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FACULDADE DE ODONTOLOGIA DE PIRACICABA
UNIVERSIDADE ESTUDAL DE CAMPINAS

JULIANA TRINDADE CLEMENTE

Cirurgiã – Dentista

Participação dos receptores opióides capa periféricos na modulação da resposta nociceptiva induzida pela administração de formalina na ATM de ratos de diferentes sexos e fases do ciclo estral.

Dissertação apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas, para obtenção do Título de Mestre em Odontologia, Área de Fisiologia Oral.

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Orientadora: Profa. Dra. Cláudia Herrera Tambeli

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A Comissão Julgadora dos trabalhos de Defesa de Tese de MESTRADO, em sessão pública realizada em 10 de Fevereiro de 2004, considerou a candidata JULIANA TRINDADE CLEMENTE aprovada.

1. Profa. Dra. CLAUDIA HERRERA TAMBELI _____

2. Prof. Dr. ROBSON FIDALGO AMUÍ _____

3. Prof. Dr. CARLOS AMÍLCAR PARADA _____

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"O sinal mais seguro da Sabedoria é a constante Serenidade".

(Montaigne, séc. XIV)

RESUMO:

Este estudo avaliou as diferenças sexuais na resposta nociceptiva induzida pela administração de formalina na articulação temporomandibular (ATM) com ou sem a co-administração do U50,488 (agonista do receptor opióide capa). As fases do ciclo estral das fêmeas foram citologicamente determinadas e apenas aquelas que apresentavam-se na fase diestro ou proestro, e machos foram incluídos. A formalina induziu um comportamento nociceptivo maior nas fêmeas em diestro do que nas fêmeas em proestro ou machos. O U50,488 reduziu significativamente as respostas nociceptivas induzidas pela formalina, e esta redução foi maior nas fêmeas, especialmente nas fêmeas da fase diestro do ciclo estral. A injeção do U50,488 na ATM contralateral não afetou na magnitude do comportamento induzido pela formalina, e o pré-tratamento com o antagonista seletivo do receptor opióide capa nor-binaltorphimine (norBNI) na ATM ipsilateral reduziu os efeitos antinociceptivos do U50,488. Estes resultados demonstram a ação dos receptores opióides capa periféricos na modulação da dor inflamatória. Além disso, considerando que os níveis plasmáticos dos hormônios ovarianos são baixos durante a fase diestro, estes resultados são consistentes com a hipótese de que os hormônios sexuais femininos podem ter uma ação analgésica na redução da dor inflamatória induzida pela formalina, assim como, também ter uma ação anti-analgésica nos efeitos mediados pelos receptores opióides capa.

Palavras-chave: Articulação temporomandibular; teste da formalina; nocicepção; diferenças sexuais; receptor capa opióide; ciclo estral.

ABSTRACT:

This study examined sex differences in nociceptive responses induced by intra-temporomandibular joint (TMJ) formalin with and without co-administration of the κ-opioid receptor agonist U50,488. The estrous phase of females was cytologically determined; only those in either proestrus or diestrus, and males, were included. Formalin elicited significantly greater nociceptive behavior in diestrus females than in either proestrus females or males. U50,488 significantly reduced formalin nociceptive responses, and this reduction was significantly greater in females, especially in the diestrus phase of the estrous cycle. U50,488 injection into the contralateral TMJ failed to affect the magnitude of formalin-induced behavior, and preinjection of the selective kappa-opioid receptor antagonist nor-binaltorphimine (nor-BNI) into the ipsilateral TMJ significantly reduced the antinociceptive effect of U50,488. These findings support a role for peripheral kappa-opioid receptors in the modulation of inflammatory pain. Furthermore, since plasma levels of ovarian hormones are lowest during diestrus, these findings are consistent with the suggestion that female sex hormones may play an analgesic role in reducing formalin-induced inflammatory pain, and may also play an anti-analgesic role, at least in κ-mediated effects.

Keywords: Temporomandibular joint; formalin test; nociception; sex differences; kappa opioid receptor; estrous cycle

1. INTRODUÇÃO:

A dor sempre foi repleta de significados, no entanto, em qualquer época da história, a dor vem sendo descrita com um fator em comum: é uma experiência que envolve os aspectos biológicos, a alma e o psiquismo (Castillo Ojuras, 1999) .

Atualmente, a Associação Internacional para o Estudo da Dor (IASP) conceitua dor como "uma experiência sensorial e emocional desagradável que é associada a lesões reais ou potenciais, ou descritas nestes termos. A dor é sempre subjetiva. Cada indivíduo aprende a utilizar este termo através de suas experiências" (Mersk, 1986).

O indivíduo que sofre de dor tem seu comportamento modificado, tendendo ao isolamento, tornando-se pouco sociável e muitas vezes, reagindo de forma agressiva ou inadequada perante estímulos habituais. Sendo assim, desde seu início, a humanidade sempre buscou soluções para a eliminação da dor , das mais simples que lhe oferece a natureza até as técnicas mais sofisticadas (Castillo Ojuras, 1999). Hoje cada vez mais se avança nos estudos dos mecanismos da dor e conseqüentemente na elaboração de tratamentos terapêuticos mais eficazes (Millan, 1999).

Das condições dolorosas crônicas da região orofacial, a dor proveniente da ATM é uma das mais freqüentes (Adler, 1992; Irving *et al.*, 1999). Uma análise mais precisa a respeito das condições dolorosas da ATM nos revela que a maioria dos pacientes que apresentam dor associada às disfunções temporomandibulares (DTMs) é do sexo feminino (Von Korff *et al.*, 1988; Krogstad *et al.*, 1992; Johansson *et al.*, 2003). Tem sido demonstrado que a prevalência de DTMs em mulheres, especialmente durante o período reprodutivo, é 1.5 a 2 vezes maior que em homens (Le Resche, 1997a ; Warren & Fried, 2001).

No entanto, apesar da alta incidência das disfunções temporomandibulares, principalmente em mulheres (Von Korff *et al.*, 1988; Dworkin *et al.*, 1990) durante o período reprodutivo (Le Resche, 1997a ; Warren & Fried, 2001), o número de insucessos no tratamento das mesmas ainda é elevado.

Conseqüentemente, o estudo dos mecanismos periféricos, particularmente dos receptores envolvidos no processo de modulação da dor da ATM em ambos os sexos, assim como a utilização de drogas analgésicas que atuem seletivamente em receptores localizados na ATM é de grande relevância clínica.

