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The Effect of Carrageenan-Induced Inflammatory Nociception on Distress Vocalizations and the Anxiolytic Efficacy of Drugs in the Chick Separation Stress Paradigm

Ralph Brooks Vance Jr.
The University of Mississippi

THE EFFECT OF CARRAGEENAN-INDUCED INFLAMMATORY NOCICEPTION ON DISTRESS VOCALIZATIONS AND THE ANXIOLYTIC EFFICACY OF DRUGS IN THE CHICK SEPARATION STRESS PARADIGM

By
Ralph Brooks Vance, Jr.

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Approved by

Advisor: Professor Kenneth J. Sufka

Reader: Professor Karen Sabol

Reader: Professor Jay Watson

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ABSTRACT

RALPH BROOKS VANCE, JR: The Effect of Carrageenan-Induced Inflammatory Nociception on Distress Vocalizations and the Anxiolytic Efficacy of Drugs in the Chick Separation Stress Paradigm.

(Under the direction of Kenneth J. Sufka)

The effects of stress on pain perception have been well studied, but only limited research has been conducted on the opposite relationship, the effect of pain on stress level. Fernandez-Guasti (2005) found that nociception caused by uric acid injections, attenuated the anxiety-like behavior of rats in both the elevated plus maze and in the burying behavior test. Additionally, they discovered that the anxiolytic effectiveness of buspirone and diazepam were weakened by the mild nociception. The purpose of this study was to apply the same research question to a different animal model in order to test whether the effects of pain on stress and drug efficacy found by Fernandez-Guasti (2005) generalize to an alternate animal model. Six-day-old chicks received intra-plantar injections of .25% carrageenan based on the inflammatory model published by Roach and Sufka (2003). Two hours post carrageenan injection, the chicks underwent 180 sec testing session either in isolation or with conspecifics in the chick separation stress paradigm (Sufka and Weed, 1994; Sufka and Hughes 1994; Feltenstein et al 2004). Fifteen minutes before the test session chicks received i.p. injections of either vehicle (.9% physiological saline), chlordiazepoxide (8 mg/kg), imipramine (10 mg/kg) or prophylactically (two hours before carrageenan injection) administered dexamethasone (2 mg/kg). After the stress test session, the chicks underwent both an edema test, which measures paw volume, and a nociception test, which measures paw withdrawal latency to a noxious

thermal stimulus. Carrageenan produced robust edema and nocicetive effects while also attenuating distress vocalizations in the separation stress paradigm. Dexamethasone and imipramine attenuated both carrageenan-induced hyperalgesia in the nociception test and distress vocalizations of non-inflamed chicks, but not those of carrageean inflamed chicks, in the separation stress paradigm. Chlordiazepoxide was the only drug that attenuated the distress vocalizations of both inflamed and non-inflamed chicks. From this study it appears that carrageenan inflammation causes a competing response with isolation from conspecifics in the separation stress paradigm, causing fewer distress vocalizations. Additionally, the anti-nociceptive and anxiolytic capablities of drugs such as dexamethasone and imipramine compete with one another, making the ability to adequately measure their anxiolytic efficacy difficult under inflammatory nociception and separation stress.

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INTRODUCTION

The correlation between stress and pain perception can be observed from two fronts. One observation is the effect of stress on pain perception, and the reverse, the effect of perceived pain on stress level. The first of these observations has been considered in both human and animal models. Clinical reports (Cornwall and Donderi 1988; Jones and Zachariae 2002) reveal that under highly stressful conditions, humans perceive pain more intensely, and while in relaxed situations, they report less intense pain perception. The human data are equivocal as stress-induced analgesia is a well studied aspect of the relationship between stress and pain (Nishith, Griffin, and Poth 2002; Girdler et al. 2005). Lee and Rodgers (1990) and Gameiro et al (2006) studied the effect stress has on nociception—stress-induced analgesia—in an animal model and found that exposure to anxiety producing cues produced antinociceptive responses in rats. The antinociceptive action—increased tail flick latency—can be reversed with certain anxiolytics (Rodgers and Randall 1987; Rodgers and Shephard 1989; Nunes-de-Souza et al. 2000); consequently, the relationship between stress and pain perception might be more closely linked than was previously thought. With different results stemming from human and animal models, there is still research necessary in order to make a solid assessment of the effect stress has on nociception.

Since there has been significant research on the effect of stress on pain perception in both human and animal models, it is surprising that there is a dearth of research based on the other side of the relationship—the effect of pain on stress. Fernandez-Guasti (2005) systematically analyzed the ways in which increasing levels of nociception affect

