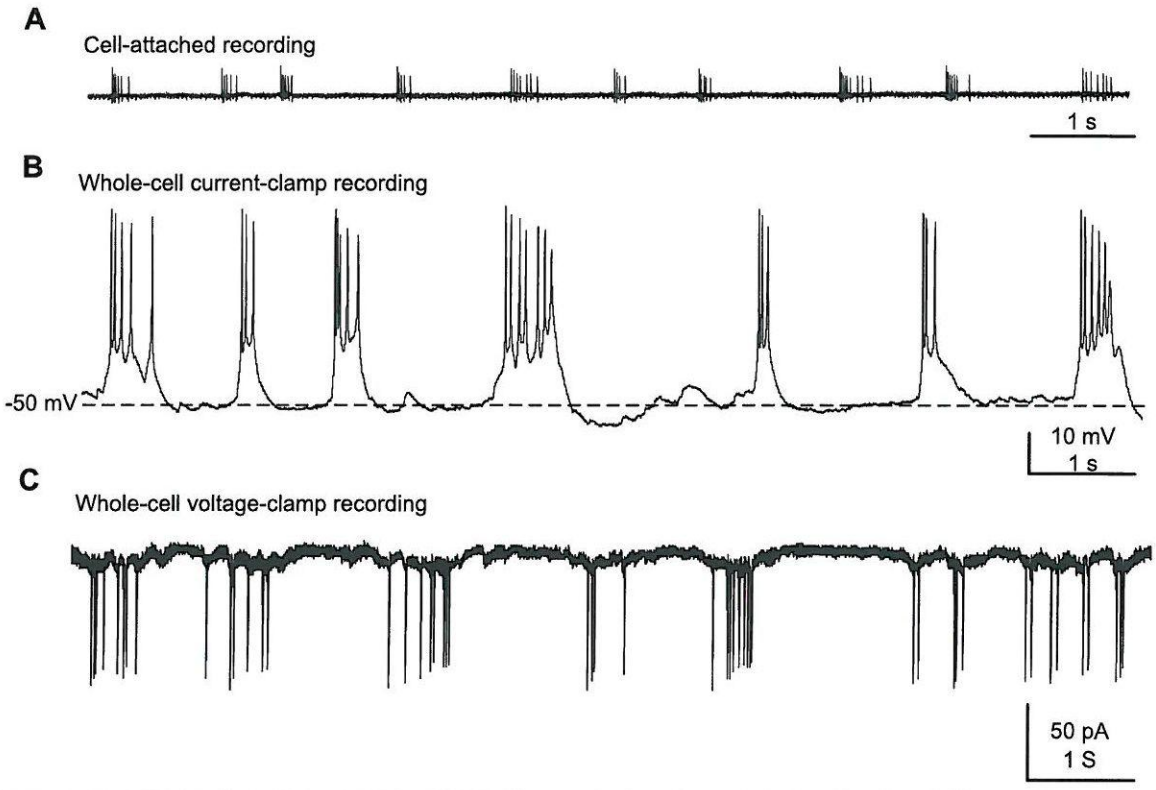


Fig. 2. Spontaneous activities recorded in the soma of a large LN. *A*: 10-s recording trace in cell-attached configuration. *B*: 10-s recording trace in whole cell current-clamp configuration. *C*: 10-s recording trace in whole cell voltage-clamp configuration.

quency of extracellular spikes decreased significantly from 3.02 ± 0.47 Hz in the control to 1.49 ± 0.41 Hz in the 3.0-T SMF treatment ($P < 0.05$, Mann-Whitney test; Fig. 5*G*). Additionally, the extracellular activities of three cells ($n = 13$) were silent after SMF exposure even though gigaohm seals were achieved (Fig. 5*A*). The effects of 3.0-T SMF on the properties of APs were further investigated (Fig. 5*B*). In whole cell configuration, the mean RMP of large LNs was −48.22 ± 1.80 mV in the control. Even though RMP seemed to decrease to −50.05 ± 3.65 mV after exposure to 3.0-T SMF for 8 h, it was not significantly different ($P = 0.633$, independent *t*-test). The mean frequency of APs was significantly reduced from 3.39 ± 0.92 Hz to 1.87 ± 0.85 Hz ($P < 0.05$, Mann-Whitney test; Fig. 5*C*), and the mean amplitude of APs decreased significantly from 32.09 ± 3.40 mV to 20.78 ± 3.21 mV ($P < 0.05$, independent *t*-test; Fig. 5*D*) after 3.0-T SMF treatment. Moreover, the mean frequency of bursts decreased from 0.58 ± 0.08 Hz to 0.20 ± 0.09 Hz ($P < 0.01$, Mann-Whitney test; Fig. 5*E*), while the mean duration of bursts decreased from 119.58 ± 14.12 ms to 50.97 ± 18.86 ms ($P < 0.05$, Mann-Whitney test; Fig. 5*F*). During dual whole cell recording performed on the ipsilateral large LN/LN pairs, the mean cross-correlation function value of low-frequency oscillation decreased from 0.79 ± 0.17 ($n = 16$) in the control group to 0.69 ± 0.26 ($n = 11$) in the SMF group ($P < 0.01$, ANOVA) after exposure (Fig. 5, *H* and *I*).

These results indicate that 3.0-T SMF can interfere with rhythmic spontaneous activities of large LNs and correlated activity in large LN/LN pairs, which may influence the activities and functions of the olfactory circuit in *Drosophila* antennal lobe.

DISCUSSION

LNs are essential for information coding and processing in neural circuits (Chou et al. 2010; Lledo et al. 2008; Palhalmi et al. 2004). Here we described a cluster of large LNs located laterally to each antennal lobe of *Drosophila*, exhibiting extensive arborizations throughout the whole antennal lobe. The significantly large size of this cluster of LNs makes it possible to target the recording electrode to the same type of neurons every time. These large LNs exhibited rhythmic spontaneous activities that correlated with the activities of other LNs and PNs in the olfactory circuit. Since correlated activity plays important roles in neural functions of the intact brain, the results further suggest that *Drosophila* antennal lobe can be an ideal model to investigate the network of neural circuits. Additionally, the activities of these large LNs can partially reveal the activities of local olfactory circuits in the antennal lobe.

With the development of superconducting magnets, the chances of exposure to intense SMFs have increased. However, safety concerns related to magnetic field exposure remain unclear, especially its effects on the central nervous system (Schenck 2000; Silva et al. 2006). Evidence has shown that membrane calcium channels are the primary sites of moderate SMF effects, and sodium channels may also be involved. The channel activation kinetics can be altered, while channel inactivation is not expected to be influenced (Miyakoshi 2005; Rosen 2003a). Both of these two channels are key structures for excitability and

