UNIVERSIDADE ESTADUAL DE CAMPINAS

INSTITUTO DE BIOLOGIA

FABIANO POLITTI

"A eletromiografia como ferramenta de estudo da ação da auriculoacupuntura"

Tese apresentada ao Instituto de Biologia para obtenção do Título de Doutor em Biologia Celular e Estrutural, na área de Anatomia

Orientadora: Profa. Dra. Evanisi Teresa Palomari

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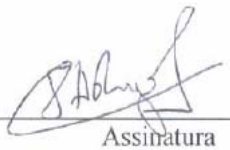

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AOS MEUS PAIS,

JOSÉ E LOURDES

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(Pitágoras)

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Índice

Lista de abreviaturas

AA	Acupuntura auricular
ACP	Momento antes do tratamento com AA
ACP1	Momento após 1 minuto de tratamento com AA
ACP2	Momento após 5 minutos de tratamento com AA
CVM	Contração voluntária máxima
EMG	Eletromiografia
RMS	Root mean square
MF	Median frequency
Sinal EMG	Sinal Eletromiográfico

1.0 Resumo

Os avanços nos conhecimentos em neurofisiologia permitiram definir que a acupuntura é um método de estimulação neural periférica, que provoca respostas reflexas, locais e sistêmicas. Portanto, o objetivo deste trabalho foi verificar se a eletromiografia de superfície pode ser utilizada como ferramenta de estudo da ação da acupuntura auricular (AA) sobre o músculo estriado esquelético e se a AA interfere na freqüência do sinal eletromiográfico (EMG) do músculo. Assim, foi analisado a amplitude sinal EMG normalizado das porções clavicular, acromial e escapular do músculo deltóide e da porção descendente do músculo trapézio com 20%, 40% e 60% da contração voluntária máxima, em 15 indivíduos voluntários saudáveis, após o tratamento com AA. A freqüência média do sinal EMG foi obtida do músculo trapézio descendente. Na coleta dos dados foi utilizado um eletromiógrafo de 8 canais com freqüência de amostragem de 1.33 kHz. Para verificar a diferenças entre os valores em RMS (*root mean square*) obtidos por meio de janela móvel de 200 ms, foi utilizado o teste de Friedman. Nos casos em que os resultados se apresentaram significantes o teste de Wilcoxon foi utilizado para comparações múltiplas. O nível de significância adotado foi de $p < 0,05$ e para as comparações Nesse estudo concluiu-se que a eletromiografia de superfície pode ser utilizada como ferramenta na análise dos efeitos da AA sobre a atividade do músculo e que o método utilizado para gravar o sinal EMG pode influenciar os resultados. Além disso, a AA pode atuar como mecanismo modulador da atividade do músculo, determinando o numero de unidades motoras recrutadas e a sua média da freqüência de disparo de acordo com o nível de força adotada durante uma tarefa realizada com contração isométrica.

2.0 Abstract

The advancement of knowledge in neurophysiology has demonstrated that acupuncture is a method of peripheral neural stimulation that promotes local and systemic reflexive responses. The purpose of this study was to determine if surface electromyography can be used as a tool to study the action of auricular acupuncture (AA) on the striated skeletal muscle and if auricular acupuncture interferes with the frequency of the EMG signal of the muscle. The EMG amplitudes of the anterior, middle and posterior deltoid muscle and the upper trapezium muscle with 20%, 40% and 60% of maximal voluntary contraction (MVC) of 15 healthy volunteers, were analyzed after the individuals were submitted to the AA treatment. The median frequency (MF) surface electromyography (EMG) recordings were obtained from the upper trapezius muscle. The non-parametric Friedman test was used to compare Root Mean Square (RMS) values estimated by using a 200 ms moving window. Significant results were further analysed using the Wilcoxon signed rank test. In this exploratory study, the level of significance of each comparison was set to $p < 0,05$. It was concluded in this study that a surface EMG can be used as a tool to investigate possible alterations of electrical activity in muscles after AA, however there is still a lack of adequate methodology for its use in this type of study, being that the method used to record the EMG signal can also influence the results. The AA peripheral stimulus can act as a modulator mechanism of muscle activity, as a result of the number of motor units recruited and their mean discharge frequency of excitation according to the level of force adopted during a task carried out with isometric contraction.

3.0 Introdução

Durante muito tempo, a acupuntura esteve isolada do mundo ocidental, pela sua forma de raciocínio e linguagem sendo, muitas vezes, desconsiderada por se apresentar de forma mística e sem base científica (Scagnamillo-Szabó & Bechara, 2001).

A técnica de curar diversas enfermidades através da inserção de agulhas em pontos específicos sobre a pele como forma de estímulo, teve sua origem na China à mais de 5000 anos (Rigol, 1992) e não é um procedimento isolado, uma vez que agrega conhecimentos profundos sobre filosofia, fisiologia dos órgãos denominados pelos chineses de *Zang Fu*, etiopatologia, fisiopatologia, diferenciação de síndromes, diagnóstico e princípios de tratamento (Farber, 1997).

Outra importante contribuição dessa medicina de prática milenar, tem como base o mapeamento de áreas que representam os órgãos e os sistemas em diferentes partes do organismo, como os demarcados nas plantas dos pés, palmas das mãos, escalpe e nas orelhas, onde cada uma atua como um terminal periférico que conecta o corpo ao cérebro (Oleson, 1996). Essas áreas são chamadas de microsistemas (Fig. 1), enquanto, a acupuntura sistêmica utiliza trajetos de energia denominados de meridianos.

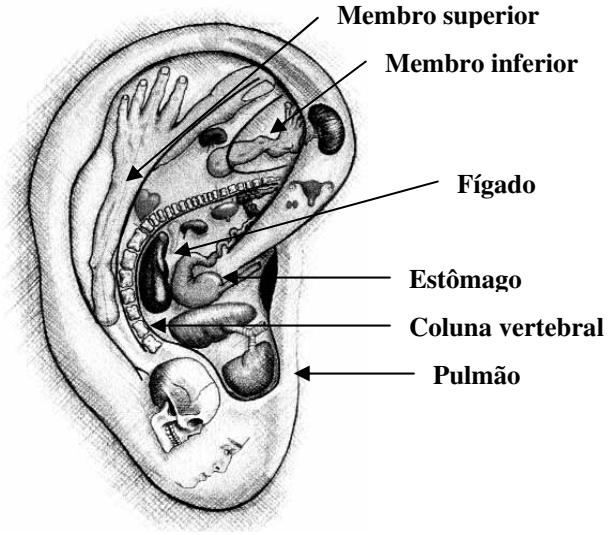

Fig. 1. Microsistema da orelha externa

No ocidente, a utilização do microsistema auricular proporcionou outra perspectiva de entendimento dos mecanismos de ação da acupuntura e isso se deve, principalmente, aos conceitos da auriculoterapia francesa, fundamentada na organização somatotópica da orelha externa e sua relação direta com o Sistema Nervoso Central, que inerva através dos ramos de alguns de seus pares de nervos cranianos todo o pavilhão auricular (Nogier, 1998).

Nessa organização somatotópica fazem parte a endoderme, a mesoderme e a ectoderme (Fig. 2. a, b, c), que de maneira geral representam respectivamente: órgãos e vísceras; aparelho músculoesquelético; sistema nervoso central e periférico (Nogier, 1998).

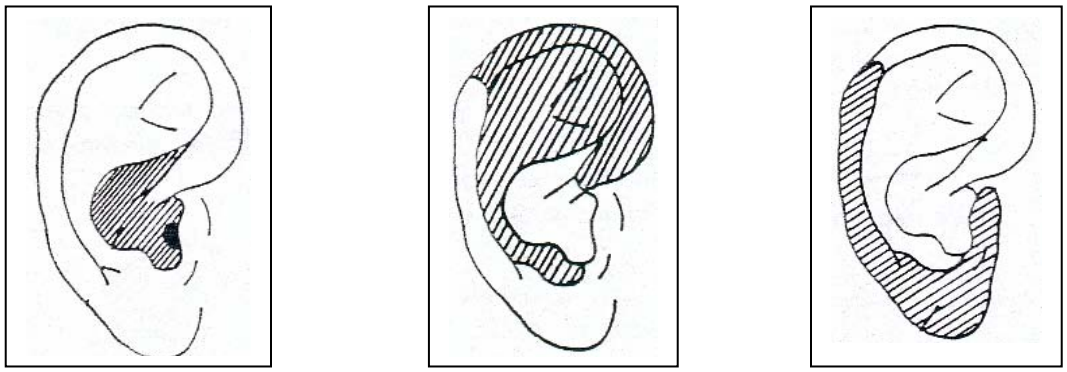

Fig. 2. (a) Endoderme. (b) Mesoderme. (c) Ectoderme.

A conexão entre o Sistema Nervoso Central e a periferia do corpo pode ser realizada por meio de terminais periféricos ligados a ramos nervosos (Farber, 1997). Esses terminais, denominados de acupontos, podem ser identificados por detectores eletrônicos de microcorrentes, por apresentam como característica a baixa resistência elétrica quando comparados com outras regiões da pele (Hyvarinen & Karlson, 1977; Starwynn, 2001).

Inicialmente, algumas hipóteses foram criadas para tentar explicar os mecanismos fisiológicos que envolvem a acupuntura dos microsistemas. Dale (1976) descreveu que os acupontos controlam de forma reflexa as conexões de outras partes do corpo por meio de caminhos neuronais do Sistema Nervoso Central, e que esses reflexos organo-cutâneos permitem revelar e delinear a patologia do corpo e, também, curá-las por meio do estímulo em pontos específicos desses microsistemas.

De acordo com a teoria dos neurônios talâmicos, as conexões reflexas entre os pontos de acupuntura e o sistema nervoso central, as eventuais mudanças patológicas que ocorrem no sistema nervoso periférico, percorrem caminhos correspondentes aos microcircuitos neuronais no cérebro e na medula espinhal. A estimulação dos pontos de acupuntura no corpo e na orelha servem para induzir a reorganização desses caminhos do cérebro frente à patologia (Lee, 1977; Lee 1994).

Com os efeitos elétricos e da estimulação de neurônios no cérebro relacionados ao alívio da dor, concluiu-se que, o sistema de inibição desta percorre o cérebro no sentido descendente e ativa os neurônios de supressão da dor, que se localizam na coluna posterior da medula espinhal (Liebeskind et al., 1974). Outra possibilidade pode estar na inibição da entrada do neurônio nociceptivo para a entrada de um neurônio tático interagindo através de um interneurônio da medula espinhal, permitindo assim, que um portão supra-espinhal no cérebro produza mensagens que irão inibir os interneurônios e com isso bloquear a ascendência do sinal de dor (Melzack & Wall, 1965).