Os opióides são os analgésicos de mais antigo relato na literatura. Já no século IIIaC, encontra-se relatos sobre o suco da papoula (*opium* – do grego, suco) nos escritos de Theophratus. O uso dos opióides sempre esteve presente no decorrer da história, merecendo destaque: (1) Paracelso – no século XVI, grande difusor desses produtos e, portanto, responsável por seu emprego em massa, utilizava soluções e tinturas de ópio, que definia como a "chave da imortalidade", obtendo ressonantes êxitos terapêuticos; (2) Frederico Guilhermo Sertürner, em 1803, isolou a morfina, chamada assim em honra ao deus do sono Morpheus, pois a sedação da dor se acompanhava da plácida tranqüilidade, ficando o paciente profundamente adormecido; devido a estes efeitos seu maior consumo inicia apenas em 1853, quando (3) Alexander Wood, médico alemão, frente aos efeitos colaterais, foi o primeiro a idealizar a administração periférica da morfina (Castillo Ojuras, 1999).

Martin & Sloan (1977), estudando os diversos efeitos da morfina levantam a hipótese da existência de várias classes de receptores opióides. Atualmente é bem conhecido na literatura que os receptores opióides *mu, delta e capa* estão implicados na modulação da resposta dolorosa. Considerando que eles encontram-se distribuídos não só no sistema nervoso central como também nos neurônios dos gânglios trigeminais (Li *et al.*,1998), espinhais (Kalyuzhny *et al.*,1995) e nas terminações periféricas das aferências somáticas (Fields *et al.*, 1980; Coggeshall *et al.*, 1997), tem sido proposto que os agonistas desses receptores podem influenciar diretamente nos nociceptores aferentes produzindo antinocicepção (Ferreira & Nakamura, 1979; Ferreira *et al.*, 1991; Stein *et al.*, 1993). Nesse contexto, tem sido demonstrado que a administração periférica de morfina na pata (Granados-Soto *et al.*,1997), no lábio (Eisenberg *et al.*, 1996) e na ATM de ratos (Bakke et al., 1998; Cai *et al.*, 2001) produz efeitos antinociceptivos.

Apesar do efeito antinocieptivo produzido pela administração de morfina na ATM de ratos sugerir a presença de receptores opióides funcionais na ATM (Bakke *et al.*, 1998; Cai *et al.*, 2001), não se sabe se existem receptores opióides funcionais do tipo capa na mesma, uma vez que a morfina não é seletiva para nenhum tipo específico de receptor opióide. No entanto, é importante ressaltar que além dos agonistas do recepetor opióide capa produzirem efeitos antinociceptivos na hiperalgesia inflamatória na pata (Machelska *et al.*, 1999; Binder *et al.*, 2001) e na cauda (Ko *et al.*, 2000), tem sido demonstrado que esses efeitos são maiores em fêmeas quando comparadas com machos no modelo de hiperalgesia térmica induzida pelo adjuvante de Freund (Binder *et al.*, 2000; Tershner *et al.*, 2000). Este mesmo efeito tem sido demonstrado em humanos, a administração sistêmica de agonistas do receptor opióide capa produz melhor analgesia na dor pós-operatória em mulheres do que em homens que se submeteram à remoção de terceiros molares inclusos (Gear *et al.*, 1996a; 1996b) e a dose ótima dos mesmos para as mulheres foi menor do que a preconizada (Gear *et al.*, 1999).

Introduzido inicialmente por Dubuisson & Dennis em 1977, o teste da formalina é largamente usado na avaliação de drogas analgésicas sendo considerado um modelo experimental animal de dor inflamatória tônica, válido e seguro (Tjolsen *et al.*, 1992; Teng & Abbott, 1998). A formalina é um agente inflamatório que produz hiperalgesia pela liberação de serotonina e histamina, que estimulam sinergicamente os receptores 5-HT e H1 (respectivamente) nas fibras aferentes primárias (Parada *et al.*, 2001). Quando administrado na ATM produz comportamentos nociceptivos esterotipados quantificáveis como o coçar a região orofacial e o levantar reflexamente a cabeça, podendo ser utilizados como índice de dor (Roveroni *et al.*, 2001). No entanto, estas respostas comportamentais nociceptivas, apresentam-se de forma alternada, sendo complementares, ou seja, os animais que ao longo do período de observação passam a maior parte do tempo coçando a região orofacial, apresentam o comportamento de levantar reflexamente a cabeça em freqüência proporcionalmente menor e vice-versa. Diante disso, essas respostas são avaliadas conjuntamente, somando-se o

período de tempo em que os animais apresentam o comportamento de coçar com o número de vezes em que os animais levantam reflexamente cabeça ao longo do período de observação, reproduzindo de forma mais adequada à intensidade de dor. Para isto, estabeleceu-se que cada movimento caracterizado pelo ato de levantar rapidamente a cabeça equivale a um segundo (Roveroni *et al.*, 2001).

No entanto, apesar da alta incidência das condições dolorosas da ATM em mulheres (Le Resche, 1997b) e da maior eficácia analgésica dos agonistas dos receptores capa no sexo feminino (Gear *et al*, 1996a, 1996b, 1999), não há nenhuma investigação científica até então, sobre a existência de receptores opióides capa funcionais na região da ATM ou sobre o papel desses receptores na modulação da dor proveniente da ATM em ambos os sexos.

Sendo assim, este trabalho teve como objetivo verificar se existem receptores opióides capa funcionais na ATM de ratos, e se o sexo e as diferentes fases do ciclo estral influenciam os efeitos mediados por esses receptores durante a resposta nociceptiva induzida pela administração de formalina na ATM.

2. CAPÍTULO:

O presente artigo foi submetido ao periódico "Pain" e se encontra em avaliação (Anexo 5).

Sexual dimorphism in the antinociception mediated by kappa opioid receptors in the rat TMJ.

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Abstract

This study examined sex differences in nociceptive responses induced by intra-temporomandibular joint (TMJ) formalin with and without co-administration of the κ-opioid receptor agonist U50,488. The estrous phase of females was cytologically determined; only those in either proestrus or diestrus, and males, were included. Formalin elicited significantly greater nociceptive behavior in diestrus females than in either proestrus females or males. U50,488 significantly reduced formalin nociceptive responses, and this reduction was significantly greater in females, especially in the diestrus phase of the estrous cycle. U50,488 injection into the contralateral TMJ failed to affect the magnitude of formalin-induced behavior, and preinjection of the selective kappa-opioid receptor antagonist nor-binaltorphimine (nor-BNI) into the ipsilateral TMJ significantly reduced the antinociceptive effect of U50,488. These findings support a role for peripheral kappa-opioid receptors in the modulation of inflammatory pain. Furthermore, since plasma levels of ovarian hormones are lowest during diestrus, these findings are consistent with the suggestion that female sex hormones may play an analgesic role in reducing formalin-induced inflammatory pain, and may also play an anti-analgesic role, at least in κ-mediated effects.