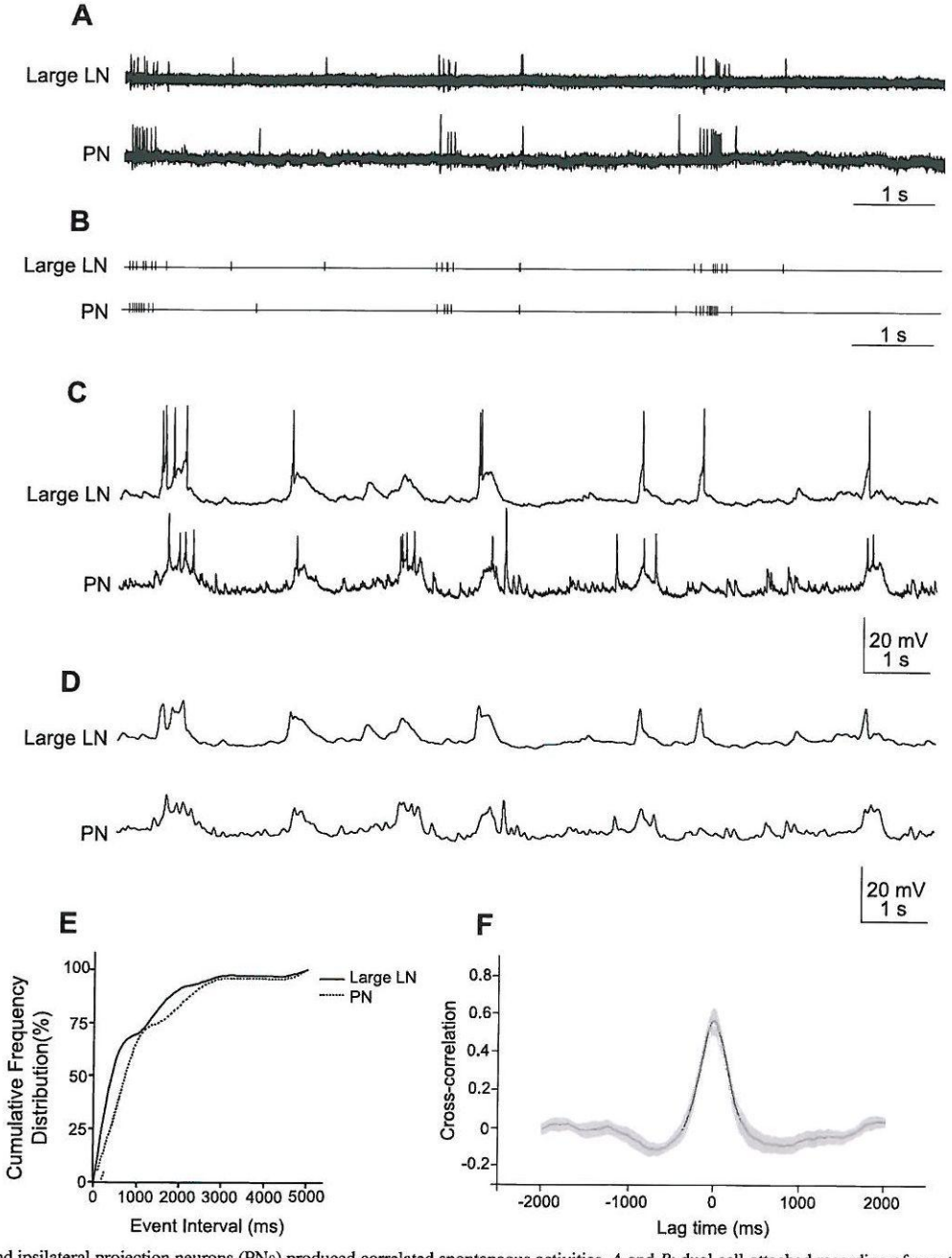


Fig. 3. Large LNs and ipsilateral projection neurons (PNs) produced correlated spontaneous activities. *A* and *B*: dual cell-attached recording of spontaneous spikes from a pair of ipsilateral large LN/PN. Rasters of spikes are shown in *B*. *C*: dual whole cell current-clamp recording of the action potentials from a pair of ipsilateral large LN/PN. *D*: low-frequency oscillations <10 Hz isolated by low-pass filtering from voltage traces acquired in dual whole cell current-clamp configuration from a pair of ipsilateral large LN/PN. *E*: cumulative frequency distribution of interval time between events acquired in cell-attached configuration from a pair of ipsilateral large LN/PN. There was no significant difference in cumulative probability distribution between large LN and PN ($P = 0.095$, Kolmogorov-Smirnov test). *F*: cross-correlation function (CCF) for low-frequency oscillations <10 Hz filtering from voltage traces acquired in whole cell current-clamp configuration from a pair of ipsilateral large LN/PN. The mean value was 0.62 ± 0.10 (lag time = 0 ms) ($n = 12$). The gray band represents ±SE across pairs.

activity in the central nervous system (Catterall and Few 2008), modulating a wide range of cellular events. Various studies carried out to detect the effects of SMF on neural function have mainly investigated cultured neurons and brain slice preparations. However, the effects of intense SMF on intact neural circuit functions still need to be further studied. Therefore, the present study used the antennal lobe of isolated *Drosophila* whole brain as a model neural circuit in order to reveal the effects of 3.0-T intense SMF on the activities of olfactory circuit. This was done by monitoring the neural activity alterations of the cluster of large LNs induced by SMF.

The properties of APs are important bioelectric parameters for characterization of spontaneously firing neurons. APs can be blocked by TTX in the large LNs, indicating that sodium channels mediate the process of AP generation. Our data demonstrate that 3.0-T SMF can interfere with the rhythmic

