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Eletroacupuntura Crônica do Ponto E36 Melhora a Função Barorreflexa e Parâmetros Hemodinâmicos em Ratos com Insuficiência Cardíaca

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DEDICATÓRIA

À minha filha amada, por me fazer querer ser uma pessoa melhor a cada dia.

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“Há momentos em que a maior sabedoria é parecer não saber nada”

Sun Tzu

RESUMO

A ativação do Sistema Nervoso Simpático, que acontece em humanos com Insuficiência Cardíaca Crônica (ICC), tem implicações tanto na progressão da doença como na sobrevivência desses indivíduos. A acupuntura tem sido utilizada como tratamento coadjuvante de várias doenças, incluindo a insuficiência cardíaca, surgindo como uma alternativa de baixo custo no acompanhamento desses pacientes. Dentro desse contexto, a Acupuntura e Eletroacupuntura (EA) vêm ganhando espaço nas publicações devido à sua ação direta no sistema nervoso autonômico (SNA). Portanto, o objetivo deste estudo foi avaliar os efeitos da eletroestimulação crônica do ponto de acupuntura E36 na função hemodinâmica e barorreflexa em ratos com Insuficiência Cardíaca (IC). Os parâmetros cardiovasculares avaliados foram a frequência cardíaca (FC), a pressão arterial (PA) e a resposta cardiovascular reflexa de FC e PA desencadeada pela estimulação de barorreceptores em animais com IC, após o infarto do miocárdio (IAM). Para isso, ratos machos Wistar foram divididos nos grupos: "Sham Controle" – animais sem IC e sem EA; "IC Controle" – animais com IC e sem EA; "IC EA" – animais com IC e que receberam protocolo de EA. Seis semanas após a cirurgia de indução do IAM, foi realizado o protocolo de EA (8 semanas, 5 vezes por semana). O protocolo de EA foi aplicado no ponto E36 na frequência de 2 Hz, pulso de 0,3 ms e intensidade de 1-3 Ma, por 30 minutos. Ao final do tratamento, os ratos foram submetidos à avaliação hemodinâmica e da função dos barorreceptores. Os dados foram comparados pelo teste de variância (ANOVA) de uma via e pelo teste *post hoc* de Bonferroni. O nível de significância estatística foi estabelecido em $p < 0,05$. Não houve diferença entre os grupos estudados nas variáveis FC, pressão arterial sistólica (PAS), pressão arterial diastólica (PAD) que foram avaliadas com os animais acordados ($p > 0,05$). Houve aumento na pressão arterial média (PAM) no grupo IC EA quando comparado ao grupo IC Controle ($p < 0,05$). Nas variáveis amplitude, *upper* platô e ponto de maior inclinação da curva (PA_{50}) referentes à função dos barorreceptores, não houve diferença significativa entre os grupos. A variação da sensibilidade média do barorreflexo (Ganho) foi maior no grupo IC EA quando comparado aos grupos IC Controle e Sham Controle. A EA crônica do ponto E36 aumentou a PAM e a sensibilidade média do barorreflexo, melhorando o quadro cardiovascular em animais com IC.

Palavras-chaves: Infarto agudo do miocárdio; Eletroacupuntura; Sistema nervoso autonômico.

ABSTRACT

The sympathetic nervous activation, which occurs in humans with heart failure (HF), has implications for both disease progression and survival in these individuals. Acupuncture has been used to treat many diseases, including HF, emerging as a low cost alternative in the treatment of these patients. Within this context, acupuncture and electroacupuncture (EA) has been gaining ground in publications related to its direct action on the autonomic nervous system (ANS). Therefore, this study aimed to evaluate the effects of chronic stimulation in the ST36 acupuncture point in hemodynamic and baroreflex function in rats with heart failure (HF). The cardiovascular parameters assessed were heart rate (HR), blood pressure (BP), and the reflex cardiovascular response of HR and BP triggered by stimulation of baroreceptors in animals with HF subsequent to acute myocardial infarction (AMI). For this purpose, male Wistar rats were divided into groups: Sham Control – animals without HF and without EA; HF Control group – animals with HF and without EA; HF EA group – animals with HF that received the EA protocol. Six weeks after surgical induction of AMI, the EA protocol (8 weeks, 5 times a week) was performed. The protocol was applied with EA at the ST36 point, frequency of 2 Hz, pulse of 0.3 ms and intensity of 1-3 mA for 30 minutes. Then, the rats were subjected to a hemodynamic assessment and baroreceptor function. Data were compared by a one way analysis of variance (ANOVA) and by the Bonferroni's *post hoc* test. The statistical significance level was set at $p < 0.05$. There was no difference among groups in the studied variables HR, systolic blood pressure (SBP), diastolic blood pressure (DBP), that were evaluated with awaken animals ($p > 0.05$). There was an increase in the mean arterial pressure (MAP) in the HF EA group compared to HF Control group ($p < 0.05$). In the variable range, upper plateau, point of greatest slope of the curve (PA_{50}) for the function of baroreceptors no significant difference among groups was identified. The mean variation baroreflex sensitivity (Gain) was higher in the HF EA group when compared to the HF Control and Sham Control groups. Chronic EA in the ST36 point increased the mean arterial pressure and baroreflex sensitivity in rats with HF.

Key words: Myocardial infarct; Electroacupuncture; Autonomic nervous system.

LISTA DE FIGURAS

LISTA DE TABELAS

LISTA DE ABREVIATURAS E SIGLAS

DCV	Doenças Cardiovasculares
E36	Ponto de Acupuntura localizado no canal de Estômago, número 36
EA	Eletroacupuntura
ECG	Eletrocardiograma
FC	Frequência Cardíaca
IAM	Infarto Agudo do Miocárdio
IC	Insuficiência Cardíaca
ICC	Insuficiência Cardíaca Crônica
MTC	Medicina Tradicional Chinesa
MTO	Medicina Tradicional Oriental
PA	Pressão Arterial
PAD	Pressão Arterial Diastólica
PAM	Pressão Arterial Média
PAS	Pressão Arterial Sistólica
PC	Peso Corporal
PDFVE	Pressão Diastólica Final do Ventrículo Esquerdo
PVS	Pressão Ventricular Sistólica
SNA	Sistema Nervoso Autonômico
VD	Ventrículo Direito
VE	Ventrículo Esquerdo
GABA	Ácido Gama-aminobutírico
ON ou NO	Óxido Nítrico
PC6	Ponto de Acupuntura localizado no canal Pericárdio, número 6
FE	Fração de Ejeção

SUMÁRIO

1 INTRODUÇÃO

A Insuficiência Cardíaca (IC) é uma síndrome progressiva em que uma condição cardíaca estrutural ou funcional prejudica a habilidade que o coração tem de bombear fluxo sanguíneo suficiente para os diversos órgãos e tecidos. (Axente, Sinescu e Bazacliu, 2011). Essa condição resulta em uma baixa qualidade de vida, intolerância ao exercício, hospitalizações frequentes e elevada morbi-mortalidade. Mesmo com os avanços no controle das doenças cardiovasculares (DCV), a IC ainda possui elevada incidência e prevalência na população mundial (Ramani, Uber e Mehra, 2010).

Essa síndrome é caracterizada por uma hiperativação do Sistema Nervoso Simpático e por uma resposta atenuada do Sistema Nervoso Parassimpático (Zhang e Anderson, 2014). A hiperativação do Sistema Nervoso Simpático, que acontece em humanos com Insuficiência Cardíaca Crônica (ICC), tem implicações tanto na progressão da doença quanto na sobrevida desses indivíduos (Floras, 2009). Além disso, o controle barorreflexo, tanto para o aumento quanto para a queda da pressão arterial (PA), está significativamente diminuído (Negrão, 2010).

A prática da acupuntura (AP) consiste na inserção de agulhas em locais específicos do corpo, os chamados pontos de acupuntura, e é utilizada nas mais diversas situações clínicas (Sierpina e Frenkel, 2005). Atualmente, a acupuntura ou a eletroacupuntura (EA – estimulação dos pontos de acupuntura através de corrente elétrica) vem ganhando espaço nas publicações referentes à sua ação direta no sistema autonômico (Middlekauff *et al.*, 2002; Sugimachi *et al.*, 2007; Huang, Kutner e Bliwise, 2011). Zhou *et al.* demonstraram os mecanismos neuroendócrinos, incluindo a modulação do sistema simpático, envolvidos na diminuição da pressão arterial (PA) através da utilização da acupuntura (Zhou e Longhurst, 2012).

Nesse contexto, estudos experimentais têm demonstrado que os efeitos da acupuntura não são apenas hemodinâmicos e sim de possível modulação do sistema nervoso simpático e parassimpático, além de atuar no sistema reflexo de controle da PA, os barorreflexos (Michikami *et al.*, 2006; Sugimachi *et al.*, 2007; Uchida, Kagitani e Hotta, 2008; Li *et al.*, 2013). Estudos em animais podem elucidar os mecanismos pelos quais a eletroacupuntura é capaz de induzir bradicardia e diminuição da pressão arterial (Uchida *et al.*, 2007; Uchida, Kagitani e Hotta, 2008; Li e Longhurst, 2010; Zhou e Longhurst, 2012). Além disso, estas pesquisas têm auxiliado na compreensão dos mecanismos de ação da acupuntura bem como da especificidade de pontos de acupuntura, sendo que alguns possuem maior efeito sobre determinados sistemas do que outros (Choi, Jiang e Longhurst, 2012) como o ponto estômago 36 (E36) que atua diretamente no sistema cardiovascular e autonômico (Tjen, Li e Longhurst, 2004; Uchida *et al.*, 2007).

No entanto, a AP ainda é pouco estudada na IC. Alguns estudos relataram a hipótese de que a AP pudesse ativar o centro cardiovascular no sistema nervoso central, o que modularia diretamente o balanço simpato-vagal, causando diminuição da frequência cardíaca (FC) e da pressão arterial (PA) (Middlekauff, 2004; Uchida, Kagitani e Hotta, 2008; Li, J. *et al.*, 2012). Porém, ainda são escassas as informações acerca da duração desses efeitos, pois a maioria dos estudos são realizados com eletroestimulação aguda (Uchida, Kagitani e Hotta, 2008; Geng *et al.*, 2013).

Portanto, o objetivo deste trabalho foi apresentar os resultados referentes ao estudo experimental realizado com objetivo de avaliar o efeito da eletroestimulação crônica do ponto E36 na sensibilidade dos barorreceptores e variáveis hemodinâmicas em modelo animal de IC. Além disto, apresentamos nosso método criado para possibilitar a realização da eletroacupuntura nos animais em nosso laboratório.

2 REVISÃO BIBLIOGRÁFICA

2.1 Conceito, Epidemiologia e Caracterização da Insuficiência Cardíaca

O conceito mais amplamente aceito sobre Insuficiência Cardíaca (IC) é de que esta é uma síndrome clínica complexa, resultante de diversas desordens cardíacas estruturais e funcionais, caracterizada pela incapacidade do coração de manter níveis adequados de fluxo sanguíneo para os tecidos, com consequente prejuízo na oferta de suprimento energético para o organismo (Jessup e Brozena, 2003; Hunt *et al.*, 2005).

As Doenças Cardiovasculares (DCV) possuem elevada mortalidade no Brasil (Mansur, 2012) e no mundo (Roger *et al.*, 2012). A figura 1 apresenta o percentual de mortes pelas principais doenças cardiovasculares nos Estados Unidos.

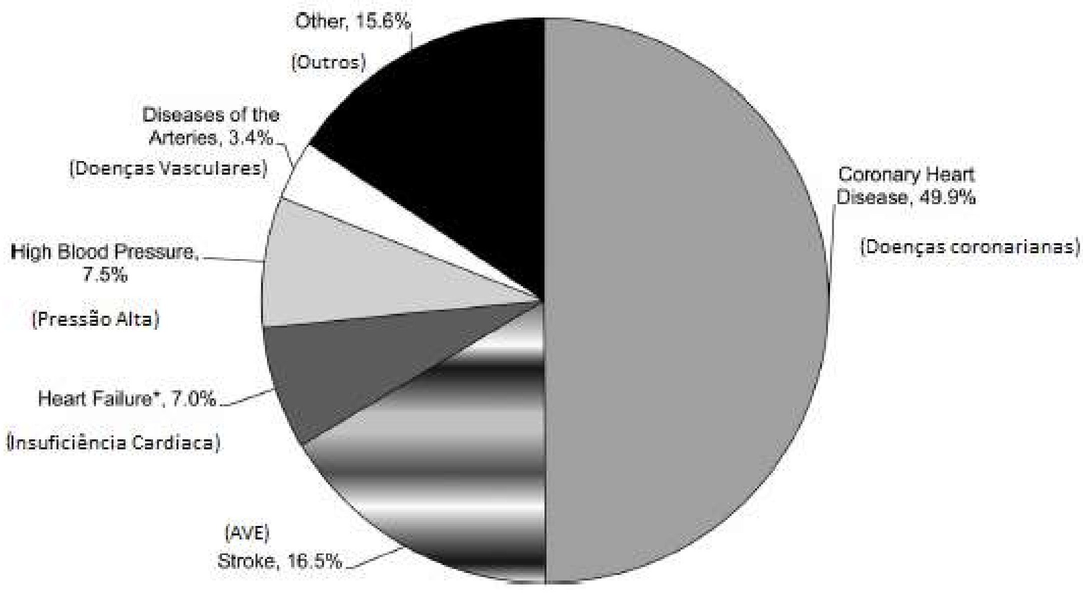

Figura 1 – Percentual de mortes atribuídas a doenças cardiovasculares (Estados Unidos, 2008). AVE: Acidente Vascular Encefálico. **Fonte**: Adaptado de National Heart, Lung and Blood Institute from National Center for Health Statistics. Modificado de (Roger *et al.*, 2012)

Em 2009, somente no Rio Grande do Sul, foram registrados 12,174 óbitos por doenças cardíacas (Caderno de Informações de Saúde do Rio Grande do Sul, 2009). Adicionalmente, o tratamento medicamentoso e a internação por DCV geram custos elevados e representam um grande impacto sobre a economia nacional (Achutti, 2008).

No Brasil, a IC é o terceiro motivo de internação entre todas as causas e o primeiro entre as doenças cardiovasculares (DCV) no Sistema Único de Saúde (SUS), em pacientes acima de 65 anos, elevando os custos com essa doença (Santos *et al.*, 2010). Assim, a IC ainda se apresenta como um importante e crescente problema de saúde pública (Braunwald, 2008), estando associada a altas taxas de mortalidade, que podem alcançar até 50% nos cinco anos que se sucedem ao diagnóstico (Levy *et al.*, 2002), e à baixa sobrevivência, média de 1,7 anos em homens e 3,2 anos em mulheres, após seu diagnóstico inicial (Kannel, 2000).

Os principais sintomas da IC são dispneia e fadiga, que podem limitar a tolerância ao exercício, e retenção hídrica, que pode levar à congestão e ao edema periférico (Jessup e Brozena, 2003; Ferreira *et al.*, 2009; Hunt *et al.*, 2009).