Keywords: Temporomandibular joint; formalin test; nociception; sex differences; kappa opioid receptor; estrous cycle

Introduction

Although the role of opioid receptors in the central nervous system in pain modulation has been extensively studied, the role of peripheral opioid receptors is not as well established. Opioid receptors have been found in trigeminal (Li *et al.*,1998), dorsal root ganglion neurons (Kalyuzhny *et al.*,1995), and in the peripheral endings of somatic afferents (Fields *et al.*, 1980; Coggeshall *et al.*, 1997), and agonists applied to these peripheral receptors produce antinociception (Ferreira and Nakamura, 1979, Ferreira *et al.*, 1991; Stein *et al.*, 1993). Morphine application into peripheral tissues such as hindpaw (Granados-Soto *et al.*, 1997), lips (Eisenberg *et al.*, 1996) and TMJ (Bakke *et al.*, 1998; Cai *et al.*, 2001), produces antinociception in rats. The antinociceptive effect produced by a TMJ injection of morphine suggests the presence of functional opioid receptors in the TMJ. However it is not known whether kappa opioid receptors are among the opioid receptors located into the TMJ, since morphine is not selective for any specific subtype of opioid receptors. There is now evidence that peripherally acting κ-opioid receptor agonists can produce antinociception in different models of nociception (Hong and Abbott, 1995; Machelska *et al.*, 1999; Ko *et al.*, 2000; Binder *et al.*, 2001). Gender differences in the analgesic response to κ-opioid receptor agonists have also been reported. For example, it has been demonstrated that peripherally acting κ-opioid receptor agonists induce greater antinociception in female than male rats in a model of Freund's adjuvant-induced thermal cutaneous hyperalgesia (Binder *et al.*, 2000) and that systemic κ-opioid analgesics produce

significantly better postoperative analgesia in females than in males in patients who underwent surgery for the removal of their third molars (Gear *et al.*, 1996a; 1996b; 1999). Thus if there are functional kappa opioid receptors into the rat TMJ it is possible that κ-opioid receptor agonists could also induce greater antinociception in females. The aim of the current study was to determine if κ-opioid receptors in the TMJ of the rat play a role in modulating inflammatory pain resulting from formalin injection. We also examined for sex differences by comparing the responses of females in different phases of the estrous cycle and males.

Methods

Animals

This study was carried out in male (n = 76) and female (n = 70) Wistar rats (150 to 250g). The animals were housed in plastic boxes with soft bedding on a 12:12 light cycle (lights on at 6 AM), with food and water available *ad libitum*. They were maintained in a temperature-controlled room (±23°C) and handled for at least one week prior to the experiments. Experimental protocols were approved by the Committee on Animal Research of the University of Campinas and conformed to IASP guidelines for the study of pain in animals (Zimmermann, 1983).

Determination of estrous phase

Estrous phase was determined by daily microscope examination of vaginal smears taken by gentle lavage, between 7 AM and 8 AM. Proestrus and diestrus phases were identified by the predominance of nucleated epithelial cells and leukocytes, respectively (Adler, 1981; Bereiter *et al.*, 2002) in rats that had been observed for at least two consecutive regular 4-day cycles before the day of the experiment (Smith *et al.*, 1975). Proestrus and diestrus phases were chosen because they represent the phases of highest and lowest ovarian hormonal level, respectively (Butcher, *et al.*,1974).

General Procedures

Testing sessions took place during light phase (between 9 AM and 5 PM) in a quiet room maintained at 23°C. Prior to the experiments, each animal was placed in the test chamber (30x30x30cm mirrored-wood chamber with a glass at the front side) for a 15 min habituation period in order to minimize the effects of stress (Abbott *et al.*, 1986).

Drugs

Formalin solutions were prepared from commercially (Sigma) available stock formalin (an aqueous solution of 37 % of formaldehyde) further diluted in 0.9% NaCl (saline) in different concentrations (1, 1.5 and 3% / 15 μl). The κ-opioid receptor agonist, U50,488 (0.3, 3 and 30 μg / 15 μl, Ko *et al.*,2000) and the κ-opioid antagonist nor-binaltorphimine (nor-BNI) (200 μg / 15 μl, Binder *et al.*,2001) were obtained from Sigma. Because it has been reported that nor-BNI may not be selective for κ-opioid receptors until several hours after administration, that is, activity at μ-opioid receptors has been reported (Horan et al., 1992; Spanagel and Shoaib, 1994; Wettstein and Grouhel, 1996), nor-BNI was administered one day prior to the experiment (Schmidt et al., 2002). U50,488 and nor-BNI were dissolved in distillated water.

TMJ injections

The animals were removed from the test chamber and lightly anaesthetized by inhalation of halothane to allow the TMJ injection, which was performed with a 30-gauge needle connected to a 50 µl Hamilton syringe. At the conclusion of the experiments, animals were anesthetized by intraperitoneal injection of a mixture of urethane (1 g/kg) and α-chloralose (50 mg/kg). Evans blue dye (0.1%, 5 mg/Kg) was then intravenously administered in order to visualize formalin-induced plasma extravasation upon *post-mortem* examination of the injected TMJs (Hass *et al.*, 1992). This procedure also allowed confirmation that the plasma extravasation induced by the TMJ injection at the correct site was restricted to the immediate TMJ region.

Testing procedure for TMJ pain

Following the TMJ injection the animal regained consciousness approximately 30 seconds after discontinuing the anesthetic and was returned to the test chamber for a 45 min observation period. The recording time was divided into 15 blocks of 3 minutes and the nociceptive response was determined for each block by measuring the number of seconds that the animal spent rubbing the orofacial region asymmetrically with the ipsilateral fore or hindpaw and/or flinching the head in an intermittent and reflexive way characterized by high frequency shakes of the head as previously described (Roveroni *et al.*, 2001). The observer who recorded the formalin-induced nociceptive behaviors was unaware of the estrous phase during the formalin test. Rats did not have access to food or water during the test.