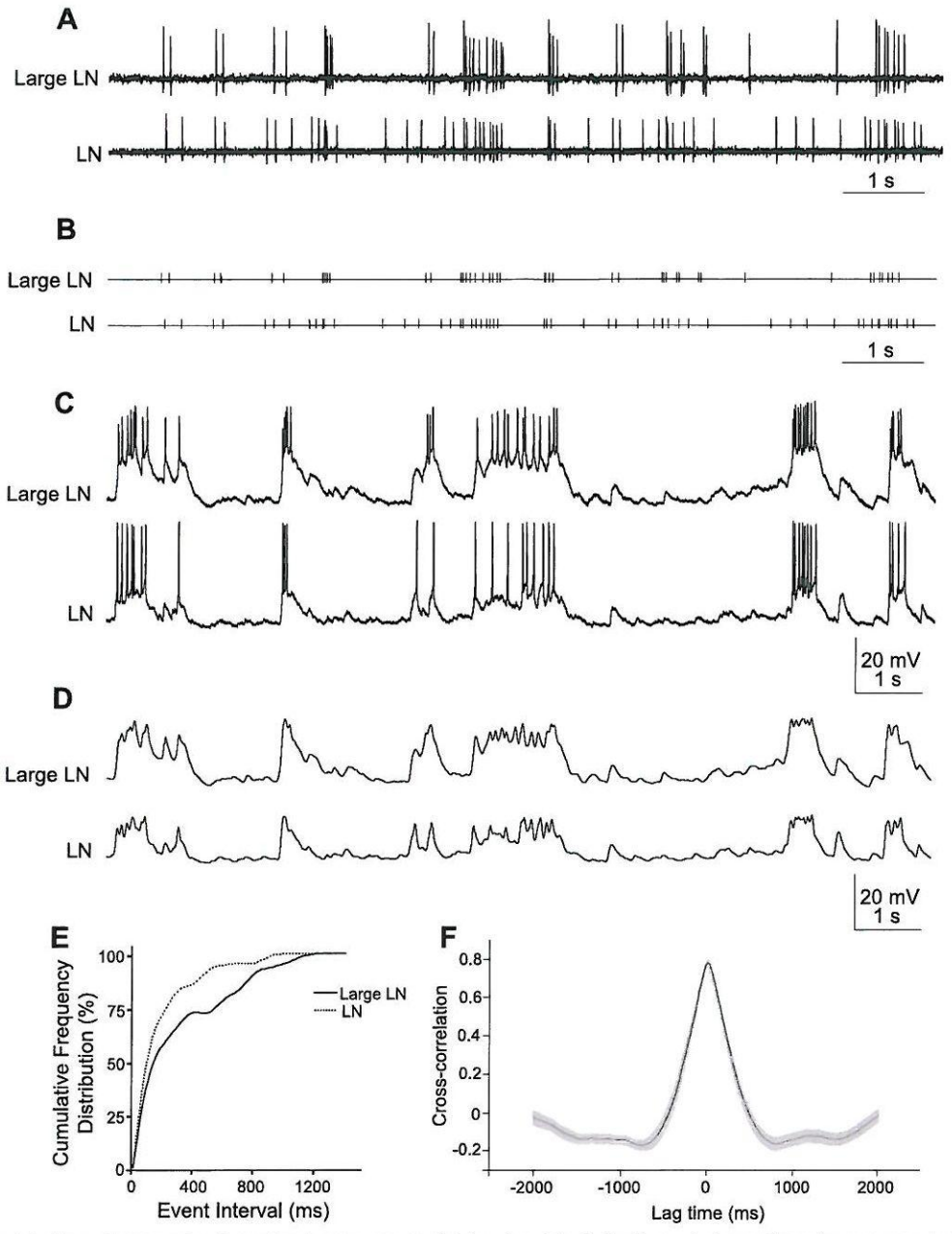


Fig. 4. Large LNs and ipsilateral LNs produced correlated spontaneous activities. *A* and *B*: dual cell-attached recording of spontaneous spikes from a pair of ipsilateral large LN/LN. Rasters of spikes are shown in *B*. *C*: dual whole cell current-clamp recording of the action potentials from a pair of ipsilateral large LN/LN. *D*: low-frequency oscillations <10 Hz isolated by low-pass filtering from voltage traces acquired in dual whole cell current-clamp configuration from a pair of ipsilateral large LN/LN. *E*: cumulative frequency distribution of interval time between events acquired in cell-attached configuration from a pair of ipsilateral large LN/LN. There was no significant difference in cumulative probability distribution between large LN and LN ($P = 0.12$, Kolmogorov-Smirnov test). *F*: cross-correlation function for low-frequency oscillations <10 Hz filtering from voltage traces acquired in whole cell current-clamp configuration from a pair of ipsilateral large LN/LN. The mean value was 0.79 ± 0.17 (lag time = 0 ms) ($n = 16$). The gray band represents ±SE across pairs.

spontaneous activities and the properties of APs, as the mean frequency of extracellular activities was significantly reduced after exposure to 3.0-T SMF for 8 h. RMP showed no significant difference, indicating that the potassium channel-mediated current may be intact. Previous studies have shown that the amplitude of the evoked APs of neurons increased after exposure to mT-level SMF (Nikolic et al. 2008; Ye et al. 2004). However, the results of this study showed that the frequency and amplitude of spontaneous APs, as well as the burst frequency and duration of these large LNs, were significantly reduced after exposure to 3.0-T SMF. This was consistent with a prior study that examined the effects of a 1,230-G SMF on spontaneous discharge frequency and discharge pattern of principal cells in the cat's lateral geniculate body in a whole brain preparation (Rosen and Lubowsky 1990). Furthermore, the detected changes could persist for 4 h after SMF