A compreensão acerca da fisiopatologia da IC progrediu enormemente no último século. O modelo cardiocirculatório ou hemodinâmico de “bomba cardíaca insuficiente” tem avançado para um modelo mais complexo, que envolve conceitos de ativação de vias neuroendócrinas, imunológicas e metabólicas e visa o entendimento da progressão da síndrome da IC (Packer, 1993; Kapadia *et al.*, 1998). Dessa forma, não surpreendentemente, a Insuficiência Cardíaca é, atualmente, reconhecida como uma desordem multissistêmica que afeta não apenas isoladamente o sistema cardiovascular, mas também outros sistemas, como o renal, o musculoesquelético, o imunológico e o neuro-humoral (Seta *et al.*, 1996; Sharma, Coats e Anker, 2000; Yndestad *et al.*, 2006; Von Haehling, 2009).

A etiologia da IC varia de uma doença isquêmica do coração até longos períodos sustentados de hipertensão arterial e patologias cardíacas valvares. Teoricamente, qualquer patologia cardíaca pode resultar, em ultima instância, na IC. Entretanto, em muitos casos, o insulto causador permanece desconhecido (Von Haehling, 2009).

2.2 INSUFICIÊNCIA CARDÍACA E O SISTEMA NERVOSO AUTONÔMICO

A disfunção autonômica presente na IC é caracterizada pela predominância do sistema nervoso simpático e concomitantemente, redução do tônus vagal (Rydlewska *et al.*, 2011). Essa, por sua vez, interfere na modulação da FC enquanto que a ativação do simpático está relacionada diretamente ao controle da pressão arterial sistólica (PAS) (Elenkov *et al.*, 2000).

Inicialmente, após o IAM, existe um aumento da atividade simpática com o objetivo de manter o débito cardíaco (DC). Porém a manutenção da ativação simpática causa efeitos deletérios em todo sistema cardiovascular (Jaenisch *et al.*, 2011). Assim, a IC é caracterizada por um desequilíbrio no fluxo fisiológico simpato-vagal com uma ativação adrenérgica crônica (La Rovere, Pinna e Raczak, 2008) e esta hiperativação está estreitamente relacionada com anormalidades nos reflexos cardiovasculares como os barorreflexos (Zhang e Anderson, 2014).

Em condições fisiológicas, a manutenção da PA é realizada pelo barorreflexo arterial através dos barorreceptores encontrados no seio carotídeo e arco aórtico. Os barorreceptores respondem ao estiramento da parede dos vasos. Com o aumento da PA, há o estiramento das paredes do vaso. Esse, gera potenciais de ação que são transmitidos pelo nervo depressor aórtico e são levados ao núcleo do trato solitário (NTS), no bulbo. Esses neurônios estimulam o núcleo dorsal do vago e núcleo ambíguo, aumentando o tono vagal ao coração. Concomitantemente, outros neurônios do NTS estimulam o bulbo ventro lateral caudal, que tem efeito inibitório sobre o bulbo ventro lateral rostral. Diminuindo assim, o tono simpático ao coração (Aires, 2008) gerando menor contratilidade cardíaca, diminuindo o tônus vasoconstritor e a FC e (Osborn, Jacob e Guzman, 2005; Joyner, Charkoudian e Wallin, 2008) por fim, reduzindo a PA.

Na IC, o controle barorreflexo, tanto para o aumento quanto para a queda da pressão arterial, está significativamente reduzido. Esta evidência foi demonstrada por pesquisadores que ao infundir uma droga vasoconstritora para elevar a PA, obtiveram uma resposta atenuada de bradicardia reflexa. Da mesma forma, ao reduzir a pressão arterial pela infusão de um vasodilatador, a resposta reflexa de elevação da FC e a atividade simpática muscular também estavam atenuadas (Negrão, 2010).

Desse modo, a resposta dos barorreceptores pode ser uma ferramenta tanto para avaliação do tônus autonômico (Lahiri, Kannankeril e Goldberger, 2008) como para prognóstico em pacientes com IC (La Rovere, Pinna e Raczak, 2008; La Rovere *et al.*, 2013).

2.3 Estudos com Modelo Animal de IC

Modelos animais em Insuficiência Cardíaca são extensivamente utilizados na literatura com o objetivo de estudar a fisiopatologia dessa síndrome e de avaliar drogas experimentais e terapias não-farmacológicas (Hasenfuss, 1998; Batista *et al.*, 2010).

Dentre os modelos experimentais de indução de IC, um dos mais difundidos e com maior número de evidências sobre as alterações cardíacas e periféricas em decorrência da IC e por resultar em áreas de infarto de tamanho mais uniformes, é o modelo de ligadura da artéria coronária descendente anterior esquerda ou “modelo de infarto agudo do miocárdio” (IAM). Nesse modelo, três a seis semanas após a ligadura da coronária, já podem ser observadas alterações ventriculares decorrentes da área de infarto, caracterizando esses animais como “insuficientes”, com a presença de hipertrofia cardíaca e aumento da pressão diastólica final de ventrículo esquerdo (PDFVE) (Pfeffer *et al.*, 1979).

Pfeffer *et al* relataram as principais alterações hemodinâmicas que podem ocorrer de acordo com a área de infarto obtida. Quanto maior a área de infarto, maior a PDFVE, sendo que os maiores valores encontram-se em animais com áreas de infarto maiores que 50% da área total do coração. Porém, em animais com área de infarto superior a 20% já podem ser observadas alterações características da IC com valores aumentados de PDFVE e menor pressão sistólica ventricular (PSV). Outra alteração observada foi que áreas de infarto maiores foram compatíveis com níveis mais reduzidos de PAM desses animais (Pfeffer *et al.*, 1979). Além disso, animais com IC induzidos pelo IAM apresentam significativa redução na capacidade funcional (Batista *et al.*, 2007).

Quanto às alterações neuro-humorais, nesse modelo, os animais com IC apresentaram maior atividade do nervo simpático renal como descrito no estudo de Francis *et al.* e uma relação linear da PDFVE com a atividade no nervo simpático renal em animais IC, seis semanas após a ligadura da coronária. Ainda, encontraram atenuação da resposta barorreflexa nos animais IC comparados ao Sham (Francis *et al.*, 2001).

O uso de modelo animais, evidentemente, tem suas limitações e não abrange totalmente as alterações da IC encontrada em humanos. Contudo, são ferramentas eficazes para estudos detalhados dos mecanismos que envolvem as alterações na IC e acima de tudo, possibilitam o estudo de terapêuticas a fim de colaborar com o tratamento desta síndrome para que, no futuro, possamos diminuir o impacto econômico e epidemiológico da IC sociedade (Klocke *et al.*, 2007).

2.4 ACUPUNTURA

A acupuntura é uma das técnicas utilizadas pela Medicina Tradicional Chinesa (MTC) caracterizada pela inserção de agulhas em locais específicos do corpo, os chamados pontos de acupuntura (Sierpina e Frenkel, 2005).

A fundamentação desta técnica está na filosofia Taoísta que leva em consideração os conceitos de *Qi* (energia), *Yin e Yang* (opostos e complementares) entre outros (Márcia Valéria Rizzo Scognamillo-Szabó, 2010), com o objetivo de promover o livre fluxo de energia, equilibrando *Yin e Yang*. A antiga tradição oriental concebe o homem como uma entidade energética e descreve esses pontos em canais de energias ou meridianos que estão distribuídos pelo corpo (Corral, 2006).

A anatomia energética segundo a Medicina Tradicional Chinesa (MTC) pode ser descrita por conter 12 “canais” ou “meridianos energéticos” distribuídos pelo corpo, que são: Canal da Bexiga, Canal do Rim, Canal do Fígado, Canal da Vesícula Biliar, Canal do Coração, Canal do Intestino Delgado, Canal Triplo Reaquecedor, Canal do Pericárdio, Canal de Estômago, Canal de Baço-Pâncreas, Canal do Pulmão e Canal do Intestino Grosso. Os pontos de acupuntura estão distribuídos por cada canal (Corral, 2006) Na Figura 2 está representado o trajeto do canal do Estômago com destaque para localização do ponto estômago número 36 (E36). O ponto E36 está localizado na porção anterolateral da tuberosidade da tíbia. É utilizado na medicina tradicional chinesa para desordens do estômago (Corral, 2006) e tem sido utilizado em pesquisa para desfechos cardiovasculares por seus efeitos bradicardicos e hipotensores (Choi, Jiang e Longhurst, 2012).

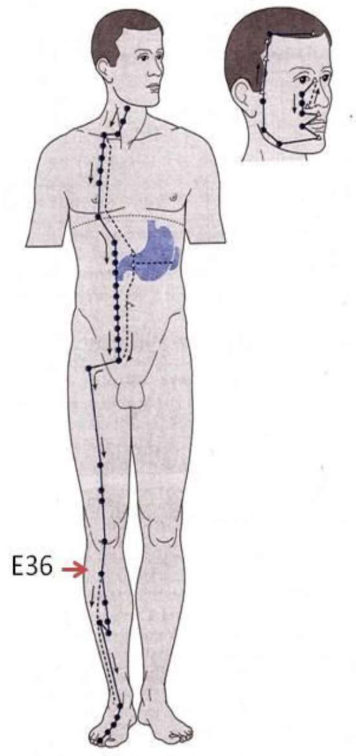

FIGURA 56.1 – Canal Principal do Estômago.

Figura 2 – Representação do Canal de Estômago e localização do ponto E36 em humanos.
Fonte: Adaptado de (Auteroche, 2000; Corral, 2006; Yu-Lin Lian, 2006))

Há poucas décadas, a acupuntura era vista como uma terapia empírica e não baseada em evidências científicas. Recentemente, estudos vêm sendo realizados a fim de explicar fisiologicamente como a acupuntura atua em diferentes sistemas e em condições patológicas (Gardani *et al.*, 2006; Li, F. *et al.*, 2012; Pach *et al.*, 2013).

Atualmente, os mecanismos de atuação da acupuntura estão bem descritos. Sabe-se que a acupuntura estimula as fibras aferentes sensoriais tipo III (Aδ) e IV (C), as quais respondem a estímulos mecânicos. No caso específico da acupuntura, respondem à sensação do próprio agulhamento. Ao serem estimuladas, dois mecanismos são acionados, um chamado de segmentar e outro de heterossegmentar. O mecanismo segmentar é caracterizado pelo efeito ao nível de medula espinhal (Figura 3). As fibras tipo III (Aδ) e IV (C) atingem as células pedunculadas (Pd), na região das grandes células superficiais da medula, as quais inibem a substância gelatinosa (SG) pela liberação do neurotransmissor encefalina (ENC), um transmissor inibitório, opióide, impedindo assim que as células de Ampla Variação Dinâmica (AVD) transmitam a informação de dor ao córtex cerebral (Filshie, 2002).

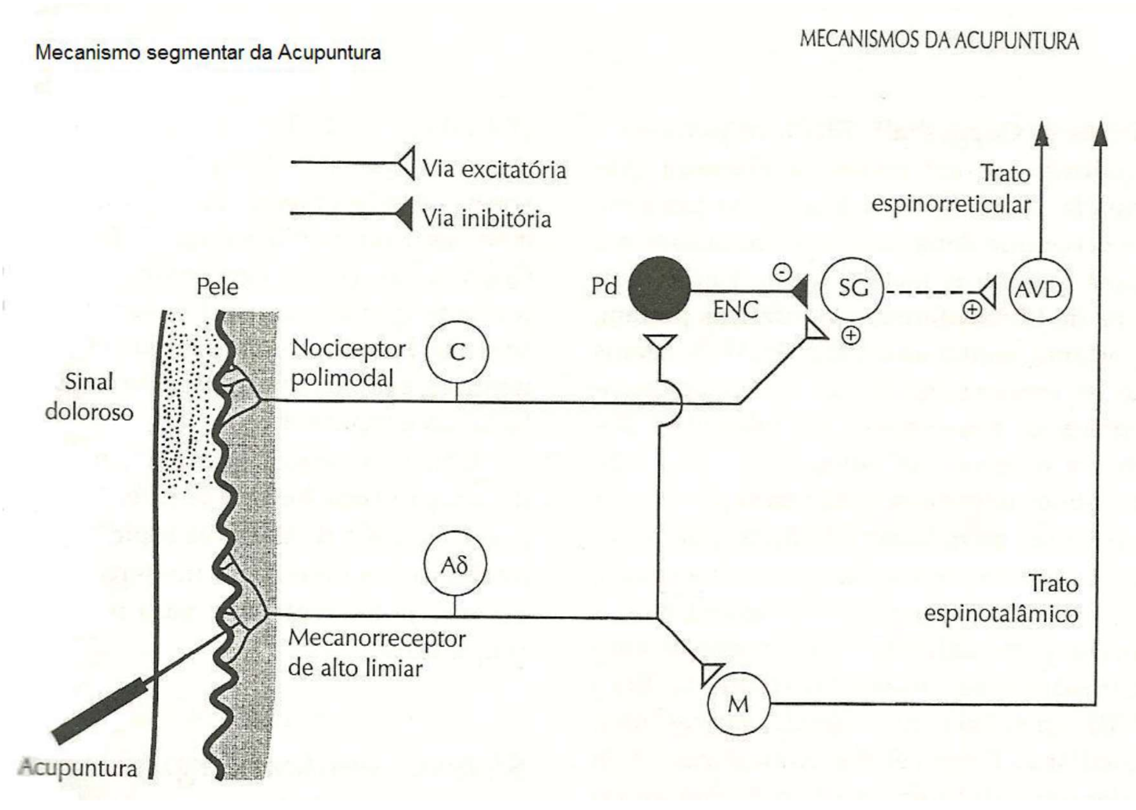

Figura 3 – Mecanismo segmentar do efeito de analgesia da acupuntura. Pd: Células Pedunculadas. ENC: Encefalina. SG: Substância Gelatinosa. AVD: Ampla Variação Dinâmica. M: Células Marginais. **Fonte:** Adaptado de (Filshie, 2002)

Já o mecanismo heterossegmentar, refere-se aos mecanismos centrais da acupuntura. As informações sensoriais geradas pelas fibras tipo III e IV são transmitidas até as células marginais (M) e pelo trato espinotalâmico chegam até o córtex pré-frontal. No hipotálamo, mais precisamente, no núcleo arqueado, fibras projetam-se à Substância Cinzenta Periaquedutal (SCPA) liberando β-endorfina, substância semelhante à morfina, inibindo a sensação de dor (Filshie, 2002).

2.4.1 Eletroacupuntura

Os pontos de acupuntura podem ser estimulados de várias formas como por meio da pressão manual, da eletroestimulação transcutânea, do calor ou ainda por meio da colocação das agulhas. Na utilização das agulhas, essas podem ser manipuladas manualmente gerando um estímulo no ponto ou por meio de uma corrente elétrica (Ulett, Han e Han, 1998), caracterizando a eletroacupuntura (EA). Alguns estudos foram feitos a fim de verificar qual técnica de estimulação é mais eficaz para obtenção de uma determinada resposta. Os resultados ainda não são conclusivos e isto depende muito do desfecho estudado em cada estudo (Wang, Mao e Han, 1992; Streitberger, Ezzo e Schneider, 2006; Witzel *et al.*, 2011).

