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Dissertation to obtain the degree of Master in Veterinary Medicine in the course of Integrated Master in Veterinary Medicine given by Universidade Lusófona de Humanidades e Tecnologias

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Dedicatory

This work is to be dedicated primarily to my closest family, especially, my parents and also grandmother "Mami" which is not alive to witness the completion of my studies with this study. I hope they may be proud since all the effort they endured to pay and to support my studies merge into what I am and will be as a professional upon completion of my degree. A high school professor once said to me that the world does not give you opportunities but it is you who give yourself opportunities to become what you want. So with that said, I had the luck to be able to pursue my dreams thanks to my family and for that I will be eternally grateful.

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Resumo

Introdução. A acupuntura representa uma parte integral e muito importante da medicina tradicional Chinesa, sendo utilizada à mais de 2500 anos. A técnica consiste em introduzir agulhas extremamente finas em pontos específicos do corpo denominados pontos de acupunctura, induzindo múltiplas respostas biológicas que activam o sistema nervoso. Os efeitos benéficos da fármacopuntura já foram provados em estudos científicos, dentro dos quais, o efeito analgésico, é reconhecido no tratamento de dor pela World Health Organization e National Institutes of Health. O mecanismo de acção da acupunctura por detrás da analgesia permanece ainda um pouco incerto.

A medicina tradicional Chinesa representa mais um tipo de filosofia que procura equilibrar o corpo através de uma perspectiva que não é baseada em evidências anatómicas, fisiológicas ou bioquímicas. Este facto, torna a compreensão e aceitação da acupuntura muito difícil por parte dos praticantes de medicina ocidental.

Teorias e estudos científicos, dos quais alguns envolvem imagiologia cerebral, aludem ao facto do sistema nervoso estar envolvido na transmissão dos sinais criados pela acupuntura a áreas-alvo.

Pomeranz em 1987, sugeriu uma teoria onde a estimulação da acupuntura activa as fibras aferentes A-δ e C no músculo levando à transmissão do sinal à medula espinal causando a libertação local de opióides endógenos. Os nervos aferentes continuam a propagação do sinal para o mesencéfalo desencadeando a libertação para a medula espinal, de neurotransmissores como a serotonina, dopamina e noradrenalina que por sua vez levam à inibição e supressão da transmissão da dor. O sinal ao chegar ao hipotálamo e à glândula pituitária desencadeia a libertação de hormona adrenocorticotrófica (ACTH) e endorfinas. Esta teoria foi fortemente confirmada por outros estudos científicos.

A libertação de opióides endógenos em resposta à acupuntura é uma das principais teorias para a explicação do mecanismo de acção da acupuntura e é denominada pela comunidade científica como a teoria neurohormonal.

Técnicas recentes de imagiologia do sistema nervoso central permitiram avaliar padrões de expressão aquando a estimulação pela acupuntura. Hiesh *et al*., 2001 através do uso de tomografia de emissão de positron, desvendou que durante a estimulação eléctrica do acuponto *Hegu* ocorria activação do hipotálamo, periaqueducto cinzento e da insula. A

activação dessas áreas ocorria com menor intensidade com o uso de estimulação mecânica, no entanto, a estimulação em pontos falsos de acupuntura não desencadeou a activação. Outras regiões foram descobertas por Biella *et al*., 2001 que demonstrou, através de estimulação dos pontos *Zusanli* e *Chize*, a activação do cíngulo anterior esquerdo, giro frontal superior, giro frontal inferior medial direito e ainda a activação bilateral do cerebelo e insula.

Hui et al., 2000, usando ressonância magnética funcional, reforçou a hipótese de que a dor e a acupuntura possuem vias sobrepostas no sistema nervoso central. Wu *et al*., 2002 suportou também esta hipótese demonstrando que através de estimulação elétrica do acuponto *Yanglingquan* e de um falso ponto, ocorria activação das vias centrais da dor. No entanto, só a estimulação elétrica no verdadeiro acuponto *Yanglingquan* é que causou activação do hipotálamo, córtex somatosensorial primário e desactivação do segmento rostral do córtex cingular anterior, sugerindo que o sistema límbico-hipotalâmico é modelado pela electroacupuntura. Ainda que importante, estudos científicos de neuroimagem em acupuntura representam apenas meras explorações da rede de sinalização neuronal causada pela acupuntura.

Na acupuntura diferentes pontos causam diferentes efeitos terapêuticos. Zhang *et al*., 2004 usando ressonância magnética funcional examinou a activação de diferentes regiões do sistema nervoso central por diferentes acupontos, *Zusanli/Sanyinjiao* e *Yanglingquan/Chengsan.* Observações demonstraram que ambas as combinações de pontos causaram activação de áreas somatosensoriais primárias e secundárias, a insula, o cerebelo, o tálamo e o putámen. No entanto, cada combinação de pontos possuía para além das áreas comuns, activação de áreas específicas.

Na acupuntura existem vários métodos para estimular os pontos sendo que a fármacopuntura é o método mais comum de injecção utilizado em acupuntura. De acordo com a medicina tradicional Chinesa este método combina a eficácia da estimulação do acuponto com as propriedades farmacológicas das drogas aplicadas, potenciando o estímulo mecânico no acuponto e produzindo efeitos similares àqueles causados pelo uso dessas drogas em terapias convencionais. A fármacopuntura usa subdoses de drogas ou pequenas quantidades de extractos herbais medicinais. Este uso de micro doses provou-se útil em medicina veterinária, Alvarenga *et al*., 1998 injectou uma subdose de prostaglandina (0.5mg/kg) no acuponto *Bai-Hui*, induzindo luteólise em éguas tão eficazmente como a dose convencional

(5mg/kg) aplicada intramuscularmente, contribuindo ainda, para a redução de efeitos secundários associados à dose convencional.

Na medicina tradicional Chinesa o acuponto *Yin Tang* tem efeito sedativo em humanos e animais. Em 2002, Luna *et al*., demonstraram que a injecção de 0.01 mg/kg de acepromazina no acuponto *Yin Tang* reduzia em 32% a quantidade necessária de tiopental para indução de anestesia em cães, enquanto que a dose convencional de 0.1 mg/kg de acepromazina causava uma redução de 51%. Cassu *et al*., 2014 demonstrou que 0.01 mg/kg de xilazina, administrada no acuponto *Yin Tang*, produzia efeitos sedativos clinicamente relevantes com a vantagem de reduzir os efeitos secundários associados ao uso de α_2-agonistas quando comparado com um grupo tratado com a dose convencional de 0.1 mg/kg de xilazina.

O mecanismo de acção da fármacopuntura continua por esclarecer apesar desta técnica ser amplamente utilizada na prática clínica. A injecção de substratos líquidos nos acupontos causa uma alteração da configuração espacial dos tecidos. Esta alteração aliada às características do substrato, estimulam o acuponto, o que por sua vez desencadeia a activação do sistema nervoso. Chen *et al*., 2014, demonstrou que a distribuição de neurónios expressores de *c-fos* em ratos, aos diferentes substratos injectados no acuponto *Zusanli* era semelhante entre substratos embora a intensidade da expressão dependesse do tipo de substrato utilizado. A distribuição dos neurónios expressores de *c-fos* observou-se primariamente na lamina II do corno dorsal da medula espinal.

Sedação é um estado caracterizado por uma depressão central acompanhada de sonolência. O paciente normalmente não está ciente dos estímulos exteriores mas responde a estímulos dolorosos. É importante que a sedação ajude a aliviar a ansiedade e a diminuir o stress associado à manipulação e todo o processo associado à anestesia, ou seja, é uma parte essencial de um protocolo anestésico completo.

Diferentes classes de sedativos produzem uma extensa variedade de respostas comportamentais entre as espécies de animais. Fenotiazínicos são drogas eficientes na sedação de cães.

A acepromazina é o sedativo mais comumente utilizado na medicina veterinária, e sendo mais potente que outros derivados fenotiazínicos, produz sedação a partir de baixas doses. A sua administração produz algum relaxamento muscular mas não produz qualquer

tipo de efeito analgésico, sendo necessário adicionar um opióide para bloquear a resposta nociceptiva em casos de procedimentos dolorosos.

Fenotiazínicos causam diferentes níveis de efeitos anticolinérgicos, antiespasmódicos, anti-histamínicos e ainda de bloqueio α-adrenérgico. O bloqueio dos receptores α_1 causa a hipotensão normalmente associada ao uso destas drogas. O bloqueio de receptores de dopamina causa depleção de catecolaminas no centro de termorregulação do hipotálamo, originando assim a perda da termorregulação que por sua vez, pode levar à hipotermia. Os fenotiazínicos produzem sedação e tranquilização por inibição dos receptores centrais dopaminérgicos (D_2). Perifericamente, os fenotiazínicos bloqueiam a noradrenalina nos receptores α-adrenérgicos.

A administração de acepromazina causa efeitos dramáticos no sistema cardiovascular tanto em animais conscientes, como em animais anestesiados, no entanto, tem pouco efeito sobre a função pulmonar. O uso desta droga deve ser feito com precaução, especialmente quando se procura usar a acepromazina como agente de contenção em animais agitados, já que esta pode fazer os animais mais propensos a reagir a estímulos sensoriais.

Objectivo. Este estudo procura avaliar o nível de sedação e os efeitos sobre a frequência cardíaca, frequência respiratória, temperatura rectal e pressão arterial resultantes da injecção no acuponto *Yin Tang*, de 0.005 mg/kg de acepromazina e comparar os resultados com aqueles da administração intramuscular de 0.05 mg/kg de acepromazina.

Materiais e Métodos. Foram submetidos ao estudo um total de 6 cães de raça indeterminada com idades compreendidas entre 1 e 5 anos de idade e pesos entre 4 e 33 kg. 3 cães foram submetidos à técnica de fármacopuntura com injecção no acuponto *Yin Tang* de 0.005 mg/kg de acepromazina e 3 cães à técnica de injecção intramuscular de 0.05 mg/kg de acepromazina. O ponto de acupuntura *Yin Tang* encontra-se na depressão presente na linha dorsal média entre as sobrancelhas do cão.

Foram recolhidos, para todos os animais, valores basais para a frequência cardíaca, frequência respiratória, temperatura rectal, pressão arterial sistólica e pressão arterial diastólica antes de aplicar a técnica de sedação. 30 minutos após a injecção do fármaco uma

nova recolha de parâmetros foi efectuada e um score de sedação atribuído a cada animal conforme a tabela de score de sedação de Vainio *et al*., 1989; Kuusela *et al*., 2001.

Os resultados foram submetidos a análise estatística através do software IBM® SPSS® statistics versão 23. Aos dados foi aplicado o teste de independência não-paramétrico Kruskal-Wallis para testar as hipóteses. As correlações entre dados foram avaliadas através do teste não paramétrico de Spearman.

Resultados. Os scores de sedação obtidos para cada grupo estão descritos na tabela 5. As medições dos parâmetros de frequência cardíaca, frequência respiratória, temperatura rectal, pressão arterial sistólica e pressão arterial diastólica recolhidas antes da sedação e 30 minutos após a sedação estão descritas na tabela 6 para o grupo de fármacopuntura e na tabela 7 para o grupo de injecção intramuscular.

Tabela 5. Método de Sedação aplicado e respectivo Score de Sedação.

Método de Sedação	ID	Score de sedação
Injecção intramuscular (G1)	P1	14/22
	P4	1/22
	P6	7/22
Fármacopuntura no Yin Tang (GPP)	P2	2/22
	P3	4/22
	P5	1/22

ID, identificação do animal; G1, grupo de injecção intramuscular; GPP, grupo de injecção no acuponto *Yin Tang*

Tabela 6. Parâmetros medidos no grupo de fármacopuntura antes e após sedação.

Fármacopuntura GPP							
	P2 BL*	P2 AS**	P3 BL	P3 AS	P5 BL	P5 AS	
HR	100	100	124	120	165	111	bpm
RR	48	26	58	28	16	16	cpm
RT	39,4	38,8	39	38,5	39,7	38,6	ºC
mSP	216,4	147,4	185,8	148,6	139,8	129,8	mm/Hg
mDP	134,6	77,8	92	69	79,6	79,6	mm/Hg

HR, frequência cardíaca; RR, frequência respiratória; RT, temperatura rectal; mSP, média da pressão arterial sistólica; mDP, média da pressão arterial diastólica; *, basal; **, após sedação; GPP, grupo de injecção no acuponto *Yin Tang*

Tabela 7. Parâmetros medidos no grupo de injecção intramuscular antes e após sedação.

Injecção Intramuscular G1							
	P1 BL*	**P1 AS****	**P4 BL**	**P4 AS**	**P6 BL**	**P6 AS**	
HR	80	84	150	129	110	104	**bpm**
RR	28	16	24	18	40	16	**cpm**
RT	38,9	38,4	40	38,6	39,5	38,4	**ºC**
mSP	120,4	127,2	140,2	135	155,6	106	**mm/Hg**
mDP	64	71,2	83,2	75,8	72	45,8	**mm/Hg**

HR, frequência cardíaca; RR, frequência respiratória; RT, temperatura rectal; mSP, média da pressão arterial sistólica; mDP, média da pressão arterial diastólica; *, basal; **, após sedação; G1, grupo de injecção intramuscular.

Análise estatística não mostrou diferença significativa (valor $p < 0.05$) entre os scores de sedação do grupo de injecção no acuponto *Yin Tang* e o grupo de injecção intramuscular. No entanto, os resultados no grupo de fármacopuntura apresentam de uma forma generalizada um score de sedação mais baixo. A análise estatística não mostrou diferença significativa (valor $p < 0.05$) entre os parâmetros recolhidos após sedação no grupo de injecção no acuponto *Yin Tang* e no grupo de injecção intramuscular.

Discussão. A amostra presente neste estudo demonstra-se insuficiente para uma correta análise estatística dos dados. Devido a restrições impostas em prol do bem estar animal, apenas cães da rotina diária do hospital poderiam ser alvos deste estudo, tornando a amostra relativamente heterogénea. As restrições também não permitiram a presença de um grupo de controlo, no entanto, estudos anteriores por Luna *et al*., 2002; 2008; Godoi *et al*., 2013, usando técnicas semelhantes observaram que a injecção das drogas ou de solução salina num ponto falso, não produzia efeito ou apenas um efeito reduzido quando comparado com a injecção num verdadeiro ponto de acupuntura.

Manipulação do animal levaria certamente à adulteração do próximo score de sedação e como tal, o score de sedação foi medido apenas uma vez aos 30 minutos. No estudo a avaliação do score seguiu uma ordem específica, começando na avaliação do aspecto geral, seguido da postura, resistência à contenção física, posição do globo ocular, reflexo palpebral, relaxamento da mandíbula e língua e resposta ao som. A escala de score cumulativo de sedação em cães usada neste estudo foi escolhida porque permitia avaliar objetivamente características que também são usadas para monitorizar animais durante a anestesia. Permitiu

ainda, quantificar eficazmente o nível de consciência, o estado de alerta e o controlo motor dos animais. Outras escalas de sedação, como a escala de sedação Ramsay, poderiam também ser usadas no estudo com o mesmo nível de eficácia e fiabilidade, no entanto, estas apresentam um maior nível de subjetividade e apenas detetam mudanças moderadas no comportamento dos animais. A escala de sedação usada no estudo não possui critérios para avaliar o nível de agitação dos animais e isto representa um problema no uso desta escala. Os cães P2 e P3 no grupo de fármacopuntura demonstraram, antes da sedação, sinais de agitação e reatividade excessiva para com os estímulos sensoriais. A técnica de fármacopuntura, tendo em conta que nenhum animal apresentou sinais de agitação após a sedação, pode ter sido eficaz no alívio da agitação, reduzindo a reactividade a estímulos sensoriais.

Estatisticamente, não houve diferença significativa entre métodos de sedação sugerindo assim, que de um ponto de vista teórico, os métodos de sedação são similares. No entanto, a observação clínica durante o estudo denotou uma diferença entre grupos, sendo que o grupo de fármacopuntura apresenta, comparativamente ao grupo de injecção intramuscular, um score de sedação mais baixo, ou seja, uma sedação mais leve.

A variação do score de sedação no grupo de injecção intramuscular de acepromazina é grande, já que o score mínimo foi de 1 num total de 22 e o score máximo de 14 em 22. Está descrito por BSAVA Small Animal Formulary (2011) que a acepromazina não é fiável para sedação, quando usada sozinha, podendo assim explicar a variação tão ampla dos valores obtidos neste estudo.