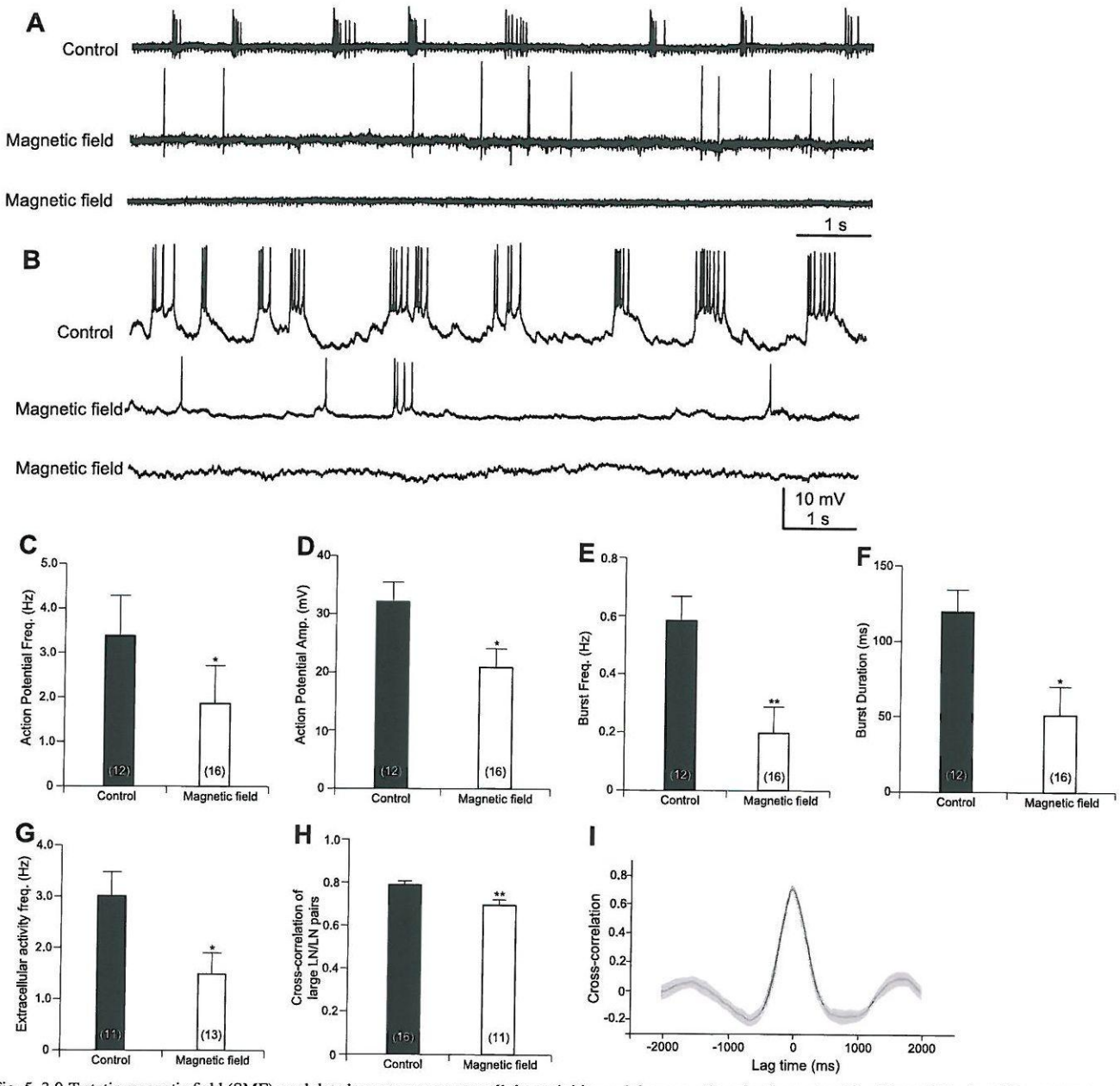


Fig. 5. 3.0-T static magnetic field (SMF) modulated spontaneous extracellular activities and the properties of action potentials of large LNs. *A* and *B*: spontaneous activities of large LNs acquired in cell-attached and whole cell current-clamp configuration in control (*top*) and in SMF treatment (*bottom*), respectively. Note that the spontaneous activities of some cells were silent after exposure to 3.0-T SMF. *C–F*: frequency (*$P < 0.05$, Mann-Whitney test) and amplitude (*$P < 0.05$, *t*-test) of action potentials and frequency (**$P < 0.01$, Mann-Whitney test) and duration (*$P < 0.05$, Mann-Whitney test) of bursting spikes decreased significantly after SMF treatment. Each bar indicates the mean ± SE from the indicated number of neurons (in parentheses). All synaptic currents were recorded at a holding potential of −70 mV. *G*: in cell-attached configuration, the mean frequency of extracellular spikes decreased significantly from 3.02 ± 0.47 Hz in control to 1.49 ± 0.41 Hz in 3.0-T SMF treatment (*$P < 0.05$, Mann-Whitney test). Each bar indicates the mean ± SE from the indicated number of neurons (in parentheses). *H* and *I*: cross-correlation function for low-frequency oscillations <10 Hz filtering from voltage traces acquired in whole cell current-clamp configuration from pairs of ipsilateral large LN/LN after 3.0-T SMF exposure. The mean value decreased from 0.79 ± 0.17 ($n = 16$) in the control group to 0.69 ± 0.26 ($n = 11$) in the SMF exposure group ($P < 0.01$, ANOVA). The gray band represents ±SE across pairs.

exposure, indicating that some metabolic processes such as enzyme activities may be involved in the observed changes induced by SMF. These results suggest that the inconsistent changes of neuronal activity induced by SMF may be related to the intensity of SMF and the different membrane properties of neurons. A previous study proposed that reorientation of membrane phospholipids during SMF exposure will result in the deformation of imbedded ion channels (Rosen 2003b). Therefore the decrease in AP amplitude suggests that sodium channels, which participate in the depolarizing phase of AP, may be affected more easily by SMF exposure. The reduction in the frequency of APs indicates that the 3.0-T SMF can inhibit large LN activities, which is consistent with previous findings (McLean et al. 1995; Nikolic et al. 2008). The bursting pattern

with consecutive spikes of neuron activity is related to calcium-dependent ion currents (Izhikevich 2000). Our results demonstrated that burst firing frequency and duration were significantly decreased after exposure to 3.0-T SMF. It is possible that alterations of bursting patterns of large LNs may result from the effects of the intense SMF on the calcium-dependent ion currents in some direct or indirect ways. Correlated spontaneous activities are important in information coding and processing by neural circuits. A previous study demonstrated that there were correlated activity and reciprocal chemical/electrical connections between olfactory neurons in *Drosophila* antennal lobe (Huang et al. 2010). A recent study showed that neuropeptide PDF (pigment-dispersing factor)-secreting large ventrolateral neurons in the *Drosophila* brain regulating daily patterns of rest and arousal exhibit synchronous rhythmic membrane activity, and cholinergic input was required for synchronous membrane activity whereas GABA can modulate firing pattern (McCarthy et al. 2011). In this study, we found that 3.0-T SMF decreased the correlated activity in pairs of ipsilateral large LN/LN. Whether this relates to the inhibited effects of SMF on single large LN activity, as we showed, the potential interaction between SMF and reciprocal chemical and/or electrical connections of neuron pairs, or possible effects of SMF on the synaptic input to large LNs needs to be studied further.

Local neurons are essential for transformation and integration of olfactory information through the glomerular relay between PNs and ORNs in antennal lobe, playing important roles in the functions of neural circuits (Ng et al. 2002; Wilson and Laurent 2005). The results of the present study demonstrate that 3.0-T intense SMF can modulate the rhythmic spontaneous activities of large LNs and correlated activity of ipsilateral pairs of large LN/LN in *Drosophila* antennal lobe, indicating that the activities of the local olfactory circuit may be affected by SMF. The data also suggested that calcium channels and sodium channels may be related to the activity alterations induced by SMF, which also needs to be investigated further. Our findings are, in a sense, the first step in detecting the effects of intense SMF on neural circuit functions. *Drosophila* antennal lobe, with genetic tractability and functional organization, provides an ideal model not only for investigating the network of neural circuits but also for linking the gaps between neural alterations and SMF stimulation.

ACKNOWLEDGMENTS

We acknowledge Naya Huang and Jiayao Lei for help in data analysis and Dongzhi Ran for fly strain support.

GRANTS

This work was supported by the Hundred Talents Program of Sun Yat-sen University (50000-3181308) and NSF 30970980.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES

Catterall W, Few A. Calcium channel regulation and presynaptic plasticity. *Neuron* 59: 882–901, 2008.

Chou Y, Spletter M, Yaksi E, Leong J, Wilson R, Luo L. Diversity and wiring variability of olfactory local interneurons in the *Drosophila* antennal lobe. *Nat Neurosci* 13: 439–449, 2010.

Das A, Sen S, Lichtneckert R, Okada R, Ito K, Rodrigues V, Reichert H. *Drosophila* olfactory local interneurons and projection neurons derive from a common neuroblast lineage specified by the empty spiracles gene. *Neural Dev* 3: 33, 2008.

Galan R, Fourcaud-Trocme N, Ermentrout G, Urban N. Correlation-induced synchronization of oscillations in olfactory bulb neurons. *J Neurosci* 26: 3646–3655, 2006.

Gelperin A. Olfactory computations and network oscillation. *J Neurosci* 26: 1663–1668, 2006.

Gowland P. Present and future magnetic resonance sources of exposure to static fields. *Prog Biophys Mol Biol* 87: 175–183, 2005.

Gu H, O'Dowd D. Cholinergic synaptic transmission in adult *Drosophila* Kenyon cells in situ. *J Neurosci* 26: 265–272, 2006.

Gu H, O'Dowd D. Whole cell recordings from brain of adult *Drosophila*. *J Vis Exp* 2007: 248, 2007.

Huang J, Zhang W, Qiao W, Hu A, Wang Z. Functional connectivity and selective odor responses of excitatory local interneurons in *Drosophila* antennal lobe. *Neuron* 67: 1021–1033, 2010.

Izhikevich E. Neural excitability, spiking and bursting. *Int J Bif Chaos* 10: 1171–1266, 2000.

Kashiwadani H, Sasaki YF, Uchida N, Mori K. Synchronized oscillatory discharges of mitral/tufted cells with different molecular receptive ranges in the rabbit olfactory bulb. *J Neurophysiol* 82: 1786–1792, 1999.

Kazama H, Wilson RI. Origins of correlated activity in an olfactory circuit. *Nat Neurosci* 12: 1136–1144, 2009.

Kreiter AK, Singer W. Stimulus-dependent synchronization of neuronal responses in the visual cortex of the awake macaque monkey. *J Neurosci* 16: 2381–2396, 1996.

Lledo P, Merkle F, Alvarez-Buylla A. Origin and function of olfactory bulb interneuron diversity. *Trends Neurosci* 31: 392–400, 2008.

McCarthy E, Wu Y, deCarvalho T, Brandt C, Cao G, Nitabach MN. Synchronized bilateral synaptic inputs to *Drosophila melanogaster* neuropeptidergic rest/arousal neurons. *J Neurosci* 31: 8181–8193, 2011.