A eletroacupuntura (EA) vem sendo frequentemente utilizada tanto na prática clínica quanto na pesquisa (Zhou *et al.*, 2005b) por ser uma técnica que possibilita a padronização do estímulo no ponto de acupuntura, o que não ocorre na acupuntura manual. Além disso, estudos demonstraram que dependendo da frequência utilizada, a resposta ou a intensidade da resposta podem variar (Kim *et al.*, 2008; Uchida, Kagitani e Hotta, 2008).

A utilização de parâmetros de frequência de dois Hertz (HZ) reduziram de forma significativa os valores da frequência cardíaca quando comparada a 10 e 0,5 Hertz no estudo de Uchida e colaboradores, com a eletroestimulação no ponto E36 em ratos (Uchida, Kagitani e Hotta, 2008). Outro estudo, realizado em camundongos, encontrou ação anti-inflamatória e diminuição de dor quando aplicada a frequência de um Hz ou 120 Hz, diferenciando apenas as vias de ativação, sendo uma ativando os neurônios simpáticos pós ganglionares e outra o eixo medular simpato-adrenal, respectivamente (Kim *et al.*, 2008). Em um modelo de hipertensão reflexa em ratos, a aplicação da eletroacupuntura no ponto E36 quando aplicada em dois Hz foi capaz de inibir o reflexo de hipertensão já quando aplicado 100 Hz não houve inibição da pressão elevada (Zhou *et al.*, 2005a; Zhou, Tjen e Longhurst, 2005).

2.4.2 Eletroacupuntura e Sistema Cardiovascular

Atualmente, alguns estudos experimentais têm sido realizados mostrando os efeitos da acupuntura diretamente no sistema cardiovascular (Middlekauff, 2004; Wang *et al.*, 2008). O ponto de acupuntura Estômago 36 (E36) vem sendo estudado e os resultados apontam que a sua eletroestimulação é capaz de reduzir significantemente a frequência cardíaca (FC) e a pressão arterial (PA) em ratos (Uchida *et al.*, 2007; Sugimachi *et al.*, 2008; Uchida, Kagitani e Hotta, 2008).

Em um estudo, com utilização de protocolo crônico de eletroestimulação no ponto E36, os resultados demonstraram redução nos valores de PA, redução do espessamento da parede da aorta e aumento dos níveis plasmáticos e no miocárdio de Oxido Nítrico em ratos espontaneamente hipertensos, indicando um remodelamento cardíaco nesses animais. (Xiong *et al.*, 2011)

Recentemente, estudo experimental utilizando como indução de IC o modelo de ligadura da artéria coronária esquerda, aplicou eletroacupuntura no ponto pericárdio 6 (PC6), outro ponto utilizado para desfechos cardiovasculares, nesses animais. Os principais resultados obtidos após uma semana de estimulação foram um significativo aumento na fração de ejeção e função sistólica, diminuição na pressão diastólica final do ventrículo esquerdo (PDFVE) e diminuição nas áreas de infarto quando comparados com animais que não fizeram o tratamento ou animais de um grupo controle da acupuntura (Ma *et al.*, 2014).

Porém, ainda são escassos os estudos relatando os efeitos da EA em modelos de doenças cardíacas como a IC, por exemplo. Por outro lado, os estudos clínicos em humanos ainda são com uma amostra pequena e com uma heterogeneidade muito grande de condições clínicas desses pacientes, bem como a etiologia da lesão cardíaca (Middlekauff, 2004). A tabela 1 resume os principais estudos experimentais sobre eletroacupuntura e sistema cardiovascular.

2.4.3 Eletroacupuntura e Sistema Nervoso Autonômico

Estudos recentes vêm demonstrando que a EA é capaz de modificar parâmetros relacionados ao sistema nervoso autonômico como PAS, FC, variabilidade da frequência cardíaca (VFC) (Yang *et al.*, 2002; Bilchick e Berger, 2006; Sugimachi *et al.*, 2008; Uchida, Kagitani e Hotta, 2008; Lee *et al.*, 2010). Atualmente, pesquisas experimentais tentam elucidar os mecanismos envolvidos nessas alterações (Li e Longhurst, 2010; Zhou e Longhurst, 2012).

Estudos experimentais têm mostrado as correlações entre a localização dos pontos de acupuntura e a inervação das fibras musculares (miótomos) e pele (dermátomos). As terminações nervosas do músculo tibial anterior estão concentradas na porção proximal da tíbia que é onde se concentram também os pontos E36, 37, 38 e 39 (Li, Zhang e Xie, 2004). Uchida *et al.* demonstrou que as repostas bradicárdicas achadas em seu estudo pela estimulação na porção ântero-lateral da tíbia de ratos correspondente ao E36 se devem provavelmente a estimulação de fibras do tipo III e IV (Uchida *et al.*, 2007) (Figura 4).

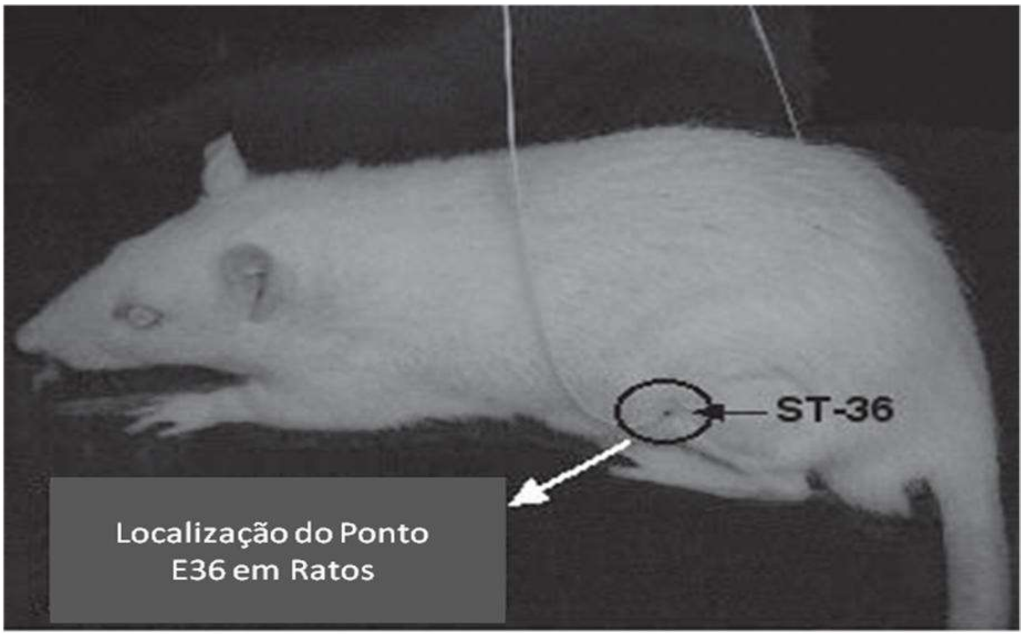

Figura 4 – Localização do ponto E36 em ratos.
Fonte: Adaptado de (Iwa *et al.*, 2006)

Essa estimulação provoca uma resposta reflexa ativando mecanismos centrais de regulação como ativação de sinapses gabaérgicas que são simpatoinibitórias (Sugimachi *et al.*, 2007; Uchida *et al.*, 2007; Uchida, Kagitani e Hotta, 2008). Neste estudo, foram elucidados os mecanismos pelos quais a eletroacupuntura provoca bradicardia ao realizar testes com animais anestesiados que receberam a EA no ponto E36. Ao verificar a resposta bradicárdica dos animais com a EA, foi realizada a secção do nervo vago, o que não alterou a resposta de diminuição da FC. Já ao seccionar o nervo simpático e a medula no nível da primeira vértebra cervical, a bradicardia foi abolida. Desta forma, os autores concluíram que, provavelmente, a via pela qual a EA diminui a FC seja via medula espinhal, por diminuição da ativação do nervo simpático e não por uma ativação do nervo vago (Uchida, Kagitani e Hotta, 2008).

Além disso, nesse mesmo estudo, foi demonstrado que a administração em ratos da naloxona (antagonista de receptor opioide não específico) foi capaz de abolir o efeito analgésico, mas não o de bradicardia. Já ao infundir bicuculina (antagonista do receptor GABA), a bradicardia foi abolida (Uchida, Kagitani e Hotta, 2008), mostrando que o efeito de induzir bradicardia ocorre por vias gabaérgicas.

Masuda *et al.*, por sua vez, demonstraram que ao infundir bicuculina no bulbo ventrolateral rostral (BVLR) onde neurônios simpato-excitatórios estão localizados, o efeito de bradicardia também foi abolido. Desta forma, concluíram que a inibição simpática produzida pela estimulação das fibras aferentes é mediada pela ativação de neurônios do bulbo ventrolateral caudal (responsáveis pela inibição do BVLR) via GABA e com componente barorreflexo (Masuda, Ootsuka e Terui, 1992). Já em animais hipertensos, a EA, por esta mesma via de ação neural, reduziu os níveis de PA (Li e Longhurst, 2010; Zhou e Longhurst, 2012). A partir destes experimentos, foi possível identificar por quais vias a EA tem efeito bradicárdico e hipotensor. Ao se estimular os pontos E36 ou PC6 é acionado o mecanismo heterossegmentar por meio da via Núcleo Arqueado – Substância Cinzenta Periaquedutal – Bulbo Ventrolateral Rostral como demonstrado na Figura 5.

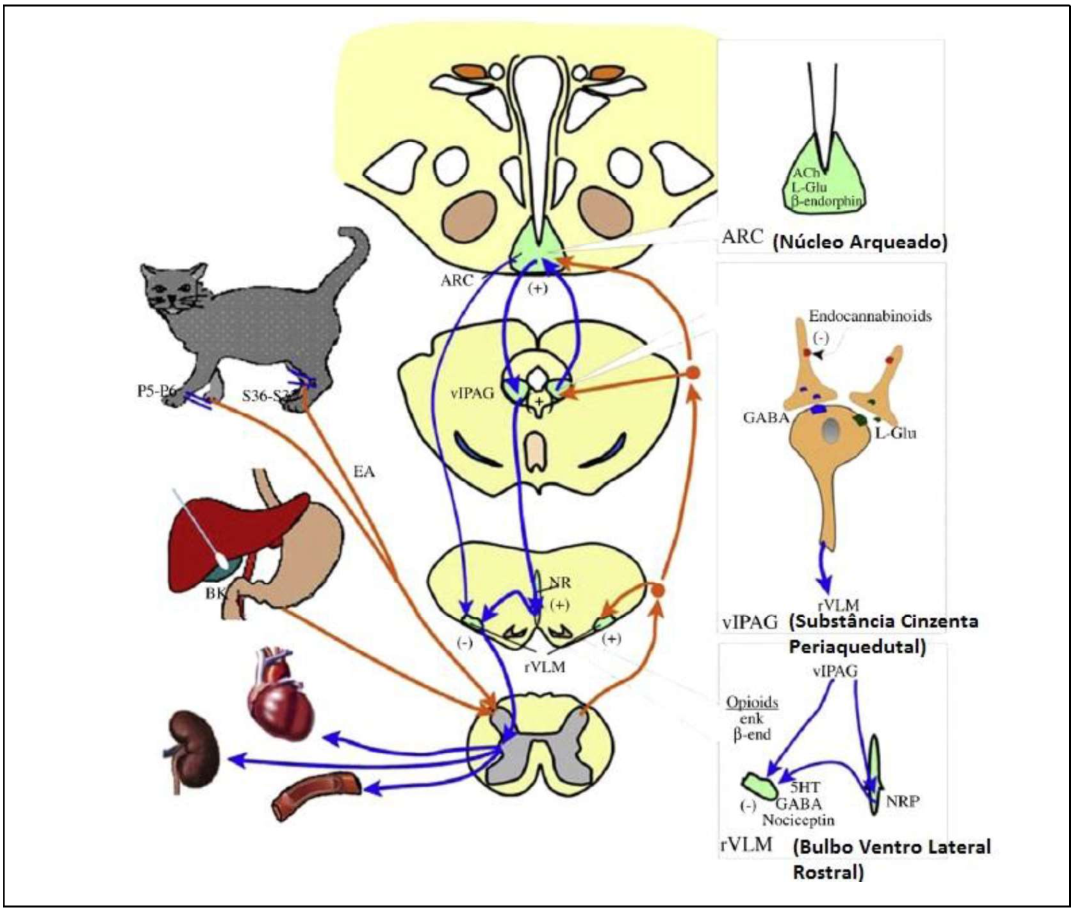

Figura 5 – Representação do circuito neural da ação da acupuntura no efeito hipotensivo em um modelo felino de hipertensão. S36: Stomach 36 (Estômago 36). P5: Pericárdio 5. **Fonte:** Adaptado de (Zhou e Longhurst, 2012).

Já quanto à função barorreflexa, a eletroestimulação do ponto E36 diminuiu a ativação do nervo simpático e a variação da amplitude da FC durante o reflexo barorreceptor em coelhos anestesiados. Quando realizada denervação peroneal, todos esses efeitos foram abolidos (Michikami *et al.*, 2006; Sugimachi *et al.*, 2007; Sugimachi *et al.*, 2008).

O estudo de (Ma *et al.*, 2014), avaliou os efeitos da EA no sistema nervoso simpático em ratos com IC. O tratamento com EA reduziu significativamente a atividade do nervo simpático renal, demonstrando que a eletroacupuntura foi capaz de reduzir ou modular a atividade simpática mesmo em situações patológicas como a IC.

Todos esses mecanismos de regulação de PAS e FC estão presentes para manter a homeostasia do corpo em indivíduos hígidos. Na presença de doenças como a IC, essa regulação falha em manter esse equilíbrio. Portanto, intervenções capazes de recuperar essa regulação intrínseca são benéficas para esses pacientes (Sugimachi *et al.*, 2007). A tabela 2 resume os principais estudos experimentais sobre eletroacupuntura e sistema nervoso autonômico.

Tabela 1 – Principais estudos experimentais de EA com desfechos cardiovasculares.