A acepromazina, devido a ser o sedativo mais amplamente usado na medicina veterinária, foi a droga de escolha para o protocolo deste estudo. Outros sedativos mais recentes poderão ter um potencial maior na sedação de cães. Um estudo anterior por Cassu *et al.*, 2014 administrou, em cães, 0.01 mg/kg de xilazina no acuponto *Yin Tang*, induzindo um efeito sedativo clinicamente relevante em cães, reduzindo ainda os efeitos secundários associados ao uso de α_2-agonistas.

A sedação leve que ocorreu no grupo de fármacopuntura pode ser resultado de uma dose ineficaz de acepromazina. Como tal, o ajuste da dose deve ser feito em estudos posteriores de forma a determinar a dose ideal de acepromazina, necessária para atingir um nível eficaz de sedação.

Ainda que anedótico, a comparação dos resultados obtidos após sedação entre o grupo de fármacopuntura e o grupo de injecção intramuscular pode ser útil e como tal, elações clínicas podem ser feitas a partir da evidência. As variações nos parâmetros de frequência cardíaca, frequência respiratória, temperatura rectal, pressão arterial sistólica e pressão arterial diastólica medidos após sedação em ambos os grupos, demonstram de um ponto de vista clínico, uma semelhança de efeito, pois ambos causaram uma diminuição dos valores basais para limites fisiológicos mais compatíveis com um cão em repouso. Este efeito similar entre técnicas, originado pelo uso da fármacopuntura é demonstrado por Alvarenga *et al*., 1998; Silva & Luna, 1999; Nie *et al*., 2001; Luna *et al*., 2008; Cassu *et al*., 2014.

Neste estudo, a injecção de 0.005 mg/kg de acepromazina administrada no ponto de acupuntura *Yin Tang* causou, com sucesso um efeito sedativo leve nos animais, moldando o comportamento dos mesmos para um estado mais relaxado. O grupo de fármacopuntura não apresentou nenhum dos efeitos secundários descritos para a acepromazina. Tendo em conta este facto e o facto da amostra ser pequena, podemos apenas especular que este método parece ser seguro de usar na prática clínica.

A fármacopuntura combina o efeito da acupuntura com os efeitos dos fármacos. Neste estudo o fármaco usado foi a acepromazina, uma droga que causa inibição dos receptores centrais dopaminérgicos (D_2) que são responsáveis pela modulação do comportamento e por sua vez, a sedação. O ponto *Yin Tang* é indicado para sedação mas o mecanismo envolvendo essa sedação é vago. Perifericamente, os fenotiazínicos como a acepromazina bloqueiam a noradrenalina nos receptores α-adrenérgicos. Estudos experimentais por Han *et al.*, 1979; Wang, Jiang & Can, 1994; Zhu *et al*., 1997, demonstram, em ratos, níveis reduzidos de noradrenalina no cérebro após electroacupuntura. Isto demonstra que tanto a droga como o efeito causado pela acupuntura vão actuar nos receptores α-adrenérgicos. Fenotiazínicos tem grande afinidade para receptores α1-adrenérgicos, que por sua vez, facilitam a sinalização nociceptiva. Isto pode explicar o porquê da acepromazina não produzir efeito analgésico. Por outro lado a acupuntura causa a libertação de opióides endógenos, sugerindo um efeito analgésico aditivo no uso da técnica de fármacopuntura. Estudos posteriores seriam necessários para confirmar esta teoria e comparar a resposta a estímulos nocivos entre o grupo de fármacopuntura e o grupo de injecção intramuscular de acepromazina.

As alterações dos parâmetros no grupo da fármacopuntura sugerem que o método desencadeia actividade parasimpática e inibe a actividade simpática, e que estas alterações são

similares àquelas observadas no grupo de injecção intramuscular, sugerindo então, que ambos os métodos alteram o sistema nervoso autónomo.

Tendo em conta as observações, a injecção de acepromazina no ponto *Yin Tang* pode provar-se útil no dia-a-dia da prática clínica se futuros estudos científicos forem efetuados com vista em obter uma melhor compreensão sobre o método e a sua segurança. Este método mostra-se promissor já que produziu efeitos sedativos clinicamente relevantes e reduziu em 90% os custos relativos á acepromazina, o que permite uma melhor gestão dos recursos pelas clínicas e ainda permite ao animal a metabolização e excreção de uma menor quantidade de droga.

Conclusão. Este estudo apenas serve como uma análise preliminar para futuras investigações científicas na área da fármacopuntura já que todas as conclusões retiradas das observações são meras especulações resultantes de um modelo animal deficiente disponível na altura do estudo. Ainda assim, elações podem ser feitas baseadas nas evidências clínicas observadas.

Um efeito sedativo leve foi observado nos animais usando o método de injecção de acepromazina no acuponto *Yin Tang* e consequentemente ocorreram mudanças na frequência cardíaca, frequência respiratória, temperatura rectal, pressão arterial e comportamento, levando os animais a retornar a um estado de repouso e relaxamento, sugerindo que este método talvez possa ser utilizado na prática clínica para sedação de cães. No entanto, este método não deve ser utilizado quando o objetivo é a contenção de animais agressivos, já que ambas as técnicas usadas no estudo demonstraram-se ineficazes neste ponto.

Devem ser realizados futuros estudos científicos com amostras fortemente controladas para avaliar os benefícios e efeitos secundários que podem advir do uso das técnicas de fármacopuntura.

Palavras-chave: Acepromazina, Acupuntura, Fármacopuntura, Sedação em Cães, Yin Tang.

Abstract

Acupuncture is part of Chinese medicine and has been used for more than 2000 years. Pharmacopuncture is a more recent way of practicing acupuncture by converging the acupuncture effect with the drug effect. This method still needs much scientific research but studies already show that is possible to administer a small amount of drug in specific acupuncture points to obtain a similar effect than that of western medicine treatments where a conventional dose is administered. This study, tried to scratch the surface of pharmacopuncture's research by administering 1/10 of the acepromazine dose at *Yin Tang*, a acupuncture point indicated for sedation in dogs, to a group of 3 dogs (GPP) and comparing the sedation score, heart rate, respiratory rate, rectal temperature and arterial blood pressure, before and after sedation, with a group of 3 dogs (G1) that received a intramuscular injection of acepromazine at the dose of 0.05 mg/kg. The results, in a clinical perspective show that pharmacopuncture method caused a similar effect when compared to the conventional dose. Sedation by this method seems promising but further extensive scientific research must be made do bring this methods to western practitioners in clinical practice.

Keywords: Acepromazine, Acupuncture, Pharmacopuncture, Sedation in Dogs, Yin Tang.

Abbreviations & Symbols

ABP - arterial blood pressure

ACTH - adrenocorticotropic hormone

AS - after sedation

BA - broadmann area

BL - baseline

BL57 - bladder meridian point nº57

bpm - beats per minute

CNS - central nervous system

cpm - cycles per minute

CSF - cerebral spinal fluid

c-fos - proto-oncogene

DRG - dorsal root ganglion

DVC - dorsal vagal complex

EA - electroacupuncture

EMA - European Medicines Agency

FDA - Food & Drug Administration

fMRI - functional magnetic resonance imaging

GAP43 - growth-associated protein

GB34 - gallbladder meridian point nº 34

GPP - *Yin Tang* pharmacopuncture group

G1 - intramuscular injection group

GV1 - governor vessel meridian point nº 1

HR - heart rate

Hz - hertz

ID - identification

IM - intramuscular

IV - intravenous

kg - quilogram

LI4 - large Intestine meridian point nº 4

LU5 - lung meridian point nº5

MAC - minimum alveolar concentration

MDR1 - multi drug resistance gene

mg - milligram

mm/Hg - millimetres of mercury

mDP - mean diastolic pressure

mSP - mean systolic pressure

NIH - National Institutes of Health

PCO_2 - partial pressure of carbon dioxide

PET - positron emission tomography

phospho Erk 1/2 - phosphorylation of extracellular-signal-regulated kinases

PO_2 - partial pressure of oxygen

RNA - ribonucleic acid

RR - respiratory rate

RT - rectal temperature

SA - sinoatrial

SC - subcutaneous

ST36 - stomach meridian point nº 36

TCM - traditional Chinese medicine

TENS - transcutaneous electric nerve stimulation

TSH - thyroid stimulating hormone

Vd - volume of distribution

WHO - World Health Organization

ºC - Celsius degree

(n) - number

22G - gauge measure

25G - gauge measure

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1. Anaesthesia

The term anaesthesia comes from Greek an-, "without" and -aisthēsis, "sensation" meaning insensibility (Thurmon & Short, 2007). It is used to describe the reversible process of depression of the central nervous system (CNS) with drugs that produce unconsciousness and a reduced or absent response to noxious stimuli (Jones, 1999).

Anaesthesia is a wide subject including many concepts like analgesia, tranquilization/sedation, narcosis, hypnosis, local analgesia, regional analgesia, limb analgesia, general anaesthesia, balanced anaesthesia and dissociative anaesthesia amongst others.

For the purpose of this work, the focus will be on the concept of sedation.

Sedation is a state characterized by central depression accompanied by drowsiness. The patient is generally unaware of its surroundings but responsive to painful manipulation (Thurmon & Short, 2007).

Analgesia is also an important concept, meaning a state of relief, reduced or abolished perception of pain (Jones, 1999). It's important since some sedatives like acepromazine do not possess analgesic effect (Lukasik, 1999), so its use, should be complement with analgesic drugs like opioids for minor painful procedures (Lemke, 2007).

1.1. Premedication and Sedation

Premedication is an essential part of a complete anaesthetic protocol. It is important to help relieve anxiety and decrease stress associated with handling and the whole process of anaesthesia. One of the major advantages is, the increased safety of the process and of the staff during restraint and introduction of intravenous catheters. The drugs used in premedication contribute to smooth induction and recovery from anaesthesia (Lukasik, 1999).

Premedication and sedation should be used to reduce anxiety, produce mild to moderate sedation and provide analgesia if required for painful manipulations or before surgery. It must also target an increase in muscle relaxation, decrease saliva and airway secretions, reduce side effects of subsequently administered drugs, suppress vomiting and regurgitation, decrease the amount of drug needed to cause unconsciousness for induction and contribute to analgesia postoperatively (Lukasik, 1999).

The different classes of sedatives produce a wide variety of behavioural responses among the species. Phenothiazines and α2-agonists are effective sedatives in dogs and cats. Benzodiazepines are effective sedatives in ferrets, rabbits, swine and birds but are not reliable for cats and young dogs. Dose requirements vary widely among species (Lemke, 2007). It's important for sedatives to take effect before anaesthesia induction otherwise an overdose of induction and maintenance drugs may occur. Patients should be given 15 to 30 minutes in a quiet area to let the drugs take effect (Lukasik, 1999). Commonly used drugs for premedication and sedation are the phenothiazines, benzodiazepines and α2-agonists. They can also be combined with opioids to produce the added effect of analgesia (Lemke, 2007).

Since 1950's phenothiazines are used in veterinary medicine as tranquilizers, even though this class, originally was used as an antipsychotic drug for treatment of schizophrenia. (Posner & Burns, 2009).

Acepromazine is the most commonly used phenothiazine in veterinary medicine (Lukasik,1999; Posner & Burns, 2009). Other phenothiazines used on rare occasions include chlorpromazine, promazine, promethazine, trimeprazine and methotrimeprazine (Lukasik, 1999).

1.1.1. Evaluating Sedation

Sedation is a drug induced state for the patient. Patient needs, differ upon clinical circumstances and can be specific for each patient changing over time even for the same patient. All must be done to ensure patient safety and comfort, including preventing excessive and prolonged sedation. Excessive or prolonged sedation is problematic leading to increased risk of complications. To prevent such consequences, sedation must be measured accurately and that has to be done using robust tools that assess precise and accurately the state of the patient. For that tools must include a wide range of behaviours. This approach leads to a patient-directed approach increasing safety for patient and practitioners (Sessler, Grap & Ramsay, 2008).

1.1.2. Sedation Scales

In the past a subjective tool called the Ramsay Sedation scale was introduced, allowing to evaluate precisely the level of consciousness during titration of sedative medications in an ICU (Ramsay *et al*., 1974). Ever since, more and more tools have been developed, validated, and applied in clinical and research environments to

monitor level of consciousness or arousal, as well as to evaluate cognition, agitation and other parameters (Sessler, Grap & Ramsay, 2008). These include the Sedation Agitation Scale (SAS) (Riker, Picard & Fraser, 1999), the Motor Activity Assessment Scale (MAAS) (Devlin *et al*., 1999), the Richmond Agitation-Sedation Scale (RASS)(Sessler *et al*., 2002) and others.

In order for such tools to be effective, they must be accurate, reliable, easy to apply and repeatable by multiple evaluators (Sessler, Grap & Ramsay, 2008). Desirable features of a good sedation scale have been enumerated and include: rigorous multidisciplinary development, ease of administration, recall and interpretation, well defined discrete criteria for each level, sufficient sedation levels for effective drug titration, assessment of agitation and demonstration of inter-rater reliability and evidence for validity in relevant patient populations (Sessler, 2004).

Implementation of a sedation assessment tool can have a positive effect on precision of sedative administration (Costa et al., 1994; Botha & Mudholkar, 2004), with greater frequency of appropriate sedation level and lower incidence of over-sedation, reduction in sedative and analgesic drug doses, shorter duration of mechanical ventilation, and even reduced use of vasopressor drugs. Implementation of strategies that incorporate scheduled assessment for agitation, have been associated with a reduction in agitation and even fewer nosocomial infections (De Jonghe, 2005; Chanques et al., 2006). Use of a sedation scale is an integral part of most patient-focused management algorithms (Sessler, Grap & Ramsay, 2008).

1.2. Acepromazine

Acepromazine is the most widely used sedative in veterinary medicine. (Lemke, 2007)

The chemical name of acepromazine is 2-acetyl-10(3-dimethylaminopropyl)-phenothiazine (fig.1).

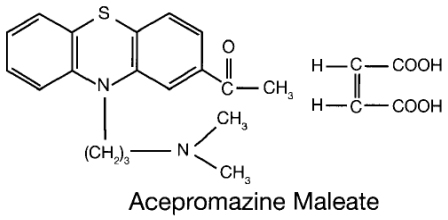


Figure 1. Chemical Structure of acepromazine (Gross, 2001)

Acepromazine is more potent than other phenothiazine derivatives and produces sedation at relatively low doses. Administration produces some muscle relaxation but has no analgesic effect (Lemke, 2007). Phenothiazine derivatives have little or no analgesic activity. Tranquilization must be supplemented with analgesics and/or general anaesthetics to block nociceptive responses during painful procedures (Posner & Burns, 2009).

1.2.1. Mechanism of action

Phenothiazines produce a wide spectrum of autonomic, endocrine and behavioural effects. The behavioural effects caused by this drugs are caused primarily by blockade of dopamine receptors in basal ganglia and in the limbic system. They inhibit conditioned avoidance behaviour and decrease spontaneous motor activity in therapeutic doses and may cause extrapyramidal effects like tremor, rigidity and catalepsy in higher doses (Lemke, 2007). Furthermore, phenothiazines have varying degrees of anticholinergic, antihistaminic, antispasmodic and α-adrenergic blocking effects (Plumb, 2008). These type of sedatives have also a great binding affinity for other types of receptors: adrenergic and muscarinic, as shown in table 1. (Lemke, 2007).

Table 1. Relative receptor-binding affinities of phenothiazines (adapted from, Lemke, 2007).

	D_1	D_2	α1	5-HT_2	M_3	H_1
Phenothiazines	+	++	+++	+++	+	+

α_1, alpha receptor; D, dopamine receptor; H, histamine receptor; 5-HT_2,5-hydroxytryptamine (serotonin) receptor; M3, muscarinic receptor; +, weak; ++, moderate; +++, strong

Phenothiazines have more affinity for α1-receptors than to dopaminergic receptors. Blockade of α1-receptors causes the hypotension effect typically associated with the use of these drugs. Dopamine receptors in the hypothalamus control the tonic inhibition of prolactin secretion, meaning that, blockade of such receptors causes an increase in prolactin secretion which is responsible for most of the endocrine effects experienced by phenothiazines. Also, the blockade of dopamine receptors in the chemoreceptor trigger zone of the medulla produces a desirable antiemetic effect, but also, an undesirable depletion of catecholamines in the thermoregulatory center of the hypothalamus leading to the loss of thermoregulation (Lemke, 2007).