Atualmente existem quatro formas de pesquisas envolvendo a acupuntura com algumas teorias neurofisiológicas. Essas pesquisas verificam se: (1) a existência da AA ou sistêmica se dá através do reflexo dos pontos, e isso pode ser sustentado por medidas eletrofisiológicas; (2) as

áreas do cérebro associadas com o estímulo para produção de analgesia também podem ser afetadas através de estímulos dos acupontos; (3) as evidências do desenho somatotópico encontrados na orelha externa são especificamente associados com as mudanças da atividade neuronal em diferentes partes do cérebro e; (4) as mudanças naturais dos opióides, endorfinas e encefalinas, relatam a realidade do alívio da dor através da auriculoterapia e da acupuntura sistêmica (Oleson, 2000).

Uma conclusão aceita sobre essa técnica, fundamenta-se nos efeitos da inserção de agulhas que, estimulam as fibras tipo A, em especial as Aβ, responsáveis pela percepção mais fina (tato) e as fibras do tipo C, responsaveis pela condução da dor com característica difusa as quais levam os estímulos até o corno posterior da medula e este ascende pelo trato espino-talâmico (Lewith & Kenyon, 1984).

Outro ponto bem estabelecido sobre os efeitos da acupuntura é que na medula, em especial nas lâminas I, II, III e V do corno posterior são liberadas substâncias analgésicas como a substância P, somatostatina e encefalina e no tálamo, são liberadas a endorfina, encefalina e neurotransmissores. Além do efeito analgésico dessas substâncias, o reflexo víscero – somáticos e intersegmentares também facilitam o relaxamento muscular (Lewith & Kenyon, 1984).

A maior contribuição da acupuntura é no alívio da dor (Ezzo et al., 2000) e uma das explicações desse efeito é que, a dor gerada durante a inserção da agulha de acupuntura, ativa sistemas moduladores, especialmente os opióides, noradrenérgicos e serotoninérgicos (Wall & Melzack, 1994).

Na tentativa de melhor compreender as respostas fisiológicas da acupuntura e de aumentar a confiabilidade dos resultados obtidos em experimentos controlados, algumas técnicas modernas

de diagnóstico como a ressonância magnética funcional tem sido utilizada para demonstrar possíveis correlações entre os pontos de acupuntura e zonas específicas no cérebro (Zang et al., 2003; Wu et al., 1999).

Além disso, alguns estudos com eletromiografia (EMG) de superfície começam a correlacionar a atividade elétrica do músculo estriado esquelético com os pontos de acupuntura (Tought, 2006), mas os resultados obtidos com o emprego dessa técnica, ainda não oferecem nenhuma conclusão sobre sua viabilidade no emprego da EMG no estudo dos mecanismos fisiológicos da acupuntura.

De forma geral, a EMG de superfície é um método aceito para investigar a função do músculo em diversos tipos de análises como na biomecânica (Finley et. al., 2005), nas desordens neuromusculares (Hogrel, 2005), na fadiga musculoesquelética (Ebaugh et al., 2006), na força (Kamibayashi & Muro, 2006) e na reabilitação (Barak et al., 2006). Assim, por fornecer uma representação global da atividade muscular de forma não invasiva (De Luca, 1993; Duchene & Goubel, 1993), a EMG de superfície também pode ser utilizada para analisar se a acupuntura interfere na atividade do músculo.

4.0 Objetivos e Justificativa

A acupuntura é um procedimento utilizado na medicina complementar, mas os mecanismos fisiológicos que envolvem essa técnica, ainda não são totalmente conhecidos. Vários métodos de estudo têm sido utilizados com a finalidade de esclarecer muitas questões que ainda continuam sem resposta. Dessa forma, o objetivo desse trabalho foi verificar se a EMG de superfície pode ser utilizada como ferramenta de estudo da ação da AA sobre o músculo estriado esquelético.

5.0 Material e Métodos

5.1 Sujeitos

Para esse estudo foram selecionados 40 estudantes de graduação e pós-graduação do Departamento de Anatomia da Universidade de Campinas. Por meio de sorteio duplo cego, foram selecionados quinze voluntários, destros, com idade entre 20 e 28 anos, sendo 8 mulheres (média de idade 20.63 ± 2.97) e 7 homens (média de idade 26.33 ± 5.68). Foram incluídos nesse estudo somente indivíduos saudáveis, não obesos, sedentários e sem história prévia de dor em cintura escapular.

Antes da realização do exame físico e da coleta dos dados, os indivíduos foram devidamente informados sobre os objetivos e os procedimentos a serem adotados durante o experimento. Posteriormente, assinaram um termo de Consentimento de Participação previamente autorizado (parecer nº 194/2006) pelo Comitê de Ética em Pesquisa da Universidade Estadual de Campinas – Unicamp, de acordo com os termos da Resolução n.º 196/96, de Outubro de 1996, do Conselho Nacional de Saúde do Ministério da Saúde (Anexo I e II). Todos os voluntários foram analisados por um fisioterapeuta especialista em desordens musculoesqueléticas de membro superior e cintura escapular.

Posteriormente, para comprovar a normalidade destes indivíduos foram utilizados testes específicos de avaliação do ombro como os de *Jobe* (Jobe & Jobe, 1983), *Neer* (Neer & Welsh, 1977), *Hawkins,* (Hawkins & Kennedy, 1980), Rockwood (Rockwood & Matsen, 1990), *Teste de Apreensão anterior e de Apreensão Posterior* (Davis et al., 1981) e da cintura escapular, teste de Compressão do Ombro (Palmer & Epler, 2000). Para a coluna cervical, foram utilizados os

seguintes testes: Teste de Compressão da coluna cervical (Teste de Spurling), Teste de Separação (Decoaptação), Teste de Maigne e *Testes de Retração Muscular* (Palmer & Epler, 2000).

5.2 Equipamento

Para a captação do sinal EMG foi utilizado o sistema de aquisição com 8 canais (*EMG System do Brasil Ltda* ®), composto por eletrodos de superfície ativos bipolar, filtro analógico passa banda de 20 a 500 Hz e modo comum de rejeição > 100 dB (Fig.1a). Os sinais eletromiográficos amostrados com freqüência de 1.33 kHz, digitalizados por placa de conversão A/D (analógico-digital) com 16 bits de resolução, e armazenados no Notebook *pentium 4* (Toshiba®) para posterior análise. Um canal do sistema de aquisição foi habilitado para a utilização da célula de carga (Fig. 1b), com saída entre 0 a 20mV e alcance até 1 kN (*Alfa Instrumentos*®).

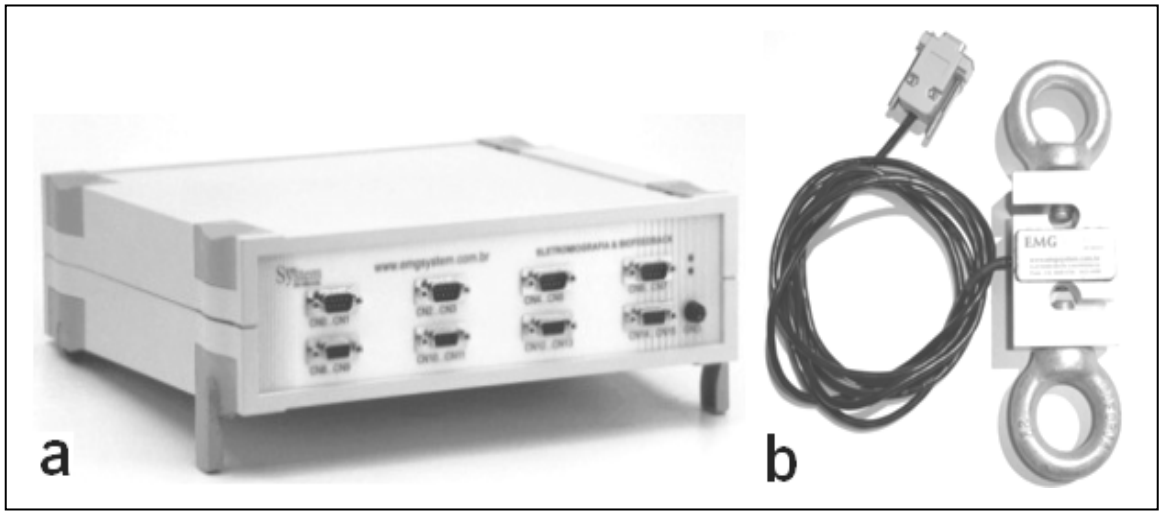

Fig. 1. (a) Eletromiógrafo – 8 canais (EMG System do Brasil®), (b) Célula de carga (Alfa Instrumentos®).

5.3 Procedimento e coleta dos dados

O músculo trapézio descendente foi selecionado devido a sua função de auxiliar a elevação do membro superior (Michels & Boden, 1992; Campos et al., 1994) e por estar sempre relacionado com dores tensionais da cabeça e do pescoço. As porções clavicular, acromial e escapular do músculo deltóide, foram escolhidos por auxiliarem na elevação e estabilização do ombro durante a elevação do membro superior (Hagberg, 1981; Kronberg et al., 1991; McCann et al., 1993).

Na coleta do sinal EMG foi utilizado eletrodos de superfície auto-adesivos circulares de prata cloreto de prata (Ag/AgCl) descartáveis (Fig. 2a), com diâmetro de 20 mm (*Medical Trace*®), e distância inter-eletrodos centro a centro de 20 mm. Os locais de fixação dos eletrodos foram previamente preparados com álcool 70% para a eliminação de resíduos gordurosos, seguida de esfoliação da pele por meio de uma lixa específica (Fig. 2b) para pele (*Bio-logic Systems Corp*®) e nova limpeza com álcool.

Como eletrodo de referência, foi utilizado um eletrodo retangular de metal, com 3 cm de comprimento e 2 cm de largura, untado com gel eletrocondutor (*Pharmaceutical Innovations*® - Fig. 2c) e fixado no punho esquerdo dos voluntários.

Nas fibras descendentes do músculo trapézio, os eletrodos foram fixados a 2 cm lateral do ponto médio da linha traçada, entre a borda póstero-lateral do acrômio e a sétima vértebra cervical (Mathiassen et al., 1995). Para as porções clavicular, acromial e escapular do músculo deltóide, foi utilizado o ponto médio entre a origem e inserção de cada porção desse músculo (Nannucci et al., 2002) como demonstrado na Fig. 3.