Autores	Amostra	Protocolo	Ponto Utilizado	Objetivos	Variáveis	Resultados	Conclusão
Uchida *et al.*, 2007	Ratos Wistar hígidos (anestesiados)	Agudo	E36	Avaliar os mecanismos das respostas bradicárdicas pela eletroestimulação.	Frequência Cardíaca	↓FC em 33,4±6,3 bpm antes e após estimulação. Diminuição abolida com secção do gânglio estrelado (C7) [p<0,05]	A bradicardia ocorrida parece ter efeito por uma diminuição da atividade dos nervos eferentes simpáticos cardíacos.
Uchida, Kagitani e Hotta, 2008	Ratos Wistar hígidos (anestesiados)	Agudo	E36	Identificar quais vias são responsáveis pela diminuição da frequência cardíaca, bem como qual frequência de EA é mais eficaz para esta resposta	Frequência Cardíaca	↓FC significativa antes e depois do estímulo de 2HZ comparado com 0,5 Hz e 10Hz [p<0,05]. Diminuição abolida pela infusão de bicuculina (inibidor do GABA) [p<0,05]	A bradicardia parece ser mediada pelo neurotransmissor GABA, a melhor resposta é na frequência de 2 HZ.
Xiong *et al.*, 2011	Ratos espontaneamente hipertensivos (não especifica se os animais foram anestesiados para serem estimulados)	Crônico (8 semanas)	E36 (uso de eletrodo)	Avaliar se a EA é capaz de diminuir a PA, a hipertrofia ventricular ou o espessamento vascular da aorta e níveis plasmáticos e cardíacos de ON	PA Hipertrofia ventricular Espes-samento da Aorta Níveis de ON	↓PA em mmHg na 3ª semana de EA (173,8±8,1 vs 167,3±8,5 p<0.05)comparado ao valor basal. Na 8ª semana, a PA no grupo EA é menor comparada ao grupo controle(173,5±8,7 vs 191±9 p<0,05)↓espessura média da aorta em µm(102,5±9,1 vs 119,8±7,2 p<0,05) EA comparado ao controle. ↑ NO no plasma (23,44±5,3 vs 32,65±6,43p<0,01) e no miocárdio (1,18±0.17 vs 1,68±0.15p< 0,05) antes e depois da EA	EA previne o aumento progressivo da PA. Redução do espessamento da aorta. Aumento nos níveis de NO no plasma e no miocárdio.
Ma *et al.*, 2014	Ratos com IC após indução de IAM	Crônico (1 semana)	PC6	Avaliar estrutura e função cardíaca	Área de infarto, FE, PDFVE	↓Área de infarto (p<0,05) ↑FE(p<0,05) e ↓ PDFVE (p<0,05) comparado aos animais IC sem EA.	EA melhora a estrutura e a função cardíaca em animais com IC

Fonte: Elaborada pelo autor. FC: Freqüência Cardíaca. C7: Cervical 7. ON ou NO: Óxido Nítrico. FE: Fração de Ejeção. PDFVE: Pressão Diastólica Final do Ventrículo Esquerdo. IAM: Infarto Agudo do Miocárdio.

Tabela 2 – Estudos que relatam o efeito da EA sobre o sistema nervoso autonômico

Autores	Amostra	Protocolo	Ponto Utilizado	Objetivos	Variáveis	Resultados	Conclusão
Sugimachi *et al.*, 2008	Coelhos brancos japoneses (anestesiados)	Agudo	E36	Avaliar o barorreflexo com uso da EA	Atividade do Nervo Simpático, parâmetros logísticos da função barorreflexa	↓Atividade do Nervo Simpático(162±31 para 130±29 p<0,005) ↓amplitude (144±35 vs 112,6±9.2p<0,005), ↓ do ponto médio da amplitude (11,4±6.5 vs 103,3±10 p<0,005) comparado ao grupo controle	EA no ponto E36 reduz atividade do nervo simpático e tem efeito no barorreflexo a nível central
Ma *et al.*, 2014	Ratos com IC após indução de IAM	Crônico (1 semana)	PC6	Avaliar efeito do EA no reflexo simpático cardíaco aferente na PAM e na atividade no nervo simpático renal	PAM, atividade do nervo simpático renal e reflexo simpático cardíaco	↓PAM e da atividade do nervo simpático renal [p<0,005]comparados aos ratos sham e aos do grupo controle da EA. Inibição do reflexo cardíaco pela acupuntura nos ratos com IC comparados aos Sham.	EA pode alterar função e estrutura cardíaca além de diminuir a atividade simpática em animais com IC.

Fonte: Elaborada pelo autor. PAM: Pressão Arterial Média

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4 ARTIGOS

4.1 ARTIGO 1

ELECTROACUPUNCTURE APPLICATION MODEL IN AWAKEN RATS
(A ser submetido ao periódico *Journal of Visualized Experiments*)

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KEYWORDS

Acupuncture, Stress, Immobilization, Burrowing, Restraint, Application Device, Rats

SHORT ABSTRACT

This research was conducted in order to present a new proposal for an electroacupuncture application model in awaken rats. For that, a device was created in order to better administer the technique. Made with plastic bottles attached to a wooden base, the device enables animals to remain inside the bottle spontaneously during the application of the protocol.

LONG ABSTRACT

Due to the difficulty encountered during the application of electroacupuncture, a device was created in order to enable the procedure without the need of anesthesia or restraints. Made of PET bottles attached to a wooden base, the device allows animals to remain still during the protocol. The bottles simulate holes, which make it more appealing for the animals to stand still and in place. Heart Rate (HR), Systolic Arterial Pressure (SAP), Diastolic Arterial Pressure (DAP) and Mean Arterial Pressure (MAP) were assessed among animals that used the bottles device and among animals that used a restraint model, which is a vest that keeps rats still and suspended. The statistical analysis used was the Student's T test. The results have shown that there was statistically significant reduction in the HR [338.2±14.3 vs. 380.5±31.9, p=0.0167], MAP [101.6±2.6 vs. 108.2±2.5, p=0.0029], SAP [125±2.1 vs. 130.6±3.8 p=0.0135] and DAP [82.2±2.9 vs. 88.5±2.5, p=0.0043] values of animals that used the bottles device (n = 6) compared to animals that used the restraint model (n = 5) during the implementation of the treatment protocols. This device presents as an advantage the application of electroacupuncture without using anesthesia or restraints, in addition to being made of recyclable materials, which makes it inexpensive and easy to reproduce. Further studies are needed to compare it to other control models.

INTRODUCTION

Acupuncture is an ancient Chinese practice that uses the insertion of needles into specific points of the body (the so-called acupuncture points) and is used to treat various diseases as well as to relieve pain[1]. Whereas the electroacupuncture is the stimulation of the acupuncture point with an electrical current as opposed to the manual stimulation used in traditional acupuncture.[2] Recently, experimental studies have been conducted in order to physiologically explain how the electroacupuncture operates and what its effects are[3, 4]. Also studies have been carried out aimed at elucidating the action mechanisms of this practice in animal models[5, 6].

However, the primary focus of these studies is based on the effects of electroacupuncture and not on how the application of the needles is carried out in animal models. Additionally, most acupuncture protocols are related to acute effects[7,8] having very few studies to approach chronic protocols[9,10], which can be explained by the difficulty of implementation and application of electroacupuncture in animals. Most experimental studies on electroacupuncture uses anesthetized animals for the application of needles and electrical stimulation[7,11] or uses restraining devices when the animals are awake [12, 13]. Acute protocols usually keep the animals under anesthesia during application[14]. On the other hand, chronic protocols apply lower depth level anesthesia for the placement of needles, just to decrease the stress level of the restraint necessary for the placement of needles[8].

However, animals' confinement may generate stress and their confinement is one of the stress induction models[15, 16]. Nonetheless, it is known that anesthesia may be capable of leading to different hemodynamic changes such as changes in heart rate, blood pressure and cardiac output[17]. Experimental and clinical studies show that electroacupuncture alters hemodynamic parameters[14, 18, 19].

However, very seldom the detailed description of the experimental electroacupuncture protocol is provided, hindering or even preventing the reproducibility of the methods[10, 12, 20, 21]. Based on this, the importance of the accomplishment of the detailed and suitable electroacupuncture protocol should be verified in order to isolate the real effects of this technique, without the interference of the two main factors: restraint and anesthesia.

As known, remaining in holes is a natural behavior portrayed by rodents and it is well evidenced in behavioral studies involving laboratory rats[22, 23]. Based on this, we developed a device where the animals stay during the application of the electroacupuncture protocol. In this sense, the intent of this paper is to present a new electroacupuncture method in awaken rats without the restraint in order to depict the clinical conditions of this technique's application.

PROTOCOL

1. Electroacupuncture Application Device

1.1 Equipment

1.1.1 Pet bottles (500ml)

1.1.2 Wood or mdf base measuring approximately 80x40cm

1.1.3 Insulating tape

1.1.4 Scissors

1.1.5 Glue

1.1.6 Brass plated pushpin

1.1.7 Hammer

1.2 Construction of the Electro-Stimulation Device

1.2.1 Cut the bottom of the plastic bottles leaving 12 cm of bottle to be used as a hole.

1.2.2 Paste the insulation tape on the opening of the hole for the animal to be comfortable while entering the device.

1.2.3 Position the hole on the PET bottle on the wood surface using glue and the brass plated pushpin with a hammer.

2 Using the Device

2.1 Material

2.1.1 Intradermal acupuncture needles with the required length to attain the acupuncture point chosen for the stimulation.

2.1.2 Electroacupuncture device with the specification for the stimulation.

2.2 Procedure

2.2.1 Adaptation of the animals to the device

2.2.1.1 Take the animals to the device and let them walk freely.

2.2.1.2 Repeat the procedure at least 2 times more until the beginning of the protocol.

3 Insertion of the Acupuncture Protocol

3.1 Hold the tail of the animal with the right hand.

3.2 Pinch the skin of the neck just below the ears with the thumb and forefinger of the left hand.

3.3 Pinch the skin on the back with the 3rd, 4th and 5th fingers against the palm.

3.4 Raise the rat from the device. Turn the animal with its paws up.

3.5 Insert the needle into the acupuncture point to be stimulated.

4 Placement of Electroacupuncture

4.1 Release the animal and let it go alone to the hole of preference.

4.2 Connect the wires to the needles.

4.3 Connect the electroacupuncture device according to the desired parameters.

RESULTS

Figures 1 demonstrate how the device can accommodate more than one animal for the stimulation, optimizing the time of the researcher, and moreover, the animals can remain without restraint voluntarily, inside the bottles and in a position of relaxation during the stimulation.

Bottle devices that enable the stimulation of more than one animal.

[Place figure 1 here]

Figure 6. PET Bottles Device that Enables the Stimulation of More than one Animal in the Same Protocol, Without the Use of Restraint or Anesthesia.

Rat being electrically stimulated in the bottle device.

[Place figure 2 here]

Figure 7. The Animal Remains Voluntarily in the Bottle During the Electro-Stimulation Protocol of the ST36 Point.

Rat in a relaxed position on the bottle device.

[Place figure 3 here]

Figure 8. Relaxed Animal During the Protocol.

For statistical analysis, Student T test was used for the comparison between the groups; The value of $P < 0.05$ was considered as statistically significant. Hemodynamic Parameters were assessed by catheter introduction in femoral artery. There was a reduction in the values of the Heart Rate (HR) [338.2±14.3 vs.

380.5±31.9, p=0.0167] of animals that used the bottle device (n = 6) compared to animals who received the restraint model, a vest that maintains the rat in the position and suspended (n = 5) during application of the treatment protocols (Fig. 4.) Values of the Mean Arterial Pressure (MAP) [101.6±2.6 vs. 108.2±2.5, p=0.0029], systolic blood pressure (SBP [125±2.1 vs. 130.6±3.8 p=0.0135] and diastolic blood pressure (DBP) [82.2±2.9 vs. 88.5±2.5, p=0.0043] were significantly lower in comparison with the vest group.

[Place figure 4 here]

Figure 9. Restraining model used for the animals with the use of vests. The animals are in the suspended position during the application of the protocol.

Result of the HR values.

[Place graph 1 here]

Graph 1. Values of the mean ±SD of the HR (mmHg) of the group that used the vest and the group that used the bottle device. Student T test (380.5±31.9 vs. 338.2±14.3, p=0.0167).

MAP values results.

[Place graph 2 here]

Graph 2. Values of the mean ±SD of the MAP (mmHg) of the group that used the vest and the group that used the bottle device. Student T test (108.2±2.5 vs. 101.6±2.6, p=0.0029).

Results of the SAP values.

[Place graph 3 here]

Graph 3. Values of the mean ±SD of the SAP (mmHg) of the group that used the vest and the group that used the bottle device. Student T test (130.6±3.8 vs. 125±2.1, p=0.0135).

Results of the DAP values.

[Place graph 4 here]

Graph 4. Values of the mean ±SD of the DAP (mmHg) of the group that used the vest and the group that used the bottle device. Student T test (88.5±2.5 vs. 82.2±2.9, p=0.0043).

DISCUSSION

The vast majority of electroacupuncture studies are based on acute protocols[14, 24] with the use of the anesthesia technique chosen to reduce the stress caused by the restraining of the animals for stimulation[7, 25, 26].

Studies using chronic protocols do not clearly describe how electroacupuncture applications are performed[9]. Recently, studies in awaken animals have proposed a new form of needle that would allow the needles to remain in place in the correct acupuncture point during the protocol[11, 12]. However, even in this case, the animals received a dose of anesthesia to minimize the restraining stress.

However, it is known that anesthesia can alter hemodynamic parameters in cardiodepressant terms, that is, it decreases the blood pressure and heart rate[17, 27, 28]. Although most studies uses chloral hydrate, which would not cause changes in these parameters[29] to anesthetize animals during the insertion of the needles, we believe that the fact that animals are submitted to anesthesia does not correlate to the acupuncture practice. Therefore, it was essential to create a method where the animals could receive electroacupuncture without the use of anesthetics or restraints.

Wang et al, discloses that in their study, that the animals were anesthetized or not restrained since they were relaxed during the application of the electroacupuncture and remained in a polyethylene cylinder during the application[30]. The use of these cylinders in experimental studies has been widely used[31] as environmental enrichment device in order to provide the possibility of rat to remain hidden or in the hole, which is a normal behavior presented by rodents[32, 33].

Based on this, we created this device for the stimulation of more than one animal at the same time without restraints or anesthesia for the application of electroacupuncture. As stated in Wang *et al's study,* the animals remain relaxed during electroacupuncture, as took place in our experiment. During the protocol clear signs of stress as piloerection or signs of pain cannot be seen. In addition, the hemodynamic evaluation of animals using our device presented lower HR, MAP, SAP, DAP values when compared with animals included in the protocols, showing that the restraints can increase these parameters.

Therefore, the use of the bottles device seems to be an effective alternative to be used in the stimulation of rats and it is also a cheap tool which is easy to use and enables the application of electroacupuncture. In the future, more studies should be accomplished comparing this device to other models of electroacupuncture application or even animals with controls that are only in their habitat. Furthermore, the prospect of converting this device into a durable material such as polyethylene may grant a useful life to the device as well as better conditions for the maintenance of the same.

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DISCLOSURES:

The authors declare that they have no competing financial interests.