Dopamine is mostly an inhibitory neurotransmitter which is responsible for the regulation of fine motor control, prolactin secretion and behavioural regulation (Lemke, 2007). Dopamine receptors are included in the family of G-protein-coupled receptors (Lemke, 2007), (Posner & Burns, 2009) and are divided in two types: dopamine 1 (D_1) receptors located post-synaptically and dopamine 2 (D_2) receptors located pre- and post-synaptically (Lemke, 2007). They were classified based on their ability to inhibit or enhance the adenylate cyclase activity (Lachowicz, 1997). Activating D_1 receptors causes an increase in adenylate cyclase activity and in intracellular levels of cyclic adenosine monophosphate (cAMP). Counterwise, activation of D_2 receptors causes a decrease in both, and can also activate other pre-synaptic signal transduction pathways, decreasing calcium conduction and post-synaptic signal transduction pathways, increasing potassium conduction. Behavioural effects are mainly mediated by the D_2 receptors family (Lemke, 2007). Phenothiazines produce sedation and tranquilization by inhibition of central dopaminergic receptors (D_2). Peripherally, phenothiazines block norepinephrine at α-adrenergic receptors. (Posner & Burns, 2009).

1.2.2. Indication

Acepromazine is approved by Food and Drug Administration (FDA) for use in dogs, cats and horses in North America and by European Medicines Agency (EMA) for cattle, sheep, goats, pigs and horses in the European Union.

Clinical uses of acepromazine are usually restricted to healthy animals. The drug is administered alone as a sedative for non-painful diagnostic procedures or in combination with an opioid for painful diagnostic and minor surgical procedures. Acepromazine is also given alone or in combination with opioids as a preanaesthetic to facilitate placement of IV catheters and to reduce the dose of injectable and inhalation anaesthetics required to induce and maintain anaesthesia. Small doses of acepromazine can also be given postoperatively, provided that patients are hemodynamically stable and that pain has been managed effectively. Acepromazine can be given SC, IM, IV, but the IM and IV routes are preferred because uptake from SC sites can be erratic in patients with altered peripheral circulation.(Lemke, 2007)

1.2.3. Pharmacokinetics and Pharmacodynamics

In dogs given acepromazine and an opioid IM, onset of sedation is observed within 15 minutes, peak effects are observed within 30 minutes, and sedation lasts for 2 to 3 hours (Cornick & Harstfield, 1992; Smith *et al*., 2001).

Acepromazine is metabolized by the liver, and unconjugated and conjugated metabolites are excreted in the urine (Dewey *et al*., 1981).

In a comparative study, IM administration of acepromazine (0.2 mg/kg) to dogs anesthetized with halothane or isoflurane decreased the MAC by 28% and 48% respectively (Webb & O'Brien, 1988).

Acepromazine administration produces dramatic effects on the cardiovascular system in both conscious and anaesthetized animals. In conscious dogs, stroke volume, cardiac output, and mean arterial pressure decrease 20% to 25% after IV administration of acepromazine (0,1 mg/kg), and mean arterial pressure is reduced for at least 2h (Coulter et al., 1981; Stepien et al., 1995). Preanaesthetic administration of acepromazine (0.1mg/kg IM) also decreases mean arterial pressure by 24% in dogs anaesthetized with isoflurane as shown in fig.2 (Bostrom *et al*., 2003).

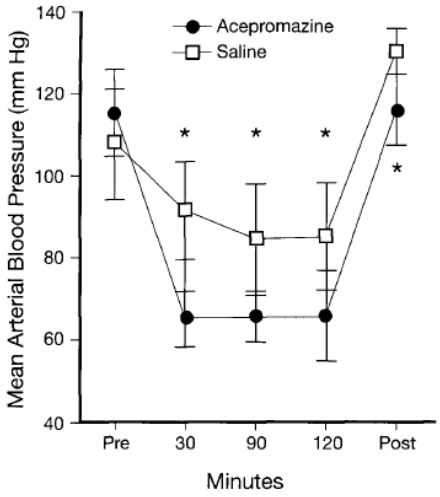


Figure 2. Effect of acepromazine (0.1 mg/kg intramuscularly) on mean arterial pressure in dogs anaesthetized with isoflurane (2%) (Bostrom et al., 2003).

Heart rate does not change considerably in conscious dogs and horses administered with acepromazine at the dose of 0.1 mg/kg through IV route (Muir & Sheehan, 1979; Coulter *et al.*, 1981). Increases in heart rate and sinus tachycardia can occur in some patients. At very high doses (1mg/kg), bradycardia and sinoatrial (SA) block can occur in dogs given

acepromazine but these arrhythmias are not usually observed at lower doses (Popovic & Mullane, 1972).

Acepromazine administration to conscious or anaesthetized animals has little effect on pulmonary function. In conscious dogs and horses, respiratory rate decreases, but arterial pH, partial pressure of carbon dioxide (PCO2), partial pressure of oxygen (PO2), and haemoglobin saturation do not change after IV administration of acepromazine (Popovic & Mullane, 1972; Muir & Sheehan, 1979).

The drug produces considerable gastrointestinal and urogenital effects. In dogs, administration of acepromazine 15 min before administration of morphine, hydromorphone, or oxymorphone lowers the incidence of vomiting from 45% to 18% (Valverde *et al*., 2004) In the other hand, administration of acepromazine alone or in combination with a opioid reduces lower oesophageal sphincter tone, delays gastric emptying, and may increase the incidence of gastric reflux (Strombeck & Harrold, 1985; Hall, Magne & Twedt, 1987; Scrivani, Bednarski & Myer, 1998). Glomerular filtration is maintained in dogs premedicated with acepromazine and anaesthetized with isoflurane (Bostrom *et al*., 2003).

Acepromazine administration can produce significant haematological adverse effects in animals. In dogs and horses, haematocrit decreases by 20 to 30% within 30 min of acepromazine administration and remains well below baseline values for at least 2h (Lang, Eglen & Henry, 1979; Ballard *et al*., 1982; Marroum *et al*., 1994). It also inhibits platelet aggregation but does not appear to alter haemostasis in normal dogs (Barr *et al*., 1992).

Many textbooks and formularies consider phenothiazines, and particularly acepromazine, contraindicated for animals with seizure histories; however, there is little scientific evidence to support this, and a recent retrospective has disputed this and has even suggested that acepromazine may have anticonvulsant properties (Tobias *et al*., 2006).

1.2.4. Dosage

Acepromazine dosage varies among the textbooks and formularies.

IM - doses for small dogs range from 0.05 to 0.2 mg/kg and those for larger dogs range from 0.04 to 0.06 mg/kg (Lemke, 2007).

IM, IV - doses for dogs range from 0.01 to 0.1 mg/kg (Posner & Burns, 2009).

IV - dose 0.01 to 0.02 mg/kg administered slowly; **IM** - 0.01 to 0.05 mg/kg (BSAVA, 2011)

1.2.5. Contraindications

Acepromazine due to its hypotensive effect is relatively contraindicated in patients with hypovolemia, shock (Plumb, 2008), trauma, cardiovascular disease (BSAVA, 2011), severe dehydration or active bleeding (Posner & Burns, 2009). Acepromazine should be avoided in animals below 3 months of age and animals with liver disease (BSAVA, 2011). Paediatric patients are very susceptible to the hypotensive effect of acepromazine (BSAVA, 1999). Phenothiazines are relatively contraindicated in patients with tetanus or strychnine intoxication due to effects on the extrapyramidal system. In dogs, acepromazine effects may be individually variable and breed dependent. Dogs with the MDR1 mutations may develop a more pronounced sedation that persists longer than normal. It could be prudent to reduce initial doses 25% to determine the reaction of a patient identified or suspect of having the mutation (Plumb, 2008).

Acepromazine should be used very cautiously as a restraining agent in aggressive dogs as it may make the animal more prone to startle and react to noises or other sensory stimulus . In geriatric patients, very low doses have been associated with prolonged effects of the drug (Plumb, 2008).

1.2.6. Adverse effects

Hypotension due to acepromazine administration is well described and an important consideration in therapy. Cardiovascular collapse secondary to bradycardia and hypotension has been described in all major species. Dogs may be more sensitive to these effects than other animals (Plumb, 2008). If profound hypotension occurs after acepromazine administration, cardiovascular function should be supported by aggressive administration of intravenous fluid. Treatment with vasopressors or catecholamines may be indicated if

cardiovascular compromise is severe. Adrenaline is contraindicated in patients overdosed with acepromazine. In the presence of α1-adrenergic blockade, adrenaline administration may lead to unopposed β2-receptor activity. This effect augments vasodilatation and hypotension may become more severe (BSAVA, 1999).

A resume of adverse effects are listed below: (BSAVA, 1999)

- Hypotension
- Hypothermia (by loss of thermoregulation)
- α1- adrenergic blockade
- Excessive vagal tone
- Bradycardia
- Organophosphate toxicity potentiation
- Haematocrit decrease

2. Acupuncture

Acupuncture is an important and integral part of traditional Chinese medicine (TCM) for more than 2500 years (Wang, Kain & White, 2008; VanderPloeg & Yi, 2009; Chang, 2012). Among acupuncture therapies, the acupuncture induced analgesia effect has been widely used to alleviate diverse pains (Zhao, 2008), this therapy was recognized as a treatment for pain by World Health Organization (WHO) in 1996 (WHO, 2003) and by the National Institutes of Health (NIH) in 1997 (NIH, 1998).

Acupuncture is a technique that makes use of hair thin needles introducing them at specific locations in the body denominated acupuncture points or acupoints. Acupuncture originates from the latin words *acus* meaning "needle" and *pungere* meaning "prick" (Vanderloeg & Yi, 2009). This technique can induce multiple biological responses through the activation of the neuronal system, and the therapeutic benefits of acupuncture treatments have already been proven (Chien *et al*., 1998; Chao *et al.*, 1999; Peng, 2002; Moazzami *et al*., 2010; Chen *et al*., 2013). Acupuncture is widely used for pain management (Wang, Kain & White, 2008) and even recognized for it by WHO and NIH. Nevertheless, the mechanism for acupuncture-induced analgesia remains uncertain (Vanderloeg & Yi, 2009).

Traditional Chinese medicine is more a philosophy which focuses on balancing the body. Its perspective is not based in anatomical, physiological, or biochemical evidence, thus lacking on the evidence to be comprehended by western medicine practitioners (Wang, Kain & White, 2008). In a research view, metaphors will continue to evade rigorous testing and offer little evidence for the understanding of true acupuncture action mechanisms (Robinson, 2009). Numerous theoretical and experimental studies including brain imaging studies allude to the nervous system being involved in transmission of acupuncture signals into target areas, in which traditional nerve-reflex theory, gate control theory, and the neurotransmitter theory are considered (Perlow, 1973; Dhond *et al*., 2008).

The traditional acupuncture mechanisms belonging to TCM are based on concepts such as meridian, acupoint, Qi, yin-yang along others (Lindley & Cummings, 2006). Cheng, (2014) suggested a possible interpretation of some concepts based on neurobiological and fascia network models.

2.1. Traditional Chinese Medicine approach

2.1.1. Meridians

Meridians are the major channels in the connective tissue fascia network in the body. Acupuncture stimulation at sites along this network tends to produce stronger response than other sites because of concentration of connective tissues and nerve endings (Cheng, 2014).

2.1.2. Acupuncture points

The traditional acupoints are the sites where acupuncture stimulation produces a stronger response than neighbouring sites due to higher concentration of connective tissues and nerve endings. These points were discovered through painstaking observation of ancient researchers and clinicians. However, the difference between a traditional acupuncture point and a non-acupuncture point lies on the intensity of response rather than structural components (Cheng, 2014).

2.1.3. Qi

Qi refers to the signals within the channels that mediate the effects of acupuncture. Although the nerve signal is the best understood one, other possible signals are the propagation of mechanical force (Langevin & Yandow, 2002; Wang *et al*., 2007) and the movement of paracrine-signalling molecules (Bai *et al*., 2011).

2.1.4. Yin-Yang

It refers to the maintenance of homeostasis true the balance between sympathetic and parasympathetic branches from the autonomic nervous system (Cheng, 2014).

2.2. Scientific research

2.2.1. Neurophysiology approach

In the interest of closing the gap between TCM and western medicine for a better understanding of how acupuncture works, a review of scientific literature focused on clinical research, theories and clinical evidences will be presented.

It is important to state that there is no unified theory of acupuncture mechanism, but rather multiple models and theories (Cheng, 2014)

Early western theories were mainly dependent upon acupuncture ability to induce neurologic signals along afferent nerves that in turn modulated spinal cord signal transmission of pain (Wang, Kain & White, 2008; VanderPloeg & Yi, 2009).

In 1987, Pomeranz suggested a theory on acupuncture signalling based on his findings in 1976, that naloxone administration is able to block analgesic effect of acupuncture in a mouse. He suggested that acupuncture needle stimulation activates A-δ and C afferent fibers in muscle which cause signal transmission to the spinal cord, resulting in local release of endogenous opioids, dynorphin and enkephalin. Afferent pathways further propagate signalling to the midbrain, triggering a sequence of excitatory and inhibitory mediators that cause release of neurotransmitters, such as serotonin, dopamine, and norepinephrine onto the spinal cord leading to pre and postsynaptic inhibition and suppression of pain transmission. When this signal reaches the hypothalamus and pituitary, it triggers the release of adrenocorticotropic hormone (ACTH) and endorphins (Pomeranz & Chiu, 1976). This theory was strongly confirmed by several studies (Research Group of Acupuncture Anaesthesia, 1973,1974; Pomeranz & Chiu 1976; Lim *et al*., 1977; Clement-Jones *et al*., 1980; Cheng & Pomeranz, 1981; Han & Terenius, 1982; Wu, 1995; Horrigan, 1996).

One of the main obstacles in the experimental study of the mechanism of acupuncture anaesthesia is the difficulty in developing suitable animal models (Research Group of Acupuncture Anaesthesia, 1974).

In 1973, Han *et al*. applied acupuncture stimulation to a rabbit for 30 minutes to achieve an analgesic effect, then the cerebrospinal fluid (CSF) was removed and infused into the lateral ventricle of an acupuncture-naive recipient rabbit. This resulted in an increased pain threshold in the recipient rabbit. No increase was noted when saline or CSF from a nonacupuncture control rabbit was infused into a acupuncture-naive recipient rabbit, concluding that acupuncture induced analgesia was associated with the release of neuromodulatory substances in the CSF (Research Group of Acupuncture Anaesthesia, 1974). In the same year, Clement-Jones *et al.* demonstrated that 10 patients with chronic pain who received electroacupuncture (EA), increased CSF levels for beta-endorphin significantly ($p<0.02$) when compared to controls (Clement-Jones *et al.*, 1980). Similarly, Sjolund and Eriksson as well as Mayer, also showed increased levels of endorphins in CSF after EA stimulation and the reversal of acupuncture analgesia by naloxone (Sjolund & Eriksson, 1977; Mayer, 1977).

Several subsequent studies supported the theory that acupuncture triggers the release of endorphins and other endogenous opioids within the central nervous system (CNS) which seems to be responsible for the analgesic properties of acupuncture (Peets & Pomeranz, 1978; Lee & Beitz, 1993; Han, 2003)

Guo *et al.* in 1996 displayed that EA at low (2Hz) and high frequency (100Hz) caused an increased *c-fos* expression in the arcuate nucleus of the rat. Low-frequency EA resulted in a much higher *c-fos* expression when compared to high-frequency EA stimulation, and also when compared to dry-needling in a control group. In situ hybridization studies showed that low-frequency stimulation increased expression of messenger RNA (mRNA) for enkephalin precursor protein, whereas, high-frequency stimulation caused a increased expression of mRNA for dynorphin precursor protein (Guo *et al*., 1996 a,b). Even though it seems that differential effects on *c-fos* expression occur by low and high-frequency EA stimulation, *c-fos* expression can also be caused by non specific stimulations. Furthermore, mRNA levels may not represent actual peptide levels (Wang, Kain & White, 2008).

The release of endogenous opioids in response to acupuncture is one of the leading theories behind acupuncture's mechanism of action, and is denominated by the scientific community as the "Neurohormonal Theory"(Wang, Kain & White, 2008).