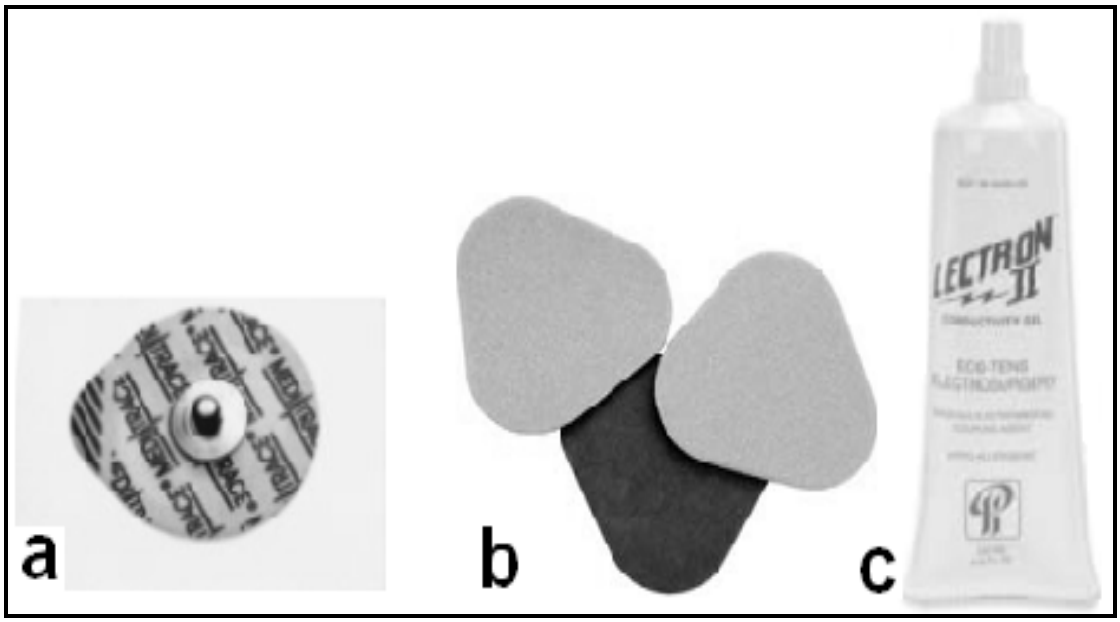

Fig. 2. (a) Eletrodo descartável de Ag/AgCl (MedicalTrace®), (b) Lixa para esfoliação (Bio-logic Systems Corp®), (c) Gel eletrocondutor (Pharmaceutical Innovations®).

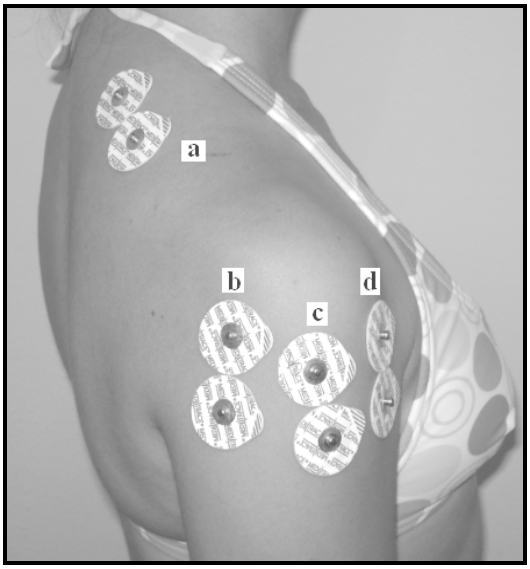

Fig. 3. Locais de fixação dos eletrodos de superfície: (a) músculo trapézio descendente e porção (b) escapular, (c) acromial e (d) clavicular do músculo deltóide.

Durante o experimento, o indivíduo permaneceu sentado, de maneira confortável na cadeira de teste. Elevando-se o ombro ispi-lateral á orelha tratada com AA, cada indivíduo realizou 3 contrações voluntárias máximas (CVM) de 3 segundos a partir da contra-resistência oferecida pela célula de carga (*Alfa Instrumentos*®) presa na base da cadeira (Fig.4). Entre as coletas foi respeitado um intervalo de 2 minutos para que houvesse a recuperação do músculo (Serger & Thorstensson et al., 1994; Serger & Thorstensson et al., 2000).

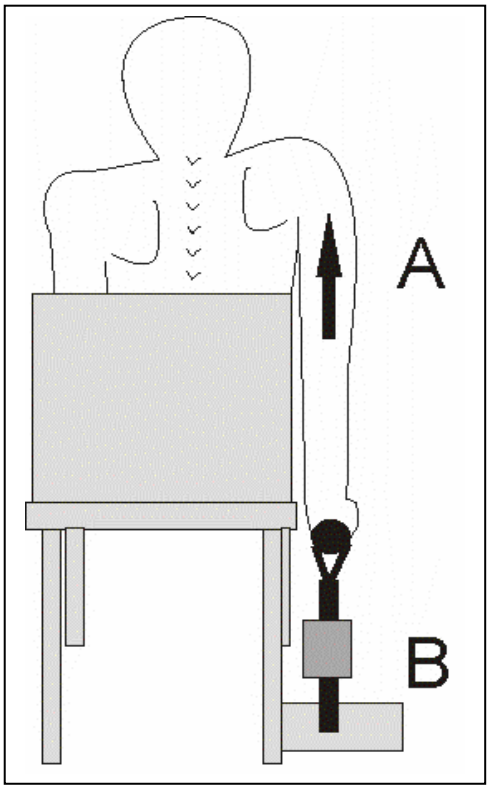

Fig. 4. Posição de teste com o indivíduo elevando o ombro (A) contra a resistência da célula de carga (B).

A média dos valores obtidos entre essas três elevações representou 100% da força de contração voluntária máxima (CMV) obtida pela tração da célula de carga (Fig. 5) e, a partir desse valor, foram calculadas as contrações submáximas de 20%, 40% e 60% da CVM. Essas três

contrações submaximas (20%, 40% e 60% da MVC) foram usadas para analisar a ação da AA nos músculos trapézio descendente e deltóide (porção clavicular, acromial e escapular).

Devido à necessidade de um grupo controle apropriado para esse tipo de estudo, foram utilizados os mesmos indivíduos como controle. Assim, o experimento foi realizado em duas etapas, com intervalo fixo de 7 dias entre cada teste. As coletas realizadas com as agulhas nos pontos específicos para o tratamento de distúrbios na região do ombro, foram atribuídas ao grupo experimental e as coletas que serviram para controle constituíram o grupo placebo. A ordem da coleta foi definida por sorteio cego e os participantes mascarados sobre a real função de cada ponto da acupuntura, como sugerido por Sherman & Cherkin (2003).

Os sinais EMG foram coletados em três diferentes momentos, sendo o primeiro denominado de ACP, serviu para comparação com os sinais obtidos após a inserção da agulha de acupuntura na orelha dos voluntários. Após 5 minutos da primeira coleta (ACP), foram inseridas agulhas estéreis de acupuntura 0,25 x 13 mm (*Suzhou Huanqiu Acupuncture Medical Appliance Co. Ltd.*®), na orelha, nos pontos previamente estabelecidos, de acordo com a proposta do estudo. Com intervalo de 1 minuto foi realizada a segunda coleta do sinal EMG (ACP1) e após 5 minutos realizou-se a terceira e última coleta EMG (ACP2), com imediata retirada da agulha.

Os critérios para coleta dos sinais EMG foram sempre os mesmos para todas as etapas do experimento. Em cada momento (ACP, ACP1 e ACP2) foi realizado coletas com 20%, 40% e 60% da CVM, mantidos por meio de feedback visual da tela do computador durante 5 segundos. Para evitar efeitos de aprendizagem, a ordem das coletas também foi definida por sorteio cego. Os possíveis riscos de compensações com o corpo durante a tração da célula de carga e a padronização de todo experimento, foram prevenidos com um treinamento que antecedeu todos os testes.

5.4 Locais de inserção das agulhas na orelha

No grupo experimental as agulhas foram inseridas na orelha em pontos correspondentes à cintura escapular, localizados no sexto dos sete espaços compreendidos entre o sulco posterior do anti-trago (em sua região de junção com a anti-hélice e a segunda depressão encontrada na anti-hélice) e ao ombro, localizado a aproximadamente 3 mm acima do sulco que separa a anti-hélice do anti-trago como indicado na Fig. 5 (Nogier & Boucinhas, 2001).

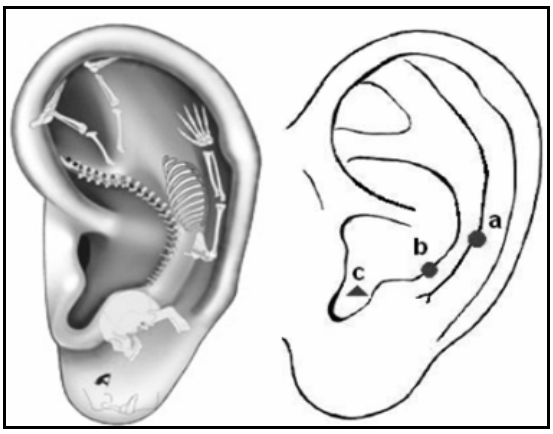

Fig. 5. Locais de inserção das agulhas na orelha. Pontos que correspondem ao ombro (a) e à cintura escapular (b) utilizados no Grupo Experimental. (c) Ponto localizado na concha da orelha utilizado para o Grupo Placebo.

Com relação ao grupo placebo a agulha foi inserida na concha da orelha como tratamento placebo (Fig. 5), uma vez que essa região não apresenta nenhuma relação somatotópica com o ombro e a cintura escapular (Nogier & Boucinhas, 2001). Para a aplicação das agulhas de acupuntura, foram realizadas assepsias nos locais por meio de álcool 70%. O tratamento com AA foi realizado por um fisioterapeuta com certificado em acupuntura pelo Conselho Regional de Fisioterapia (CREFITO-3).

5.5 Processamento e Análise dos Sinais

Para o estudo, os sinais EMG obtidos durante as contrações submaximas de 20%, 40% e 60% referentes a CVM, nas condições ACP, ACP1 e ACP2, foram normalizados pelos valores médios de três repetições com 100% da CVM com resistência fixa para cada músculo, como utilizado por McLean, 2005. Cada coleta foi realizada com 3 segundos de duração e intervalo de descanso de 2 minutos. Abaixo segue a posição e a ação de cada músculo estudado:

a) trapézio descendente e deltóide porção acromial: indivíduo sentado, realizando contração estática do membro em abdução de 90 graus e rotação neutra de ombro, contra a resistência de uma faixa localizada próxima a articulação do cotovelo (McLelan, et al., 2003);

b) deltóide porção escapular: indivíduo sentado, com leve abdução, extensão e rotação medial do ombro, realizando extensão do membro com contração isométrica durante a tração de uma faixa fixa fixa próxima a articulação do cotovelo (Kendal, et al., 1993);

c) deltóide porção clavicular: indivíduo sentado, com leve abdução, flexão e rotação lateral do ombro, realizando flexão do membro em contração isométrica durante a tração de uma faixa fixa próxima à articulação do cotovelo (Kendal, et al., 1993).

Na análise da amplitude do sinal EMG normalizado pela CVM, foram utilizados valores em RMS (*root mean square*) obtidos por uma janela móvel de 200ms, por meio do software EMG-Analysis *Ver. 1.01* (*EMG System do Brasil Ltda* ®).

5.6 Análise estatística

Os dados são apresentados como média e desvio padrão. Para a comparação intra-sujeitos dos valores em RMS do sinal EMG entre a condição sem acupuntura (ACP) e a condição com acupuntura (ACP1 e ACP2) com 20%, 40% e 60% da MVC, foi utilizado o teste não paramétrico

de Friedman. Nos casos em que os resultados se apresentaram significantes o teste de Wilcoxon foi utilizado para comparações múltiplas. O nível de significância adotado foi de $p < 0.05$. Toda a análise foi realizada pelo software estatístico SPSS ® (Versão 12.0) .