McLean M, Holcomb R, Wamil A, Pickett J, Cavopol A. Blockade of sensory neuron action potentials by a static magnetic field in the 10 mT range. *Bioelectromagnetics* 16: 20–32, 1995.

Miyakoshi J. Effects of static magnetic fields at the cellular level. *Prog Biophys Mol Biol* 87: 213–223, 2005.

Ng M, Roorda R, Lima S, Zemelman B, Morcillo P, Miesenböck G. Transmission of olfactory information between three populations of neurons in the antennal lobe of the fly. *Neuron* 36: 463–474, 2002.

Nikolic L, Kartelija G, Nedeljkovic M. Effect of static magnetic fields on bioelectric properties of the Br and N1 neurons of snail *Helix pomatia*. *Comp Biochem Physiol A* 151: 657–663, 2008.

Palhalmi J, Paulsen O, Freund T, Hajos N. Distinct properties of carbachol- and DHPG-induced network oscillations in hippocampal slices. *Neuropharmacology* 47: 381–389, 2004.

Ravel N, Chabaud P, Martin C, Gaveau V, Hugues E, Tallon-Baudry C, Bertrand O, Gervais R. Olfactory learning modifies the expression of odour-induced oscillatory responses in the gamma (60–90 Hz) and beta (15–40 Hz) bands in the rat olfactory bulb. *Eur J Neurosci* 17: 350–358, 2003.

Rosen A. Effect of a 125 mT static magnetic field on the kinetics of voltage activated Na^+ channels in GH3 cells. *Bioelectromagnetics* 24: 517–523, 2003a.

Rosen A. Inhibition of calcium channel activation in GH3 cells by static magnetic fields. *Biochim Biophys Acta* 1282: 149–155, 1996.

Rosen A. Mechanism of action of moderate-intensity static magnetic fields on biological systems. *Cell Biochem Biophys* 39: 163–173, 2003b.

Rosen AD, Lubowsky J. Magnetic field influence on central nervous system function. *Exp Neurol* 95: 679–687, 1987.

Rosen AD, Lubowsky J. Modification of spontaneous unit discharge in the lateral geniculate body by a magnetic field. *Exp Neurol* 108: 261–265, 1990.

Saunders R. Static magnetic fields: animal studies. *Prog Biophys Mol Biol* 87: 225–239, 2005.

Schenck J. Physical interactions of static magnetic fields with living tissues. *Prog Biophys Mol Biol* 87: 185–204, 2005.

Schenck J. Safety of strong, static magnetic fields. *J Magn Reson Imaging* 12: 2–19, 2000.

Shang Y, Claridge-Chang A, Sjulson L, Pypaert M, Miesenböck G. Excitatory local circuits and their implications for olfactory processing in the fly antennal lobe. *Cell* 128: 601–612, 2007.

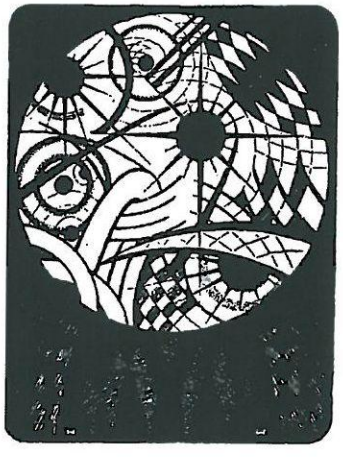
Silva A, Silva L, Egito E, Carriço A. Safety concerns related to magnetic field exposure. *Radiat Environ Biophys* 45: 245–252, 2006.

Wieraszko A. Dantrolene modulates the influence of steady magnetic fields on hippocampal evoked potentials in vitro. *Bioelectromagnetics* 21: 175–182, 2000.

Wilson R, Laurent G. Role of GABAergic inhibition in shaping odor-evoked spatiotemporal patterns in the *Drosophila* antennal lobe. *J Neurosci* 25: 9069–9079, 2005.

Wilson R, Turner G, Laurent G. Transformation of olfactory representations in the *Drosophila* antennal lobe. *Science* 303: 366–370, 2004.

Ye S, Yang J, Chen C. Effect of static magnetic fields on the amplitude of action potential in the lateral giant neuron of crayfish. *Int J Radiat Biol* 80: 699–708, 2004.



Szerzett multifokális myoclonus pathomechanizmusa – Beteg bemutatás

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Összefoglalás

A közlemény multifokális myoclonusok miatt szenvedő, 48 éves férfibeteg betegségtörténetét mutatja be és elemzi. A szérumban talált feszültségfüggő kálium csatorna (VGKC) elleni ellenanyagok alapján a betegség autoimmun mechanizmusa merült fel. A klinikai tünetek agytörzsi eredetű myoclonusokra utaltak, míg az izombiopszia vizsgálata felvetette annak a lehetőségét, hogy a myoclonusok kialakulásában neuromuszkuláris, perifériás tényezők is szerepet játszottak. Plasmaferesis kezelés hatékonynak bizonyult, azonban a tünetek visszatérése miatt szükséges volt a kezelések ismétlése. Ismert, hogy krónikus fájdalom szindrómákban a feszültségfüggő kálium csatorna elleni ellenanyagoknak ugyancsak pathogenetikai szerepük van, és ezen állapotok gyakran eredményesen kezelhetők komplex módon, orvosok, fizioterápiás szakemberek, pszichoszociális támogatók bevonásával. Joggal merül fel tehát a gondolat, hogy betegünknél az immunkezelés komplex kezelési stratégiákkal történő kiegészítése tartósabb panasz- és tünetmentességhez vezethetne.

Kulcsszavak: myoclonus, feszültségfüggő kálium csatorna, plasmaferesis, fizioterápia

Pathomechanism of acquired multifocal myoclonus – Case history

Gáti I, Danielsson O, Pálhalmi J.

Summary

A history of a 48-old male patient with acquired multifocal myoclonus have been presented. An autoimmune mechanism was postulated on the base of serum voltage-gated potassium channel (VGKC) antibodies. The clinical symptoms pointed to a possible brain stem origin, while muscle biopsy findings supported a possibility of peripheral, neuromuscular system involvement. Plasma exchange resulted in substantial recovery, but repeated therapy had to be used. Chronic pain also is a syndromic manifestation of VGKC-complex autoimmunity, and complex therapy by participation of neurologist, physiatrists and psychosocial interventions usually successful in these conditions. In our case - besides the immunotherapy – one may consider similar complex treatment strategies, which might lead to longer symptom free periods.

Keywords: myoclonus, voltage-gated potassium channel, plasma exchange, physiotherapy

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Bevezetés

A myoclonusok hirtelen, akaratlan szabálytalan izom összehúzódások. A tünetek hátterében különböző anatómiai lokalizációk és kórélettani mechanizmusok ismertek (1, 2). Elkülönítendők más izom hiperaktivitásával járó központi idegrendszeri betegségektől, mint dystoniáktól, tremoroktól, choreatól, vagy epilepsziás görcsöktől (3), valamint perifériás eredetű kórképektől, mint a myokymiától, fasciculatiótól, rippling szindrómától és a pseudomyotoniától (4, 5). Középkorú férfibeteg betegségtörténtét mutatjuk be, akinél szerzett, multifokális myoclonusok voltak észlelhetők, erős verítékezéssel és izomgörcsökkel. Sem központi -, sem perifériás idegrendszeri károsodást nem igazolódott. A klinikai tünetek alapján agytörzsi lokalizáció és autoimmun pathomechanizmus látszott valószínűnek, ugyanakkor nem volt kizárható, hogy perifériás neuromuszkuláris mechanizmus is szerepet játszott a panaszok és tünetek krónikussá válásában.