Pan *et al*. observed if there was an overlap of central pathways in rats by comparing noxious stimulation and electroacupuncture stimulation. They found that both induced a *c-fos* expression in the anterior lobe of the pituitary gland, arcuate nucleus and in nearby hypothalamic nuclei. A similar *c-fos* expression in the anterior lobe of the pituitary gland, was shown in immobilization stress in awake rats. Although they all seem to have similar pituitary gland activation, noxious simulation and EA involved different hypothalamic nuclei (Pan, Castro-Lopes & Coimbra, 1996). In a follow up study, researchers found that *fos*-immunoreactive cells activated by noxious stimulation and EA, co-localized with adrenocorticotropic hormone (ACTH) or thyroid-stimulating hormone (TSH) and that noxious stimulation and EA were associated with a similar increase in plasma ACTH and β-endorphin. EA stimulation showed distinct increase *c-fos* expression at the hypothalamic level in the mediobasal-nuclei and in the paraventricular nucleus. This researchers also confirmed that a intact nociceptive primary afferent input is needed to transmit signal from both, noxious stimulation and EA. They found that there was no activation of the hypothalamic-pituitary-adrenocortical axis or increased plasma ACTH in rats, when afferent

input was eliminated by sensory deafferentation. Thus, it seems to be an overlap in pain and acupuncture central pathways (Pan, Castro-Lopes & Coimbra, 1997).

In 2010, Goldman *et al*. showed that stimulation at ST36, *Zusanli* acupoint in mice induces purinergic receptor activation, which, in turn, inhibits pain transmission to the CNS (Goldman *et al*., 2010). Purinergic receptor activation in the sciatic nerve increases the synthesis of axonal growth-associated protein (GAP-43) in dorsal root ganglion (DRG) sensory neurons (Arthur, Akassoglou & Insel, 2005). GAP-43 is the neural-specific protein known to play a role in neuronal development and activity-dependent synaptic plasticity (Aigner et al., 1995; Benowitz & Routtenberg, 1997). A recent study by Kim *et al*., 2012 investigating the effects of acupuncture stimulation on nervous system activation in mice and rats, showed that acupuncture stimulation at ST36, *Zusanli* acupoint generates increased neuronal response in terms of increased expression of GAP-43 and phospho Erk 1/2 activation in DRG sensory neurons and induction in *c-fos* expression in neurons of the dorsal vagal compex (DVC) area. Sham acupuncture also causes a certain level of neuronal response, although the extent of the responsiveness is weaker than that of acupuncture stimulation.

2.2.2.Central Nervous System Imaging

Researchers, in an attempt to try to unfold the more complex aspects of the acupuncture's effect, have focused in expression patterns in the CNS in order to examine regions of the brain that are directly influenced by acupuncture stimulation (VanderPloeg & Yi, 2009).

Nowadays technology advancement as allowed researchers to examine those patterns through positron emission tomography (PET) and functional magnetic resonance imaging (*f*MRI) (Wang, Kain & White, 2008).

2.2.2.1.PET

Using this technology, Hiesh *et al*., in 2001 studied the central activation caused by acupuncture stimulation at LI4, *Hegu* acupoint, as well as stimulation by a non-classical needling at a non-acupoint (sham acupuncture). The study consisted in PET imaging in 4 groups, one with needle insertion up to 1 cm at LI4 with EA stimulation at 4Hz, a second had same EA stimulation at a sham-acupoint; a third had superficial insertion at LI4 with minimal mechanic stimulation and a fourth had superficial insertion of the needle at a sham-acupoint.

In the study they found that only the true acupuncture with the *De-Qi* sensation (EA 4Hz) at LI4, significantly activated the hypothalamus, the periaqueductal gray and the insula. Minimal stimulation at LI4 also activated both regions but in a lesser way. No activation was show in both sham-acupuncture stimulations (Hiesh *et al*., 2001). "De Qi" sensation is frequently described by patients as soreness, numbness, ache, fullness, or warm sensation that is achieved during manipulation of acupuncture needles (Liu & Akira, 1994; Pomeranz, 1998).

Evidence of other regions involved in the acupuncture mechanism were discovered by Biella *et al*. also in 2001, where they sequentially applied acupuncture and sham-acupuncture at bilateral ST36, *Zusanli* and LU5, *Chize* acupoints during a pet scanning sequence. They observed that acupuncture but not sham, activated the left anterior cingulum, superior frontal gyrus, bilateral cerebellum and insula as well as the right medial and inferior frontal gyri (Biella et al., 2001).

In 2005, Pariente *et al*., suggested that belief and anticipation could affect the therapeutic outcome in humans. Using PET imaging, they reported that true and sham acupuncture activated the right dorsolateral prefrontal cortex, anterior cingulated cortex and the midbrain. They also found that only true acupuncture caused a greater activation in the insula ipsilateral.

2.2.2.2. *f*MRI

Wu *et al*. established that traditional acupuncture stimulation caused activation in the hypothalamus and nucleus accumbens but on the other hand caused deactivation in the rostral part of the anterior cingulated cortex, the amygdale formation, and the hippocampus complex. Superficial pricking caused activation at the primary somatosensory cortex, the thalamus and the anterior cingulated cortex (Wu *et al*., 1999).

Hui *et al*. reported that acupuncture stimulation associated with *De Qi* sensation deactivated the nucleus accumbens, hypothalamus, amygdale, hippocampus, para hippocampus, ventral tegmental area, anterior cingular gyrus, caudate, putamen, temporal lobe, and insula (Hui *et al*., 1999). In a follow up study, they found that subjects experiencing *De Qi* sensation deactivated the frontal pole, ventromedial prefrontral cortex, cingulated cortex, hypothalamus, reticular formation, and the cerebellar vermis. Furthermore, subjects who experienced pain activated the anterior cingular gyrus, caudate, putamen, and the anterior thalamus. Subjects that experienced both, pain and *De Qi* sensation had a mix of CNS

responses with predominant activation of frontal pole, anterior, middle, and posterior cingulate (Hui *et al*., 2000) reinforcing the hypothesis that pain and acupuncture have overlapping central pathways. Other studies support this hypothesis. Wu *et al*.,2002 reported that both true and sham EA at the GB34, *Yanglingquan* acupoint activated pain central pathways on *f*MRI, but that only true EA activated hypothalamus, the primary somatosensory cortex, and deactivated the rostral segment of the anterior cingulate cortex which led to their insight that the hypothalamus-limbic system was modulated by EA (Wu *et al.*,2002). Zhan *et al*., (2003) showed that true EA stimulation and not sham, can modify signals generated by experimental cold pain stimulation. Only patients who received true EA reported a decrease in pain ($p<0.01$) displaying a acupuncture-induced increase activity in the bilateral somatosensory areas and medial prefrontal cortices and Brodmann area (BA32) and also, a decrease in the contralateral primary somatosensory areas BA7 and BA24 (anterior cingulated gyrus)(Zhang *et al*., 2003). Furthermore, in another study, Zang *et al*., showed that low frequency (2Hz) and high frequency (100Hz) EA stimulation appear to be mediated by different brain networks.

Napadow *et al.*, 2005 study used *f*MRI to compare manual acupuncture with EA at 2 and 100 Hz and tactile control stimulation at ST36 in a group of volunteers. They reported that EA produced more widespread *f*MRI signal changes than manual acupuncture, and the latter, more than simple tactile stimulation. EA produced a considerable signal increase in the anterior middle cingulate cortex, but only, low frequency EA (2Hz) activated the raphe area (Napadow *et al*., 2005).

Essentially, neuroimaging studies in acupuncture are mere explorations of acupuncture signal networking (Wang, Kain & White, 2008).

2.3. Acupuncture Point Specificity

TCM teaches that different acupoints produce different reactions and with the common CNS patterns in the imaging studies involving acupuncture stimulation, a question is born. Do different points produce different results?

Zhang et al., (2004) using *f*MRI, examined the different activation of CNS regions by different TCM acupoints, ST36 *Zusanli/* SP6 *Sanyinjao* acupoints or the GB34 *Yanglingquan/*BL57 *Chengsan* acupoint. They observed that both acupoints caused activation of primary and secondary somatosensory areas, the insula, cerebellum, thalamus, and the

putamen. Nevertheless, each acupoint had a specific pattern of activation in addition to the common one. *Zusanli/Sanyinjao* acupoint activated the orbital frontal cortex along with deactivation of the amygdala, while *Yanglingquan/Chengsan* acupoint activated the dorsal thalamus with deactivation of the primary motor area and pre motor cortex (Zhang *et al*., 2004).

2.4. Acupuncture Point Stimulation Methods

There are several ways to stimulate an acupoint according to TCM and each method has particular characteristics and indications. Some methods are dry-needle, hemoacupuncture, aquapuncture/pharmacopuncture, pneumoacupuncture, moxibuston, electroacupuncture and implants (Xie's & Preast, 2007). For this work the focus will be in the pharmacopuncture method.

2.4.1. Pharmacopuncture

It is the most common method of injection in acupuncture. It is the injection of fluids and soluble products into acupoints. Sterile saline (aquapuncture), vitamin B12, homeopathic remedies, patient's own blood, and local anaesthetics are the most commonly used in western acupuncture practice. A variation using herbal medicines is used in China (Xie's & Preast, 2007).

According to Chinese authors who follow TCM, this technique combines the efficacy of acupoint stimulation with the pharmacological effect of drugs, enhancing the mechanical stimulus at acupoints and producing similar effects to those, with conventional drugs (Zhang, Wu & Jiang, 2005; Zhu & Chen, 2005; Jin, Xu & Zheng, 2006) Pharmacopuncture uses subclinical doses of drugs or small amounts of extracts of medicinal herbs (Kim & Kang, 2010). Pharmacopuncture's use of micro doses of drugs has proven useful in veterinary practice. A subclinical dose of prostanglandine 0.5 mg/kg (1/10 of the conventional dose) injected at the *Bai-Hui* acupoint induced luteolysis in mares as effectively as the conventional dose (5 mg/kg) injected intramuscularly (Alvarenga *et al*., 1998) while significantly decreasing the side effects associated with conventional dose (Nie *et al*., 2001). However, in a follow up study, glucose or distilled water had no effect, suggesting a specific drug effect on the acupoint (Luna et al., 1999). Using the same micro dose approach, Silva & Luna, (1999) obtained the same weight gain in calves born from cows treated with 1/10 of the conventional

dose of growth hormone (somatotrophin) when compared to a control group treated with the conventional dose (500 mg) injected intramuscularly (Silva & Luna, 1999).

In TCM, the acupoint *Yin Tang*, has a sedative effect in humans and animals (Shoen, 2001; Ovechkin *et al*., 2003; Dos Santos *et al*., 2005). In 2002, Luna *et al*., study in dogs showed that even though not statistically significant the injection of 0.01 mg/kg of acepromazine (1/10 of the conventional dose) reduced in 32% the dose of thiopenthone necessary for induction of anaesthesia when compared to the 51% reduction in animals treated with the conventional dose of acepromazine (Luna *et al*., 2002).

The *Ho-Hai* acupoint, also named *Chang Qiang* (or GV1) has too a sedative indication, specially for horses in alternative to the *Yin Tang* acupoint. Luna *et al*, (2008) study in horses, demonstrated that injection of 1/10 of the conventional dose of acepromazine (0.01 mg/kg) in GV1 caused sedation effect at 30 min. with long lasting effect (60 min.) when compared with control groups. The conventional dose (0.1 mg/kg) given intramuscularly caused a greater sedation but also a decrease in respiratory rate which supports the fact that pharmacopuncture as the advantage to reduce undesirable side effects while producing similar desirable effects (Luna *et al*., 2008).

Cassu *et al*., (2014) studied the sedative and clinical effects of pharmacopuncture with xylazine in dogs. Pharmacopuncture with 1/10 of the conventional dose of xylazine (0.01 mg/kg) produced a clinically relevant sedative effect in dogs with the advantage of reducing undesirable side effects associated with α2-agonists, including bradycardia, arrythmias, and emesis shown in the group treated with the conventional dose (Cassu *et al*., 2013).

Santos Godoi *et al*., (2014) study in horses, compared the administration of 1/10 of the conventional dose of acepromazine (0.01 mg/kg) at GV1 with acepromazine (0.1 mg/kg) given intramuscularly to reduce stress-induced responses during transport. Pharmacopuncture at GV1 reduced the stress induced response in the heart rate of horses, suggesting a possible autonomic effect. However, it was not able to change other variables, such as transport induced increases in cortisol, body temperature, and respiratory rate. On the other hand, acepromazine given intramuscularly produced significant sedation and reduced the stress-induced increase in respiratory rate during transportation without reducing the stress-induced increase in cortisol.

2.4.1.1. Mechanism

Pharmacopuncture mechanism is still very unclear even though this technique has been widely used in clinical practice. Injection of the liquid substrates in the acupoints causes local spatial configuration changes. Both, spatial configuration changes and the liquid substrate characteristics stimulate the acupoint and activate the neuronal system (Chen *et al*., 2014).

Chen *et al*., (2014) studied the number and distribution of neurons expressing *c-fos* protein following the changes in spatial configuration caused by liquid substrate stimulation in rats at the acupoint ST36 (*Zusanli*). These researchers used dry-needle and different liquid substrates like, bee venom, normal saline, vitamin B1 and vitamin B12. The different liquid substrates injected and the dry-needle insertion into ST36 induced *c-fos* expression in the L3, L4 and L5 segments of the spinal cord. The group injected with bee-venom had more *c-fos*-positive neurons in the dorsal horn than the other groups. All groups showed more c-*fos* expression than the sham control group (dry needle in sham location). The study showed that the distribution of *c-fos*-expressing neurons following stimulation by dry needle or the liquid substrates was similar despite the difference in intensity and that it was primarily observed in lamina II of the dorsal horn of the spinal cord (Chen *et al*., 2014).

3. Objective

The study objective is to evaluate the level of sedation and the effects caused on parameters like heart rate (HR), respiratory rate (RR), rectal temperature (RT) and arterial blood pressure (ABP) resultant from administration of 1/10 of the acepromazine dose 0,05 mg/kg (0,005 mg/kg) in the *Yin Tang* acupoint and compare it to the administration of acepromazine intramuscular dose of 0,05 mg/kg in dogs.

4. Hypothesis

Null Hypothesis **H0 -** Sedative effect is equal between the injection of 0,005 mg/kg of acepromazine at the *Yin Tang* acupoint and the intramuscular injection of 0,05 mg/kg of acepromazine.

Alternative Hypothesis **H1 -** Sedative effect is different between the injection of 0,005 mg/kg of acepromazine at the *Yin Tang* acupoint and the intramuscular injection of 0,05 mg/kg of acepromazine.

5. Materials and Methods

The study protocol was approved by the Animal Welfare Committee from Faculdade de Medicina Veterinária belonging to Universidade Lusófona de Humanidades e Tecnologias and all experiments were conducted in the University Veterinary Hospital.

5.1. Animals

In this study, six (6) dogs were used with ages between one (1) and five (5) years old. The dogs were mixed breed, had a body condition of 5 in the scale of 9 from the body condition score chart from WSAVA(2013) with weights ranging from 4 to 33 kg. In the study sample, 4 dogs were female and 2 dogs were male. All dogs were evaluated before the study in a pre anaesthetic exam making them suitable for surgical procedure: orquiectomy or ovariohisterectomy, respectively. All animals had a signed consent by the owner. Detailed information for each dog can be seen in table 2. No dog had history or symptoms of cardiac or neurologic disease and all were in an normal state of hydration. No dog had alterations on haematological and biochemical parameters. Dogs preformed a 12 hours fasting for solid food and a 2 hours for liquids. Dogs (n=6) were divided in 2 groups, one group (G1) (n=3)

receiving a 0.05 mg/kg intramuscular acepromazine injection at the *longissimus dorsi* muscle (lombar region) and the other group (GPP) (n=3) receiving a 0.005 mg/kg acepromazine injection in the *Yin Tang* acupoint. Dogs were assigned to a group randomly without knowledge of the evaluator by the veterinarian who applied the injection.

Table 2. General information of all dogs included in the study.

Study ID	Age	Breed	Sex	Weight	BCS**
P1	2 y.	mbd*	male	5.1kg	5/9
P2	1y.	mbd	female	32.1kg	5/9
P3	1.5 y.	mbd	female	5.4kg	5/9
P4	2y.	mbd	female	4.0kg	5/9
P5	2y.	mbd	female	4.6kg	5/9
P6	5y.	mbd	male	29.1kg	5/9

y., years; *mbd - Mixed breed dog; ** BCS - Body Condition Score.