Statistical analysis

For Fig. 1 a t test or one-way analysis of variance (ANOVA) was used. Tukey post hoc tests were performed to determine the basis of the significant differences between more than two groups. A probability level of less then 0.05 was considered to indicate statistical significance. For Figs. 2 and 3 a two way ANOVA with two between subject factors (dose with 3 and 2 levels, respectively, and sex with three levels) was used. If there was a significant dose x sex interaction, a one-way ANOVA with α-level adjusted by a Bonferroni-type correction for each dose was used to determine at which doses the largest difference in sexes occurred. Data are presented in figures and text as means $\pm$ S.E.M.

Results

Effect of peripheral U50,488 on the nociceptive behavior induced by formalin application into the TMJ region of male rats

Ipsilateral U50,488 (0.3, 3, 30μg) administration attenuated in a dose-related fashion nociceptive behavior induced by formalin ($p<0.05$, Fig. 1A). Contralateral U50,488 (30 μg) administration had no significant effect (Fig. 1A) Prior ipsilateral administration of the κ-receptor antagonist nor-BNI significantly diminished this antinociceptive effect of U50,488 (Fig. 1B).

Sexual dimorphism of the antinociceptive effect of peripheral U50,488

Injection of formalin (1, 1.5 or 3%) into the TMJ region induced dose-dependent nociceptive behavior in males as well as diestrus and proestrus females (Fig. 2). The two-way ANOVA demonstrated a significant main effect of dose ($p<0.001$) and sex ($p<0.001$) but not a significant dose x sex interaction ($p = 0.117$). The behavioral nociceptive response induced by 1.5% formalin was significantly greater in diestrus females than males and proestrus females ($p<0.05$, Tukey test). When 1% formalin was administered in diestrus females and 1.5% formalin was administered in males and proestrus females the magnitude of the behavioral nociceptive response was similar among the groups.

To compare the three sex groups for antinociceptive responsiveness to local application of a κ-opioid agonist, U50,488 (0.3 μg) was co-administered with formalin into the TMJ. Equi-nociceptive doses of formalin, as determined above

(Fig. 2), were administered to the three sex groups; that is, males and proestrus females received 1.5% formalin and diestrus females received 1.0% formalin. The two-way ANOVA demonstrated a significant dose x sex interaction ($p=0.01$) as well as a significant main effect of dose ($p<0.001$) and sex ($p<0.001$). To determine the basis of the significant interaction, one-way ANOVAs (sex with three levels) were performed for each dose. Because this required a separate ANOVA for each of the 2 doses administered (the vehicle group was not included in the analysis), a Bonferroni-type correction was applied such that the α-level was set to 0.025 (*i.e.* $0.05 \div 2$). These analyses demonstrated significant differences between the groups at both the 0.3 μg and the 3 μg doses. Post-hoc analyses revealed that U50,488 attenuated the nociceptive behavior of both diestrus and proestrus females significantly more than males at the dose of 0.3 μg. At a higher dose (3 μg) U50,488 attenuated the nociceptive behavior of diestrus females significantly more than both males and proestrus females at the dose of (Fig. 3). These findings indicate that activation of κ-opioid receptors in the TMJ induces a significantly greater antinociceptive effect in females, especially in the diestrus phase of the estrous cycle.

Discussion

In the present study formalin, injected into the TMJs of female and male rats, resulted in concentration-dependent nociceptive behavior. Females were grouped according to their estrous phase; only those in either diestrus or proestrus were included in the study. Females showed significantly greater nociceptive behavior than males. This finding and the findings of others (Bereiter, 2001; Cairns, 2001, 2002) indicate that females exhibit greater TMJ nociception than males. Despite the findings that TMJ injury produces a greater number of Fos-positive neurons in the trigeminal brainstem complex of proestrus females than diestrus females or males (Bereiter, 2001) we showed that the behavioral nociceptive response induced by a TMJ application of formalin was greater in diestrus females. Since ovarian hormones are lowest during diestrus (Butcher et al., 1974), this finding suggests that the higher levels of these hormones occurring during proestrus may play an antinociceptive role in inflammatory pain. This suggestion is supported by a recent report that the female sex hormone estrogen can suppress inflammatory hyperalgesia (Joseph *et al.*, 2003). Formalin is an inflammatory agent that produces hyperalgesia by the release of serotonin and histamine, which synergistically stimulate nociceptive primary afferent fibers via 5-HT and H1 receptors, respectively (Parada et al., 2001). In contrast, the jaw muscle nociceptive reflex induced by a TMJ injection of glutamate, a non-inflammatory agent, is enhanced by estrogen (Cairns *et al.*, 2002 ; Fiorentino et al., 1999). Although these data suggest that estrogen increases nociceptor sensitivity, it is possible that ovarian hormones indirectly attenuate the TMJ formalin-induced

inflammatory hyperalgesia by reducing the levels of inflammatory mediators released at the injured site (Joseph *et al.*, 2003).

Direct administration of the selective κ-agonist U50,488 into the TM joint significantly attenuated formalin-induced behavior in males and females. Moreover, this effect was probably the result of activation of TMJ κ-receptors since U50,488 injection into the contralateral joint did not reproduce the effect and also because prior injection of the selective κ-antagonist nor-BNI into the ipsilateral joint significantly diminished the antinociceptive effect of U50,488. Although the presence of functional opioid receptors in the rat TMJ has been previously suggested (Bakke *et al.*, 1998; Cai *et al.*, 2001) the current results demonstrate the presence of the κ-opioid receptor subtype in this joint, which is consistent with the expression of κ-opioid receptor mRNA in the trigeminal ganglia (Schafer *et al.*, 1994; Xie *et al.*, 1999).

In order to compare the magnitude of κ-mediated antinociception among males, proestrus females and diestrus females, equi-nociceptive concentrations of formalin as determined from the formalin dose response data were administered in combination with U50,488. That is, 1.5% formalin was administered to males and proestrus females, and the 1% formalin was administered to diestrus females. Under these conditions, U50,488-induced antinociception was significantly greater in females than males, especially in the diestrus phase of the estrous cycle.

The present results suggest that peripheral activation of κ-opioid receptors contributes to the increased sensitivity of females to the effects of systemically

administered κ-opioid receptor agonists (Bartok &Craft, 1999; Gear *et al.*, 1996a, 1996b, 1999; 2003). It is important to point that in contrast to the κ-opioid agonist U50,488, it has been shown that morphine application into the rat TMJ induces greater antinociception in male than female rats (Cai *et al.*, 2001). However, unlike U50,488, morphine is a non selective opiate agonist that acts predominantly at μ-opioid receptors. It is possible that the relative contribution of different opioid receptor subtypes expressed on the primary afferent fibers of the TMJ may reflect the sexual dimorphism of morphine-mediated antinociception.