6.0 Artigos

6.1 The use of surface EMG for the study of auricular acupuncture

Submeted to: Complementary Therapies in Medicine, Elsevier, EUA.

6.2 Spectral analysis of the electromyography of the upper trapezius after auricular acupuncture

A ser submetido na revista: Clinical Neurophysiology, Elsevier, EUA.

The use of surface EMG for the study of auricular acupuncture

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Keywords: Electromyography, auricular acupuncture, muscle.

Abstract

The advancement of knowledge in neurophysiology has demonstrated that acupuncture is a method of peripheral neural stimulation that promotes local and systemic reflexive responses. The purpose of this study was to determine if surface electromyography can be used as a tool to study the action of auricular acupuncture (AA) on the striated skeletal muscle. The amplitudes EMG of the anterior, middle and posterior deltoid muscle and the upper trapezium muscle with 20%, 40% and 60% of maximal voluntary contraction (MVC) of 15 healthy volunteers, were analyzed after the individuals were submitted to the AA treatment. The non-parametric Friedman test was used to compare Root Mean Square (RMS) values estimated by using a 200 ms moving window. Significant results were further analysed using the Wilcoxon signed rank test. In this exploratory study, the level of significance of each comparison was set to $p < 0.05$. It was concluded in this study that a surface EMG can be used as a tool to investigate possible alterations of electrical activity in muscles after AA, however there is still a lack of adequate methodology for its use in this type of study, being that the method used to record the EMG signal can also influence the results.

1. Introduction

Acupuncture was recently recognized by western science as a procedure that can be used in supplementary medicine, especially in the treatment of chronic pain syndrome. [1] Advances in the knowledge of neurophysiology have made it possible to establish that this is a neural peripheral stimulation method that promotes local and systemic reflexive responses, mediated by endocrine and immune systems and by superior centers of central control.[2,3]

A reflexive response is caused by a gauging stimulus located in the somatic nerve fibers, which is triggered by insertion of a needle[4] in specific points of the skin and muscles, called acupoints. [5] These acupoints, found in greater concentration in specific areas such as the ear, can be used in a combined fashion with points on the body.[6]

The most frequent use of this acupuncture treatment is in muscular relaxation,[7] in the systemic regulation of motor apparatus dysfunctions[8] and in the control of the skeletal muscle pain, considering that its analgesic effects are mediated by central mechanisms that involve neural standards. [9,10]

However, results are still questionable regarding acupuncture's action in pain control. Studies carried out on the effects of chronic pain have indicated that the placebo group demonstrated better results than the group receiving conventional treatment, generating much criticism about the methodology applied in these types of studies.[11]

In general, the methodology utilized to demonstrate the effects of acupuncture has concerned several authors,[12,13] who rate many of these studies inconsistent, partial and likely to overestimate the positive effects of the treatment.[11,14, 15]

Due to the existence of different techniques for the insertion of the needle, combined with different strategies for the localization of the insertion points and the existence of several

treatment methods for the same type of diseases, [12] it is essential for this type of study to consider the appropriate selection of a specific treatment for each type of pathology, and the selection of the control group, the choice of the participants and the choice of the acupuncturists.[16]

Recent review articles have concluded that acupuncture still requires investigation[17] and methodological standards in order to receive greater scientific credibility.[16]

Since it is an acceptable method in the investigation of muscular function in various types of analysis such as biomechanics,[18] muscular skeleton fatigue, [19] strength, [20] rehabilitation[21] and neuromuscular disorders, [22] surface electromyography can be a reliable tool to validate the effects of acupuncture, because it is then possible to investigate the amplitude and occurrence of electrical activity in the muscle while a task is being performed.[23]

The aim of the present study was to determine if the surface electromyography can be utilized as a tool to study the action of auricular acupuncture (AA) on striated skeletal muscles. Additionally, the behavior of the electrical activity was verified from anterior, middle and posterior deltoid muscles and upper trapezium muscle at different levels of exertion in a single test position.

2. Methods

2.1. Subjects

The volunteers taking part in the study were selected by means of a blind draw from the population of undergraduate and graduate students from the Anatomy Department of the University of Campinas Biology Institute. Fifteen volunteers, between ages 20 and 28, were selected and of those, eight were females (average 20.63 ± 2.97) and seven were males (average

26.33 ± 5.68). Included in the study were healthy, non-obese, sedentary volunteers having no history of previous shoulder pain. The volunteers were examined by a physiotherapist familiar with muscle skeletal disorders of the upper limbs and scapular waist and neck. All volunteers signed a Term of Consent as required by resolution 196/96 issued by the National Health Council and previously approved by the Ethical Committee in Research from the State University of Campinas. Each subject was informed of the purpose and potential risks of the study before their written voluntary consent was obtained.

2.2. Equipment

Myoelectric signals were obtained using an 8-channel module (EMG System do Brazil Ltda. ®), with the following characteristics: band pass filter of 20–500 Hz, amplifier gain of 1000, and common rejection mode ratio > 100dB. All data were acquired and processed using a 16-bit Analog to Digital converter (EMG System do Brazil Ltda. ®), with a sampling frequency of 1.33 kHz. The system was composed of active bipolar electrodes yielding a pre-amplification gain of 20x on the raw EMG signal. A channel of the acquisition system was enabled for the utilization of the load cell (Alfa Instruments®), having an output between 0 a 20 mV and a range up to 1 kN.

2.3. Procedure and data collection

Muscle activity was recorded from the upper trapezius, selected because they are the primary muscles used to elevate the arm [24,25] and because they are always related to tension pain of the

head and neck. The anterior, middle and posterior deltoid muscles were chosen because they assist in the elevation and stabilization of the shoulder during this elevation.[26-28]

The bipolar surface circular electrodes (Ag/AgCl – Medical Trace®) witch 20mm in diameter, were used for the surface recording of EMG with a center to center distance of 20 mm. Prior to the fixation of the electrodes, the skin was cleansed for the elimination of residual fat; cleansing was followed by exfoliation using a specific sand paper for skin (Bio-logic Systems Corp®) and a second cleaning with alcohol. The electrodes were affixed 2 cm laterally in reference to the mid point of a line traced from the posterior lateral edge of the acromion to the 7th cervical vertebra[29] in the upper trapezium muscle. For the anterior, middle and posterior deltoid electrodes were positioned in the lower half of the distance from the acromion to the deltoid tuberosity.[30] A reference rectangular electrode (3 cm x 2 cm), was lubricated with electro-conductor gel (Pharmaceutical Innovations®) and fastened to the left wrist of the volunteers. During the experiments, the individuals remained comfortably seated in the test chair.

Before beginning the recording of EMG signals, each individual subject was asked to carry out a series of three maximum force elevations of the shoulder ipsi-lateral to the AA, with duration of 3 s each, against the resistance offered by the load cell (Fig. 1). A 2-min rest period was given between efforts. Verbal encouragement was given to the subject especially during the task.

The mean value from the three trials obtained against the resistance offered by the load cell, represented a subject's 100% maximum voluntary contractions (MVC) force, and the 20%, 40% and 60% values of MVC were calculated from that number. Mean muscle output was used to determine MVC as it was believed to be a more accurate representation of a subject's strength

than a single contraction. The three sub-maximal contractions (20%, 40% and 60% MVC) were used in the analysis of the AA action on the shoulder muscle.

Due to the necessity of having an appropriate control group for this type of study, the same volunteers served as the control. Consequently, the experiment was run in two stages with a fixed, 7-day interval between the two tests. The results obtained with the needles placed in the points specific to the treatment of shoulder region problems were assigned to the experimental group and the placebo group served as the control. A blind draw determined the order of the individual subjects sampled. As suggested by Sherman and Cherkin, [16] the real function of each acupuncture point was masked from the participants.

EMG signals were recorded at three distinct moments, the first, pre-acupuncture (ACP), served as comparison to the signal obtained after the insertion of the acupuncture needle. Five minutes after the first sample (ACP), disposable sterile acupuncture needles 0.25 x 13 mm (Suchou Huanqiu Acupuncture Medical Appliance Co. Ltd.®) were inserted at previously established points of the outer ear and were maintained without manipulation until the end of the experiment. After a 1-min interval, a second EMG signal sample was recorded with acupuncture (ACP1), and after 5 min, the third and last sample EMG signal (ACP2) was recorded, followed by immediate removal of the needle.

The criteria for the recording of the EMG signals were always the same for all stages of the experiment. At each moment (ACP, ACP1 and ACP2), 20%, 40% and 60% sub-maximum MVC samples were collected and maintained through visual feedback provided by a line drawn on the computer screen. The duration of each EMG signal sample was 5 s. In order to avoid a learning effect, the order of the sample collection was also determined by blind draw. Possible risks of

bodily compensation during the traction of the load cell and of patterning in the whole experiment were prevented through training before all the tests. Of greatest concern during the experiment was that the head and neck be always maintained in the same position, so as to avoid interference from the upper trapezius muscle in the activity.

2.4. Needle insertion points on the ear

In the experimental group, the needles were inserted on the ear at the points corresponding to the scapular waist, located in the sixth of seven spaces contained between the posterior fold of the anti-tragus (in the region of its junction with the anti-helix and the second depression located on the anti-helix), and to the shoulder, located approximately 3 mm above the furrow which separates the anti-helix from the anti-tragus as indicated in Fig. 2. [31]

In relation to the placebo group, the needles were inserted on the shell of the ear as placebo treatment (Fig. 2), being that this region does not present any somatotropic relationship to the shoulder and the scapular waist.[31] Asepsis by means of alcohol was provided at the location of the acupuncture needle insertion. A physiotherapist, certified in acupuncture by the Regional Physiotherapy Council (CREFITO-4), performed the treatment with AA.

2.5. Processing and analysis of the signals

For this study, the EMG signals obtained during the 20%, 40% and 60% sub-maximal MVC contractions under the conditions ACP, ACP1 and ACP2, were normalized by the mean values of the three repetitions at maximal effort with fixed resistance for each muscle analyzed, as used by McLean.[32] Each sample lasted 4 s with a rest interval of 2 min. The position and action of each muscle studied was thus: i) upper trapezium and medium deltoid: individual subject seated,

performing isometric contraction of the limb in abduction of 90 degrees and neutral rotation of the shoulder, against the resistance of a strap positioned near the elbow joint;[33] ii) posterior deltoid: individual subject seated, with a slight abduction, extension and medial rotation of the shoulder, performing extension of the limb with isometric contraction during the traction of a fixed strap attached near the elbow joint;[34] iii) anterior deltoid: individual subject seated, with slight abduction, flexion and lateral rotation of the shoulder, performing flexion of the limb in isometric contraction during the traction of a fixed strap attached near the elbow joint.[34]

After data were normalized for each muscle the root mean square (RMS) was calculated using a 200 ms moving window. EMG Analysis Software, Version 1.01 (EMG System do Brasil, Ltda. ®) was used.