Beteg ismertetés

A 48 éves férfi beteg tünetei 6 évvel ezelőtt kezdődtek, fájdalmas görcsökkel kísért akaratlan izom összehúzódásokkal, a nyak és a karok izomzatában, naponta 5-10 alkalommal, változó 5-30 perces időtartamokra. A tünetek gyakorisága fokozódott, és rángások jelentkeztek a has izomzatában, és az alsó végtagokban is, bal oldali hangsúlyozottsággal. Akaratlagos izom kontrakció, tapintás és fájdalmas bőr stimulusok, valamint erős lelki hatás kontrakciókat provokáltak, ugyanezen stimulusok a rángások frekvenciáját és amplitődóját jelentősen fokozták. A tüneteket intenzív izzadás kísérte. A panaszok alvásban nem jelentkeztek.

Kórházi felvétele alkalmával intenzív verítékezésen kívül kóros belszervi eltérés nem volt észlelhető. Neurológiai vizsgálata alkalmával myoclonusoknak minősíthető rángások voltak láthatók a nyakizmokban, a hasi izomzatban és a végtagokban, bal oldali hangsúlyozottsággal. Fenti stimulusok az izom összehúzódásokat provokálták.

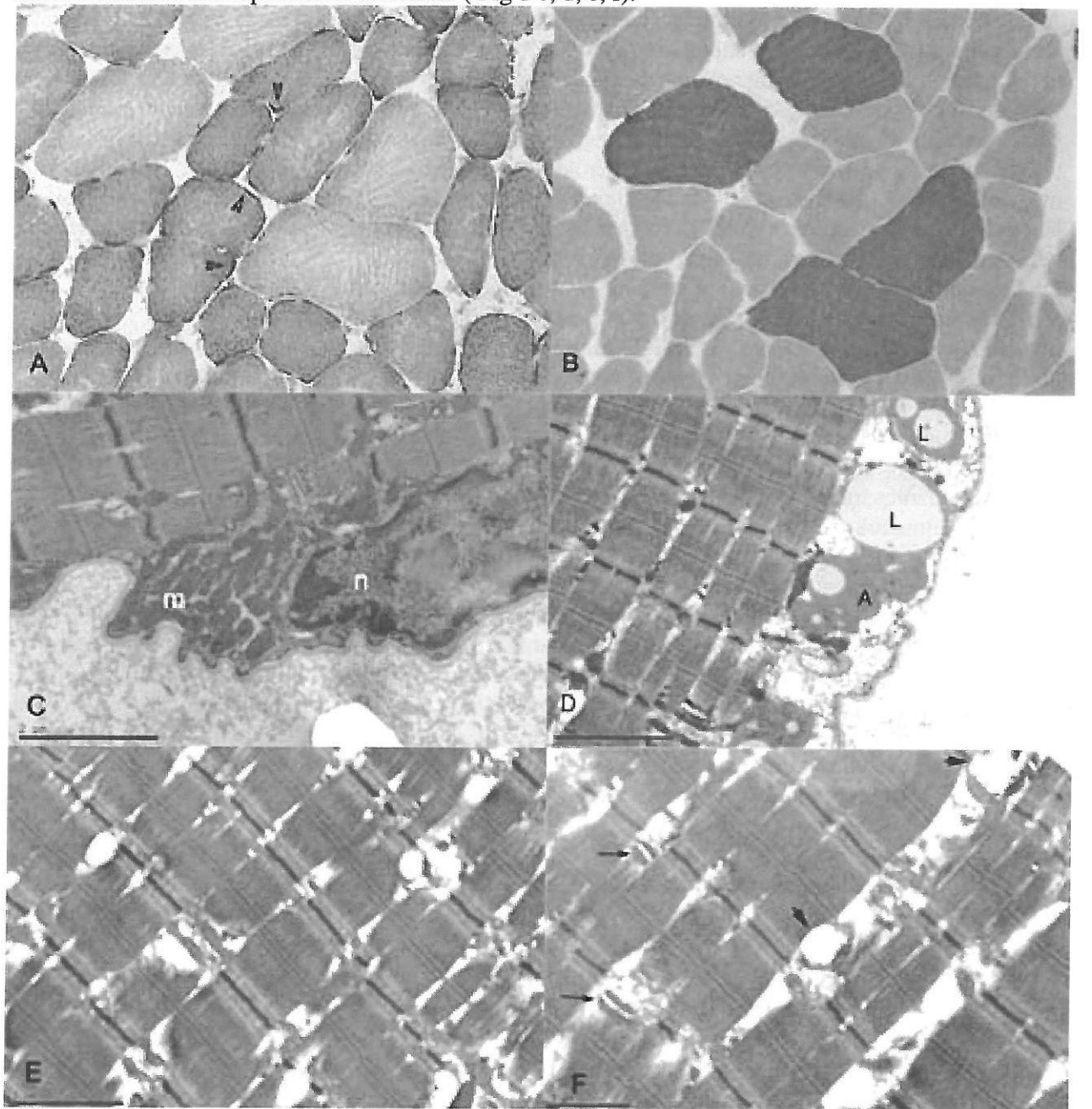
A rutin laboratóriumi vizsgálatok során kórosat nem találtunk. Radioprecipitációs módszerrel történtek a következő fehérjék elleni antitest vizsgálatok: glutamic acid decarboxylase GAD-65 (IgG), Hu, Amphiphysin 1, Yo, Ri, Purkinjecell, Ma 2, anti-neuronal nuclear antigén (ANNA3), N-methyl D-aspartate (NMDA)-receptor (NR1), leucine-rich glioma inactivated1 protein (LG-1), contactin associated protein2 (CASPR2), VGKC kv 1.1, 1.2, 1.6. A fentiek közül csupán a feszültségfüggő kálium csatorna (kv.1.2 VGKC) elleni ellenanyag küszöbszintű emelkedése volt mérhető. Két évvel később ugyanezen kálium csatorna ellen jelentős mértékű ellenanyagszint volt észlelhető: VGKC- komplex antitest: 130,2 pmol/l (norm: : < 85 pmol/l). A likvor vizsgálata alkalmával intrathechalis IgG szintézis igazolódott. Izoelektromos fókuszálással a szerumban és likvorban azonos gammaglolulin szubfrakciók jelentek meg.

Vírus szerológiai vizsgálatok a következő vírusokkal történt korábbi infekciókat igazolták: HSV 1. típus, VZV, TBE, CMV and EBV (VCA and EBNA). EEG és SEP vizsgálatok kórosat nem igazoltak. A tibiális MEP vizsgálat megnyúlt latenciát mutatott, mely pyramis pálya károsodására utalt. Az EMG vizsgálat nem jelzett spontán aktivitást, emellett maximális izom összehúzódás alkalmával normális interferencia kép ábrázolódott. Koponya MRI és SPECT vizsgálatok kórosat nem ábrázoltak. Tumorkutatás negatív eredménnyel zárult.

Carbamazepine, gabapentine, levetiracetam antiepileptikus kezelések, valamint intravénás immunoglobulin kezelés nem eredményeztek javulást. Plasmeferezis, 5 egymást követő napon történt kezelésekkel a tüneteket jelentősen csökkentették, azonban kb. 1-3 hónap után ismételt kezelések váltak szükségessé.