5.2. Data Collection

For this study, each dog was submitted to a pre-assessment which would determine the basal line for specific parameters like heart rate in beats per minute (bpm), respiratory rate in cycles per minute (cpm), rectal temperature in celsius degrees (ºC) and arterial blood pressure in millimetres of mercury (mm/Hg). Heart rate and respiratory rate were both measured using a 3M ™ Littmann® pediatric or classic II stethoscope depending on the weight of the animal. Temperature was measured using a Hartmann Thermoval® rapid flex thermometer. HR, RR and RT measurements were repeated twice to minimize error. Arterial blood pressure was measured with a Hifarmax™ VET HDO® (high definition oscillometry). Five (5) measurements were made from the median artery in the right forelimb. A basal result was calculated from the median of the data collected.

Sedation score was established using a cumulative score chart (table 3) from Vainio e*t al.*, 1989; Kuusela *et al*. 2001. Score minimum value is 0 and maximum value is 22 being the dog sedation score calculated from the sum from each assessment.

Table 3. Evaluation of the level of sedation in terms of a cumulative score in dogs
(adapted from Vainio *et al*., 1989; Kuusela *et al*. 2001).

Evaluation of the level of sedation in terms of a cumulative score in dogs	
Assessment	**Score and definition**
Posture	**0** - Standing position, normal proprioception (animal walks without ataxia) **1** - Animal remains in sternal or lateral position but is able to stand when verbally stimulated **2** - Remains in sternal recumbency **3** - Lateral recumbency, eventually move or lifts its head **4** - Lateral recumbency, if not verbally stimulated does not move or lifts is head.
Eyelid reflex	**0** - Strong lateral and medial eyelid reflexes* **1** - Lateral and medial eyelid reflexes presented but reduced **2** - Lateral eyelid reflex absent and medial eyelid reflex present **3** - Lateral and medial eyelid reflexes absent
Eye globe position	**0** - Eye centrally positioned **1** - Partial rotation of the eye globe **2** - Full rotation of the eye globe
Relaxation of the tongue and mandible	**0** - Normal tone of the mandible and tongue **1** - Reduced tone of the mandible and tongue, allowing opening of the mandible with little difficulty; tongue can be exposed with some difficulty and is readily retracted after being released **2** - Reduced tone of the mandible, tongue can be easily exposed but is readily retracted after being released. **3** - Mouth can be easily opened with jaw tone markedly reduced, tongue can be easily exposed, but animal retracts the tongue a few seconds after being released **4** - Mandible and tongue fully relaxed, tongue can be exposed and its is not retracted after being released
Response to sound (clapping)	**0** - Alert attitude, readily reacts (looks, lifts head) to the stimulus **1** - Reduced reaction (discrete movement, lifting of the head), however the animal appears sedated **2** - No reaction or movement
Resistance to physical restraint in lateral recumbency	**0** - Animal resists; readily returns to standing position or sternal recumbency after being released **1** - Offers little resistance, but readily returns to standing position or sternal recumbency after being released **2** - Does not offer resistance, but eventually moves or lifts its head and returns to sternal recumbency **3** - Remains in lateral recumbency, does not offer resistance
General appearance	**0** - Alert, normal consciousness **1** - Animal lightly sedated, promptly reacts or moves in response to environmental stimulation **2** - Animal moderately sedated, eventually reacts to environmental stimulation **3** - Animal appears to be moderately to deeply sedated, reduced reaction to environmental stimulation **4** - Animal appears deeply sedated, does not react to environmental stimulation

Sedation Score was established 30 minutes after injection due to the peak effect of acepromazine. At this time, all others parameters were also collected for comparison with the basal line, respecting the same collecting methods for each parameter.

All parameters and sedation score were taken by the same evaluator at both, t<0 and t=30min. with no knowledge of the applied method. There was no sharing of data between the evaluator and the veterinarian who applied the injections until the end of the study.

5.3.Timeline

Timeline can be seen in fig.3. Dogs were examined to set the parameters basal line right before the injection. The injection of acepromazine was given intramuscularly at the *longissimus dorsi* muscle or in the *Yin Tang* acupoint at t=0, at respective doses. It was given a waiting period of 30 minutes to let the drug take effect. At t=30min. score of sedation was taken using the chart provided in fig.2, after which, the remaining parameters were taken to compare with the basal line.

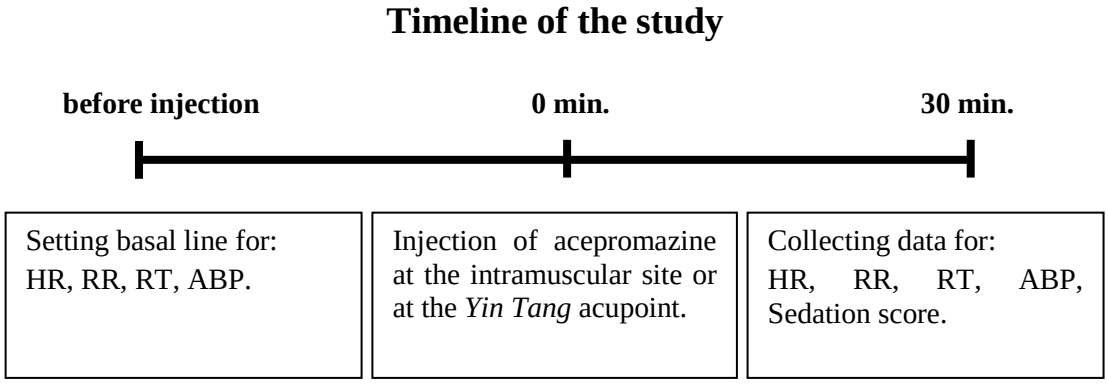


Figure 3. Timeline of the study. HR, heart rate; RR, respiratory rate; RT, rectal temperature; ABP, arterial blood pressure.

5.4. Intramuscular Injection

The injection was given in the *longissimus dorsi* muscle at the lombar region. The dose of acepromazine was 0.05 mg/kg. The solution was taken from the available Calmivet™ Injectable solution with 5mg/ml of acepromazine maleate. Injection was made with a 22G hypodermic needle by a licensed veterinarian. No reactions to the injection were reported.

5.5. Pharmacopuncture Injection

The injection of 0.005 mg/kg of acepromazine in the *Yin Tang* acupoint was made by a veterinarian certified by the IVAS (International Veterinary Acupuncture Society) for the practice of acupuncture. This was the same veterinarian for both groups. The injection was made with a 25G hypodermic needle. Preparation of the dilution from the available Calmivet™ Injectable solution with 5mg/ml of acepromazine maleate was made before application in the *Yin Tang* acupoint. The dilution was made with sterile solution of sodium chloride at 0,9% concentration. No reactions to the injection were reported.

Yin Tang acupoint is located in a depression on the dorsal midline between the eyebrows on the dog (fig.4) (Cassu *et al*., 2014).

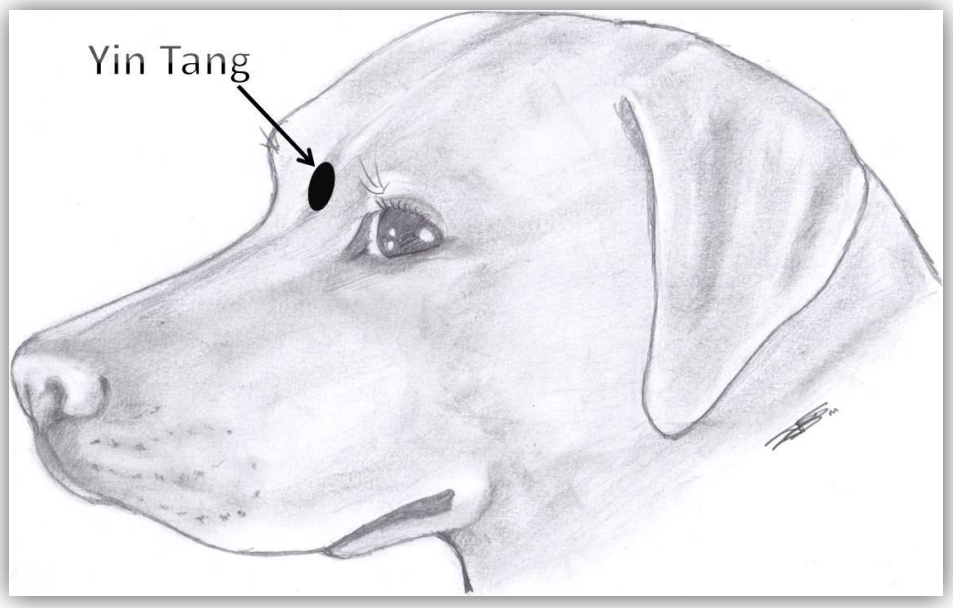


Figure 4. Location of the *Yin Tang* acupoint in dogs. Drawing by Tomás Pinto de Sousa, 2015.

5.6. Data analysis

Statistical analysis was made using the IBM® SPSS® statistics version 23 software. The data analysis was made using a non-parametrical Kruskal-Wallis independence test to test the hypothesis. Application of the non-parametrical Spearman test was also used to check correlation between the data. Tables and figures were created using Microsoft Office Excel 2007® and Microsoft Office Power Point 2007®.

6. Results

In the end of the study the sedation method was revealed to the evaluator for each animal as shown in table 4.

Table 4. General information for all dogs included in the study.

Study ID	Age	Breed	Sex	Weight	BCS**	Sedation method
P1	2 y.	mbd*	male	5.1kg	5/9	IM(1)
P2	1y.	mbd*	female	32.1kg	5/9	PP(2)
P3	1.5 y.	mbd*	female	5.4kg	5/9	PP(2)
P4	2y.	mbd*	female	4.0kg	5/9	IM(1)
P5	2y.	mbd*	female	4.6kg	5/9	PP(2)
P6	5y.	mbd*	male	29.1kg	5/9	IM(1)

y., years; *mbd - Mixed breed dog; ** BCS - Body Condition Score; (1), intramuscular injection group; (2), pharmacopuncture group.

Sedation score results are shown for each animal in its respective group in table 5. Baseline parameters and its after sedation results are shown for the pharmacopuncture injection at *Yin Tang* acupoint group in table 6. and for the intramuscular injection group at table 7, respectively. Study ID's order in each group is random because animals were only assigned to each group after all data had been collected from the entire population sample.

Table 5. Individual animal sedation score and respective applied sedation method.

Sedation method	Study ID	Sedation score*
Intramuscular injection (G1)	P1	14/22
	P4	1/22
	P6	7/22
Pharmacopuncture at Yin Tang (GPP)	P2	2/22
	P3	4/22
	P5	1/22

ID, animal identification; G1, intramuscular injection group; GPP, *Yin Tang* acupoint injection group

Table 6. Parameters collected in the pharmacopuncture group.

Pharmacopuncture GPP							
	P2 BL*	P2 AS**	P3 BL	P3 AS	P5 BL	P5 AS	
HR	100	100	124	120	165	111	bpm
RR	48	26	58	28	16	16	cpm
RT	39,4	38,8	39	38,5	39,7	38,6	ºC
mSP	216,4	147,4	185,8	148,6	139,8	129,8	mm/Hg
mDP	134,6	77,8	92	69	79,6	79,6	mm/Hg

HR, heart rate; RR, respiratory rate; RT, rectal temperature; mSP, mean systolic pressure; mDP, mean diastolic pressure; *, baseline; **, after sedation; GPP, *Yin Tang* acupoint injection group.

In the GPP group in table 6, maximum values after sedation for HR, RR, RT, mSP, mDP parameters were 120 bpm, 28 cpm, 38,8ºC, 148,6 mm/Hg and 79,6 mm/Hg, respectively. Minimum values after sedation for HR, RR, RT, mSP, mDP parameters were 100 bpm, 16 cpm, 38,5ºC, 129,8 mm/Hg and 69 mm/Hg, respectively.

Table 7. Parameters collected in the intramuscular injection group.

Intramuscular injection G1							
	P1 BL*	P1 AS**	P4 BL	P4 AS	P6 BL	P6 AS	
HR	80	84	150	129	110	104	bpm
RR	28	16	24	18	40	16	cpm
RT	38,9	38,4	40	38,6	39,5	38,4	ºC
mSP	120,4	127,2	140,2	135	155,6	106	mm/Hg
mDP	64	71,2	83,2	75,8	72	45,8	mm/Hg

HR, heart rate; RR, respiratory rate; RT, rectal temperature; mSP, mean systolic pressure; mDP, mean diastolic pressure; *, baseline; **, after sedation; G1, intramuscular injection group.

In the G1 group in table 7, maximum values after sedation for HR, RR, RT, mSP, mDP parameters were 129 bpm, 18 cpm, 38,6ºC, 135 mm/Hg and 75,8 mm/Hg, respectively. Minimum values after sedation for HR, RR, RT, mSP, mDP parameters were 84 bpm, 16 cpm, 38,4ºC, 106 mm/Hg and 45,8 mm/Hg, respectively.

A graphical overview on the evolution of the measured parameters, heart rate, respiratory rate, rectal temperature and arterial blood pressure is shown in fig.5 for the acepromazine intramuscular injection group (G1) and for the *Yin Tang* pharmacopuncture injection group (GPP) in fig.6, respectively.