FIGURES

Figure 1:

Figure 2:

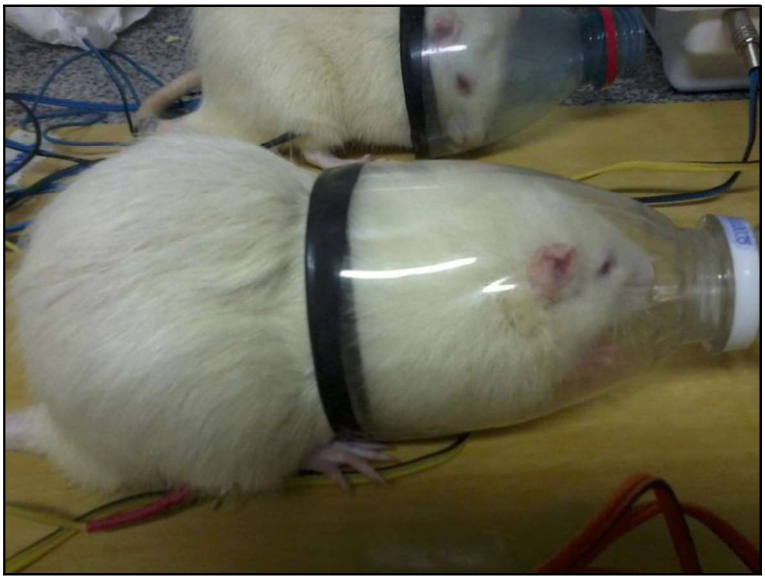


Figure 3:

Figure 4:

Figure 5:

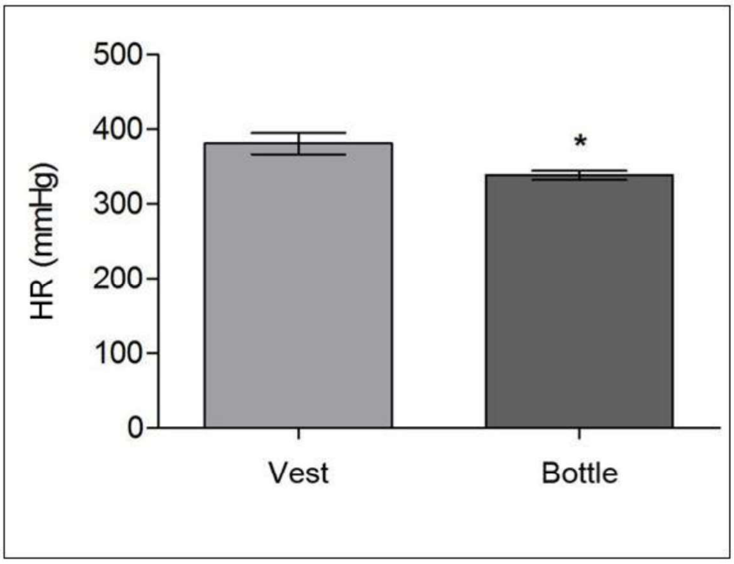


Figure 6:

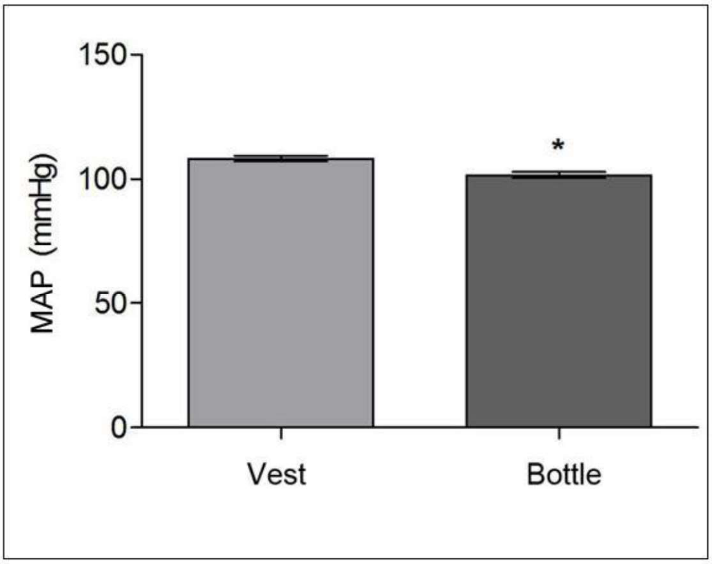


Figure 7:

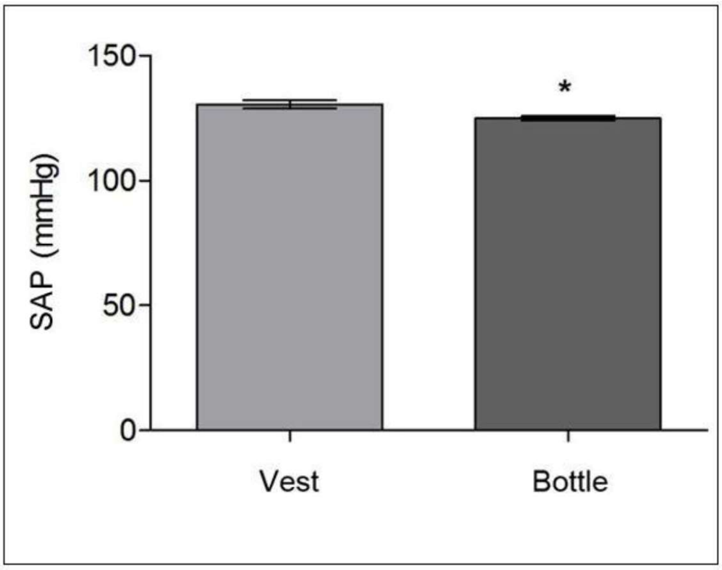
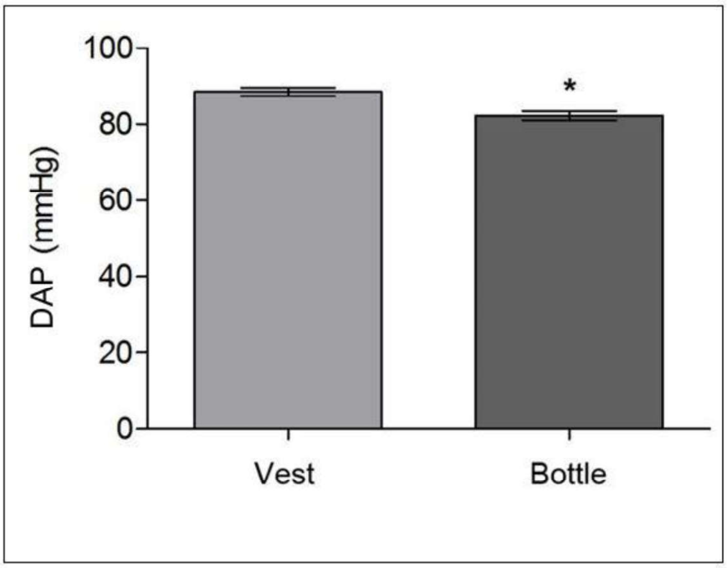


Figure 8:



4.2 ARTIGO 2

Chronic Electroacupuncture of the ST36 Point Improves Baroreflex Function and Haemodynamic Parameters in Heart Failure Rats

(A ser submetido ao periódico *Autonomic Neuroscience: Basic and Clinical*)

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ABSTRACT

Electroacupuncture (EA) has been used to treat many diseases, including heart failure (HF). This study aimed to evaluate the effects of chronic stimulation in the ST36 acupuncture point on haemodynamic parameters and baroreflex function in rats with HF. Cardiovascular parameters assessed were heart rate (HR), blood pressure (BP), and the reflex cardiovascular response of HR and BP triggered by stimulation of baroreceptors in animals with HF subsequent to acute myocardial infarction (AMI). Male Wistar rats were divided into three groups: Sham Control – animals without HF and without EA; HF Control group – animals with HF and without EA; HF EA group – animals with HF that received the EA protocol. Six weeks after surgical induction of AMI, the EA protocol (8 weeks, 5 times a week) was performed. The protocol was applied with EA at the ST36 point, frequency of 2 Hz, pulse of 0.3 ms and intensity of 1–3 mA for 30 min. Haemodynamic parameters and baroreceptor function were assessed. There was no difference between groups in the variables HR, systolic blood pressure (SBP) and diastolic blood pressure (DBP), which were evaluated with awake animals ($p > 0.05$). There was an increase in the mean arterial pressure (MAP) in the HF EA group compared to the HF Control group ($p < 0.05$). The maximum gain of the baroreflex curve (Gain) was higher in the HF EA group than the HF Control and Sham Control groups. Chronic EA in the ST36 point increased the MAP and baroreflex sensitivity in rats with HF.

Keywords: myocardial infarct; electroacupuncture; autonomic nervous system.

1 INTRODUCTION

Heart failure (HF) is a progressive syndrome in which a structural or functional cardiac condition impairs the heart's ability to adequately supply blood flow to the various organs and tissues (Axente et al., 2011).

Acute Myocardial Infarction (AMI) is the most common etiologic factor for the development of HF (Von Haehling, 2009). Initially, there is an increase in sympathetic cardiac activity in order to maintain the cardiac output (CO). The most important symptoms that appear in the early stage of HF are dyspnoea and exercise intolerance (Antunes-Correa et al., 2010).

However, the maintenance of this sympathetic activation leads to deleterious effects throughout the cardiovascular system (Jaenisch et al., 2011). In this sense, HF is characterised by an imbalance in the sympathetic–vagal physiological activity with a chronic adrenergic activation, together with impairment in the baroreflex sensitivity (La Rovere et al., 2008).

The baroreflex is one of the most important cardiac reflexes able to regulate the heart rate (HR) and blood pressure (BP) since there is a direct influence on the autonomic nervous system (Quagliotto et al., 2008). Baroreceptors are sensitive to mechanical deformations of the vascular walls and promote short-term blood pressure control (Irigoyen, 2004). Changes in the characteristics of the baroreflex function, such as a reduction in the sensitivity due to hyperactivation of the sympathetic system, may alter the autonomic control of the cardiovascular system (La Rovere et al., 1998; Zhang et al., 2014). Thus, the evaluation of the sensitivity of the baroreflex has become an important prognostic marker for cardiovascular disease (La Rovere et al., 2013) and cardiac death after acute myocardial infarction (La Rovere et al., 1998).

Experimental studies have been conducted to elucidate the effects of acupuncture on the cardiovascular system (Li et al., 2012; Middlekauff, 2004; Wang et al., 2008) and on the autonomic system function (Huang et al., 2011; Middlekauff et al., 2002; Sugimachi et al., 2007), including changes in the sympathetic and parasympathetic activity and also in the baroreflex function (Middlekauff et al., 2002; Sugimachi et al., 2007; Tsou et al., 2004; Xiong et al., 2011). Studies conducted on animals may elucidate the mechanisms by which electroacupuncture is capable of generating bradycardia and decreased blood pressure (Li et al., 2010; Uchida et al., 2008; Uchida et al., 2007; Zhou et al., 2012). Besides that, these studies have helped in the understanding of the acupuncture mechanisms, demonstrating the specificity of each acupuncture point (Choi et al., 2012), such as the stomach point 36 (ST36), which has an effect on the cardiovascular and autonomic system (Tjen et al., 2004; Uchida et al., 2007).

However, this evidence is based mostly in healthy animals and little is known about the effects of acupuncture in HF (Middlekauff, 2004). Furthermore, most studies show only short-period acupuncture applications, which makes it difficult to prove the long-lasting effects of acupuncture in chronic situations. Our hypothesis is that a protocol of chronic electroacupuncture is capable of improving baroreflex sensitivity in rats with heart failure. Therefore, the primary objective of this study was to evaluate the effect of chronic electroacupuncture in the ST36 point in baroreflex sensitivity, and a secondary objective was to evaluate the haemodynamic parameters of rats with heart failure.

2 METHODS

2.1 Animals

Male Wistar rats (n = 32), weighing between 200 and 300 g (approximately 60 days old), from the animal breeding centre of the Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA) were used. Animals were kept in a cage with ad libitum food and water in a room with a 12:12 h dark/light cycle. The study followed the Law 11.794 of 10/08/2008 and Decree No. 6.899 of 07/25/2009, which currently regulates research activities conducted with animals, as well as the guidelines contained in the International Guiding Principles for Biomedical Research Involving Animals from the Council for International Organizations of Medical Science (CIOMS). All procedures performed in this study were previously approved by the UFCSPA Ethics and Research Committee (Protocol 088-12).

2.2 Surgery to Induce Myocardial Infarction

Rats were anaesthetised by inhalation of 2% isoflurane (Isoforine, 100 mL - Cristália) and 98% oxygen (Munkvik et al., 2011) and intubated, and anaesthesia was maintained at the same concentration of isoflurane through the tracheal tube with 100% oxygen and the use of a vaporizer with 1 L/min^{-1} flow of gas (rotometer for veterinary anaesthesia, BR-200 Vetcare, Incontec Científica LTDA, Brazil). Animals were maintained at Guedel's anaesthetic level III (Colman et al., 2002; Hecker et al., 1983). Myocardial infarction was induced as previously described (Hentschke et al., 2013; Pfeffer et al., 1979). The thoracotomy was performed at the level of the left fourth intercostal space, the pericardium was cut and the left coronary artery was ligated with a 6.0 mono nylon wire. The chest incision was closed using 3.0 mono nylon wires, and the pneumothorax was drained by a continuous suction system. Then, the animals were removed from artificial ventilation and encouraged to breathe (Francis et al., 2004). As a prophylaxis against infections, single doses of penicillin

(20,000 units) were administered (Andrade, 2002). For the Sham HF groups, the same procedures were conducted except for the performance of the coronary ligature.

2.3 Experimental Groups

After the myocardial induction, the animals remained in the cages for at least 6 weeks (time necessary to develop the HF state). After this period, animals were assigned to one of three groups: Sham Control (Sham Control, n = 9), HF Control (HF Control, n = 13) and HF Electroacupuncture (HF EA, n = 10).

2.4 Electroacupuncture Protocol

Animals were adapted and gently handled for 30 min, for 2–3 days before the beginning of the protocol, always by the same researcher (Lao et al., 2004). For the EA protocol, acupuncture needles made of stainless steel, size 0.5 mm (Complementar Agulhas, Brazil), were inserted bilaterally in the stomach point 36, ST36 (Su Zan Li), which is located in the anterolateral portion of the hind limb, near the anterior tibial tuberosity, in the tibialis anterior muscle and is innervated by the deep fibular nerve (Li et al., 2009; Tjen et al., 2004). The needling was performed at a depth of 5mm and confirmed by a slight muscle contraction (Wu et al., 2010) or by slight and repeated movement of the paw (Zhou et al., 2005). The NKL electro-stimulator (Model EL608) with eight channels was used. The parameters used are described in Table 1.

The control group was placed in the same device developed in our laboratory for animal stimulation (unpublished data), in which the animals received EA but without needle insertion or the electrical stimulation for the same 30-minute period (Fig. 1).

2.5 Functional Capacity Test (VO_2 max)

After the completion of the EA protocol, animals were subjected to a functional capacity test. To determine maximum oxygen consumption (VO_2max), each animal accomplished the maximum testing capacity of Batista's adapted exercise (Batista et al., 2007). The parameters were measured using a gas analyser system for animals (AVS Projects, São Carlos, SP, Brazil). The air volume supplied was of 4.5 L/min. The analyser was calibrated with a mix of a known quantity of gas prior to each test. After a period of 15 min of acclimatisation, the test protocol was initiated at a speed of 10 m/min. The protocol consisted of a gradual increase in the speed of the treadmill with an increment of 5 m/min every 3 min. It was considered as exhaustive when the animal remained on the shock platform for more than 15 seconds.

2.6 Surgical Preparation for Cardiovascular Measures on Animals

After functional capacity test , animals were anaesthetised with ketamine (90 mg/kg ip) and xylazine (12 mg/kg ip). Two catheters filled with saline solution (NaCl 0.9%, pH 7.40, 06 L) and heparin (0.01 mL) were inserted into the left artery and femoral vein to directly measure arterial blood pressure and for the administration of drugs, respectively.

2.7 Baroreflex Sensitivity

On the following day after vascular catheter implantation, the cannula was connected to an extension of 40 cm (PE-50) in order to enable the animal's movement. The arterial cannula was connected to a pressure transducer (Strain-Gauge, Narco Biosystem Miniature Pulse Transducer RP-155, Houston, Texas, USA) connected to a signal amplifier (Pressure Amplifier HP 8805C). The blood pressure signals were recorded for 15 min and were used as baseline data for each animal (CODAS - Data Acquisition System, PC 486). The "beat-to-beat" recorded data were analysed to quantify the parameters of interest.