Az izombiopszia fénymikroszkópos vizsgálata során II-es rost hypertrofiát, számos rost hasadást, subsarcolemmalis mitokondrium felszaporodást találtunk (Fig 1 a, b). Spectrin, cavelin-3 és dysferlin

immunhisztokémiai vizsgálatok normális fehérje expressiot mutattak. Cav3 gén molekuláris biológiai vizsgálata nem talált gén mutációt. Az izom elektronmikroszkópos vizsgálata alkalmával tágult T-tubulusokat, glycogen, degradációs lipid strukturák, amorph anyag és a mitokondriumok subsarcolemmal felszaporodását észleltük. (Fig 1 c, d, e, f).



1. ábra. A: Succinate dehydrogenase festés intenzív subsarcolemmalis enzime aktivitást mutat a legtöbb I-es típusú izomrostban (nyílhegy), B: Rutin ATP-as festés II-es rost hypertrofiát ábrázol. C: Mag körüli subsarcolemmalis mitochondrium proliferáció (m: mitochondrium, n: mag), D: Subsarcolemmalis myofibrillum degeneráció amorph, részben electrodens anyag (A), valamint lipid (L) felhalmozódásához vezet. E: Vakuolumok és Z-lemez közeli strukturális dezintegráció. F: Triádokhoz (nyíl) közeli szöveti károsodás, T-tubulus kitágulás (vastag nyíl).

Megbeszélés

Évek óta multifokális myoclonusok miatt szenvedő, 48 éves férfi beteg betegségtörténetét elemeztük.

A tünetek elsősorban a felső testrész izomzatában jelentkeztek, lelki, fizikai stimuláció, akaratlagos erőkifejtés kiváltotta, vagy stimulálta az izom összehúzódásokat. A felső testrészen jelentkező myoclonusok jellemzőek myoclonus-dystonia szindrómára (6), de a betegünk betegségtörténete, valamint a dystoniás jelenségek hiánya miatt ez a lehetőség elvethető volt. Fej -, vagy végtagi tremor, ataxia, encephalopathia, valamint a szemmozgás zavarok hiánya miatt opsoclonus-myoclonus szindróma (7) diagnózisa sem látszott valószínűnek. Erőkifejtéssel provokálható izomgörcsök, a has izomzatban megjelenő izom összehúzódások betegünknél észlelhetők voltak, mely tünetek a "rippling muscle" betegségben is jellemzőek. Molekuláris genetikai vizsgálatok, valamint az izombiopszia vizsgálata nem támogatta ennek a szindrómák öröklött, vagy szerzett formáját (8, 9). Betegünk esetében, a klinikai tünetek alapján agytörzsi eredetű myoclonusokat valószínűsítettünk.

Myoclonus lehet agykérgi, agytörzsi, vagy gerincvelői eredetű. A kortikális myoclonusokat myoclonus-szinkron tüske-hullám EEG jelek jellemzik (3), míg az agytörzsi myoclonusok és a gerincvelői myoclonusok elektromosan nem detektálhatók. A perifériás hyperexcitáblitással járó kórképekben, mint a neuromyotoniában, a myoclonusok dupla-tripla motoros egység kisülésekkel kísértek, vagy fasciculatiok, fibrillatiok észlelhetők (4, 5). Betegünknél az ismételt EEG és EMG vizsgálatok nem mutatott kóros elektromos aktivitást. A mágneses ingerléses vizsgálat megnyúlt latenciát igazolt, mely a kortikospinalis pálya károsodását valószínűsítette. A gyakori alsóvégtagi fájdalmas tónusfokozódások, a külső ingerekkel stimulálható myoclonusok valamint a megnyúlt motoros latencia az agytörzsi eredetű myoclonusok egyik klinikai formájára, hyperekplexiára (startl betegség) utaltak (10, 11).

Izombiopsziás vizsgálatot végeztünk perifériás neuromuszkuláris betegség lehetőségének megerősítése, vagy kizárása céljából. Az izombiopszia fénymikroszkópos vizsgálata sem neuropathia, sem myopathia jeleit nem mutatta. A II-es típusú rost hipertrófiája és a rosthasadás a folyamatos, vagy gyakori, intenzív izommunka következménye lehet (12). Az ultrastruktúrális jelenségek, mint a T-tubulusok kitágulása, a mitochondrium proliferáció, subsarcolemmalis myofibrillaris degeneráció ugyancsak megjelenhetnek fokozott izommunka következményeiként (13). Ugyanakkor, a jelentős mértékű subsarcolemmalis degenerativ jelenségek perifériás, neuromuszkuláris károsodásra utalhatnak. Az észlelt izomszöveti elváltozások – a fokozott izommunka következményén túl – felvetik annak a lehetőségét, hogy az idegi és izomszöveti struktúrákon kívül más szöveti elemek, így valószínűsíthetően az extracellularis mátrix alkotói is részt vesznek a pathomechanizmusban, mely alkotóelemek jelentős szereppel bírnak a neuromuszkuláris jelátvitelben, valamint a sarcolemmalis membran funkciókban(14).

A fokozott verítékezés, és a szinte folyamatos myoclonusok a perifériás hyperexcitabilitással járó neuromyotonia betegségben jellemzőek (4). Betegünknél feszültségfüggő kálium csatorna elleni antitesteket találtunk, mely a neuromyotonia pathogenesisében is szerepet játszik. Betegünknél azonban nem voltak észlelhetők nyugalmi motoros egység kisülések, továbbá az alvás alatti tünetmentesség is ellentmondanak a neuromyotonia valószínűségének. Feszültségfüggő kálium csatornák elleni ellenanyagok azonban a központi idegrendszeri Morvan szindrómában és limbikus encephalitisben is pathogenetikai szerepet bírnak (5, 15, 16, 17), így továbbra is elsősorban az agytörzsi eredetű myoclonusokat tartottuk valószínűnek.

Autoimmun pathogenesisű, agytörzsi myoclonusokban igazoltak intrathecalis IgG szintézist (18), mely esetünkben is észlelhető volt. A szérumban és a likvorban talált azonos immunoglobulin szubfrakciók alapján egy korábbi infekció indukálta ellenanyag termelés gyanúja merült fel. Betegünknél számos vírussal történt korábbi infekció igazolódott, melyek bármelyike lehetett az immunválaszt indukáló ágens.

A fenti vírusok közül a kullancs encephalitis vírus esetleges pathogenetikai szerepe említhető. Betegünk kullancs encephalitis vírus fertőzések endémiás területén él (19). A kullancs encephalitis

vírusa gerincvelő mellsőszarvi - , agytörzsi-, vagy középagyi területeket is károsíthat (20, 21). Néhány közlemény multifokális vagy palatális myoclonusok hátterében ezen vírus pathogenetikai szerepét igazolta (22, 23, 24). Így betegünk esetében sem vethetjük el annak a lehetőségét, hogy a kullancs encephalitis vírusa direkt agytörzsi károsodást okozott, vagy valószínűbben, ezen vírus indukálta az immunválaszt, mely a kálium csatornák blokkolásán keresztül vezetett a tünetek megjelenéséhez.