The physiological basis for the sex-related difference in κ-mediated TMJ antinociception is not presently known. However, our finding that this effect was highest during the diestrus phase of the estrous cycle suggests that female sex hormones may attenuate κ-mediated responses possibly by modulating the function or the expression of κ-opioid receptors within the TMJ (Maggi *et al*, 1999).

Taken together, our findings suggest, perhaps somewhat paradoxically, that the higher ovarian hormone levels in proestrus females may play an antinociceptive role in that diestrus females exhibited significantly greater nociceptive responses to formalin alone, but may also play a partial anti-analgesic role, at least in κ-mediated responses, in that diestrus females exhibited greater attenuation of formalin behavior after U50,488 administration.

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Figure Legends

Figure 1.

Effect of peripheral κ- opioid receptor agonist U50,488 on the TMJ formalin-induced nociceptive response in male rats.

1A - Co-application of increasing concentrations of U50,488 (0.3, 3, 30μg) significantly reduced the magnitude of 1.5% formalin-induced behavioral nociceptive response from the concentrations of 3μg ($p<0.05$, ANOVA, Tukey test). Application of U50,488 (30μg) into contralateral (ct) TMJ did not affect the magnitude of 1.5% formalin-induced behavioral nociceptive response ($p>0.05$, t test). Values are plotted as mean (± S.E.M) and the number of rats in each group is shown in parentheses. Significant differences between groups ($p<0.05$, ANOVA, Tukey test) are indicated by matching pair (s) of symbols above the bars.

1B - Pretreatment of the TMJ with the selective κ-opioid receptor antagonist nor-BNI (200 μg) significantly attenuated the antinociceptive effect of U50,488 (200 μg, $p<0.05$, ANOVA, Tukey test), but did not affect the formalin-induced nociceptive response ($p>0.05$, ANOVA, Tukey test). Values are plotted as mean (± S.E.M) and the number of rats in each group is shown in parentheses. Significant differences between groups ($p<0.05$, ANOVA, Tukey) are indicated by matching pair (s) of symbols above the bars.

Figure 2.

Effect of increasing concentrations of formalin in rats of both sexes

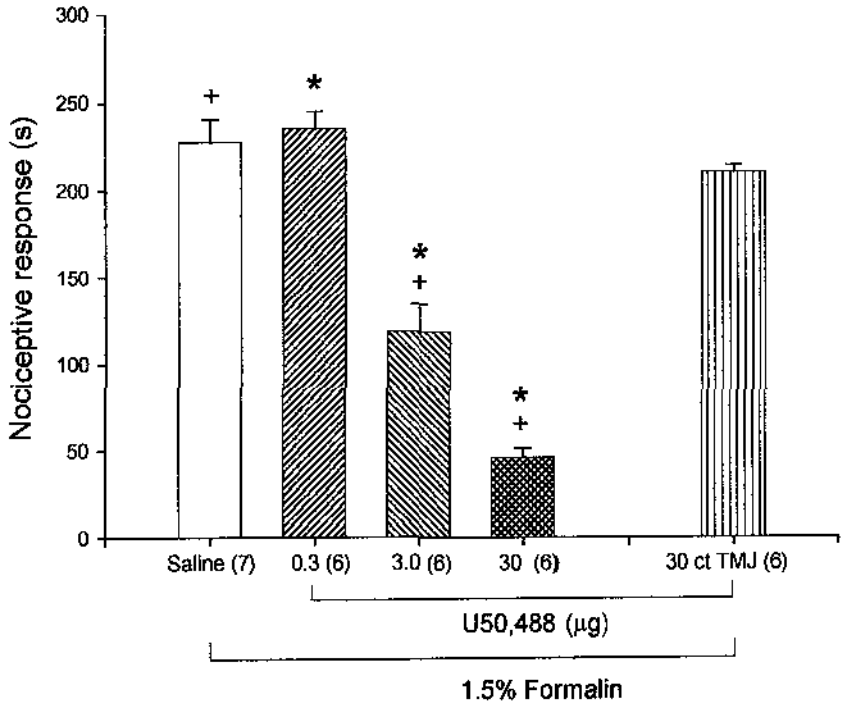
Administration of formalin (1, 1.5, 3%) into the rat TMJ induced a dose dependent behavioral nociceptive response which was significantly higher in diestrus females than males and proestrus females at the concentration of 1.5% ($p<0.05$, two-way ANOVA, Tukey test). The magnitude of nociceptive response was similar among the groups when 1% formalin was administered in diestrus females and 1.5% formalin was administered in males and proestrus females. The significant differences between male, proestrus female and diestrus female rats ($p<0.05$,Tukey) are indicated by matching pair (s) of symbols above the bars. Values are plotted as mean (± S.E.M) of 4 to 8 rats per group.

Figure 3.

Sexual dimorphism of the antinociceptive effect of peripheral U50,488

Co-application of the κ-opioid receptor agonist, U50,488, at the dose of 0.3μg significantly reduced the TMJ formalin-induced nociceptive response in females but not in males. The highest dose of U50,488 (3μg) that completely blocked the TMJ formalin-induced nociceptive response in diestrus female, significantly decreased nociception in males rats ($p<0.001$, Tukey). The significant differences between male, proestrus female and diestrus female rats ($p<0.025$, ANOVA, Tukey) are indicated by matching pair (s) of symbols above the bars. Values are plotted as mean (± S.E.M) of 5 to 6 rats per group.