The baroreflex sensitivity was tested by phenylephrine intravenous infusion (Sigma Chemical Company, USA) at a dose of 8 μg/ml, and sodium nitroprusside (Sigma Chemical Company) at a dose of 100 μg/ml. The phenylephrine was used to induce a BP increase, followed by a reflex bradycardia controlled by baroreceptors. Sodium nitroprusside was used as a potent vasodilator to reduce the BP, followed by a reflex tachycardia response, which is also controlled by baroreceptors. Drugs were injected only after the cardiovascular parameters were at baseline levels of the animal at rest, and were performed randomly within each experimental group. Subsequently, the HR values corresponding to the MAP values of the baseline period, as well as BP increases or reduction, were used for the analysis of the baroreceptor reflex-mediated responses. These HR responses were evaluated by a specific computer program for the adjustment of the sigmoidal curve as described previously (Sigma Plot, USA) (Head et al., 1987; Quagliotto et al., 2008).

2.8 Surgical Preparation for Haemodynamic Evaluation

On the following day, after baroreflex sensitivty assessment, animals were anaesthetised with ketamine (90 mg/kg ip) and xylazine (12mg/kg ip), and a small incision was made on the neck to insert a catheter into the right carotid artery. The blood pressure was recorded by the same aforementioned acquisition system. After recording the blood pressure for 5 min, the same catheter was positioned in the left ventricle to record the ventricular pressure for 5 min. These records were used to determine the left ventricular systolic pressure (LVSP), achieved from the contraction (+dP/dt) and the relaxation (-dP/dt) of the LV , respectively, and the left ventricular end diastolic pressure (LVEDP).

2.9 Cardiac Hypertrophy and Pulmonary and Hepatic Congestion

After the haemodynamic evaluation, animals were sacrificed using an anaesthetic overdose (100 mk/kg pentobarbital) for tissue collection. Heart, liver and lungs were collected. The left ventricle (LV) and right ventricle (RV) were dissected, separated and weighed. The ratios of left ventricular weight were determined according to the body weight (LV/BW) and right ventricle weight in comparison with the body weight (RV/BW) in order to establish the cardiac hypertrophy. Liver and lungs of each animal were dehydrated (80°C) for 48 hours and weighed again to determine the percentage of water available in the tissue to determine liver and lung congestion.

2.10 Infarction Area

The intermediate ring (~3 mm) of the left ventricle was sectioned in a microtome in sections of 5 μm with 100 μm intervals between the sections (Frantz et al., 2003). Sections were stained with haematoxylin and eosin (HE) on the histologic blades. The Image Pro-plus 6.1 computer program (Media Cybernetics, Silver Spring, USA) was used to determine the size of the infarction area from scanned photographs of the slides containing tissue sections. The infarction area, expressed as a percentage of the total area of the left ventricle, was calculated by dividing the sum of the infarcted area by the sum of all sections of the left ventricular area (including those without infarction) and multiplied by 100, as previously described (Pfeffer et al., 1979).

2.11 Statistical Analysis

The Kolmogorov–Smirnov test was used to assess the normal distribution of all variables. The values were expressed as average values and standard deviation (SD). The one-way ANOVA test was performed to analyse all variables between the groups, followed by Bonferroni's post hoc test. The value of $P < 0.05$ was considered as statistically significant. The software SigmaPlot 11.0 (Systat Software Inc., San Jose, USA) for windows was used as a computational tool for statistical data analysis. The GraphPad Prism 5 software (GraphPad Software, San Diego, CA, USA) for Windows was used as a computational tool for further analysis and elaboration of graphs.

3 RESULTS

Tables 2 and 3 feature the HF model presenting the main tissue and the physiological changes caused by HF.

Table 2 summarises the data on body weight, infarction size, full heart hypertrophy, left ventricular hypertrophy, right ventricular hypertrophy, and pulmonary and hepatic congestion in mice of the Sham Control (n = 9), HF Control (n = 13) and HF EA (n = 10) groups. Only animals in the HF Control group presented significantly higher values for total heart hypertrophy in comparison with the Sham Control group [$F(2,31) = 8.643$; $p = 0.0011$]. Regarding the left ventricular hypertrophy, animals of the HF EA group presented higher values in comparison with the Sham Control group and even lower values when compared with the HF Control [$F(2,31) = 20.28$; $p < 0.0001$]. Only the HF Control animals showed pulmonary congestion when compared to the Sham Control [$F(2, 31) = 4.895$, $p = 0.0145$].

Regarding the exercise capacity test, there was no difference in VO_2 max, basal VO_2 and reserve VO_2 between groups. HF animals showed significantly lower values regarding the distance travelled and the duration of the test [$F(2,14) = 6.288$; $p = 0.0136$ and $F(2,14) = 6.337$; $p = 0.0132$, respectively] in comparison with the animals from the Sham Control group (Table 3).

3.1 Haemodynamic Variables

Table 4 presents the data regarding the haemodynamic variables of Sham Control (n = 8), HF Control (n = 9) and HF EA (n = 9) groups. Rats with HF presented higher end-diastolic pressure than Sham Control animals [$F(2,25) = 10.37$; $p = 0.0006$] and a smaller left ventricular systolic pressure in comparison with Sham Control animals [$F(2,27) = 11.94$; $p = 0.0002$]. On the other hand, the contraction and relaxation variables of the ventricles of the HF Control group had lower values in comparison with the Sham Control group [$F(2,26) = 5.057$; $p = 0.0147$] and [$F(2,23) = 6.401$; $p = 0.0068$], respectively.

3.2 Haemodynamic Data of the Animals While They are Awake

The haemodynamic data in the awake condition of Sham Control animals (n = 9), HF Control (n = 9) and HF EA (n = 8) are depicted in Table 5. There was no difference in HR [$F(2,27) = 1.107$; $p = 0.3461$] and dyastolic arterial pressure (DAP) [$F(2,23) = 2.739$; $p = 0.0877$] between the groups. The animals of the HF Control group presented a systolic arterial pressure (SAP) that was lower than that in the

Sham animals [F(2,25) = 8.998; p = 0.0013] and although there was no significant difference, the SAP values of the HF EA group were similar to the Sham Control group. HF EA animals had significantly higher MAP values in comparison with the HF control and similar values compared to the Sham animals [F(2,25) = 7.438; p = 0.0032).

3.3 Baroreflex Response

Regarding the reflex mediated by the baroreceptors of the Sham Control (n = 6), HF Control (n = 5) and HF EA (n = 5) groups, there was no difference in the values of the mean arterial pressure at the half of the heart rate change (MAP_{50}) in the baroreflex curve, which represents the level of the BP presented by the maximum baroreflex gain after the infusion of phenylephrine and sodium nitroprusside to stimulate the reflex response [F(2,15) = 0.8540; p = 0.4483] between groups. Also, no difference was found in the values regarding the amplitude equivalent to the operating range of the baroreceptor reflex [F(2,16) = 0.5336; p = 0.5980], the upper plateau which is the maximum HR response induced by the decrease of the BP [F(2,15) = 1.224;p = 0.3258], as well as the values of the lower plateau that represent the maximum decrease of the HR induced by the BP increase [F(2.15) = 1.830; p = 0.1994], after the baroreceptor reflex stimulation, respectively (Table 6).

There was a difference in the gain of the baroreflex between the HF EA group and the Sham Control group (Fig. 2 and 3).

4 DISCUSSION

As far as we know, this is the first experimental study that has been conducted in order to evaluate the chronic effects of EA directly on the baroreceptor reflex function in animals with HF. In this study, chronic electroacupuncture of the ST36 point improved the baroreflex sensitivity in animals with HF. Moreover, EA reduced the hypertrophy of the LV and increased MAP in comparison with the animals of the HF Control group.

Heart failure is a high morbidity/mortality disease that generates high costs of hospitalisation and drugs, both in Brazil and worldwide (Achutti, 2008; Roger et al., 2012). The hyperactivation of the sympathetic nervous system commonly observed in HF is accompanied by the attenuation of the baroreflex system (Zhang et al., 2014). This is an important determinant of the neural regulation of the cardiovascular system, which currently presents clinical relevance in the prediction of sudden cardiac death (La Rovere et al., 2008) and also the prognostic value in patients with heart failure (HF) (La Rovere et al., 1998).

Other studies have shown that EA may modulate the autonomic nervous system (Uchida et al., 2008; Zhang et al., 2014). This modulation appears to be related to the decreased activity of the sympathetic system, which can be shown by the maintenance of the bradycardic effect even after vagus nerve section (Uchida et al., 2008). Some studies have demonstrated the acute effect of electroacupuncture of the ST36 point in the baroreflex (Michikami et al., 2006; Sugimachi et al., 2007; Sugimachi et al., 2008). These studies were conducted in rabbits, and the main findings indicated that the electroacupuncture stimulation of the ST36 point does not affect the peripheral response, but is related to the neural response, referring to the vasomotor centre (Sugimachi et al., 2007). Moreover, these studies demonstrate that the baroreflex is preserved during the electroacupuncture stimulation, which suggests the application of this technique to treat cardiovascular diseases with sympathetic nervous system hyperactivity (Michikami et al., 2006). However, in this study, the effects of BP reduction are eliminated immediately at the end of the electroacupuncture stimulation, indicating that there is no long-term effect of the electroacupuncture. After the assessment of the chronic electroacupuncture protocol, we have demonstrated that there are likely to be long-lasting effects of acupuncture in chronic situations. Similarly, our findings in the baroreflex sensitivity are probably related to the reduced activation of the sympathetic nervous system by EA. We can attribute this mechanism to the reduction of LV hypertrophy shown in this study. Although there was no difference between the HF EA and the HF Control groups, the values of the HF EA group regarding systolic blood pressure and those achieved from the contraction and relaxation presented a similar behaviour, presenting values closer to the ones found in the Sham group and greater than those found in the HF Control group (Tables 4 and 5). Our study shows a reduction in LV hypertrophy in the EA group. These data corroborate the findings demonstrating that EA improved the cardiac structure and function in animals with this model of HF and additionally reduced the sympathetic activity (Ma et al., 2014).

A reduction in SAP and MAP in HF animals has been observed, which is directly related to the infarct size. This reduction was observed in this MAI induction model and probably occurs due to the animals' ability to survive massive heart infarction. SAP and MAP reductions are proposed as a compensatory mechanism to the loss of the left ventricular function and increased LVEDP (Pfeffer et al., 1979). However, we have found a significant increase in MAP in animals that received treatment with EA compared to the ones in the HF Control group. Therefore, we can infer that there was an improvement in the ventricular function of these animals as a result of the chronic electrostimulation of the ST36 point.

Both the hypotensive and bradycardic effects of the ST36 point demonstrated in healthy animals are related to the deep peroneal nerve stimulation, while other points did not have this same effect (point specificity) (Choi et al., 2012; Tjen et al., 2004), such as the bladder point 21 (B21), located 5 mm from the spinous process of the 12th thoracic vertebra (Iwa et al., 2006), which has no effect on the autonomic nervous system (Imai et al., 2009). Similarly, studies have shown that when compared with inactive points for cardiovascular outcomes, points located in deep somatic nerves provide more stimulus to the sympathetic premotor neurons in the rostral ventromedial medulla (RVM), which is an area that plays an important role in the regulation of the BP (Zhou et al., 2012). At the same time, RVM is also inhibited by the caudal ventrolateral medulla by the stimulation of baroreceptors (Schreihofer et al., 2002) indicated that electroacupuncture and arterial baroreflex divides a common central activation pathway (Michikami et al., 2006).

A limitation of our study was the unavailability of the EA control group, which would enable us to attribute these effects specifically to the stimulation of the ST36 point. Moreover, during the study, some animals were lost, especially during the assessment of the baroreflex function, since it is a complex technique that involves drugs infusion that causes the increase and decrease of pressure in animals that are already weakened by the HF itself, which explains the sampling difference in this variable.

In conclusion, this study demonstrates that chronic electroacupuncture of the ST36 point in animals with HF improved the baroreflex function, probably due to the decreased activation of the sympathetic nervous system, and promoted the increased of the MAP in these animals, thus improving the ventricular function. However, more studies are needed to clarify the mechanisms by which these changes occur, and direct assessments of the sympathetic nervous system are necessary for the complete understanding of this subject.

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CONFLICT OF INTEREST

The authors state that there are no known actual or potential conflicts of interest in the present report.

TABLES

Table 1. Description of treatment protocol parameters according to Xiong et al. (2011). Brand device: NKL, model EL 608. ST36 point: Stomach 36 point.

Table 2. Values are shown as mean ± SD. Sham Control (n = 9): animals without heart failure (HF) and without electroacupuncture (EA). HF Control (n = 13): animals with HF and without EA. HF EA (n = 10): animals with HF and EA. *$p < 0.05$ compared with the Sham Control group. †$p < 0.05$ compared with the HF control.

Table 3. Values are shown as mean ± SD. Sham Control (n = 5): animals without heart failure (HF) and without electroacupuncture (EA). HF Control (n = 5): animals with HF and without EA. HF EA (n = 5): animals with HF and EA. *$p < 0.05$ compared with the Sham Control group.

Table 4. Values are shown as mean ± SD. Sham Control (n = 8): animals without heart failure (HF) and without electroacupuncture (EA). HF Control (n = 9): animals with HF and without EA. HF EA (n = 9): animals with HF and EA. *$p < 0.05$ compared with the Sham Control group.

Table 5. Values are shown as mean ± SD average. Sham Control (n = 8): animals without heart failure (HF) and without electroacupuncture (EA). HF Control (n = 9): animals with HF and without EA. HF EA (n = 9): animals with HF and EA. *$p < 0.05$ compared with the Sham Control group. †$p < 0.05$ compared with the IC Control.

Table 6. Values are shown as mean ± SD. Sham Control (n = 6): animals without heart failure (HF) and without electroacupuncture (EA). HF Control (n = 5): animals with HF and without EA. HF EA (n = 5): animals with HF and EA. MAP_{50}: variation in the pressure value (BP in mmHg) at the major slope of the curve. HR Range: amplitude variation in HR in beats per minute (bpm) during the reflection of the baroreceptor. Upper Plateau: variation in the difference of the increase of the maximum response in HR (bpm) caused by the reduction of the BP (mmHg). Lower Plateau: variation of the difference of the maximum response of the decrease of the FC (bpm) caused by the increase of the BP (mmHg).

FIGURES

Fig. 1. Device Used for the Stimulation of Animals and Control Animals.

Fig. 2. Response of the Variation of the Mean Gain or Mean Baroreflex Sensitivity after the Reflex Stimulation of Baroreceptors. Values are shown as mean (± SD) of the variation of the baroreflex sensitivity (gain bpm/mmHg). Sham Control ($n = 6$): animals without heart failure (HF) and without electroacupuncture (EA). HF Control ($n = 5$): animals with HF and without EA. HF EA ($n = 5$): animals with HF and EA. There was a statistically significant difference when comparing the HF EA group with the Sham Control group ($p < 0.05$).