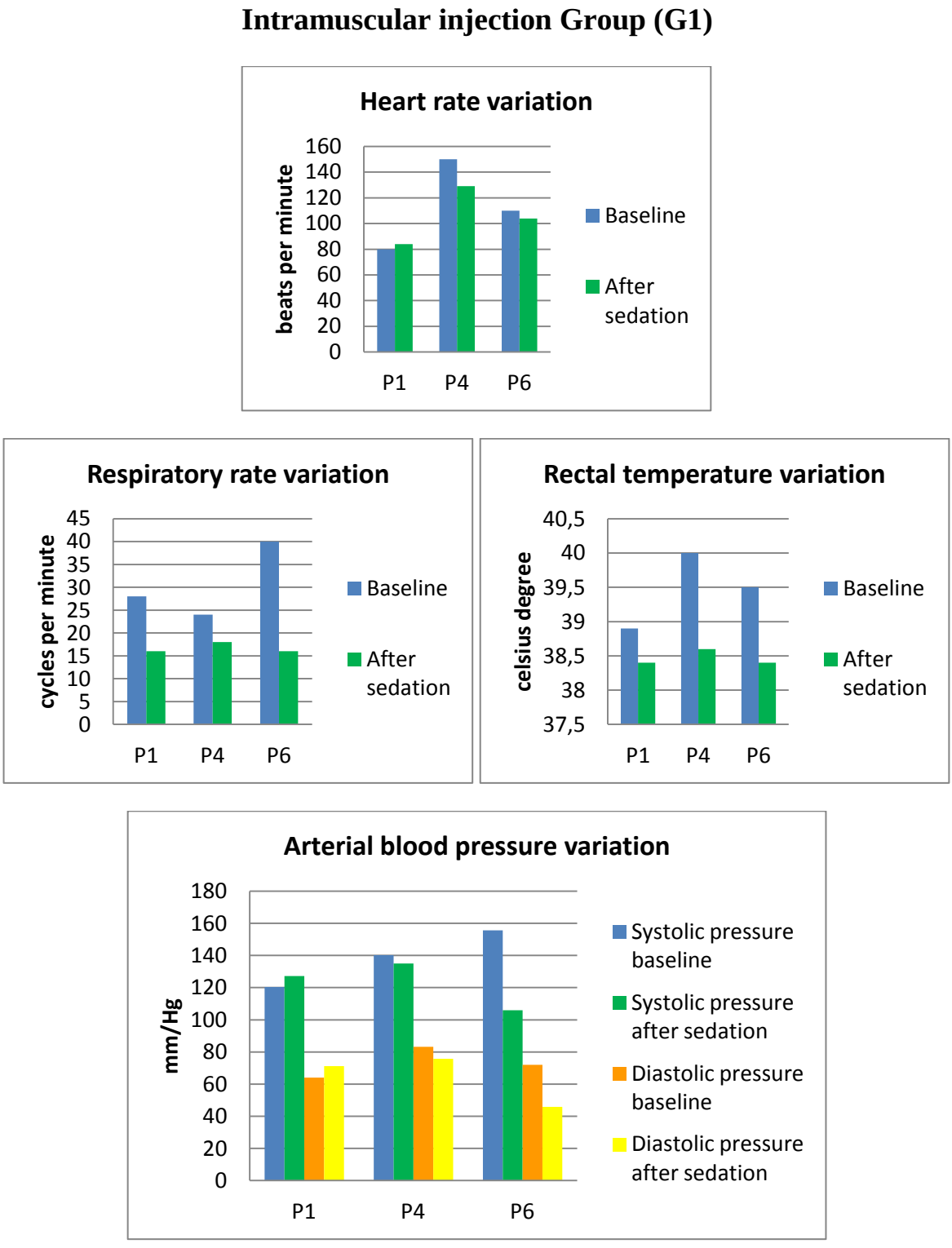
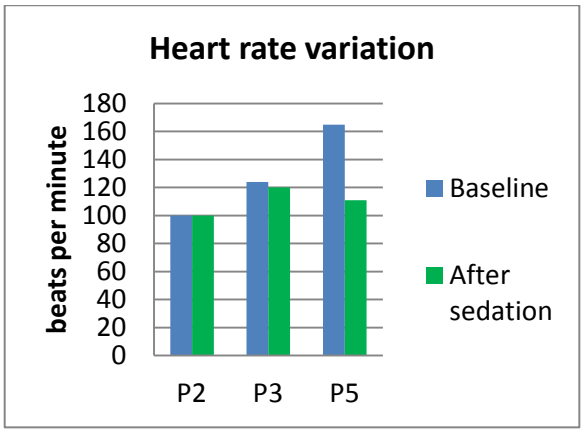
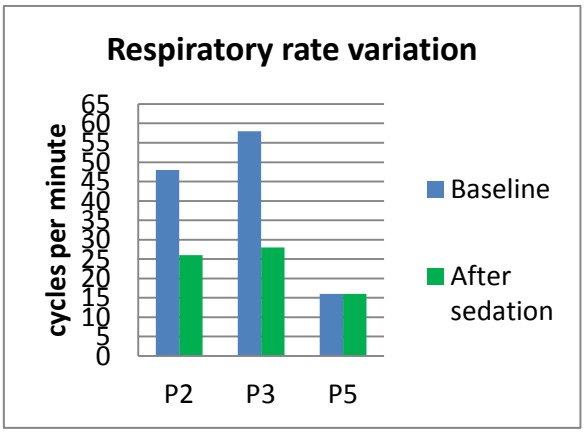
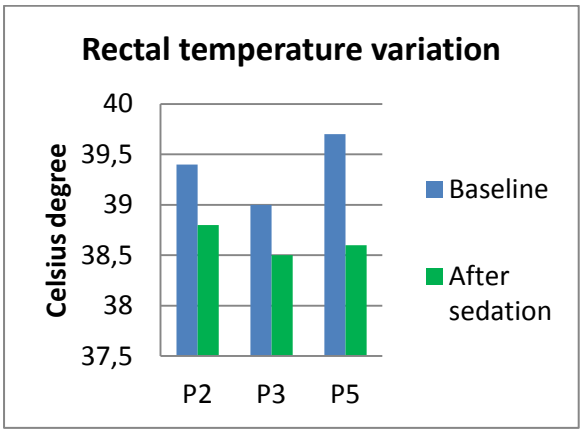


Figure 5. Parameter changes in heart rate(HR), respiratory rate(RR), rectal temperature(RT) and arterial blood pressure(ABP) of the animals in the acepromazine intramuscular injection group (G1).

Yin Tang Pharmacopuncture Group (GPP)







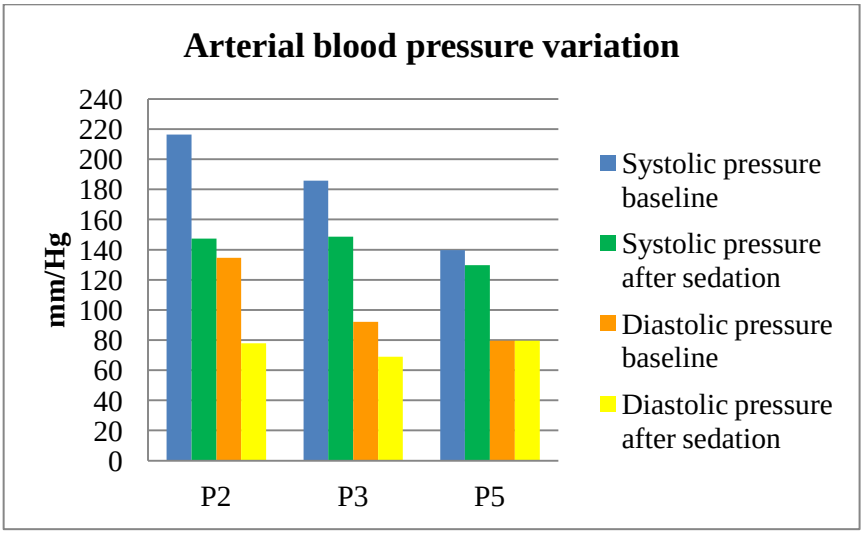


Figure 6. Parameter changes in heart rate(HR), respiratory rate(RR), rectal temperature(RT) and arterial blood pressure(ABP) of the animals in the acepromazine pharmacopuncture group (GPP).

In the study, statistically, there is no significant differences (p-value < 0.05) between the *Yin Tang* sedation method group (GPP) and the intramuscular injection sedation method (G1). However, the results show an overall lower sedation score in the pharmacopuncture group (GPP) when compared to the G1 group as shown in table 5.

Statistical analysis showed no significant differences (p-value < 0.05) between the *Yin Tang* sedation method group (GPP) and the intramuscular injection sedation method (G1) and the after sedation measurements of heart rate, respiratory rate, rectal temperature and arterial blood pressure.

7. Discussion

The use of routine animals from the hospital due to animal welfare restrictions caused the sample to be heterogeneous and may not contribute for the comparison of results between groups. This study only had permission to use healthy animals undergoing selective surgery so they could benefit from the use of the techniques applied in the study as a mean of premedication. Also, restrictions did not allow for a control group with a placebo effect so such group was not included in this study but other studies with similar techniques used control groups with saline injection at false acupuncture points and observed that this groups had no effect or a reduced effect when compared to injection at a true acupuncture point with the respective drug used in the study (Luna *et al*., 2002; 2008; Godoi *et al*, 2013).

7.1. Sedation Score

Sedation score and the other parameters were only measured once in the timeline because manipulation would most definitely alter the subsequent results after the first measurement. The decision was to only take measurements at 30 minutes after injection which is the time of the peak effect described for acepromazine by Cornick & Harstfield, 1992; Smith *et al*., 2001. Due to the complexity of the sedation score chart in table 3 a specific order for assessment was considered to avoid excitement that could alter the overall sedation score. The sedation score table shown in table 3 was applied in this study due to its ability to assess objectively different characteristics also used to monitor animals during anaesthesia. It allowed to effectively quantify consciousness, environmental awareness and motor muscle control. Other tools could be used like the Ramsay sedation scale, Riker sedation and agitation scale and Richmond agitation-sedation scale which are easier to use and rely more on observation than manipulation of the patient which can be good when applying it to daily routine. Nevertheless, they possess a more subjective overview over patients and have a smaller range that makes it hard to notice slight changes in sedation behaviour. In veterinary practice it is important to characterize patients control over motor behaviour because it implies safety for the handlers during clinical procedures. This and the ability to detect minor changes during sedation in patients, were decisive when choosing the sedation scale to apply in this study. Nevertheless, this scale does not evaluate agitation of patients representing a considerable downside when using this tool.

Statistically, there was no significant difference between both methods of sedation thus, one, could only speculate that the null hypothesis H0 that both are similar in efficacy is true, however, it was watched, clinically, a relatively noteworthy difference, whereas the injection of acepromazine at the *Yin Tang* acupoint created an overall lower sedation score in its group than the acepromazine intramuscular injection group. This can be caused by a very small sample and due to the high variation between sedation scores, especially in the intramuscular injection G1 group, where the minimum value is 1 and the maximum value is 14 in a total of 22 points. This variation can also be an effect of the unreliability of acepromazine when used alone for sedation purposes as described in BSAVA Small Animal Formulary 7th edition (2011).

This study used acepromazine because it is the most widely used sedative in veterinary medicine (Lemke, 2007) representing a good primary target for research in pharmacopuncture, since results from this study may be helpful for a wider range of practitioners worldwide. A previous study from Cassu *et al*., 2014 using 0.01mg/kg of xylazine injected in the *Yin Tang* acupoint induced clinically relevant sedative effects in dogs, reducing the undesirable side effects associated with α_2-agonists. Nonetheless, other sedatives are more recent (e.g. α_2-agonists; benzodiazepines) deserving attention in future researches with similar methods of pharmacopuncture for sedation in animals. Recent sedatives may show more potential to sedate animals considering their specific drug properties and their interaction with pharmacopuncture mechanism.

The light sedation observed in GPP group may be a result of an ineffective dose of acepromazine. This study used 1/10 of the conventional dosage 0.05 mg/kg of acepromazine in the attempt to produce a similar sedation effect. A previous study from Luna *et al*., 2002 used an acepromazine dose of 0.01 mg/kg in the *Yin Tang* acupoint in order to obtain a similar effect in thiopental reduction for induction in dogs than that of 0.1 mg/kg of acepromazine administered subcutaneously. Results demonstrated also a lighter but clinically relevant effect suggesting that, in similarity with this study, future studies should be made to determine the ideal dose of acepromazine injected in the *Yin Tang* acupoint to achieve a reliable level of sedation. Any reduction in the use of drugs to obtain similar effects than that of conventional therapies, contributes to a safer and healthier process for the patients justifying future research in the area.

7.2. Measured Parameters

Statistically, there was no significant difference (p-value < 0.05) in variations of heart rate, respiratory rate, rectal temperature and arterial blood pressure measurements between the G1 and GPP group. This statistical view is compromised by the size of the sample so a clinical perspective over the data must be made.

7.2.1. Heart Rate

Clinically, heart rate variations don´t show a visible relationship between each other inside the group subjects as shown in table 8. This may be that acepromazine has little effect over heart rate changes and the changes observed are due to another process altering normal nervous, humoral and local control of the cardiovascular function.

The lower value after sedation presented by each group is, 84 bpm in G1 and 100 bpm in GPP, however the lowest value in G1 is actually an increase in heart rate of P1 subject when compared with its baseline value of 80 bpm, demonstrating in this case no heart rate depression effect as well as the P2 subject in GPP which had the value of 100 bpm in baseline and after sedation measurements. After sedation subjects on GPP group maintained heart rate values between 120 and 100 bpm. G1 group maintained heart rate values between 129 and 84 bpm.

Table 8. Heart rate variation between baseline and after sedation values.

Heart rate variation			
GPP		G1	
P2	0%	**P1**	↑5%
P3	↓3,2%	**P4**	↓14%
P5	↓32%	**P6**	↓5,4%

↓, decrease; ↑, increase; G1, intramuscular inejction group; GPP, *Yin Tang* acupoint injection group.

The biggest variation in heart rate was shown in GPP by subject P5 lowering his baseline value of 165 bpm to an after sedation value of 111 bpm demonstrating a decrease of 32% in heart rate. In G1 the biggest variation in heart rate was shown by P4 with a decrease of 14% in heart rate. P5 from GPP and P4 from G1 obtained a sedation score of 1/22 showing that they were slightly sedated and suggest that this big

variations may be due to higher values prior to sedation developed by the presence of environmental stressors, which led to their decrease when the subjects experienced sedation and consequential relaxation to the environment. G1 and GPP heart rate values don't change considerably which was expected because a similar phenomena is also described in previous studies in dogs and horses with the use of acepromazine at the dose of 0.1 mg/kg through IV route (Muir & Sheehan, 1979; Coulter *et al.*, 1981). Although, there seems to be no evidential correlation, the overall response to both methods was a decrease in heart rate that respected the physiological range for dogs. The decrease in heart rate can be the outcome of the sedation and decrease of catecholamines in circulation as described by Posner and Burns, 2009.

7.2.2. Respiratory Rate

Respiratory rate variation between G1 and GPP is similar if we take in consideration the decrease percentage in respiratory rate for each subject as shown in table 9. When looking to minimum value after sedation both groups reached 16 cpm, although this result in the GPP group is represented by subject P5 who had no respiratory rate variation at all. P5 baseline value represents a calm respiratory rate and since the sedation score was 1/22 this may explain for the lack of variation in this parameter.

Table 9. Respiratory rate variation between baseline and after sedation values.

Respiratory rate variation			
GPP		G1	
P2	↓45,8%	**P1**	↓42,8%
P3	↓51%	**P4**	↓25%
P5	0%	**P6**	↓60%

↓, decrease; G1, intramuscular injection group; GPP, *Yin Tang* acupoint injection group.

Clinically, this variations show a similarity of effect in decreasing the respiratory rate between 1/10 of the acepromazine dose at the acupoint *Yin Tang* and that of the 0.05 mg/kg acepromazine dose injected intramuscularly. This study acts in accordance with previous studies (Popovic & Mullane, 1972) that show that acepromazine has little effect on pulmonary function but seems to decrease respiratory rate in dogs.

7.2.3. Rectal Temperature

Rectal temperature values after sedation in G1 varied from 0,5ºC to as further as 1,4 ºC reaching a minimum value of 38,4 ºC. In GPP, variation went from 0,5 ºC to 1,1 ºC reaching a minimum value of 38,5 ªC. Comparison of G1 and GPP values in table 10 show that both groups had a similar effect on thermoregulation. One of the adverse effects described for acepromazine is hypothermia and the loss of thermoregulation.

Table 10. Rectal temperature variation between baseline and after sedation values.

Rectal temperature variation			
GPP		G1	
P2	↓1.5%	**P1**	↓1.2%
P3	↓1.3%	**P4**	↓3.5%
P5	↓2.8%	**P6**	↓2.8%

↓, decrease; ↑, increase; G1, intramuscular injection group; GPP, *Yin Tang* acupoint injection group.

Observations show that the groups had similar rectal temperatures and that no subject at the time of data collection was below physiological rectal temperature values described for dogs in BSAVA Manual of Small Animal Anaesthesia and Analgesia, 1999. It would be interesting to collect more measurements over time during the sedation period to analyse if the thermoregulation behaviour is similar in both sedation methods. In G1, subject P4 showed a baseline value of 40 ºC compatible with stress induced hyperthermia, having it dropped after sedation to a value of 38,6 ºC. The same behaviour was watched in GPP, where P5 had a baseline value of 39,7 ºC having it dropped after sedation to 38,6 ºC. Although P5 had a difference of 0,3 ºC from P4, it also showed stress induced hyperthermia, which was reduced after sedation to the same value than P4 (Olivier *et al.*, 2003). Clinically, this suggests that the injection of 1/10 of the dose of acepromazine at the *Yin Tang* acupoint may have the ability to bring animals from a stress induced state to a more tranquilized state through sedation.

7.2.4. Arterial Blood Pressure

Baseline arterial blood pressure values are lower in G1 than in GPP but almost all subjects showed a slight hypertension which may be attributed to the fact that animals were in fact in a strange stressful environment. Interestingly, both methods caused a similar decrease in arterial blood pressure. In G1 the minimum values for systolic and diastolic pressure belong to P6 which went below recommend values for awaken dogs having a moderate decrease in diastolic pressure. This accounts for the only hypotensive phenomenon observed in this study. Considering this, the intramuscular injection of acepromazine at the dose 0.05 mg/kg should be done with caution always controlling arterial blood pressure. The injection of acepromazine at the dose of 0.005 mg/kg at the *Yin Tang* acupoint had no hypotensive phenomenon and caused a similar decrease in arterial blood pressure that never went below the expected physiological range for dogs. This may suggest that this method is safe to use, however more intensive study on the effects of the method should be done prior to its use in routine practice.

Table 11. Arterial blood pressure variation between baseline and after sedation values.

Arterial blood pressure variation					
GPP	**Systolic pressure**	**Diastolic pressure**	**G1**	**Systolic pressure**	**Diastolic pressure**
P2	↓31,8%	↓42,1%	**P1**	↑5,64%	↑11,25%
P3	↓20%	↓25%	**P4**	↓3,7%	↓8,9%
P5	↓7,1%	0%	**P6**	↓31%	↓36,4%

↓, decrease; ↑, increase; G1, intramuscular injection group; GPP, *Yin Tang* acupoint injection group.