1A



1B

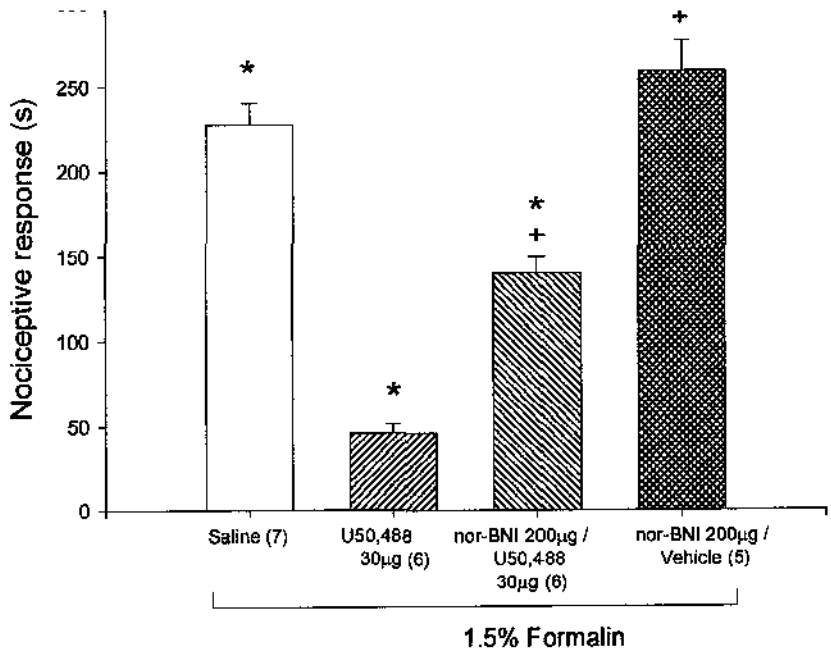


Figure 1

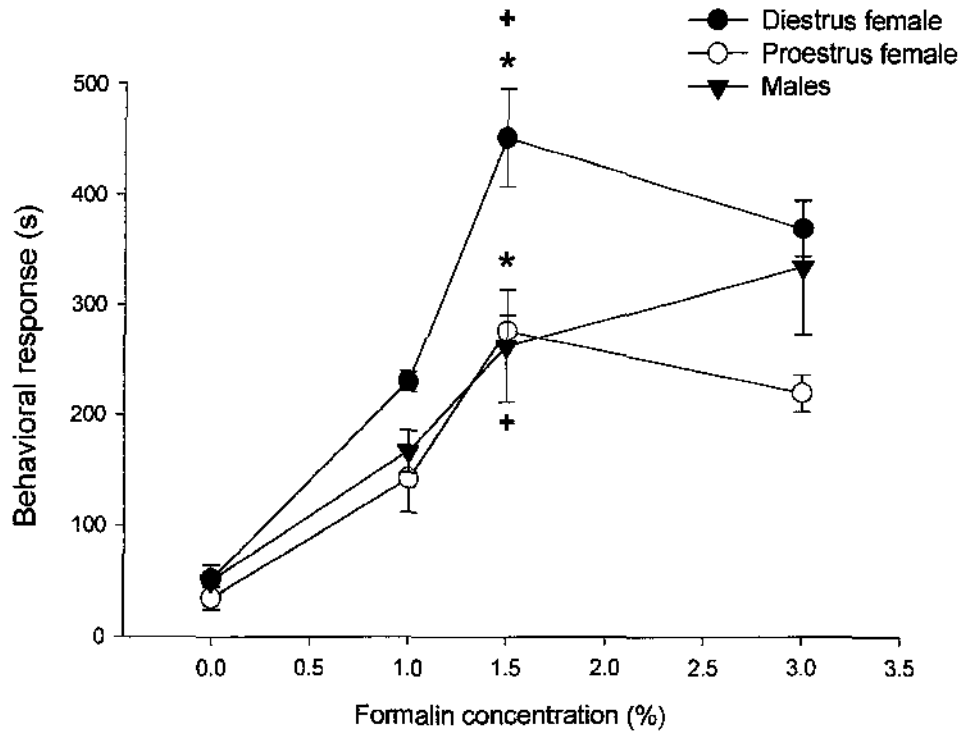

Diestrus female
Proestrus female
Males
Behavioral response (s)
500
400
300
200
100
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Formalin concentration (%)
+
*
*
+

Figure 2

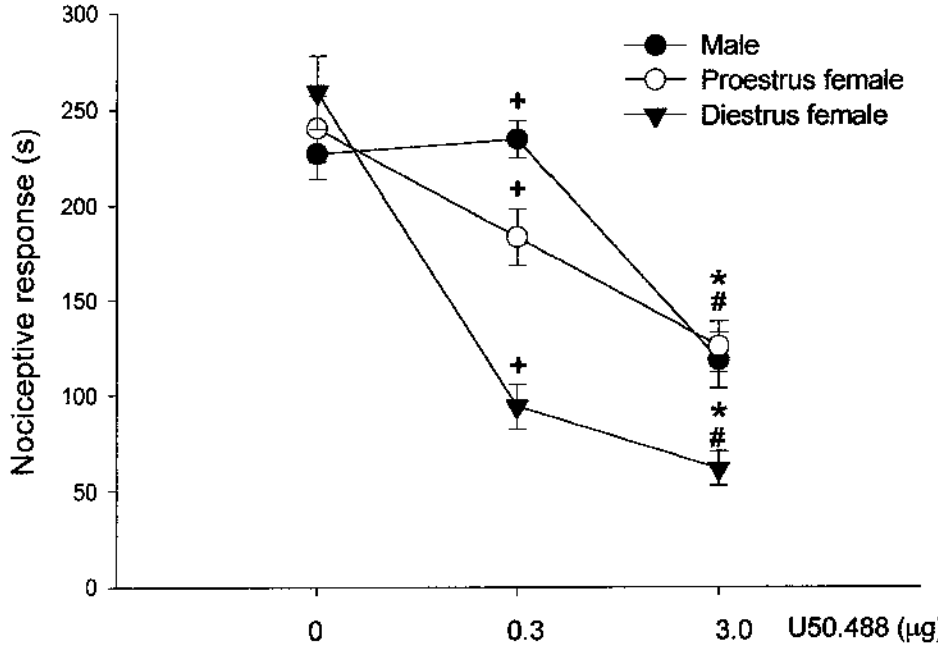

Male
Proestrus female
Diestrus female
Nociceptive response (s)
300
250
200
150
100
50
0
0
0.3
3.0
U50.488 (μg)
+
+
+
*
#
*
#

Figure 3

3. CONCLUSÃO

Este trabalho sugere, de forma paradoxical, que os altos níveis hormonais sexuais na fase proestro podem ter uma ação antinociceptiva, uma vez que as fêmeas em diestro apresentaram maior resposta nociceptiva frente à administração de formalina, porém, também podem ter uma ação anti-analgésica em respostas mediadas pelos receptores opióides capa, sendo que as fêmeas em diestro apresentaram melhor redução das respostas nociceptivas induzidas pela formalina, quando administrado o U50,488.

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ANEXO 1

Ficha utilizada para quantificar as respostas comportamentais, induzidas pela administração de formalina na região da ATM de ratos, duranto o período de observação de 45 minutos.