Fig. 3. The Curve of Mean Arterial Pressure Modification in Accordance with the Variation in Heart Rate. Modified curve analysis of mean arterial pressure (MAP in mmHg) according to the variation of heart rate (HR in bpm) as a baroreceptor reflex response test. There was a statistically significant difference in the values of the HF EA group in comparison with the Sham Control and the HF Control ($p < 0.0001$) groups. The displacement of the curve to the right in the HF EA group ($n = 5$) will be observed since the same HR value generates more MAP response.

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TABLES

Table 7
Protocol parameters and electroacupuncture treatment scheme.

Electroacupuncture Parameters	
Model Device	NKL EL 608, Manufacturer
Frequency	2 Hz
Pulse	0.3 ms
Intensity in the first 5’	1mA
Intensity at 5 to 10’	2mA
Intensity during 20’	3mA
Intensity	1-3mA
Total time	30 minutes
Duration of the treatment	5x week/ 8 weeks
ST36 Point	

Table 8
Sample Characterization and the Infarcted Tissue Characteristics in Sham Rats and Rats with Heart Failure.

Groups	**Initial Body Weight (g)**	**Final Body Weight (g)**	**Infarcted Area (%)**	**H/BW (mg/g)**	**LV/BW (mg/g)**	**RV/BW (mg/g)**	**Pulmonary Congestion (%)**	**Hepatic Congestion**
Sham Control	249.1±21.53	334.2±46		2.54±0.27	1.8±0.2	0.72±0.33	72.52±1.2	72.09±0.7
HF Control	253.2±16.61	327.5±25.22	30.67±4.4	4.6±1,7*	2.96±0.6*	1.35±0.9	76.75±3.67*	73.33±1.72
HF EA	266.3±20.54	347.5±49.48	33.43±5.9	3.4±0.5	2.47±0.3*†	0.95±0.5	76.15±3.22	73.24±0.93

Values are shown as mean ± SD. Sham Control (n=9): animals without Heart Failure (HF) e without electroacupuncture (EA). HF Control (n=13): animal with HF and without EA. HF EA (n=10): animals with HF and EA. *P <0.05 compared with the Sham Control. †P< 0.05 compared with the IC control.

Table 9
Values Related to Functional Capacity Test.

Groups	**Distance (m)**	**Time (s)**	**Max VO_2**	**Basal VO_2**	**Reserve VO_2**
Sham Control	366.7±88.20	1036±151	49.48±19.11	15.13±9.17	34.34±11.69
HF Control	219.9±97.04*	749.4±78.75*	42.10±9.6	18.69±9.57	23.42±5.57
HF EA	210.3±97.04*	695.6±224.4*	41.22±4.92	19.12±4.3	22.10±6.26

Values are shown as mean± SD. Sham Control (n=5): animals without Heart Failure (HF) and without electroacupuncture (EA). HF Control (n=5): animal with HF and without EA. HF EA (n=5): animals with HF and EA. *P <0.05 compared with Sham – Control.

Table 10
Hemodynamic Variables in Sham Rats and in Rats with IAM Induction.

Groups	**LEDP (mmHg)**	**LVSP (mmHg)**	**+dP/dtMax(mmHg/s)**	**-dP/dtMax(mmHg/s)**
Sham Control	3.6±1.9	119.1±18.8	5698±2235	-4391±1497
HF Control	18.05±8.6*	89.07±15.2*	3191±1210*	-2494±764*
HF EA	15.5±7.7*	85.3±16.7*	4307±1230	-3064±826.1

Values are shown as mean ± SD. Sham Control (n=8): animals without Heart Failure (HF) e without electroacupuncture (EA). HF Control (n=9): animal with HF and without EA. HF EA (n=9): animals with HF and EA. *P <0.05 compared with Sham – Control.

Table 11
Hemodynamic Data of the Animals Awake After the EA Protocol.

Groups	**FC (bpm)**	**PAD (mmHg)**	**PAS (mmHg)**	**PAM (mmHg)**
Sham Control	364.3±45.91	86.22±8.52	124.6±6.13	103.8±5
HF Control	355.3±35.97	79.23±8.83	106.3±12.9*	91.72±9.5*
HF EA	385.8±46.35	87.15±7.74	116.7±8.26	102.9±6.9†

Values are shown as ± DP average. Sham Control (n=8): animals without Heart Failure (HF) and without electroacupuncture (EA). HF Control (n=9): animals with HF and without EA. HF EA (n=9): animals with HF and EA. *P <0.05 compared with Sham – Control. †P< 0.05 compared with the IC – Control.

Table 12
Baroreflex Response.

Groups	PA_{50} (mmHg)	Range (bpm)	Tachycardia Plateau (bpm)	Bradycardia Plateau
Sham Control	95.15±8.26	121.3±45.9	457.4±46.26	329.1±30.48
HF Control	86.62±8.45	100.8±17.81	430.3±23.05	329.5±24.08
HF EA	93.07±5.64	101.1±41.73	465.7±25.77	371.2±39.65

Values are shown as mean ±SD. Sham Control (n=6): animals without Heart Failure (HF) e without electroacupuncture (EA). HF Control (n=5): animal with HF and without EA. HF EA (n=5): animals with HF and EA. PA_{50}: variation in the pressure value (BP in mmHg) at the major slope of the curve. Amplitude: amplitude variation in FC (FC in bpm) during the reflection of the baroreceptor. Tachycardia Plateau: variation of the difference of the increase of the maximum response in FC (bpm) caused by the reduction of the BP (mmHg). Bradycardia Plateau: variation of the difference of the maximum response of the decrease of the FC (bpm) caused by the increase of the BP (mmHg).

FIGURES

Fig. 1. Device Used for the Stimulation of Animals and Control Animals.

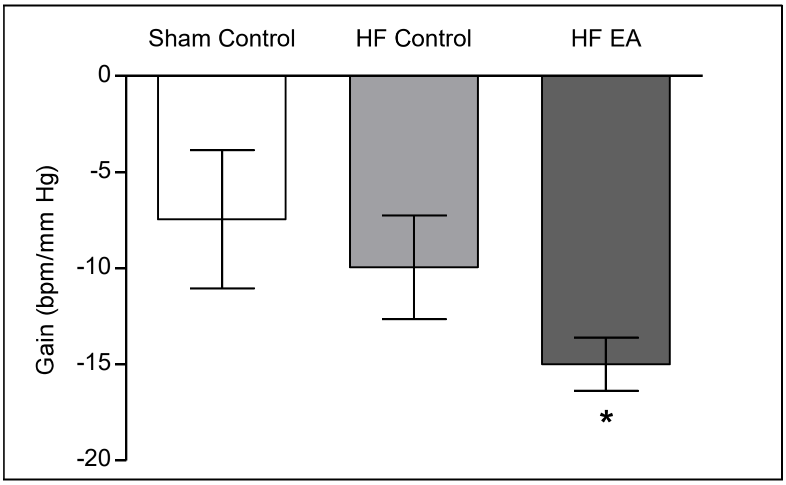

Fig. 2. Response of the Variation of the Mean Gain or Mean Baroreflex Sensitivity after the Reflex Stimulation of Baroreceptors. Values are shown as mean (± SD) of the variation of the baroreflex sensitivity (gain bpm/mmHg). Sham Control (n=6): animals without Heart Failure (HF) e without electroacupuncture (EA). HF Control (n=5): animal with HF and without EA. HF EA (n=5): animals with HF and EA. There was a statistically significant difference when comparing the HF EA group with the Sham Control group ($p < 0.05$).

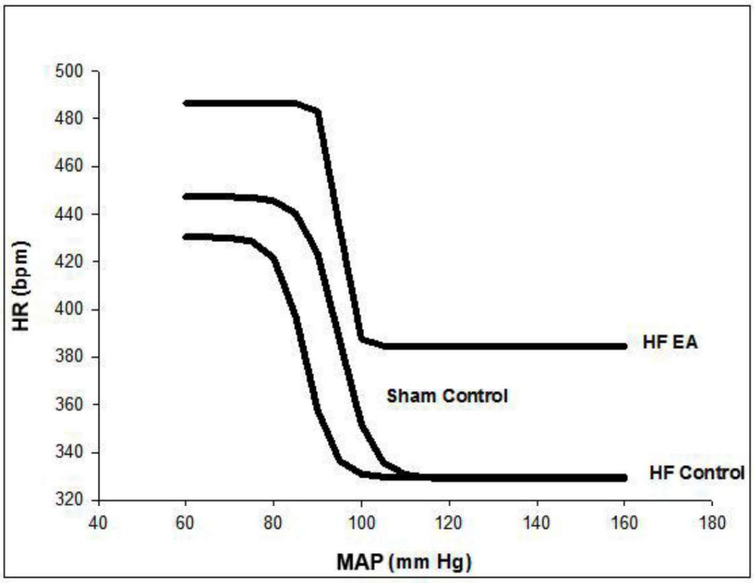

Fig. 3. The Curve of Mean Arterial Pressure Modification in Accordance with the Variation in Heart Rate. Modified curve analysis of mean arterial pressure (MAP in mmHg) according to the variation of heart rate (HR in bpm) as a baroreceptor reflex response test. There was a statistically significant difference in the values of the HF EA group in comparison with the Sham Control and the HF Control ($p < 0.0001$). The displacement of the curve to the right in the HF EA group ($n = 5$) will be observed since the same HR value generates more MAP response.

5 CONCLUSÃO GERAL

Podemos concluir com este trabalho que a eletroestimulação crônica do ponto E36 melhora a sensibilidade do barorreflexo em ratos com IC. Além disso, pudemos observar que houve uma melhora na função ventricular desses animais evidenciada pelo aumento da PAM. Mais estudos são necessários para elucidação dos mecanismos envolvidos nestes resultados bem como para que a eletroacupuntura possa se tornar uma terapêutica de uso comum no tratamento da Insuficiência Cardíaca.

Concluímos também que o modelo proposto para eletroestimular os animais durante o protocolo de acupuntura utilizando o dispositivo de garrafas pet não causa estresse aos animais, além da vantagem de não ser necessário anestesiar os animais para a colocação das agulhas e nem contê-los durante o protocolo. Desta forma, o dispositivo permite a prática da eletroacupuntura em animais o mais próximo possível da prática clínica.

ANEXO A

Normas de formatação do periódico "Journal of Visualized Experiments"

Manuscript Requirements

Required sections (Please note- a more detailed style guide is below):

- Title (maximum 150 characters)
- Authors (including affiliation(s), address and contact information for each author)
- Corresponding Author
- Keywords (6-12 words)
- Short Abstract (50 words maximum)
- Long Abstract (150-300 words)
- Introduction (3-6 paragraphs)
- Protocol (2 page minimum)
  - Protocol Content
  - Protocol Length
- Representative Results
  - Figures and Tables (1 figure or table minimum)
  - Results Text (1-3 paragraphs)
- Discussion (3-6 paragraphs)
- Acknowledgments
- Disclosures
- Table of Specific Reagents/Equipment
- References (require **at least 10**; correctly formatted)

Detailed Instructions

Your manuscript must include the following information. Failure to follow these guidelines will delay the editorial and peer review process, placement on the videographer schedule, and ultimately publication turnaround time.

Required formatting:

- *File Type:* Microsoft Word is JoVE's preferred format for text. All manuscripts must be submitted as a .doc or .docx file
- *Font:* 12 pt Arial, Calibri, or Times New Roman throughout the entire document
- *Page margins:* 1 inch on all sides
- *Line spacing:* single spaced with a space between each paragraph/bullet point

Style Guidelines:

The written submission is an integral component of your manuscript. Please take care that it is well-written using proper and formal English (American-English preferred), grammar, and syntax. Please ensure that your document has been proofread prior to submission for both content and copy. Failure to do so will delay the review of your manuscript. Please pay particular attention to the following areas:

- Avoid the use of colloquial phrases and personal pronouns
- Use consistent formatting throughout the document (e.g. consistent spacing, capitalization and punctuation)
- Avoid the use of commercial language, including any TM/R symbols or the mention of company brand names before an instrument or reagent

Technical Language

- Define acronyms upon first usage
- Use standard abbreviations for SI units: L, ml, µl *etc.*
- Abbreviate time designations: hr, min, sec, µsec *etc.*
- Italicize all Latin words and nomenclature: *et al., in vivo, in vitro, in silico, i.e., e.g., etc.*
- Abbreviate binomial nomenclature after first usage: *Caenorhabditis elegans* should be abbreviated *C. elegans*
- Include a space between the temperature and degree sign: 37 °C *etc.*
- List all centrifugation speeds in terms of centrifugal g-force: 100 x g *etc.*
- Molecular formulas should include subscripts: CO2, H2O2, O2 *etc.*

Manuscript Contents:
The following contents are **required** for all JoVE submissions. Exclusion of one or more sections will delay the review of your manuscript.

TITLE: *(maximum 150 characters)*
The title should be a specific and concise description of the technique and its application. If applicable, the title should also include the model system used or the type of study design. Please avoid the use of abbreviations.

AUTHORS:
Authorship should be assigned at the initial stages of submission and cannot be altered following editorial review. Please keep in mind that appearance in the video portion of your submission is not a requirement of authorship, but you may want to acknowledge all those who will appear in the video. Please see JoVE's Editorial Policies for more details regarding authorship.

Use the following format for **all** authors and list in the order of authorship:

Last Name, First Name Middle Initial (if appropriate)
Department
Institution
City, Country
E-mail address

Include the telephone number for the corresponding author. For multiple affiliations, denote with numerical superscripts. For equal contribution or other footnotes, indicate with an asterisk (*). Affiliations should reflect where the work was performed and the authors' current locations.

CORRESPONDING AUTHOR:
JoVE assumes that the corresponding author represents all authors during the publication process.

- The published corresponding author may differ from the one used for correspondence with the editorial office during the submission and publication process
- Authors should specify a corresponding author in their manuscript document, which will be listed on the final publication
- Please consider designating a long-term corresponding author for final publication

KEYWORDS: *(minimum 6, maximum 12)*
Keywords should highlight the most important aspects of the manuscript's content.
For example: Neuroscience, Medicine, brain, mouse, transplantation, labeling

SHORT ABSTRACT: *(10 words minimum, 50 words maximum)*
The short abstract should include a general description of the article and its applications. This description should focus around the protocol, not the results obtained by the technique.

LONG ABSTRACT: *(150 words minimum, 300 words maximum)*
The focus of the abstract should be on the technique being presented rather than the results of a specific experiment. A more detailed overview of the technique and a brief summary of its advantages, limitations, and applications are appropriate. Avoid discussing specific results acquired using the experimental approach and instead focus on the general types of results acquired.

INTRODUCTION: *(3-6 paragraphs)*
This section should include:

- A clear statement of the overall goal of this method
- The rationale behind the development and/or use of this technique
- The advantages over alternative techniques with applicable references to previous studies where the technique was used
- Description of the context of the technique in the wider body of literature
- Information that can help readers determine if the method described is appropriate for their application

PROTOCOL: (*2 page minimum, ~10 page maximum*)
The protocol text should provide a detailed description to enable the accurate replication of the presented technique (including set up, materials, actions, conditions etc.). Well-established techniques (such as Western Blotting, PCR, etc.) used within the protocol should be cited as necessary and any modification of the aforementioned procedures should be described in detail.