2.6. Statistical analysis

Data are presented as means and standard deviations (SD). The non-parametric Friedman test was used to compare intraclass results in root mean square amplitude (RMS). Significant results were further analyzed using the Wilcoxon signed rank test. In this exploratory study, the level of significance of each comparison was set to $p < 0.05$. The entire analysis was conducted using the software SPSS ® (Version 12.0).

3. Results

The intraclass analysis (Friedman test) of anterior, middle and posterior deltoid muscle (Table 1) did not present a statistically significant difference ($p > 0.05$) of the values of RMS amplitude,

under pre-acupuncture conditions (ACP) and with acupuncture (ACP1 and ACP2), tested under each sub-maximum force level (20%, 40% and 60% of MVC).

The same analytical criteria was adopted for the upper trapezium muscle, which, in accordance with the Friedman test displayed significant difference ($p < 0.003$) for values corresponding to 60% of the MVC in the experimental group. In the multiple comparisons, using the Wilcoxon test, a significant difference ($p < 0.003$), was identified in the RMS values corresponding to the ACP and ACP2 (Fig. 3). These results indicate an increase of the RMS amplitude in the upper trapezium muscle at 60% MVC, after 5 min of the insertion of the acupuncture needle in the ear. For values regarding 20% and 40% MVC in the experimental group, no significant differences were found (Friedman test, $p > 0.05$). Under these same test conditions, no statistically significant differences were found in the analysis of the trapezium muscle in the placebo group (Fig. 3).

4. Discussion

Based on the advances in neurophysiology, it is possible to define acupuncture as a neural peripheral stimulation method aimed at promoting changes in the sensorial, motor, hormonal and cerebral functions.[3,35] Such changes originate from the reflex response caused by the afferent stimuli in the somatic nerve fibers after the insertion of the needle.[4]

Knowledge about the reflex response is concentrated in experimental studies carried out on the pathways of the sensory nerve system responsible for the modulation and inhibition of pain[2,3] in the three levels of the central nervous system: spinal cord,[7,36] encephalic trunk[36] and cerebral cortex.[37] However, the action of auricular acupuncture on the motor apparatus is not frequently discussed in scientific studies, which makes difficult a discussion of the results presented in this work.

With respect to the results obtained by means of the methodology used in this study, it is not possible to state that auricular acupuncture can influence the activity of the muscles studied because, although a significant increase ($p < 0.05$) in EMG activity of the upper trapezius muscle was observed in the experimental group with 60% of MVC (Fig. 4), there was no alteration of the EMG signal with 20% and 40% of MVC. In general, during an isometric exercise at constant load, there is a time related increase of the EMG signal[38] which could be related to changes in the recruiting pattern of motor units after the first seconds of contraction, as well as to the increase of the amplitude of the action potential and to the recruitment of motor units or the firing of the motor neurons[38-40] however, this fact is not enough to justify the increased amplitude of the EMG signal in the experimental group, since the same experimental conditions were maintained for the two groups studied.

Another argument that would contradict this outcome is that acupuncture has as one of its more frequent clinical uses, muscular relaxation[7] and, in this case, the EMG signal is reduced. As such, this study opens to questioning the affirmation that AA has muscular relaxation as one of its effects.

One possible explanation for the appearance of the significant difference found in the experimental group at 60% of MVC could be related to the methodology employed during the experiment. Since the collection sequence of EMG signal was by blind draw, the data referring to the sub-maximum contraction of 60% of MVC could have randomly been the last to be collected under the ACP2 condition of the experimental group, but there is no way of proving this, each possibility of sub-maximum contraction (20%, 40% and 60% MVC) having been determined by the draw which preceded each of the samples. If this really occurred, the increased EMG signal amplitude could be related to the onset of fatigue,[41] which can happen during a repetitive or

sustained activity,[42] as was the case during the experiment. This points to a possible fault in the method used in this study.

During the development of this study, a constant source of concern for the authors was the use of methodological rigor in the collection and treatment of data. This concern arose mainly after the literature review indicated potential problems involving sample size, nature of the study, inadequate control groups and absence of long-term responses. [11,15-17,43]

To insure confidence in the results obtained, a blind clinical trial was carried out by means of a drawing. The trial aim was to compare the action of the true points in the treatment experimental group versus the action of the false points in the placebo group, in random distribution, as suggested in the literature[44,45] and described in the methodology of the present study. The use of false points is possible due to the fact that points on the ear are highly specific and there are differences between the stimuli effect from the real and false points.[46,47]

It is important to mention that it is impossible to carry out a double blind study in acupuncture since the acupuncturist cannot be blinded.[2,45] Consequently, the volunteers that participated in this study, as well as the author of the evaluations of the EMG signals, were not informed of the specific function of the AA treatments adopted in the experiment, as suggested by Filshie and White[45] and Carlsson et al. [2]

Even with all these cautionary measures, it was observed that the methodology used for EMG signal recording of the deltoid muscle was not the most appropriate. Previous studies indicate that the action of the three portions of the deltoid muscle is directly related to the movements of abduction, flexing and the dislocation of the upper limb in different angular movements.[24,25] Since this experiment was conducted without angular shoulder movement (Fig. 1), the EMG signal amplitude obtained of the three portions of the deltoid muscle was very low and possibly

not sufficient to show a possible influence of AA on this muscle. With relation to the upper trapezius muscle, the position adopted for the tests (Fig. 1) permitted that this muscle performed its functions as a prime mover during elevation and rotation of the scapula and as a stabilizer of the scapula during glenohumeral movements[48] and during glenohumeral torque production.[49] Consequently, it is important to respect the muscular function because the firing frequency of the motor units varies according to the levels of force exerted during the isometric contraction[50] and it is possible that the greater the motor engagement, the greater the chance that alterations will be observed in the pattern of the EMG signal.

5. Conclusions

Based on the results found, it is not yet possible to state that AA can influence the electrical acitivity of the muscle. A suitable methodology needs to be developed to allow the utilization of AA as a research tool in the investigation of the action of AA on the electrical activity of the muscle. Factors such as the duration of collection, main action of the muscle and the number of test repetitions during the same experiment can influence the amplitude of the EMG signal leading to wrong conclusions about the answers obtained from the tests. To avoid misleading results in future investigations that involve the use of surface EMG in the analysis of the effects of AA, the experiments should be carried out while the muscles perform their main action to increase the chances of detecting any alteration of the EMG signal caused by the use of AA. The duration of data collection and the number of repetitions of the experiment also need to be well planned to avoid alterations of the amplitude EMG signal as a result of biological factors such as muscular fatigue.

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Table and legend

Table 1. Mean and standard derivation (SD) of RMS (μV) from the anterior, middle and posterior deltoid muscle from experimental group and placebo group. ACP indicates the pre-acupuncture condition and ACP1 and ACP2 indicate the acupuncture condition tested under three levels of sub-maximum force (20%, 40% and 60% MVC).

*A Friedman test did not show a significant difference ($p > 0.05$) in the intraclass comparison.

Figures and legends

Legends

Fig. 1. Test position as the individual elevates the shoulder ispi-lateral to the AA (A) against the resistance of the load cell (B).

Fig. 2. Insertion points of the acupuncture needle in the ear, corresponding to the shoulder (a) and the scapular waist (b) used in the experimental group and the point located in the shell of the ear (c) used for the placebo group.

Fig. 3. Mean of the RMS (μV) value computed over 5s from the upper trapezium muscle (UP). The intraclass tests accomplished in the placebo group, did not demonstrate significant statistical differences (Friedman test, $p > 0.05$) between the pre-acupuncture (ACP) and with acupuncture (ACP1 and ACP2) in each one of the tree tested levels of sub-maximum force (20%, 40% and 60% MVC). * Indicates a significant difference between ACP and ACP2 (Wilcoxon, $p < 0.003$).

Table 1.

Muscles	% MVC	ACP	ACP1	ACP2	*P value
		Mean (SD)	Mean (SD)	Mean (SD)	
Experimental Group					
	60%	0.29 (0.18)	0.28 (0.18)	0.32 (0.22)	0.76
Anterior Deltoid	**40%**	0.18 (0.12)	0.21 (0.12)	0.17 (0.14)	0.42
	20%	0.14 (0.09)	0.14 (0.11)	0.15 (0.11)	0.54
	60%	0.83 (0.58)	0.80 (0.59)	0.86 (0.55)	0.81
Middle Deltoid	**40%**	0.52 (0.49)	0.56 (0.42)	0.54 (0.42)	0.81
	20%	0.47 (0.41)	0.46 (0.41)	0.48 (0.40)	0.62
	60%	0.88 (0.56)	0.86 (0.68)	0.90 (0.69)	0.93
Posterior Deltoid	**40%**	0.58 (0.52)	0.49 (0.43)	0.52 (0.41)	0.12
	20%	0.28 (0.16)	0.28 (0.19)	0.31 (0.19)	0.48
Placebo Group					
	60%	0.21 (0.06)	0.20 (0.10)	0.20 (0.06)	0.72
Anterior Deltoid	**40%**	0.17 (0.06)	0.21 (0.19)	0.20 (0.19)	0.28
	20%	0.10 (0.03)	0.11 (0.04)	0.10 (0.03)	0.76
	60%	0.67 (0.39)	0.63 (0.36)	0.67 (0.35)	0.62
Middle Deltoid	**40%**	0.42 (0.17)	0.47 (0.22)	0.46 (0.21)	0.62
	20%	0.35 (0.15)	0.39 (0.20)	0.38 (0.19)	0.86
	60%	0.71 (0.68)	0.62 (0.51)	0.63 (0.41)	0.63
Posterior Deltoid	**40%**	0.43 (0.23)	0.41 (0.28)	0.43 (0.23)	0.21
	20%	0.27 (0.12)	0.26 (0.11)	0.28 (0.12)	0.70