7.3. Synopsis

Even though anecdotal the comparison between the two groups, maximum and minimum values after sedation of HR, RR, RT, mSP, mDP parameters which received different sedation methods with the same pharmaceutical drug, acepromazine, G1 and GPP show a decrease of the values to more acceptable physiological values and that they seem to produce a similar effect on the parameters measured in this study, suggesting that maybe 1/10 of the dose of acepromazine administered in the acupoint *Yin Tang* can produce a similar effect than that of 0.05 mg/kg acepromazine dose injected intramuscularly. This effect of

pharmacopuncture is described and showed in earlier studies as Alvarenga *et al*., 1998; Silva & Luna, 1999; Nie *et al*., 2001; Luna *et al*., 2008; Cassu *et al*., 2014.

In this study, the injection of 1/10 of the dose of acepromazine administered in the acupoint *Yin Tang* successfully caused light sedation in GPP animals, shaping their behaviour to a more relaxed state. This can be shown by the variations shown by each animal for HR, RR, RT and ABP parameters.

GPP group did not show any of the described (BSAVA, 1999) undesirable side effects for acepromazine like hypotension, bradycardia and hypothermia. In view of this, and as a result of a small sample, we can only speculate that this method is safe to apply, although, clinically the sedation itself is unreliable as in G1, but shows signs of tranquilization of the animals returning them to more physiological state by shaping their behavioural response.

Acupuncture and by consequence pharmacopuncture mechanism of action remains very unclear, nevertheless, and considering the observations in this study a theory will be discussed. Pharmacopuncture combines the effects of acupuncture and drugs. In this case, the drug used was acepromazine which causes inhibition of central dopaminergic receptors (D2), that are responsible for shaping the behavioural response, thus contributing for the sedation (Lemke, 2007). The *Yin Tang* acupoint is indicated for sedation, but the mechanism that leads to sedation is vague.

Peripherally, phenothiazines block norepinephrine at alpha-adrenergic receptors (Posner & Burns, 2009). Experimental studies in rats, showed decreased levels of norepinephrine in the brain after EA (Han *et al*., 1979; Wang, Jiang & Can, 1994; Zhu *et al*., 1997). This shows that both the drug and the effect caused by acupuncture will act at α-adrenergic receptors. Phenothiazines have high affinity for α1-adrenergic receptors as shown in table 1. This α1-adrenergic receptors in spinal dorsal horn facilitate nocioceptive signalling whereas α2-adrenergic receptors suppress it (Millan, 2002). This may explain for the fact that acepromazine has no analgesic effect. In the other hand, acupuncture causes the release of endogenous opioids (e.g. β-endorphin) suggesting a possible added analgesic effect to the pharmacopuncture method that would be interesting to prove in future studies by comparing response to noxious stimuli between G1 and GPP. The alterations shown in the parameters by pharmacopuncture suggest parasympathetic activity and inhibition of sympathetic activity similar to the intramuscular injection of 0.05 mg/kg of acepromazine, thus proposing similar pathways. This show us that both methods alter the autonomous nervous system.

Acupuncture causes the release of β-endorphin a pituitary peptide (Grimm & Wagner, 2007) showing hypothalamic-pituitary activation. Acepromazine also has hypothalamic-pituitary activity by blocking dopamine receptors which cause release of prolactin in pituitary gland. The adrenal gland medulla produces cathecolamines, epinephrine, norepinephrine, dopamine (Squires, 2003) suggesting that it also plays a role in the action of both methods. Summarizing, in pharmacopuncture mechanotransduction at the acupoint causes signalling trough afferent pathways to the spinal dorsal horn, which in turn will cause central nervous system activation at hypothalamic- pituitary-axis. This in turn will create systemic effects by altering the autonomous nervous system influencing the adrenal gland and its hormones. The specific mechanism is unclear but clinical observations suggest that pharmacopuncture mechanism works by stimulation of specific acupuncture points that cause local effects and specific systemic effects which work in synergy with drugs, contributing to their systemic and/or specific local effects.

The injection of acepromazine at the *Yin Tang* acupoint may prove useful in daily clinical practice if further research is done in providing a better understanding and safety involving the method. In the eyes of this study, the method shows promising by causing similar effects and reducing the costs of the drug to both the clinician and the owner of the dog by 90% when compared with the intramuscular injection of 0.05 mg/kg of acepromazine, making it possible for the clinician to reduce possible adverse effects and for the dog to quickly metabolize and excrete the small amount of drug received. This may allow for the drug to be used more often to tranquilize dogs that show excitatory behaviours and also contribute to the economic inflow in the clinic. If a clinician was to use this method in a place or circumstance where the animal could not be well monitored, this method may prove itself to be safer to achieve tranquilization in dogs than the intramuscular injection of 0.05 mg/kg of acepromazine according to observations in this study.

8. Conclusion

This study will only serve as a preliminary approach to further studies in the areas of pharmacopuncture and induced sedation through injection of acepromazine in the *Yin Tang* acupuncture point since all conclusions are mainly mere speculations resultant from a deficient animal model available at the time. Even so and trusting that not all is a statistical truth, speculations can be made from a clinical point of view.

A light sedation effect was observed in the animals in this study using the *Yin Tang* acupoint method and consequently, heart rate, respiratory rate, rectal temperature, arterial blood pressure and behavioural changes occurred suggesting that, although, not statistically proved, this method may be used to sedate dogs and return them to a more relaxed state. However one should be very careful using this method if the aim is to control the motor behaviour in more agitated and aggressive animals since acepromazine use in both methods showed to be unreliable and unfitting for the job. Practitioners should consider maybe only using the acepromazine injection at the *Yin Tang* acupoint method to tranquilize dogs which show signs of agitation or stress response in a clinical environment since it is a relatively safe method to use and a very small amount of drug for the dog metabolism to process and eliminate.

Limitations encountered during this study, severely damaged the sample and consequent interpretation of the results. During the time span of 7 months it was hard to obtain dogs with the adequate criteria and a healthy condition prior to selective surgery. Furthermore, it was difficult to conciliate the patients with the availability of the specialized technician to apply the method. Another limitation, like the selection of the adequate dose was unfortunate and came from the principle that 1/10 of the dose should cause similar effect than that of the conventional dose. In this study, both methods caused an inadequate effect, so for future studies, maybe the doses or the proportion of such doses should be reconsidered.

Further extensive studies with more controlled samples are required to evaluate the future benefits and consequent side effects of this method but from this study perspective the method may show itself promising to tranquilize not only healthy dogs but elderly dogs and/or dogs that are somehow debilitated and/or have a concurrent disease which make them a higher risk patient for normal sedation protocols. The pharmacopuncture effect of producing similar effects using only 1/10 of conventional doses remains promising but nevertheless, it should be confirmed with extensive critical scientific research at all levels of investigation in

many areas of veterinary practice so it can be brought to the western medical point of view and allow western practitioners to use it in routine practice.

Bibliografia

Aigner, L.; Arber, S.; Kapfhammer, J.P.; Laux, T.; Schneider, C.; Botteri, F.; et al. (1995) Overexpression of the neural growth- associated protein GAP-43 induces nerve sprouting in the adult nervous system of transgenic mice. Cell; 83:269e278.

Alvarenga, M.A.; Ferreira, J.E.P.; Meira, C.; Luna, S.P.L.; Burns, P.J. (1998) Induction of luteolysis in mares using a micro-dose of prostaglandin in the sacral lumbar space (Bai Hui Acupoint). Equine Vet Sci; 18(3) 167-168.

Arthur, D.B.; Akassoglou, K.; Insel, P.A. (2005) P2Y2 receptor activates nerve growth factor/TrkA signaling to enhance neuronal differentiation. Proc Natl Acad Sci USA;102:19138e19143.

Bai, Y.; Wang, J.; Wu, J.P.; Dai, J.X.; Sha, O.; Tai Wai Yew, D.; et al. (2011) Review of evidence suggesting that the fascia network could be the anatomical basis for acupoints and meridians in the human body. Evid Based Complement Alternat Med.; 2011:260510.

Ballard, S.; Schults, T.; Knownacki, A.; Blake, J.; Tobin, T. (1982) The pharmacokinetics, pharmacological responses and behavioural effects of acepromazine in the horse. J Vet Pharmacol Ther, Mar; 5(1): 21-31.

Barr, S.C.; Ludders, J.W.; Looney, A.L.; et al. (1992) Platelet aggregation in dogs after sedation with acepromazine and atropine and during subsequent general anesthesia and surgery. Am J Vet Res; 53: 2067-2070.

Benowitz, L.I.; Routtenberg, A. (1997) GAP-43: an intrinsic determinant of neuronal development and plasticity. Trends Neurosci; 20:84e91.

Biella, G.; Sotgiu, M.L.; Pellegata, G.; Paulesu, E.; Castiglioni, I.; Fazio, F. (2001) Acupuncture produces central activations in pain regions. Neuroimage; 14:60–6.

Bostrom, I.; Nyman, G.; Kampa, N.; et al. (2003) Effects of acepromazine on renal function in anesthetized dogs. Am J Vet Res; 64:590-598.

Cassu, R.N.; Melchert, A.; Canoa, J.T.B.; de Oliveira Martins, P.D. (2014) Sedative and clinical effects of the pharmacopuncture with xylazine in dogs. Act Cir Bras; 29(1) 2014-47.

Chao, D.M.; Shen, L.L.; Tjen-A-Looi, S.; Pitsillides, K.F.; Li, P.; Longhurst, J.C. (1999) Naloxone reverses inhibitory effect of electroacupuncture on sympathetic cardiovascular reflex responses, Am J Physiol Cell Physiol, vol. 276, no. 6, pp.H2127–H2134.

Chen, C.-Y.; Chern, R.-S.; Liao, M.-H.; Chang, Y.-H.; Hsu, J.-Y. C.; Chien, C.-H. (2013) The possible neuronal mechanism of acupuncture: morphological evidence of the neuronal connection between groin A-shi point and uterus, Evid Based Complement Alternat Med, vol. 2013, ID:429186, 13 pp.

Chen, C.Y.; Lin, C.N; Chern, R.S.; Tsai, Y.C.; Chang, Y.H.; Chien, C.H. (2014) Neuronal activity stimulated by liquid substrates injection at Zusanli (ST36) acupoint: The possible mechanism of aquapuncture. Evid Based Complement Alternat Med, ID:627342, 7 pp.

Cheng, K.J. (2014) Neurobiological mechanisms of acupuncture for some common illnesses: a clinician's perspective. J Acupunct Meridian Stud; 7(3): 105-114.

Cheng, R.S.; Pomeranz, B. (1980) Electroacupuncture analgesia is mediate by sterospecific opiate receptors and is reversed by antagonists of type I receptors. Life Sci;26:631–8.

Cheng, R.S.; Pomeranz, B. (1981) Monoaminergic mechanism of electroacupuncture analgesia. Brain Res; 215:77–92.

Chien, C.-H.; Shieh, J.-Y.; Liao, M.-H.; Ling, E.-A.; Wen, C.-Y. (1998) Neuronal connections between the auricular skin and the sympathetic pre- and postganglionic neurons of the dog as studied by using pseudorabies virus, Neurosci Res, vol. 30, no. 2, pp. 169–175.

Clement-Jones, V.; McLoughlin, L.; Corder, R.; Lowry, P.J.; Besser, G.M.; Rees, L.H.; et al. (1980) Increased beta-endorphin but not met-enkephalin levels in human cerebrospinal fluid after acupuncture for recurrent pain. Lancet; 2:946–9.

Cornick, J.L.; Hartsfield, S.M. (1992) Cardiopulmonary and behavioural effects of combinations of acepromazine/butorphanol and acepromazine/oxymorphone in dogs. J Am Vet Med Assoc; 200:1952-1956.

Coulter, D.B.; Whelan, S.C.; Wilson, R.C.; et al. (1981) Determination of blood pressure by indirect methods in dogs given acetylpromazine maleate. Cornell Vet; 71:75-84.

Devlin, J.W.; Boleski, G.; Mlynarek, M.; Nerenz, D.R.; Peterson, E.; Jankowski, M.; Horst, H.M.; Zarowitz, B.J. (1999) Motor Activity Assessment Scale: a valid and reliable sedation scale for use with mechanically ventilated patients in an adult surgical intensive care unit. Crit Care Med, 27:1271-1275.

Dewey E.A.; Maylin, G.A.; Ebel J.G.; et al. (1981) The metabolism of promazine and acetylpromazine in the horse. Drug Metab Dispos; 9:30-36.

Dhond, R.P.; Yeh, C.; Park, K.;, Kettner, N.; Napadow, V. (2008) Acupuncture modulates resting state connectivity in default and sensori-motor brain networks. Pain.;136:407e418.

Dos Santos, J.G. Jr.; Tabosa, A.; do Monte, F.H.; Blanco, M.M.; de Oliveira Freire, A.; Mello, L.E. (2005) Electroacupuncture prevents cognitive deficits in pilocarpine-epileptic rats. Neurosci Lett;384: 234–8.

Goldman, N.; Chen, M.; Fujita, T.; Xu, Q.; Peng, W.; Liu, W.; et al. (2010) Adenosine A1 receptors mediate local anti-nociceptive effects of acupuncture. Nat Neurosci; 13:883e888.

Grimm, K.A.; Wagner, A.E.(2007) Nervous System. In: Tranquilli, W.J.; Thurmon, J.C.; Kurt, A.G., Lumb & Jones Veterinary Anesthesia and Analgesia (4th ed, pp. 153-168). Blackwell Publishing Professional.

Gross, M.E. (2001) Tranquilizers, a2-adrenergic agonists, and related agents. In: Adams HR. Veterinary Pharmacology and Therapeutics, (8th ed, pp.299-342). Ames: Iowa State University Press.

Guo. H.F.; Tian, J.H.; Wang, X.; Fang, Y.; Hou, Y.; Han, J. (1996) Brain substrates activated by electroacupuncture of different frequencies. I. Comparative study on the expression of oncogene c-fos and genes coding for three opioid peptides. Brain Res Mol Brain Res; 43:157–66.

Guo. H.F.; Tian, J.H.; Wang, X.; Fang, Y.; Hou, Y.; Han, J. (1996) Brain substrates activated by electroacupuncture (EA) of different frequencies. II. Role of Fos/Jun proteins in EA-induced transcription of preproenkephalin and pre-prodynorphin genes. Brain Res Mol Brain Res; 43:167–73.

Hall, J.A.; Magne, M.L.; Twedt, D.C. (1987) Effect of acepromazine, diazepam, fentanyl-droperidol, and oxymorphone on gastroesophageal sphincter pressure in healthy dogs. Am J Vet Res; 48:556-557.

Han, J.S. (2003) Acupuncture: neuropeptide release produced by electrical stimulation of different frequencies. Neuroscience; 26:17–22.

Han, J.S.; Ren, M.F.; Tang, J.; Fan, S.G.; Xu, J.M.; Guan, X.M. (1979) The role of catecholamine in acupuncture analgesia. Chin Med J (Engl); 92:793e800.

Han, J.S.; Terenius, L. (1982) Neurochemical basis of acupuncture analgesia. Annu Rev Pharmacol Toxicol; 22:193–220.

Hashem, A.; Kietzmann, M.; Scherkl, R. (1992) The pharmacokinetics and bioavailability of acepromazine in the plasma of dogs. Dtsch Tierarztl Wochenschr, Oct; 99(10): 396-8.

Horrigan, B. (1996) Acupuncture and the Raison D'etre for alternative medicine. Altern Ther Health Med; 2:85–91.

Hsieh, J.C.; Tu, C.H.; Chen, F.P.; Chen, M.C.; Yeh, T.C.; Cheng, H.C.; et al. (2001) Activation of the hypothalamus characterizes the acupuncture stimulation at the analgesic point in human: a positron emission tomography study. Neurosci Lett;307:105–8.

Hui, K.K.S.; Liu, J.; Makris, N.; Gollub, R.L.; Chen, A.J.; Moore, C.I.; et al. (2000) Acupuncture modulates the limbic system and subcortical gray structures of the human brain: evidence from fMRI studies in normal subjects. Hum Brain Mapp; 9:13–25.