All methods including the use of human or animal subjects and/or tissue or field sampling must include an ethics statement ***before*** *the numbered protocol section.*

A) **Protocol Content-** The protocol should be a step-by-step methodology written as follows:

Format:

- 12 pt font, single space, 1 space between each step, 1 inch margins, no indentations
- This section must be a **numbered** list, step 1 followed by 1.1, followed by 1.1.1 etc.
- Each step should only include 1-2 actions and be no longer than 2-3 sentences, use substeps as necessary
- DO NOT underline any text in the protocol, but **bold** text is acceptable for emphasis

Grammar:

- Use complete sentences throughout the entire protocol
- Use active/ imperative tense throughout this section
  Good Example: Add 30 µl of solution A to 30 µl of solution B.
  Bad Example: Thirty µl of solution A was added to 30 µl of solution B.
- Avoid the use of pronouns throughout this section (i.e. “you”, “your”, “we” or “our”)
- Avoid the use of commercial language, including any TM/R/C symbols or the mention of company brand names before/after an instrument or reagent

Technical Specifications:

- Use subheadings for clarity if there are discrete stages of the protocol
- Please indicate any points at which the experiment can be paused and then restarted later. For these situations, indicate the choices at that point in the protocol.
- Indicate any toxic or harmful chemicals with the word ‘CAUTION’ when they are first used and include notes that describe the hazard and appropriate handling guidelines (or appropriate references to these guidelines)

B) **Protocol Length**- There is a ~10 page limit (with proper formatting) for the amount of text you may write for the protocol section.

REPRESENTATIVE RESULTS:

All claims of the effectiveness of a technique must be backed up with data, i.e. representative results. For example, if authors claim that technique X cleanly purifies nuclear envelope proteins from a cell, they must include a figure conclusively demonstrating such.

A) **Figures and Tables** *(separate figure files, title and legends within manuscript text)*

Your manuscript must include at least one data figure or table providing representative results. If any figure is adapted or republished from a previous publication, authors must appropriately cite the original article in the figure legend and receive permissions from the publisher. All figures and results tables are placed below the representative results text unless otherwise noted; please use brackets ([place figure 1 here] etc.) to indicate alternative figure placement.

Content:

- Data from successful experiments
- Data from sub-optimal experiments (useful to demonstrate the range of outcomes possible and what could occur if critical steps were not followed)
- A diagram/ schematic of the technique is recommended but is NOT sufficient representative data and must be accompanied by at least one other figure

Legends:

- Each figure or table must have an accompanying legend including a short title, followed by a short description of each panel and/or a general description
- Legends should be included as part of the manuscript and NOT included in the figure file

Format:
Figures:
- Text: Arial, 20pt font or greater
- Axis/Axis Tick Labels/Graph Lines: 20 pt font or greater, weight 3pt or greater
- All figures should be provided as individual files, DO NOT embed in the manuscript file
- Multi-section figures (with parts A, B, C, etc.) should be submitted as a single, combined file that contains all parts of the figure
- All data figures must include measurement definitions and error bars (if applicable)
- DO NOT include your figure title or figure legend as part of your figure file

Tables:
- All tables should be provided as individual excel files and submitted as figures, DO NOT embed tables in the manuscript
- There are no specific formatting requirements for tables *except* the required Table of Specific Reagents/Materials (JoVE will reformat all tables as necessary)

Technical Specifications:
- 50MB maximum size (contact your editor or submissions@jove.com for exceptions)
- Preferred figure file types: .eps, .psd, .pdf (please save any .ai files as .pdf for submission but maintain .ai files for production purposes)
- If submitting as .tif (accepted) or .jpg (not preferred), file must be at least 1440x480 pixels or 300dpi
- Preferred animated figure file types (if necessary): .mov, .mp4, .m4v (uploaded as "Animated/Video Figure")

B) **Representative Results Text** *(Paragraph form, complete sentences)*
Please add a concise, written description of a "representative" outcome following the use of this protocol, so that a viewer will have a sense of what a "good" or "bad" result looks like. This section should allude to **all of the data figures** included in the manuscript, emphasizing how the results confirm the success of the represented protocol and how to interpret the data. Include information both on likely outcomes and cases where results are ambiguous or un-interpretable.
Below is an example of a Representative Results section.

DISCUSSION: *(3-6 paragraphs)*
Please remember that JoVE articles are focused on the method and protocol. Thus, the discussion should be similarly focused. This should be written in full sentences and paragraph form. This section should discuss:
- Critical steps within the protocol
- Modifications and troubleshooting
- Limitations of the technique
- Significance of the technique with respect to existing/alternative methods
- Future applications or directions after mastering this technique

ACKNOWLEDGEMENTS:
Please list acknowledgments and all funding sources for this work here. Also consider listing any person demonstrating the protocol technique on film who does not appear in the authors list.

DISCLOSURES:
The published manuscript will include a statement at the end of the article that provides information regarding the authors' competing financial interests or other conflicts of interest. The corresponding author must ensure that all authors have been asked to disclose any and all conflicts of interest. When a conflict of interest is disclosed, either by an author or an editor, it is included in the published article. If authors have no competing financial interests, then a statement indicating no competing financial interests must be included.

If you have any concerns regarding a specific conflict of interest, please see JoVE's Editorial Policies or contact submissions@jove.com.

TABLE OF SPECIFIC MATERIALS/EQUIPMENT: *(separate spreadsheet file, DO NOT embed in manuscript)* Authors should provide a table of the essential supplies, reagents and equipment and upload as a separate excel file: Table of Materials/Equipment Template

This table should include information for viewers to obtain the materials used in the protocol such as company, web address and catalog number. It is appropriate to include the specific brand of reagent used in your experiments especially if this specificity influences the outcome of the experiment. However, it is not appropriate to mention specific brand names or company names throughout the manuscript text. Please avoid the use of any copyright or trademark symbols throughout the text, especially in the tables of specific reagents and equipment.

In the "Comment" column, please include information on multiple suppliers or if an alternative product can be used. Also in the "Comment" column, please note if any reagent is harmful or toxic. If appropriate, include the nature of the hazard and any standard precautions that should be made in the '**PROTOCOL**' text. Also highlight any custom or specialized equipment required.

JoVE will link some of the reagents and equipment to a product finder tool to connect directly to supplier sites. Please include as much information as possible so that these links are accurate (i.e. company name, company website, product code).

REFERENCES: *(10 minimum)*
Compile all references used in the text in a numbered list. List references in the order that they appear in the manuscript; each publication cited should have its own listing (multiple items listed under one reference number are not allowed). A minimum of 10, correctly-formatted references should be provided. Citations should include articles in formal, peer-reviewed journals, laboratory manuals, textbooks, and patents among others.
In-Text Formatting:
- The corresponding reference numbers should appear as superscripts after the appropriate statement(s) in the text (before punctuation but after closed parenthesis)
- Multiple references should be separated by commas, or a dash for inclusive numbers (example 2, 5 is references 2 and 5 while example 2-5 indicates references 2 through 5)

- Personal communications, unpublished data, website URLS, and conference abstracts can be cited parenthetically in the text with author last name, initials and year
- Footnotes should not be used, and grant details and personal acknowledgments should not be cited as a numbered reference (but included in the Acknowledgments section)

Citation Formatting: (in order)

- Last Name, First and Middle (if available) initials. (List ALL authors- if there are more than six authors, list the first author and then state "*et al.*")
- Include article, book, or chapter titles
  - Titles of books should be italicized, whereas article and chapter titles should not
  - Article titles should start with a capital letter and end with a period
  - The title should be exactly how it was published in the original work with no abbreviations or truncations
- Write journal names in italics and list using standard abbreviations with periods. The city or country where a journal is located can be included in parenthesis with the journal name. For books or other works, a publisher name, city and country can be included.
- Write volume numbers in bold, followed by a space, then issue number (in parentheses), a comma, then a range of page numbers (start page – last page). A single page number or publisher item identifier, [pii], can be substituted for a range of page numbers (but should not be included in the DOI field in any referencing software)
- DOI (if available), preceded by a comma and listed as "doi:" then the number.
- Year published (in parentheses), preceded by a comma. "Accepted" or "In Press" can be listed after the title or journal name and before the year.

If you use EndNote or Reference Manager software, use the JoVE EndNote style file or the JoVE Reference Manager style file, respectively. Alternatively, if you use software such as Papers or Mendeley, please use the JoVE CSL style file.

ANEXO B

Normas de formatação do periódico "Autonomic Neuroscience: Basic and Clinical"

Introduction
Formatting requirements

Article structure

Subdivision - numbered sections
Divide your article into clearly defined and numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2, ...), 1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer to 'the text'. Any subsection may be given a brief heading. Each heading should appear on its own separate line.

Introduction
State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and methods
Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described.

Experimental
Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described.

Theory/calculation
A Theory section should extend, not repeat, the background to the article already dealt with in the Introduction and lay the foundation for further work. In contrast, a Calculation section represents a practical development from a theoretical basis.

Results
Results should be clear and concise.

Discussion
This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Conclusions
The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

Appendices

If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

Essential title page information

- Title. Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.
- Author names and affiliations. Where the family name may be ambiguous (e.g., a double name), please indicate this clearly. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.
- Corresponding author. Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. Ensure that phone numbers (with country and area code) are provided in addition to the e-mail address and the complete postal address. Contact details must be kept up to date by the corresponding author.
- Present/permanent address. If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Abstract

A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself. The abstract should summarize the results obtained and the major conclusions in such a way that a reader not familiar with the particular area of work can understand the implications of the work. It should not exceed one twentieth of the length of the manuscript.

Keywords

Immediately after the abstract, provide a maximum of 6 keywords, using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

Units
Follow internationally accepted rules and conventions: use the international system of units (SI). If other units are mentioned, please give their equivalent in SI.

Footnotes
Footnotes should be used sparingly. Number them consecutively throughout the article. Many word processors build footnotes into the text, and this feature may be used. Should this not be the case, indicate the position of footnotes in the text and present the footnotes themselves separately at the end of the article. Do not include footnotes in the Reference list.
Table footnotes
Indicate each footnote in a table with a superscript lowercase letter.

Figure captions
Ensure that each illustration has a caption. A caption should comprise a brief title (not on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

Tables
Number tables consecutively in accordance with their appearance in the text. Place footnotes to tables below the table body and indicate them with superscript lowercase letters. Avoid vertical rules. Be sparing in the use of tables and ensure that the data presented in tables do not duplicate results described elsewhere in the article.

References
Citations in the text should be given in parentheses at the appropriate place by author(s) name(s) followed by the year in chronological order according to the Harvard system (Paintal, 1973; Birdsall et al., 1980). With more than two authors, name only the first followed by "et al." (Birdsall et al., 1980). When two or more papers by the same author(s) appear in one year, distinguish them by a, b, etc. after the date.

The Reference List
The reference list should be submitted in double spacing. It should be arranged in alphabetical order of the first author's name. If the first author's name appears more than once, the order is as follows: (1) single author: chronological sequence; (2) author and co-author: alphabetically according to co-author; (3) author and more than one co-author: chronological sequence (as in the text these will be referred to as "et al."). Reference must be complete including, in this order: author's name, initials, year of publication, title of article, title of the journal, volume, first and last page number of the article cited. Title abbreviations should conform to those adopted by List of Serial Title Word Abbreviations (available from International Serial data System, 20 Rue Bachaumont, 75002 Paris, France, ISBN 2-904938-02-8).

ANEXO C

Parecer da comissão de ética no uso de animais

REPÚBLICA FEDERATIVA DO BRASIL
MINISTÉRIO DA EDUCAÇÃO

UFCSPA

UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE

CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

PARECER CONSUBSTANCIADO DE PROJETO DE PESQUISA E ENSINO

1) PROTOCOLO Nº: 088/12 **Parecer 145/12**

2) DATA DO PARECER: 11/07/2012

3) TÍTULO DO PROJETO:

Influência do tratamento crônico de eletroacupuntura no sistema nervoso autônimo de ratos com insuficiência cardíaca

4) PESQUISADOR RESPONSÁVEL:

Janice Luisa L. Tartari

5) RESUMO DO PROJETO:

O projeto visa testar o efeito da eletroacupuntura sobre o controle reflexo da pressão arterial e da frequência cardíaca em uma situação de insuficiência cardíaca

6) OBJETIVOS DO PROJETO:

Avaliar o efeito da eletroacupuntura no balanço simpato-vagal

7) FINALIDADE DO PROJETO: [ ] Ensino [x] Pesquisa

8) ITENS METODOLÓGICOS E ÉTICOS DO PROJETO:

Título [x] Adequado [ ] Comentários

Introdução [x] Adequada [ ] Comentários

Objetivos [x] Adequados [ ] Comentários

Primeiro e segundo objetivos específicos abordam mesma coisa. Ambos referem-se à atividade reflexa

Relevância e Justificativa [x] Adequados [ ] Comentários

Materiais e Métodos [x] Adequados [ ] Comentários

Cronograma para execução da pesquisa [x] Adequado [ ] Comentários

Orçamento e fonte financiadora [x] Adequados [ ] Comentários

Referências Bibliográficas [x] Adequadas [ ] Comentários

9) O PROJETO ESTÁ ADEQUADO À LEGISLAÇÃO VIGENTE:
[x] Sim [ ] Não

10) INFORMAÇÕES RELATIVAS AOS ANIMAIS:

Grau de dor/estresse: **B** | **C** [ ] **D** [ ] **E** [ ]

Justifique:

Espécie: Ratos wistar **Número Amostral:** 99

Redução Amostral: [ ] Sim [x] Não

Justifique:

REPÚBLICA FEDERATIVA DO BRASIL
MINISTÉRIO DA EDUCAÇÃO

UFCSPA

UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE

Substituição de Metodologia: [ ] Sim [x] Não

Se achar necessário, justifique e sugira uma nova metodologia:

Aprimoramento da Metodologia: [ ] Sim [x] Não

Se achar necessário, justifique e sugira aprimoramentos da metodologia:

Nos aspectos apontados acima

Acomodação e manutenção dos animais: [x] Adequada [ ] Inadequada

Se achar inadequada cite abaixo as melhorias necessárias:

Manipulação dos animais: [x] Adequada [ ] Inadequada

Se achar inadequada cite abaixo as melhorias necessárias:

Analgesia dos animais (se aplicável): [ ] Adequada [ ] Inadequada

Se achar inadequada cite abaixo as melhorias necessárias com analgésico substituto:

Vide comentário acima

Anestesia dos animais (se aplicável): [ ] Adequada [ ] Inadequada

Se achar inadequada cite abaixo as melhorias necessárias com anestésico substituto:

Eutanásia dos animais (se aplicável): [ ] Adequada [x] Inadequada

Se achar inadequada cite abaixo as melhorias necessárias com metodologia substituta:

Adequar a dose à literatura disponível

Local de Realização (Biotério/Laboratório):

Outra instituição. Qual?

11) CRONOGRAMA DE UTILIZAÇÃO DE ANIMAIS

Data	Espécie	Sexo	Quantidade
Março de 2012 a Fevereiro de 2014			

12) RECOMENDAÇÃO:

[x] Aprovado

[ ] Com pendência

[ ] Não aprovado

Comentários gerais sobre o projeto:

As alterações solicitadas pelo revisor foram contempladas e o parecer é de APROVAÇÃO