Figures

Fig.1

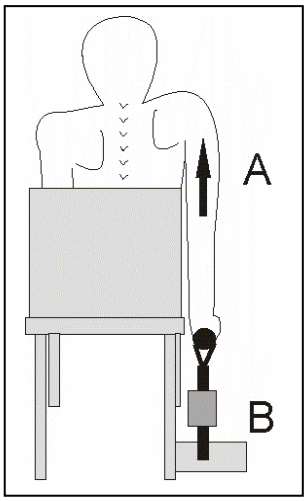


Fig.2

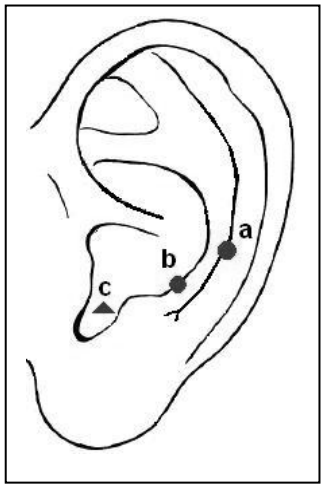
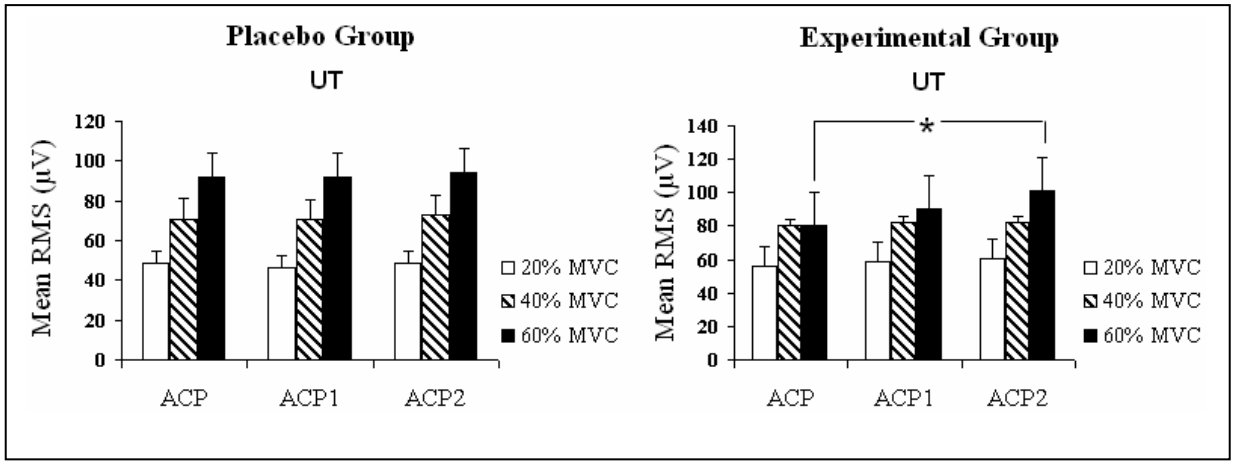


Fig. 3


Placebo Group
UT
Mean RMS (μV)
120
100
80
60
40
20
0
ACP
ACP1
ACP2
20% MVC
40% MVC
60% MVC
Experimental Group
UT
*
Mean RMS (μV)
140
120
100
80
60
40
20
0
ACP
ACP1
ACP2
20% MVC
40% MVC
60% MVC

Spectral analysis of the electromyography of the upper trapezius after auricular acupuncture

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Keywords: Electromyography, auricular acupuncture, spectral analysis.

Abstract

Objective: To investigate if auricular acupuncture interferes with the frequency of the EMG signal of the muscle fiber and if there exists correspondence between the auricular acupoints and the trapezius muscle.

Methods: The action of AA was verified in fifteen healthy subjects with 40% and 60% of the maximum force that was continuously monitored by a force transducer. The median frequency (MF) surface electromyography (EMG) recordings were obtained from the upper trapezius muscle following ipsilateral recurrent noxious needle stimulation in the ear.

Results: The intraclass analyses indicate a significant increase of the MF in the upper trapezius muscle at 60% MVC, one minute after insertion of the acupuncture needle in the ear.

Conclusion: There is correspondence of the acupoint with the trapezius muscle and the AA can act as a modulator mechanism of the muscle during an exertion in isometric contraction.

Significance: The understanding of the action of AA on the motor control of healthy subjects is important for the study and use of this treatment technique in patients suffering from motor system dysfunction

1. Introduction

Presently, acupuncture is a technique considered to be capable of stimulating the regulatory systems of the organism, such as the central nervous system, the endocrine system and the immunological system (Sims, 1997). Its effects are based on the local or systemic reflex responses, obtained by means of afferent stimuli of specific points of the skin and muscles, points known as acupoints (Li et al., 2004).

These acupoints are characterized by a concentration of "gap" junctions that facilitate intercellular communication and increased electrical conductivity (Altman, 1992) and by sensory nerve ending connected to nerve branches that make possible direct access to the central nervous system (Li et al., 2004) and that induce activation patterns in specific areas of the brain (Yan et al., 2005).

In many cases, the acupoints are found in mapped areas called microsystems, such as those on the soles of the feet, on palms of the hands and on the ears, that represent the organs and the systems in different parts of the organism. Each acupoint acts as a peripheral terminal connecting the body to the brain (Oleson, 1996).

In western culture, the use of the auricular microsystems developed in France by Dr. Nogier gave another perspective to the understanding of some of the mechanisms by which acupuncture works. The concept of the technique is based on the somatotopic organization of the external auricular pavilion and principally on its direct relation to the central nervous system, which happens through the branches of pairs of cranial nerves innervating the whole auricular pavilion (Nogier, 1998).

Serious problems with the methodology used to investigate the action of acupuncture on the organism have been found in studies of metanalysis (Birch, 2003; Hopwood and Lewith, 2003;

Sherman et al., 2003). This could be one of the most important causes for the slow rate of acceptance of acupuncture in western culture. One of many difficulties involving treatment with acupuncture has its origins in the various traditions involving different concepts of physiology and diagnosis.

In an attempt to better understand the physiological answers of acupuncture and to increase the reliability of the results obtained in controlled experiments, some modern diagnostic techniques, such as functional magnetic resonance, have been used to demonstrate possible correlations between the acupuncture points and specific zones of the brain (Zang et al., 2003; Wu et al., 1999). In addition, some studies with surface electromyography (EMG) have started to correlate the electrical activity of the striated skeletal muscles with acupuncture points (Tough, 2006).

Generally speaking, surface EMG is an acceptable method in the investigation of muscular function in various types of analysis: in muscular biomechanics (Finley et al., 2005), muscular skeleton fatigue (Ebaugh et al., 2006), strength (Kamibayashi and Muro, 2006), rehabilitation (Barak et al., 2006) and neuromuscular disorders (Hogrel, 2005). Surface electromyography, has been used to study muscle activation, can be a reliable tool to validate the effects of acupuncture due to its non-invasive nature, its ease of use, and the fact that it can provide a representation of the global level of muscle activity (De Luca, 1993, Duchêne and Goubel, 1993).

The power spectral density of the EMG signal can be a way to investigate possible changes that occur during muscle activity. The basic assumption for the use of spectral characteristics of the signal for inferring motor control strategies or changes in fiber membrane properties is the scaling effect that muscle fiber conduction velocity has on the power spectrum of the signal (Lindstrom and Magnusson, 1997; Stulen and DeLuca, 1981). Median frequency and mean

frequency are the forms most utilized to verify the possible changes happening in the power spectrum.

As it is less affected by noise and more sensitive to the biochemical and physiological processes that occur during a sustained contraction (Stulen and DeLuca, 1981), this study uses the median frequency (MF) to investigate whether the peripheral stimulus of auricular acupuncture (AA) interferes in the EMG signal frequency of muscle fiber and whether there exists a correspondence between the auricular acupoints and the trapezius muscle.

2. Methods

2.1. Subjects

The volunteers taking part in the study were selected by means of a blind draw from the population of undergraduate and graduate students from the Anatomy Department of the University of Campinas Biology Institute. Fifteen volunteers, between ages 20 and 28, were selected and of those, eight were females (average 20.63 ± 2.97) and seven were males (average 26.33 ± 5.68). Included in the study were healthy, non-obese, sedentary volunteers having no history of previous shoulder pain. The volunteers were examined by a physiotherapist familiar with muscle skeletal disorders of the upper limbs and scapular waist and neck. All volunteers signed a Term of Consent as required by resolution 196/96 issued by the National Health Council and previously approved by the Ethical Committee in Research from the State University of Campinas. Each subject was informed of the purpose and potential risks of the study before their written voluntary consent was obtained.

2.2. Electromyography

MF was assessed via the frequency spectrum for the upper trapezius muscle. Preamplifier bipolar circular surface electrodes (Ag/AgCl – Medical Trace®) were placed on muscle with a fixed interelectrode distance (center-to-center) of 2 cm. Prior to electrode placement, the skin area was shaved, cleaned with isopropyl alcohol and abraded with coarse gauze in order to reduce skin impedance and to ensure electrode adherence. Electrode placement for the 2 cm laterally in reference to the mid point of a line traced from the posterior lateral edge of the acromion to the 7th cervical vertebra (Mathiassen et al., 1995). A reference rectangular electrode (3cm x 2cm), was lubricated with electro-conductor gel (Pharmaceutical Innovations®) and fastened to the left wrist of the volunteers. EMG activity was collected by a eith-channel unit (EMG System do Brazil Ltda®) consisting of a band pass filter of 20–500 Hz, an amplifier gain of 1000, and a common rejection mode ratio > 100dB. All data were acquired and processed using a 16-bit Analog to Digital converter (EMG System do Brazil Ltda ®), with a sampling frequency 1.33 kHz. A channel of the acquisition system was enabled for the utilization of the load cell (Alfa Instruments®), having an output between 0 a 20 mV and a range up to 1 kN.

A power spectral analysis was performed on the 5 window for upper trapezius muscle. A fast Fourier transform of 512 points (Hanning window processing) was performed on 19 consecutive, 512 ms segments, overlapping each other by half their length (256 ms), for each 5 sec contraction. The FM was determined from each of the 19 overlapping windows. The mean and standard deviation of the FM during each contraction were then calculated for each muscle

Data collection

Muscle activity was recorded from the upper trapezius, selected because they are the primary muscles use to elevate the arm (Michels and Boden, 1992; Campos et al., 1994) and because they are always related to tension pain of the head and neck. In addition, the whole shoulder and cervical region are represented in an area of the ear (Nogier, 2001).

During the experiments, the individuals remained comfortably seated in the test chair.

Before beginning the recording of EMG signals, each individual subject was asked to carry out a series of three maximum force elevations of the shoulder ispi-lateral to the AA, with duration of 3 seconds each, against the resistance offered by the load cell (Fig. 1). A 2-min rest period was given between efforts. Verbal encouragement was given to the subject especially during the task.

The mean value from the three trials represented a subject's 100% maximum voluntary contractions (MVC) force, and the 40% and 60% values of MVC were calculated from that number. Mean muscle output was used to determine MVC as it was believed to be a more accurate representation of a subject's strength than a single contraction. The two sub-maximal contractions (40% and 60% MVC) were used in the analysis of the auricular acupuncture action on the upper trapezius muscle.

Due to the necessity of having an appropriate control group for this type of study, the same volunteers served as the control. Consequently, the experiment was run in two stages with a fixed, 7-day interval between the two tests. The results obtained with the needles placed in the points specific to the treatment of shoulder region problems were assigned to the experimental group and the placebo group served as the control. A blind draw determined the order of the individual subjects sampled. As suggested by Sherman and Cherkin (2003), the real function of each acupuncture point was masked from the participants.

EMG signals were recorded at three distinct moments, the first, pre-acupuncture (ACP), served as comparison to the signal obtained after the insertion of the acupuncture needle. Five minutes after the first sample (ACP), disposable sterile acupuncture needles 0.25 x 13 mm (*Suchou Huanqiu Acupuncture Medical Appliance Co*. Ltd.®) were inserted at previously established points of the outer ear and were maintained without manipulation until the end of the experiment. After a 1-min interval, a second EMG signal sample was recorded with acupuncture (ACP1), and after 5 min, the third and last sample EMG signal (ACP2) was recorded, followed by immediate removal of the needle.