Hui, K.K.S.; Liu, J.; Marina, O.; Napadow, V.; Haselgrove, C.; Kwong, K.K.; et al. (2005) The integrated response of the human cerebro-cerebellar and limbic systems to acupuncture stimulation at ST 36 as evidenced by fMRI. Neuroimage; 27:479–96

Jin, J.J.; Xu, Y.L.; Zheng Y. (2006) Effects of acupoint-injection of Kaixilai on TG, TC, MDA, SOD, AST and ALT in the patient of non-alcoholic fatty liver (Chinese). Zhongguo Zhen Jiu; 26:100–2.

Jones, R.S. (1999) The Practice of Veterinary Anaesthesia and Analgesia. In: Seymour, C.; Gleed, R., BSAVA Manual of Small Animal Anaesthesia and Analgesia (pp. 3-7). British Small Animal Veterinary Association.

Kim, J.; Kang, D.I. (2010) A descriptive statistical approach to the Korean pharmacopuncture therapy. J Acupunct Meridian Stud; 3:141–9.

Kim, M.-H.; Park, Y.-C.; Namgung, U. (2012) Acupuncture-stimulated activation of sensory neurons. J Acupunct Meridian Stud; 5(4): 148-155.

Lang, S.M.; Eglen, R.M.; Henry, A.C. (1979) Acetylpromazine administration: Its effect on canine haematology. Vet Rec; 105:397-398.

Langevin, H.M.; Yandow, J.A. (2002) Relationship of acupuncture points and meridians to connective tissue planes. Anat Rec.; 269:257e265.

Lee, J.H.; Beitz, A.J. (1993) The distribution of brain-stem and spinal cord nuclei associated with different frequencies of electroacupuncture analgesia. Pain; 52:11–28.

Lemke, K.A.(2007) Anticholinergics and Sedatives. In: Tranquilli, W.J.; Thurmon, J.C.; Kurt, A.G., Lumb & Jones Veterinary Anesthesia and Analgesia (4th ed, pp. 203-241). Blackwell Publishing Professional.

Leung, L. (2012) Neurophysiological Basis of Acupuncture-induced Analgesia - An Updated Review. J Acupunct Meridian Stud; 5(6): 261-270.

Lim, T.; Loh, T.; Kranz, H.; Scott, D. (1977) Acupuncture-effect on normal subjects. Med J Aust;1:440–2.

Liu. G.; Akira, H. (1994) Basic principle of TCM. In: Liu. G.; Akira, H. (eds.) Fundamentals of acupuncture and moxibustion. Tianjin, China: Tianjin Science and Technology Translation and Publishing Corporation.

Lukasik, V.M. (1999) Premedication and Sedation. In: Seymour, C.; Gleed, R., BSAVA Manual of Small Animal Anaesthesia and Analgesia (pp. 71-83). British Small Animal Veterinary Association.

Luna, S.P.; Angeli A.L.; Ferreira, C.L.; Lettry, V.; Scognamillo-Szabo, M. (2008) Comparison of pharmacopuncture, aquapuncture and acepromazine for sedation of horses. Evid Based Complement Alternat Med; 5:267–72.

Luna, S.P.L.; Alvarenga, M.A.; Joaquim, J.G.F.; Minichelli, M. (1999) The role of Bai-Hui accupoint for induction of luteolysis in mares. International Congress on Veterinary Acupuncture, 25., Lexington, Proceedings, p.29-32.

Luna, S.P.L.; Maiante, A.A.; Xavier, F.; Pires-Osório, D.D.; Endo, Y.; Karasawa, A.S.M. (2002) Effect of acupuncture on the thiopental dose for induction of anaesthesia in dogs. R. bras. Ci. Vet; 9(1) 289-291.

Marroum, P.J.; Webb, A.I.; Aeschbacher, G.; et al. (1994) Pharmacokinetics and pharmacodynamics of acepromazine in horses. Am J Vet Res;55:1428-1433.

Mayer, D.J. (1977) Antagonism of acupuncture analgesia in man by the narcotic antagonist naloxone. Brain Res; 121:368–72.

Maylin, G.; Dewey, F.; Ebel, J.; Henion, J. (1978) The metabolism of promazine and acetylpromazine in the horse. Drug Metab Dispos. Jan-Feb; 9(1): 30-6.

Millan, M.J. (2002) Descending control of pain. Prog Neurobiol.; 66:355e474.

Moazzami, A.; Tjen-A-Looi, S.C.; Guo, Z.-L.;. Longhurst, J.C (2010) Serotonergic projection from nucleus raphe pallidus to rostral ventrolateral medulla modulates cardiovascular reflex responses during acupuncture, J Appl Physiol, vol. 108, no. 5, pp. 1336–1346.

Muir, W.W.(2007) Consideration for General Anesthesia. In: Tranquilli, W.J.; Thurmon, J.C.; Kurt, A.G., Lumb & Jones Veterinary Anesthesia and Analgesia (4th ed, pp. 7-31). Blackwell Publishing Professional.

Muir, W.W.; Skarda R.T.; Sheehan, W. (1979) Hemodynamic and respiratory effects of a xylazine-acetylpromazine drug combination in horses. Am J Vet Res; 40:1518-1522.

Napadow, V.; Makris, N.; Liu, J.; Kettner, N.W.; Kwong, K.K.; Hui, K.K. (2005) Effects of electroacupuncture versus manual acupuncture on the human brain as measured by fMRI. Hum Brain Mapp; 24:193–205.

National Institutes of Health. (1998) The NIH consensus development panel on acupuncture. Acupuncture-NIH consensus conference. J Am Med Assoc; 280:1518e1524.

Nie, G.J.; Goodin, A.N.; Braden, T.D.; Wenzel, J.G. (2001) Luteal and clinical response following administration of dinoprost tromethamine or cloprostenol at standard intramuscular sites or at the lumbosacral acupuncture point in mares. Am J Vet Res;62:1285–9.

Olivier, B.; Zethof, T.; Pattij, T.; van Boogaert, M.; van Oorschot, R.; Leahy, C. et al. (2003) Stress-induced hyperthermia and anxiety: pharmacological validation. Eur J Pharmacol. Feb 28; 463(1-3):117-32.

Ovechkin, A.; Kim, K.S.; Lee, J.W.; Lee, S.M. (2003) Thermo-visual evaluation of the Yin-Tang acupuncture point for intracranial hypertension syndrome. Am J Chin Med; 31:455–66.

Pan, B.; Castro-Lopes, J.; Coimbra, A. (1994) C-fos expression in the hypothalamo-pituitary system induced by electroacupuncture or noxious stimulation. Neuroreport; 5:1649–52.

Pan, B.; Castro-Lopes, J.; Coimbra, A. (1996) Activation of anterior lobe corticotrophs by electroacupuncture or noxious stimulation in the anaesthetized rat, as shown by colocalization of Fos protein with ACTH and beta-endorphin and increased hormone release. Brain Res Bull;40:175–82.

Pan, B.; Castro-Lopes, J.; Coimbra, A. (1997) Chemical sensory deafferentation abolishes hypothalamic pituitary activation induced by noxious stimulation or electroacupuncture but only decreases that caused by immobilization stress. A c-fos study. Neuroscience; 78:1059–68.

Pariente, J.; White, P.; Frackowiak, R.S.J.; Lewith, G. (2005) Expectancy and belief modulate the neuronal substrates of pain treated by acupuncture. Neuroimage; 25:1161–7.

Peets, J.; Pomeranz, B.; (1978) CXBK mice deficient in opiate receptors show poor electroacupuncture analgesia. Nature; 273:675–6.

Peng, L. (2002) Neural mechanisms of the effect of acupuncture on cardiovascular diseases, Int Congr Ser, vol. 1238, pp. 71–77.

Perlow, B.W. (1973) Acupuncture: its theory and use in general practice. Proc R Soc Med; 66:426e428.

Plumb, D.C. (2008) Plumb's Veterinary Drug Handbook (6th ed, pp.3-4). Blackwell Publishing Professional.

Pomeranz, B. (1998) Scientific basis of acupuncture. In: Stux G, Pomeranz B, (eds.) Basis of acupuncture. (4th ed.) Heidelberg: Springer-Verlag.

Pomeranz, B.; Chiu, D. (1976) Naloxone blockade of acupuncture analgesia: endorphin implicated. Life Sci; 19:1757–62.

Popovic, N.A.; Mullane, J.F.; Yhap, E.O. (1972) Effects of acetylpromazine maleate on certain cardiorespiratory responses in dogs. Am J Vet Res; 33:1819-1824.

Posner, L.P.; Burns, P. (2009) Sedative Agents: Tranquilizers, Alpha-2 Agonists, And Related Agents. In: Riviere, J.E.; Papich, M.G., Veterinary Pharmacology and Therapeutics (9th ed, pp. 337-381). Wiley Blackwell.

Ramsay, M.A.; Savege, T.M.; Simpson, B.R.; Goodwin, R. (1974) Controlled sedation with alphaxalone-alphadolone. BMJ, 2:656-659.

Ramsey, I. (2011) BSAVA Small Animal Formulary (7th ed, pp.1). British Small Animal Veterinary Association.

Redman, H.C.; Wilson, G.L.; Hogan, J.E.(1973) Effect of chlorpromazine combined with intermittent light stimulation in the electroencephalogram and clinical response of the Beagle dog. Am J Vet Res 34:929-936.

Research Group of Acupuncture Anesthesia PMC. (1973) The effect of acupuncture on human skin pain threshold. Chin Med J; 3:151–7.

Research Group of Acupuncture Anesthesia PMC. (1974) The role of some neurotransmitters of brain in finger acupuncture analgesia. Sci Sin; 117:112–30.

Riker, R.R.; Picard, J.T.; Fraser, G.L. (1999) Prospective evaluation of the Sedation-Agitation Scale for adult critically ill patients. Crit Care Med, 27:1325-1329.

Robinson, N.G. (2009) Making sense of the metaphor: how acupuncture works neurophysiologically. J Equine Vet Sci; 29(8) 642-644.

Santos Godoi, T.L.O.; Villas-Boas, J.D.; Almeida, N.A.S.; Trigo, P.I.; Almeida, F.Q.; Medeiros, M.A. (2014) Pharmacopuncture versus acepromazine in stress responses of horses during road transport. J Equine Vet Sci; 34:294-301.

Schoen, A.M. (2001) Veterinary acupuncture: ancient art to modern medicine.(2nd ed.) St. Louis: MO: Mosby.

Scrivani, P.V.; Bednarski, R.M.; Myer, C.W. (1998) Effects of acepromazine and butorphanol on positive-contrast upper gastrointestinal tract examination in dogs. Am J Vet Res; 59:1227-1233.

Sessler, C.N. (2004) Sedation scales in the ICU. Chest, 126:1727-1730.

Sessler, C.N.; Gosnell, M.S.; Grap, M.J.; Brophy, G.M.; O'Neal, P.V.; Keane, K.A.; Tesoro, E.P.; Elswick, R.K. (2002) The Richmond Agitation-Sedation Scale: validity and reliability in adult intensive care unit patients. Am J Respir Crit Care Med, 166:1338-1344.

Sessler, C.N.; Grap, M.J.; Ramsay, M.A. (2008) Evaluating and monitoring analgesia and sedation in the intensive care unit. Crit Care, 12(S3):S2

Silva, C.T.L.; Luna, S.P.L. (1999) Use of micro-dose of somatotropin to increase weight gain in calves. Congresso Internacional de Acupuntura Veterinária; São Paulo. Proceedings. 1, p.87.

Sjolund, B.H.; Ericksson, M. (1977) Increased cerebrospinal fluid levels of endorphins after electroacupuncture. Acta Physiol Scand; 100:382–4.

Smith, L.J.; Yu J.K.; Bjorling D.E.; et al. (2001) Effects of hydromorphone or oxymorphone, with or without acepromazine, on preanesthetic sedation, physiologic values, and histamine release in dogs. J Am Vet Med Assoc; 218:1101-1105.

Squires, E.A. (2003). Applied Animal Endocrinology (1st ed., pp. 204-208) CAB International.

Stepien, R.L.; Bonagura, J.D.; Bednarski, R.M.; et al. (1995) Cardiorespiratory effects of acepromazine maleate and buprenorphine hydrochloride in clinically normal dogs. Am J Vet Res; 56:78-84.

Strombeck, D.R.; Harrold, D. (1985) Effects of atropine, acepromazine, meperidine, and xylazine on gastroesophageal sphincter pressure in the dog. Am J Vet Res; 46:963-965.

Thurmon, J.C.; Short, C.E.(2007) History and Overview of Veterinary Anesthesia. In: Tranquilli, W.J.; Thurmon, J.C.; Kurt, A.G., Lumb & Jones Veterinary Anesthesia and Analgesia (4th ed, pp. 3-7). Blackwell Publishing Professional.

Tobias, K.; Marioni-Henry, K.; Wagner, R. (2006) A retrospective study on the use of acepromazine maleate in dogs with seizures. J Am Anim Hosp Assoc, Aug; 42(4) 283-9.

Valverde, A.; Cantwell, S.; Hernandez, J.; et al. (2004) Effects of acepromazine on the incidence of vomiting associated with opioid administration in dogs. Vet Anaesth Analg; 31:40-45.

VanderPloeg, K.; Xiaobin, Yi. (2009) Acupuncture in modern society. J Acupunct Meridian Stud; 2(1): 26-33.

Wang, H.; Jiang, J.; Can, X. (1994) [Changes of norepinephrine release in rat's nucleus reticularis paragigantocellularis lateralis in acupuncture analgesia]. Zhen Ci Yan Jiu; 19:20e25.

Wang, J.; Dong, W.R.; Wang, C.L.; Yao, D.W.; Zhao, B.L.; Shen, B.L.; et al. (2007) From meridians and acupoints to self-supervision and control system: a hypothesis of the 10th functional system based on anatomical studies of digitized virtual human. Nan Fang Yi Ke Da Xue Xue Bao;27:573e579.

Wang, S.-M.; Kain, Z.N.; White, P. (2008) Acupuncture analgesia: I. The scientific basis. Pain Med; 106(2): 602-610.

Webb, A.I.; O'Brien, J.M. (1988) The effect of acepromazine maleate on the anesthetic potency of halothane and isoflurane. J Am Anim Hosp Assoc; 24:609-613.

World Health Organization (1991). A proposed standard international acupuncture nomenclature: Report of a WHO Scientific Group. Geneva, Switzerland: World Health Organization.

World Health Organization (2003). Acupuncture: review and analysis of controlled clinical trials. Geneva, Switzerland: World Health Organization.

Wu, B. (1995) Effect of acupuncture on the regulation of cell-mediated immunity in patients with malignant tumors. Zhen Ci Yan Jiu; 20:67–71.

Wu, M.T.; Hsieh, J.C.; Xiong, J.; Yang, C.F.; Pan, H.B.; Chen, Y.C.; et al. (1999) Central nervous pathway for acupuncture stimulation: localization of processing with functional MR imaging of the brain—preliminary experience. Radiology; 212:133–41.

Wu, M.T.; Sheen, J.M.; Chuang, K.H.; Yang, P.; Chin, S.L.; Tsai, C.Y. et al. (2002) Neuronal specificity of acupuncture response: an fMRI study with electroacupuncture. Neuroimage; 16:1028–37.

Zhang, A.L.; Wu, Y.; Jiang, X.L.; (2005) Analysis on therapeutic effect of acupoint injection on chronic hepatitis B (Chinese). Zhongguo Zhen Jiu; 25:25–6.

Zhang, W.; Jin, Z.; Huang, J.; Ahang, Y.W.; Luo, F.; Chen, A.C.; et al. (2003) Modulation of cold pain in human brain by electric acupoint stimulation: evidence from fMRI. Neuroreport; 14:1591–6.

Zhang, W.T.; Jin, Z.; Luo, F.; Zhang, L.; Zeng, Y.W.; Han, J.S. (2004) Evidence from brain imaging with fMRI supporting functional specificity of acupoints in humans. Neurosci Lett; 354:50−3.

Zhao, Z.Q. (2008) Neural mechanism underlying acupuncture analgesia. Prog Neurobiol;85:355e375.

Zhu, C.B.; Li, X.Y.; Zhu, Y.H.; Wu, G.C.; Xu, S.F. (1997) [Alteration of monoamine contents in microdialysate following droperidol enhanced electroacupuncture]. Sheng Li Xue Bao; 49:382e388.

Zhu, Y.H.; Chen, Y.H. (2005) On effects of acupoints and drugs in acupoint injection treatment (Chinese). Zhongguo Zhen Jiu; 25:46–8.