Betegünk esetében a myoclonusokat elsősorban agytörzsi szintű neuronális hiperaktivitással tudtuk összefüggésbe hozni, azonban a szokásos antiepileptikus kezelés azonban hatástalan maradt (25). Az izom szövettani vizsgálata támogatta egy esetleges perifériás, neuromuszkuláris mechanizmus szerepét is, melyben a neuromuszkuláris transzmissziót befolyásoló extracellular mátrix elemeinek a szerepe is felmerült. Feltehető, hogy ezen károsodás másodlagos, és elsősorban a kórfolyamat krónikussá válásában lehet szerepe. Ezen komplex mechanizmus hasonlít az elsősorban fájdalommal járó szerzett krónikus állapotokhoz, mint a myofascialis fájdalom szindrómához, vagy a fibromyalgiához – melyekben a fájdalmas izomtónus fokozódások, izomgörcsök ugyancsak jellemzők –, és amely betegségekben a káliumcsatornák elleni ellenanyagoknak ugyancsak pathogenetikai szerepet tulajdonítanak (26). Betegünk esetében a plasmaferesis átmeneti javulást eredményezett. Amennyiben betegünknél a krónikus fájdalom szindrómákhoz hasonló komplex pathomechanizmust tételezünk fel, nála is megfontolandó és eredményesebb lehet orvosok fizioterápiás szakemberek, pszichoszocialis támogatók részvételével végzett, a krónikus fájdalom szindrómákban hatékony, komplex kezelés. (27, 28).

Köszönetnyilvánítás: Köszönetünket fejezzük ki Dr. Arndt Rolfs, Andrea Breda Klobus PhD, Glönitz Uta PhD és Sabrina Eichler PhD, a CENTOGENE, Institute of Molecular Diagnostics, Rostock, D-18057, Germany, munkatársainak a molekuláris genetikai vizsgálatokért. valamint Gunnvor Sjöö és Liv Gröntoft munkatársainknak, a szövettani munkáért.

References

1 Shibasaki H, Thompson PD. (2011) Milestones in myoclonus. Mov Disord 6:1142-1148

2 Caviness JN, Truong DD (2011).:Myoclonus. Handb Clin Neurol 100:399-420

3 Cassim F, Houdayer E.(2006) Neurophysiology of myoclonus. Neurophysiol Clin 36:281-291

4 Maddison P. (2006) Neuromyotonia, Clin Neurophysiol 117:2118-2127

5 Hart IK, Maddison P, Newsom-Davis J, Vincent A, Mills KR (2002) Phenotypic variants of autoimmune peripheral nerve hyperexcitability, Brain 125:1887-1895

6 Kinugawa K, Vidailhet M, Clot F, Apartis E, Grabli D, Roze E. (2009) Myoclonus-dystonia: an update. Mov Disord 24:479-489

7 Verma A, Brozman B.:Opsoclonus-myoclonus syndrome following Epstein-Barr virus infection. (2002) Neurology 58:1131-1132

8 George JS, Harikrishnan S, Ali I, Baresi R, Hanemann CO. (2010) Acquired rippling muscle disease in association with myasthenia gravis. J Neurol Neurosurg Psychiatry 81:125-126

9 Lo HP, Bertini E, Mirabella M, Domazetovska A, Dale RC, Petrini S, D'Amico A, Valente EM, Barresi R, Roberts M, Tozzi G, Tasca G, Cooper ST, Straub V, North KN. (2011) Mosaic caveolin-3 expression in acquired rippling muscle disease without evidence of myasthenia gravis or acetylcholine receptor autoantibodies. Neuromuscul Disord. 21:194-203

10 Brown P, Rothwell JC, Thompson PD, Britton TC, Day BL, Marsden CD. (1991) The hyperekplexias and their relationship to the normal startle reflex. Brain 114:1903-1928

11 Hamelin S, Rohr P, Kahane P, Minotti L, Vercueil L.:Late onset hyperekplexia.(2004) Epileptic Disord 6:169-172

12 Verdijk LB, Gleeson BG, Jonkers RA, Meijer K, Savelberg HH, Dendale P, van Loon LJ. (2009) Skeletal muscle hypertrophy following resistance training is accompanied by a fiber type-specific increase in satellite cell content in elderly men. J Gerontol A Biol Sci Med Sci. 64:332-339

13 Sroka H, Bornstein B, Sandbank U (1975) Ultrastructure of the syndrome of continuous muscle fibre activity. Acta Neuropathol, 31:85-90

14 Smith LR, Meyer G, Lieber RL (2013) Systems analysis of biological networks in skeletal muscle function. Wiley Interdiscip Rev Syst Biol Med 5:55-71

15 Liguori R, Vincent A, Clover L, Avoni P, Plazzi G, Cortelli P, Baruzzi A, Carey T, Gambetti P, Lugaresi E, Montagna P. (2001) Morvan's syndrome: peripheral and central nervous system and cardiac involvement with antibodies to voltage-gated potassium channels. Brain 124:2417-2426

16 Kleopa KA, Elman LB, Lang B, Vincent A, Scherer SS. (2006) Neuromyotonia and limbic encephalitis sera target mature Shaker-type K+ channels: subunit specificity correlates with clinical manifestations. Brain 129:1570-1584

17 Vincent A, Bien CG, Irani SR, Waters P. (2011) Autoantibodies associated with diseases of the CNS: new developments and future challenges. Lancet Neurol 10:759-772

18 Salmaggi A, Carella F, Ciano C, Binelli S, Giovannini P, Palazzini E, Girotti F. (1995) Intrathecal immune activation in three patients with progressive myoclonic ataxia. Mov Disord 10:207-210

19 Johan F, Asa L, Rolf A, Barbro C, Ingvar E, Mats H, Ake L, Sirkka V, Bo S. (2006) Tick-borne encephalitis (TBE) in Skåne, southern Sweden: A new TBE endemic region? Scand J Infect Dis. 38:800-804

20 Schellinger PD, Schmutzhard E, Fiebach JB, Pfausler B, Maier H, Schwab S.(2000) Poliomyelitic-like illness in central European encephalitis. Neurology 55:299-302

21 Bender A, Schulte-Altedorneburg G, Walther EU, Pfister HW. (2005) Severe tick borne encephalitis with simultaneous brain stem, bithalamic, and spinal cord involvement documented by MRI..J Neurol Neurosurg Psychiatry 76:135-137

22 Hanajima R, Terao Y, Ugawa Y, Kwak S, Kanazawa I.(1996) Involuntary movements observed in a patient with Russian spring summer encephalitis]. Rinsho Shinkeigaku. 36:571-576

23 Pogorzelski R, Drozdowski W, Rogowski M. (2006) Symptomatic palatal myoclonus with ear click after tick-borne meningoencephalitis. Eur Arch Otorhinolaryngol 263:711-713

24 Kraemer M, Meyn H, Linden D, Berlit P. (2006) Persistent multifocal myoclonus following Russian spring summer encephalitis. J Neurol. 253:821-822

25 Dijk JM, Tijssen MA(2010) Management of patients with myoclonus: available therapies and the need for an evidence-based approach. Lancet Neurol. 9:1028-1036

26 Klein CJ, Lennon VA, Aston PA, McKeon A, Pittock SJ. (2012) Chronic pain as a manifestation of_potassium channel-complex autoimmunity. Neurology 79:1136-1144

27 Borg-Stein J(2006) Treatment of fibromyalgia, myofascial pain, and related disorders. Phys Med Rehabil Clin N Am 17:491-510

28 Mastaglia FL.(2012) The relationship between muscle pain and fatigue. Neuromuscul Disord 22 Suppl 3:S178-180