The criteria for the recording of the EMG signals were always the same for all stages of the experiment. At each moment (ACP, ACP1 and ACP2), 40% and 60% sub-maximum MVC samples were collected and maintained through visual feedback provided by a line drawn on the computer screen. The duration of each EMG signal sample was 5 sec. In order to avoid a learning effect, the order of the sample collection was also determined by blind draw. Possible risks of bodily compensation during the traction of the load cell and of patterning in the whole experiment were prevented through training before all the tests. Of greatest concern during the experiment was that the head and neck be always maintained in the same position, so as to avoid interference from the upper trapezius muscle in the activity.

2.4. Needle insertion points on the ear

In the experimental group, the needles were inserted on the ear at the points corresponding to the scapular waist, located in the sixth of seven spaces contained between the posterior fold of the anti-tragus (in the region of its junction with the anti-helix and the second depression located on

the anti-helix), and to the shoulder, located approximately 3 mm above the furrow which separates the anti-helix from the anti-tragus as indicated in Fig. 2 (Nogier and Boucinhas, 2001). In relation to the placebo group, the needles were inserted on the shell of the ear as placebo treatment (Fig.2), being that this region does not present any somatotropic relationship to the shoulder and the scapular waist (Nogier and Boucinhas, 2001). Asepsis by means of alcohol was provided at the location of the acupuncture needle insertion. A physiotherapist, certified in acupuncture by the Regional Physiotherapy Council (CREFITO-4), performed the treatment with AA.

2.6. Statistical analysis

Data are presented as means and standard deviations (SD). The non-parametric Friedman test was used to compare intraclass results in MF. Significant results were further analyzed using the Wilcoxon signed rank test. In this exploratory study, the level of significance of each comparison was set to $p < 0.05$. The entire analysis was conducted using the software SPSS ® (Version 12.0).

3. Results

An intraclass study was used for data analysis. In the placebo group, a Friedman test did not present a statistically significant difference ($p > 0.05$) between the MF values under the conditions of pre-acupuncture (ACP) and with acupuncture (ACP1 and ACP2), tested at each level of sub-maximum force (40% and 60% of MVC).

The same analytical criteria were adopted for the experimental group, which, in accordance with the Friedman test, displayed a significant difference ($p < 0.03$) for values corresponding to 60%

of the MVC. In the multiple comparisons, using the Wilcoxon test, a significant difference ($p < 0.03$), was identified in the FM corresponding to the ACP and ACP1 (Fig. 3). These results indicate an increase of the MF in the upper trapezium muscle at 60% MVC after 1 min of the insertion of the acupuncture needle in the ear. For the values referring to 40% of MVC in the experimental group, no significant differences were found (Friedman test, $p > 0.05$).

4. Discussion

Based on the advances in neurophysiology, it is possible to define acupuncture as a neural peripheral stimulation method aimed at promoting changes in the sensorial, motor, hormonal and cerebral functions (Nishijo et al., 1997; Mayer, 2000). Such changes originate from the reflex response caused by the afferent stimuli in the somatic nerve fibers after the insertion of the needle (Andersson and Lundeberg, 1995).

Knowledge about the reflex response is concentrated in experimental studies carried out on the pathways of the sensory nerve system responsible for the modulation and inhibition of pain (Mayer, 2000; Carlsson, 2002) in the three levels of the central nervous system: spinal cord (Lewith and Kenyon, 1984; Cao, 2002), encephalic trunk (Cao, 2002) and cerebral cortex (Zhang et al., 2004).

The spectral modification verified by the significant increase of MF in the experimental group at 60% MVC in the ACP1 reflects the alteration of recruitment behavior of motor units in the muscle after AA. Following this increase in ACP1, a decrease of MF in the ACP2 condition was observed. This alteration of MF indicates the existence of a time-dependent reflex action of the AA on the muscle activity.

Support for these affirmations are found in some other studies. Continuous cutaneous stimulation changes the population of motor units active during steady voluntary contraction (Stephens et al., 1978), and alters the order and threshold of motor unit recruitment during gradually increasing voluntary contraction (Garnett & Stephens, 1978). The therapeutic effect of acupuncture can be achieved by a variety of needling stimuli (Nordenstrom, 1989; Jisheng et al., 1998). Needle stimulation can be brief (a few seconds), prolonged (several minutes), or intermittent, depending on the clinical situation (Shanghai College of Traditional Medicine, 1987). This observation can possibly explain the decrease of MF in the ACP2 with 60% of MVC, being that, during the experiment, no needle stimulation of any type was used during ear puncture (ACP1 and ACP2).

Although significant alterations of MF were not found in the experimental groups with 40% MVC, the average obtained between the pre-acupuncture (ACP) conditions and the acupuncture (ACP1 and ACP2) conditions presented readings similar to those obtained in the movement with 60% MVC (Fig. 3). In general, the recruitment order has been confirmed for isometric contractions with a high correlation between recruitment force threshold and twitch force (Desmedt, 1981; Riek and Bawa, 1992). The rate of change of muscle fiber conduction velocity and spectral parameters depends on the force of contraction and this happens due to the progressive recruitment of larger motor units having higher muscle fiber conduction velocity (Lowery et al., 2002). Studies with surface EMG have already demonstrated that alterations of the peripheral systems, such as the proprioceptive system (joint receptors, golgi tendon organs and cutaneous receptors), can affect the firing order and the properties of the motor units and can interfere in the strength of the muscle contraction (Basmajian and De Lucca, 1985).

With this information it is possible to affirm that the results obtained in this study indicate that AA can act as a modulating mechanism for muscle activity, determined by the number of motor

units recruited and their mean discharge frequency of excitation in accordance with the level of force adopted during a task performed with isometric contraction. The modulating action of acupuncture has already been confirmed in studies of other systems, such as the cardiovascular system (Sun, et al., 1983; Wand and Yao, 1986) and gastrointestinal functions (Li et al., 1992).

It has been know that activation of cutaneous afferents can produce excitatory or inhibitory effects on the associated motor neurons (Perrier, et al., 2000); thus, the results of the experimental group confirm the correspondence of auricular acupuncture with specific areas of the body, as discussed in the French AA theory (NOGIER).

In Table 1, the medians of the placebo group, even without significant alterations of MF, can be seen showing a reflex answer after AA, similar to the experimental group. One possible explanation for this is that the sham acupuncture (needling of non-acupuncture points in this study), as an invasive procedure, always causes physiological reactions, e.g., triggering of neural pathways resulting in diffuse noxious inhibitory control (Le Bars et al., 1979).

5. Limitations of the study

The major limitation of this study was not having studied the EMG activity with longer fixity of the needle in the acupoint (more than 5 minutes) and during a period after removing the needle. This procedure would be sufficient to clarify if a tendency of the EMG signal observed after ACP1 would be to continue to decrease or to normalize in relation to the signal collected before the insertion of the needle (ACP), Continuous manipulation of the needles was recommended (Jisheng, et al., 1998) to achieve more consistent readings but this procedure was not adopted during the study. An analysis of the EMG of the trapezius muscle counterlateral to the ear would also have enriched the results and the discussion of this study.

6. Conclusions

The AA peripheral stimulus can act as a modulator mechanism of muscle activity, as a result of the number of motor units recruited and their mean discharge frequency of excitation according to the level of force adopted during a task carried out with isometric contraction. Besides, it was possible to verify correspondence of the auricular acupoint with the trapezius muscle

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Table and legend

Table 1. Mean (Hz) and standard deviation (SD) of the FM pre-acupuncture (ACP) and during acupuncture (ACP1, ACP2) to the ear. * Significant difference (Friedman test $p < 0.05$) in the intraclass comparison.

Figures and legends

Legends

Fig. 1. Test position as the individual elevates the shoulder ispi-lateral to the AA (A) against the resistance of the load cell (B).

Fig. 2. Insertion points of the acupuncture needle in the ear, corresponding to the shoulder (a) and the scapular waist (b) used in the experimental group, and the point located in the shell of the ear (c) used for the placebo group.

Fig. 3. Mean of median frequency (Hz) of the upper trapezius pre-acupuncture (ACP), during acupuncture (ACP1 and ACP2) from experimental group, tested under two levels of sub-maximum force (40% and 60% MVC). * Difference statistically significant compared to pre (Wilcoxon test, p=0.023)

Table 1.

% MVC	Acupuncture time	Experimental group Mean (SD)	*P Value* Freedman test	Placebo group Mean (SD)	*P Value* Freedman test
60%	**ACP**	56.6 (5.2)	0.03*	59.6 (8.8)	0.88
	ACP1	60.3 (7.6)		64.2 (10)	
	ACP2	58.3 (5.2)		62 (8)	
40%	**ACP**	56.7 (6.9)	0.62	60.7(12.1)	0.12
	ACP	58.8 (12)		62.6 (9.9)	
	ACP2	57.1 (12.4)		62.1(10.6)	

Figures

Fig.1

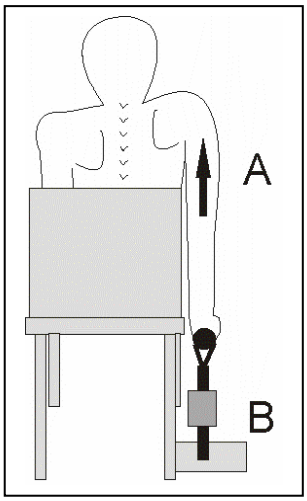


Fig.2

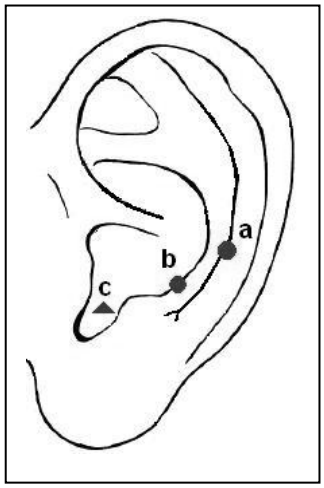
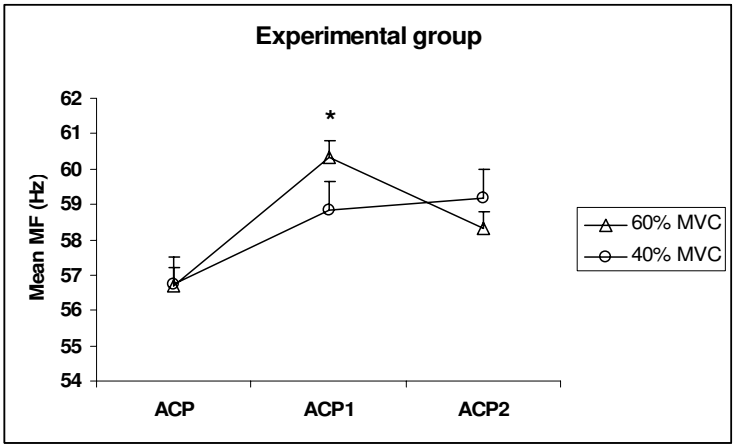


Fig. 3


Experimental group
Mean MF (Hz)
62
61
60
59
58
57
56
55
54
*
ACP
ACP1
ACP2
60% MVC
40% MVC

7.0 Conclusões Gerais

Baseado nos resultados apresentados, concluímos que a EMG de superfície pode ser utilizada para investigar um possível efeito da AA na atividade elétrica do músculo, mas ainda necessita de uma metodologia adequada para seu emprego, uma vez que o método utilizado para gravar o sinal EMG pode influenciar os resultados.