DATA: HORÁRIO:

GRUPO:

PESO (g):

	0-3	3-6	6-9	9-12	12-15	15-18	18-21	21-24	24-27	27-30	30-33	33-36	36-39	39-42	42-45
CO															
LC															

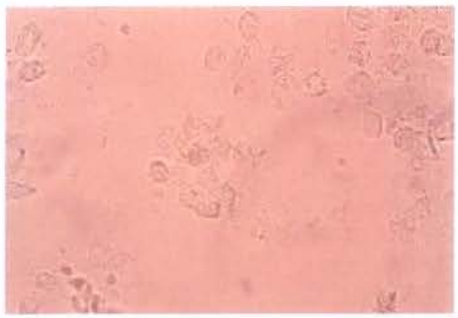
CO: comportamento de coçar a região orofacial (segundos)

LC: comportamento de levantar rapidamente a cabeça (número de vezes)

OBSERVAÇÕES:

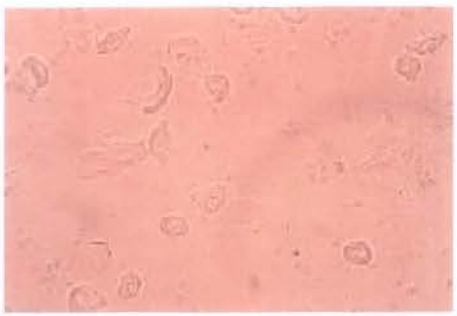
ANEXO 2

DETERMINAÇÃO DAS FASES DO CICLO ESTRAL:

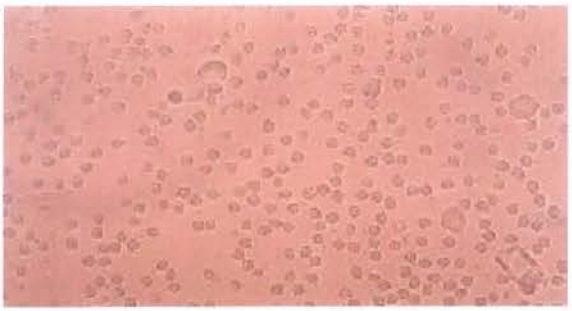


PROESTRO – predomínio de células epiteliais

ESTRO – predomínios de células queratinizadas



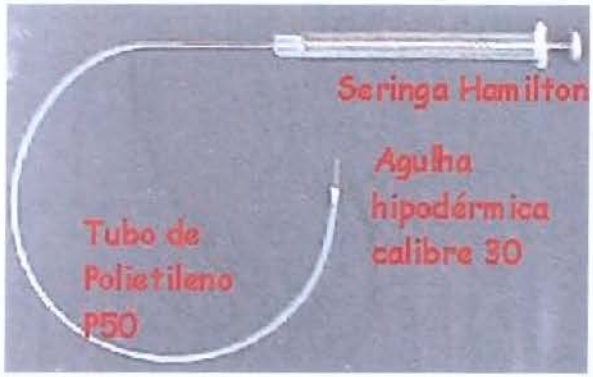
METAESTRO – proporção semelhante dos três tipos celulares



DIESTRO – predomínio de leucócitos

Imagens obtidas através de microscópio óptico, num aumento de 10 vezes.

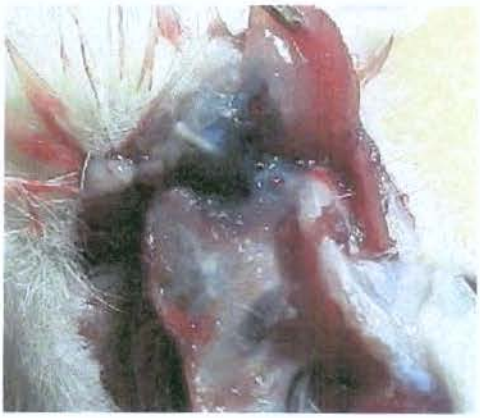
ANEXO 3



Agulha hipodérmica calibre 30 conectada a uma seringa Hamilton de 50µl, através de uma cânula de polietileno P50, utilizada para administração de drogas na região da ATM do animal.

Administração da droga na ATM do animal. Posicionamento da agulha em relação à cabeça do animal.

CONTADOR DE CÉLULAS – utilizado para quantificar o número de vezes que o animal levanta reflexamente a cabeça.
CRONÔMETRO – utilizado para quantificar o tempo, expresso em segundos, que o animal coça a região orofacial.



Identificação visual do sítio de injeção, de acordo com a aparência do corante Azul de Evans extravasado.

ANEXO 4

UNICAMP

INSTITUTO DE BIOLOGIA
UNICAMP

CEEA-IB-UNICAMP

Comissão de Ética na Experimentação Animal
CEEA-IB-UNICAMP

CERTIFICADO

Certificamos que o Protocolo nº 404-1, sobre "Influência do sexo e do ciclo estral na analgesia opióide periférica", sob a responsabilidade de Juliana Trindade Clemente, está de acordo com os Princípios Éticos na Experimentação Animal adotados pelo Colégio Brasileiro de Experimentação Animal (COBEA), tendo sido aprovado pela Comissão de Ética na Experimentação Animal (CEEA)-IB-UNICAMP em reunião de 5/7/2002.

Campinas, 5 de julho de 2002.

CERTIFICATE

We certify that the protocol nº ....., entitled "....., is in agreement with the Ethical Principles in Animal Research established by the Brazilian College for Animal Experimentation (COBEA). This project was approved by the Institutional Committee for Ethics in Animal Research (State University of Campinas – UNICAMP) on __/__/__.

Campinas 5 de julho de 2002.

Profa) Dr(a) Alba R.M. Souza Brito
Presidente – CEEA/IB/UNICAMP

UNIVERSIDADE ESTADUAL DE CAMPINAS
INSTITUTO DE BIOLOGIA
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APÊNDICE

Tabelas suplementares dos resultados demonstrados nas figuras.

TABELA 1: Efeito periférico do agonista do receptor capa opióide U50,488 na resposta nociceptiva induzida pela formalina na ATM de ratos.