Para investigações futuras que envolvam o uso da eletromiografia de superfície na análise dos efeitos da AA, sobre a atividade do músculo estriado esquelético, sugerimos que os experimentos sejam realizados com os músculos desempenhando sua ação principal, a fim de aumentar as chances de serem verificadas quaisquer alterações do sinal EMG causadas pelo uso da AA.

A carga aplicada, o tempo de coleta dos dados e o número de repetição do experimento também devem ser levados em consideração para que o aumento da amplitude do sinal EMG não seja confundido com os efeitos da fadiga muscular.

Outra importante observação é que o estímulo periférico da AA pode atuar como mecanismo modulador da atividade do músculo. Essa ação pode determinar a freqüência de disparo e o número de unidades motoras recrutadas na contração muscular, de acordo com o nível de força adotado durante uma tarefa realizada em contração isométrica. Além disso, também foi possível verificar que existe correspondência do acuponto auricular com o músculo trapézio porção descendente.

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9.0 Anexo

Anexo I

CONSENTIMENTO FORMAL DOS VOLUNTÁRIOS QUE PARTICIPARÃO DA PESQUISA: ***"Análise da atividade eletromiográfica do músculo estriado esquelético pós aplicação da auriculoacupuntura".***

RESPONSÁVEL PELO PROJETO: Profa. Dra. Evanisi Teresa Palomari (Orientadora) e Fabiano Politti (Pós-Graduando em Biologia Molecular e Estrutural, nível Doutorado, área de concentração - Anatomia)

Eu,_____,
_____anos de idade, RG: _____, residente à Rua e/ou Av: _____, Cidade _____, voluntariamente concordo em participar da pesquisa acima mencionada, que será detalhada a seguir, e sabendo que para sua realização as despesas monetárias serão de responsabilidade dos responsáveis pela pesquisa.

É de meu conhecimento que esta pesquisa será desenvolvida em caráter de pesquisa científica e objetiva avaliar as atividades elétrica dos músculos trapézio e deltóide (músculos do ombro), após a inserção de agulhas de acupuntura.

Estou ciente que esse método de diagnóstico se caracteriza pela utilização de eletrodos que serão fixados sobre a pele (em cima dos músculos já referidos). Já fui informado que esses eletrodos se assemelham a pequenos pedaços de fitas adesivas, e portanto não oferecem nenhum tipo de risco para minha pele.

Também estou informado que para a realização desse exame, terei que ficar com o ombro e o braço desnudo (sem nenhum tipo de roupa) e que serão inseridas em minha orelha agulhas estéreis de acupuntura. Concordo, sem nenhuma restrição, que essa situação não irá me causar

nenhum tipo de constrangimento e assumo qualquer risco diante da inserção das agulhas de acupuntura na orelha.

Sei que, mesmo assinando esse termo de compromisso, e tendo total conhecimento dos procedimentos que deverão ser realizados, poderei, a qualquer momento, deixar de participar da pesquisa, sem que isso possa causar-me qualquer tipo de prejuízo ou aborrecimento.

Estou ciente ainda de que, as informações obtidas durante as avaliações eletromiográficas, serão mantidas em sigilo e não poderão ser consultadas por pessoas leigas, sem a minha devida autorização. As informações assim obtidas, no entanto, poderão ser usadas para fins de pesquisa científica, desde que a minha privacidade seja sempre resguardada.

Contudo, informo que li e entendi todas as informações precedentes, sendo que eu e os responsáveis pela pesquisa já discutimos todos os riscos e benefícios decorrentes desta, onde as dúvidas futuras que possam vir a ocorrer poderão ser prontamente esclarecidas, bem como o acompanhamento dos resultados obtidos durante a realização dos exames.

Comprometo-me, na medida das minhas possibilidades, participar dessa pesquisa, vizando além do benefício do diagnóstico, colaborar para um bom desempenho do trabalho científico dos responsáveis por essa pesquisa.

Campinas, de de 2006.

_____

Voluntário

_____

Mestrando: Fabiano Politti
Dep. de Anatomia – UNICAMP
Fone: (19) 3893 - 6674

_____

Orientadora: Profa. Dra. Evanisi Teresa Palomari
Dep. de Anatomia – UNICAMP
Fone: (19) 3788 – 7391 / R: 26

Anexo II

FACULDADE DE CIÊNCIAS MÉDICAS
COMITÊ DE ÉTICA EM PESQUISA

www.fcm.unicamp.br/pesquisa/etica/index.html

CEP, 28/06/06.
(Grupo III)

PARECER PROJETO: Nº 194/2006 (Este nº deve ser citado nas correspondências referente a este projeto)
CAAE: 0142.0.146.000-06

I-IDENTIFICAÇÃO:

PROJETO: **"ANÁLISE DA ATIVIDADE ELETROMIOGRÁFICA DO MÚSCULO ESTRIADO ESQUELÉTICO PÓS APLICAÇÃO DA AURICULOACUPUNTURA".**
PESQUISADOR RESPONSÁVEL: Fabiano Politti
INSTITUIÇÃO: Departamento de Anatomia/IB/UNICAMP
APRESENTAÇÃO AO CEP: 09/05/2006
APRESENTAR RELATÓRIO EM: 28/06/07 (O formulário encontra-se no *site* acima)

II - OBJETIVOS

Avaliar como a acupuntura contribui para a diminuição ou inibição da dor para o relaxamento muscular. O pesquisador pretende realizar um estudo da atividade eletromiográfica da porção anterior, média e posterior do músculo deltóide e da porção superior do músculo trapézio em indivíduos normais submetidos à acupuntura auricular, para que sejam observados o comportamento da atividade elétrica desses músculos e correlacionar os achados com a prática clínica.

III - SUMÁRIO

Segundo o informado no projeto, o pesquisador convidará alunos da graduação e pós-graduação que freqüentam o Departamento de Anatomia do IB da UNICAMP. O estudo será randomizado duplo cego com 30 participantes 15 participantes pertencentes ao grupo experimental e 15 participantes do grupo placebo. Serão realizados testes para comprovar a normalidade destes indivíduos. Antes da realização do exame físico e da coleta de dados, os indivíduos serão informados sobre os objetivos e procedimento a serem adotados. A adequação da metodologia bem como das condições estão coerentes com o projeto proposto.

IV - COMENTÁRIOS DOS RELATORES

O projeto está bem redigido. A pesquisa não expõe o participante a nenhum tipo de constrangimento e de risco a sua integridade física. A acupuntura é um método invasivo, mas o pesquisador se compromete a utilizar materiais desacatáveis e limpeza séptica adequada, não oferece riscos previsíveis ao paciente. O pesquisador declara ser sigiloso quanto à divulgação dos dados. Os resultados poderão contribuir para outros indivíduos.
Recomendação: Colocar no Termo de Consentimento Livre e Esclarecido o telefone do Comitê de Ética em Pesquisa da FCM/UNICAMP.

Comitê de Ética em Pesquisa - UNICAMP
Rua: Tessália Vieira de Camargo, 126
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V - PARECER DO CEP

O Comitê de Ética em Pesquisa da Faculdade de Ciências Médicas da UNICAMP, após acatar os pareceres dos membros-relatores previamente designados para o presente caso e atendendo todos os dispositivos das Resoluções 196/96 e complementares, resolve aprovar sem restrições o Protocolo de Pesquisa, bem como ter aprovado o Termo do Consentimento Livre e Esclarecido, assim como todos os anexos incluídos na Pesquisa supracitada.

O conteúdo e as conclusões aqui apresentados são de responsabilidade exclusiva do CEP/FCM/UNICAMP e não representam a opinião da Universidade Estadual de Campinas nem a comprometem.

VI - INFORMAÇÕES COMPLEMENTARES

O sujeito da pesquisa tem a liberdade de recusar-se a participar ou de retirar seu consentimento em qualquer fase da pesquisa, sem penalização alguma e sem prejuízo ao seu cuidado (Res. CNS 196/96 – Item IV.1.f) e deve receber uma cópia do Termo de Consentimento Livre e Esclarecido, na íntegra, por ele assinado (Item IV.2.d).

Pesquisador deve desenvolver a pesquisa conforme delineada no protocolo aprovado e descontinuar o estudo somente após análise das razões da descontinuidade pelo CEP que o aprovou (Res. CNS Item III.1.z), exceto quando perceber risco ou dano não previsto ao sujeito participante ou quando constatar a superioridade do regime oferecido a um dos grupos de pesquisa (Item V.3.).

O CEP deve ser informado de todos os efeitos adversos ou fatos relevantes que alterem o curso normal do estudo (Res. CNS Item V.4.). É papel do pesquisador assegurar medidas imediatas adequadas frente a evento adverso grave ocorrido (mesmo que tenha sido em outro centro) e enviar notificação ao CEP e à Agência Nacional de Vigilância Sanitária – ANVISA – junto com seu posicionamento.

Eventuais modificações ou emendas ao protocolo devem ser apresentadas ao CEP de forma clara e sucinta, identificando a parte do protocolo a ser modificada e suas justificativas. Em caso de projeto do Grupo I ou II apresentados anteriormente à ANVISA, o pesquisador ou patrocinador deve enviá-las também à mesma junto com o parecer aprovatório do CEP, para serem juntadas ao protocolo inicial (Res. 251/97, Item III.2.e)

Relatórios parciais e final devem ser apresentados ao CEP, de acordo com os prazos estabelecidos na Resolução CNS-MS 196/96.

VII - DATA DA REUNIÃO

Homologado na VI Reunião Ordinária do CEP/FCM, em 28 de junho de 2006.

Profa. Dra. Carmen Silvia Bertuzzo
PRESIDENTE DO COMITÊ DE ÉTICA EM PESQUISA
FCM / UNICAMP

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DECLARAÇÃO

Declaro para os devidos fins que o conteúdo de minha **Tese de Doutorado** intitulada **"A eletromiografia como ferramenta de estudo da ação da auriculoacupuntura"**

.

( ) não se enquadra no Artigo 1°, § 3° da Informação CCPG 002/06, referente a bioética e biossegurança.

( ) está inserido no Projeto CIBio (Protocolo nº _____), intitulado _____

( ) tem autorização da Comissão de Ética em Experimentação Animal (Protocolo nº _____).

(X) tem autorização do Comitê de Ética para Pesquisa com Seres Humanos - Protocolo nº 194/2006.

_____

Aluno: Fabiano Politti

_____

Orientadora: Profa Dra. Evanisi Teresa Palomari

Para uso da Comissão ou Comitê pertinente:

(x) Deferido ( ) Indeferido

_____

Nome:
Função:

Profa. Dra. Carmen Silvia Bertuzzo
Matrícula 17464-0
Presidente do Comitê de Ética em Pesquisa
Faculdade de Ciências Médicas - UNICAMP