TABELA 1A

GRUPOS	SOMA DOS COMPORTAMENTOS (s)
Formalina 1,5% (Machos, n = 7)	227,34 ± 12,29 *
Formalina 1,5% + U50,488 (0,3μg) (Machos,n=6)	234,97 ± 8,82 #
Formalina 1,5% + U50,488 (3μg) (Machos, n = 6)	118,50 ± 14,58 * #
Formalina 1,5% + U50,488 (30μg) (Machos, n=6)	45,49 ± 5,12 * #
Formalina 1,5% ipsi e U50,488 30μg ct (Machos, n = 6)	209,97 ± 3,75

TABELA 1B

GRUPO	SOMA DOS COMPORTAMENTOS (s)
Formalina 1,5% (Machos, n = 6)	227,34 ± 12,29
Formalina 1,5% + U50,488, 30μg (Machos, n = 6)	45,49 ± 5,12 *
Nor-BNI 200μg e Formalina 1,5% + U50,488, 30μg (Machos, n = 6)	139,87 ± 8,89 *
Nor-BNI 200μg e Formalina 1,5% (Machos, n=5)	259,02 ± 16,06

Os dados estão expressos pela média ± EPM. Nesta tabela assim como nas tabelas subseqüentes símbolos iguais indicam diferença estatística ($p<0,05$; Teste Tukey).

TABELA 2: Efeito da administração de diferentes concentrações de formalina na ATM de ambos os sexos.

TABELA 2A

MACHOS (GRUPOS)	SOMA DOS COMPORTAMENTOS (S)
Salina 0,9% (machos, n = 4)	45,025 ± 5,08 [A]
Formalina 1% (Machos; n = 6)	167,12 ± 17,64 [AC]
Formlina 1,5% (Machos; n = 4)	262,17 ± 44,25 [AB]
Formalina 3% (Machos; n = 4)	334,43 ± 52,79 [ABC]

Dados expressos pela média ± EPM.

TABELA 2B

FÊMEAS EM PROESTRO (GRUPOS)	SOMA DOS COMPORTAMENTOS (s)
Salina 0,9% (Fêmea proestro; n = 4)	34,65 ± 8,89 [A]
Formalina 1% (Fêmeas proestro; n = 4)	142,52 ± 27,85 [AC]
Formalina 1,5% (Fêmeas proestro; n = 4)	276,09 ± 12,40 [ABC]
Formalina 3% (Fêmeas proestro; n = 4)	219,59 ± 14,94 [AB]

Dados expressos pela média ± EPM.

TABELA 2C

FÊMEAS DIESTRO (GRUPO)	SOMA DOS COMPORTAMENTOS (s)
Salina 0,9% (Fêmea diestro; n = 4)	51,92 ± 10,78 [A]
Formalina 1% (Fêmeas diestro; n = 6)	230,44 ± 9,20 [AC]
Formalina 1,5% (Fêmeas diestro; n = 4)	451,52 ± 38,63 [ABC]
Formalina 3% (Fêmea diestro; n = 8)	369,02 ± 23,29 [ABC]

Dados expressos pela média ± EPM.

TABELA 2D

GRUPOS	SOMA DOS COMPORTAMENTOS (s)
Salina 0,9% (machos, n = 4)	45,025 ± 5,08
Salina 0,9% (Fêmea proestro; n = 4)	34,65 ± 8,89
Salina 0,9% (Fêmea diestro; n = 4)	51,92 ± 10,78
Formalina 1% (Machos; n = 6)	167,12 ± 17,64
Formalina 1% (Fêmeas proestro; n = 4)	142,52 ± 27,85 *
Formalina 1% (Fêmeas diestro; n = 6)	230,44 ± 9,20 *
Formlina 1,5% (Machos; n = 4)	262,17 ± 44,25 #
Formalina 1,5% (Fêmeas proestro; n = 4)	276,09 ± 12,40 *
Formalina 1,5% (Fêmeas diestro; n = 4)	451,52 ± 38,63 * #
Formalina 3% (Machos; n = 4)	334,43 ± 52,79
Formalina 3% (Fêmeas proestro; n = 4)	219,59 ± 14,94 *
Formalina 3% (Fêmea diestro; n = 8)	369,02 ± 23,29 *

Dados expressos pela média ± EPM.

TABELA 3: Dimorfismo sexual nos efeitos antinociceptivos periféricos do U50,488.

TABELA 3A

MACHOS (GRUPOS)	SOMA DOS COMPORTAMENTOS (s)
Formalina 1,5% (Machos, n=7)	227,34 ± 12,28*
Formalina 1,5% + U50,488 0,3μg (Machos, n=6)	234,97 ± 8,81
Formalina 1,5% + U50.488 3μg (Machos, n=6)	118,49 ± 14,57*

Dados expressos pela média ± EPM.

TABELA 3B

FÊMEAS PROESTRO (GRUPOS)	SOMA DOS COMPORTAMENTOS
Formalina 1,5% (Fêmeas proestro, n=5)	240,34 ± 15,32 *
Formalina 1,5% + U50,488 0,3μg(Fêmeas proestro, n=6)	183,55 ± 13.64 #
Formalina 1,5% + U50,488 3μg (Fêmeas proestro, n=6)	125,49 ± 13,43 * #

Dados expressos pela média ± EPM.

TABELA 3C

FÊMEAS DIESTRO (GRUPOS)	SOMA DOS COMPORTAMENTOS
Formalina 1% (Fêmeas diestro, n=5)	259,32 ± 16,82 * #
Formalina 1% + U50,488 0,3μg (Fêmeas diestro, n=6)	94,15 ± 10,92 #
Formalina 1% + U50,488 3μg (Fêmeas diestro, n=6)	61,59 ± 8,76 *

Dados expressos pela média ± EPM.

TABELA 3D

GRUPO	SOMA DOS COMPORTAMENTOS
Formalina 1,5% (Machos, n=7)	227,34 ± 12,28
Formalina 1,5% (Fêmeas proestro, n=5)	240,34 ± 15,32
Formalina 1% (Fêmeas diestro, n=5)	259,32 ± 16,82
Formalina 1,5% + U50,488 0,3μg (Machos, n=6)	234,97 ± 8,81 *
Formalina 1,5% + U50,488 0,3μg(Fêmeas proestro, n=6)	183,55 ± 13.64
Formalina 1% + U50,488 0,3μg (Fêmeas diestro, n=6)	94,15 ± 10,92 *
Formalina 1,5% + U50.488 3μg (Machos, n=6)	118,49 ± 14,57 *
Formalina 1,5% + U50,488 3μg (Fêmeas proestro, n=6)	125,49 ± 13,43 #
Formalina 1% + U50,488 3μg (Fêmeas diestro, n=6)	61,59 ± 8,76 * #

Dados expresso pela média ± EPM