stress level using an animal model. They used the elevated plus maze as well as the burying behavior test to measure anxiety in rats. Inflammatory pain was induced by injecting (i.a.) varying percentages of uric acid. Pain was measured using the PIFIR model (Lopez-Munoz et al. 1993), and the two anxiety tests were performed 3 h post uric acid injection. Three drug challenges were conducted using two anxiolytics, diazepam and buspirone, as well as an anti-inflammatory analgesic, acetylsalicylic acid, which was chosen because it has no known anxiolytic properties. The results of the study provide interesting information about the relationship between pain on stress. Groups receiving mild nociception registered decreased cumulative burying behavior as well as more time spent in the open arms of the elevated plus maze; in other words, the mild nociception acted as an anxiolytic. In the burying behavior test, rats that received mild nociception showed no anxiolytic behavior. Also, in the elevated plus maze, diazepam showed no anxiolytic effect in rats that received both doses of nociception. The anxiolytic-like effects of both buspirone and diazepam were altered by nociception. Additionally, in the groups receiving the antinociceptive drug acetylsalicylic acid, the anti-inflammatory properties of the drug eliminated the effects of the uric acid nociception that had been measured in the elevated plus maze and the burying behavior test. Thus any anxiolytic effect produced by the uric acid was negated. If this pattern of effects holds true, then these effects should generalize to other viable stress and pain models.

The research in the areas of both pain and stress published by our laboratory was conducive to assessing the relationship between pain perception and stress. Roach and Sufka (2003) produced a viable pain study using a chick model. In this study pain was induced using carrageenan powder as the inflammatory agent. A nociceptive test, which

measures pain and analgesia through foot withdrawal latencies to a thermal stimulus, and an edema test, which computes inflammation by measuring paw volume via water displacement, were conducted. In all concentrations of carrageenan used, foot withdrawal latencies were decreased (i.e. hyperalgesia), and edema measures in the inflamed feet were significantly greater than the non-inflamed feet. Antinociceptive and anti-inflammatory drug challenges effectively attenuated edema as well as carrageenan induced hyperalgesia.

Panksepp (1980) and Panksepp et al. (1980) introduced an effective means to observe and measure separation stress in both rats and chicks, respectively, by inducing stress through isolation from conspecifics. Sufka and Weed (1994) and Feltenstein et al (2004) used the chick separation stress paradigm—an apparatus built with six isolation chambers connected to sensitive audio counters—to dependently measure stress in chicks by counting the number of distress vocalizations (dVocs). Various drugs, particularly the known anxiolytics such as imipramine and chlordiazepoxide, attenuated these distress vocalizations during the 180 second test, effectively reducing stress levels in the chicks (Feltenstein et al. 2004).

The purpose of this experiment was to combine the pain and stress models already produced by our laboratory in order to appropriately study the ways pain affects not only stress but also the efficacy of three different classes of drugs, imipramine and chlordiazepoxide with known anxiolytic properties and effectiveness in the separation stress paradigm, and dexamethasone an anti-inflammatory analgesic. The intraplantar carrageenan injections occurred two hours before separation stress test based on the Sufka and Roach (2003) study. Immediately following the separation stress test, the

nociception test (paw withdrawal latency) and the edema test were conducted to insure that the nociceptive manipulation had taken effect. If the model published by Fernandez-Guasti (2005) can be successfully applied to the chick model, the nociception produced should not only attenuate stress measures (dVocs) but also hinder the potency of the anxiolytics in the separation stress paradigm. The experiment will either validate the previous study or provide alternate explanations for their results. In any case this experiment offers further empirical information on the relationship between pain and stress.

MATERIALS AND METHODS

Subjects

Cockerels (*Gallus gallus*; strain W36; Cal-Maine Foods, Mendenhall, MS) were obtained 1-day posthatch and group housed in stainless steel cages (34x57x40 cm), 12-13 chicks per cage. Food and water were available ad libitum through 1qt. gravity fed feeders and water bottles. Room temperature was sustained at 29 ± 1 °C with a light 12h daily light-dark cycle. Daily maintenance was conducted within the first quarter of the light cycle.

Apparatuses

Based on studies conducted by Feltenstein et al. (2004), the separation stress measurement was determined using the "Sufka Chick Separation Stress Paradigm," a six-unit test apparatus containing Plexiglas viewing chambers (25 x 25 x 22 cm) situated in sound-attenuating enclosures. A 25-W light bulb lights each unit with ventilation from an 8-cm-diamter rotary fan (Commonwealth model FP-108AX S1). Miniature video cameras (SuperCircuit Model PC47MC) provided a means for observation during the test

intervals. Distress vocalizations were recorded by microphones (Lafayette Instruments Model 3-675-001) mounted on the ceiling of the Plexiglas chamber and connected to digital sound-activating relays (Lafayette Instruments Model 63040A; setting: 75% sensitivity and 0.10-s delay) that triggered electromechanical counters (Lafayette Instruments Model 58004).

A plantar analgesia meter (Model 390, IITC, Woodland Hills, CA) was used to conduct nociceptive tests. Six cylindrical Plexiglas chambers, 15 cm in length with diameters of 12.5 cm, were positioned atop the glass table in a horizontal arrangement. The radiant heat source was calibrated before the test session by focusing the heat source on a thermister taped to the surface of the glass table. The pilot studies of Roach and Sufka (2003) determined that a 44 °C glass surface temperature produced a10-s withdrawal latency, the midpoint of a 20-s peak cutoff, in healthy 7-day old chicks. Foot edema was measured with a volume meter (Ugo-Basile Plethysmometer, Model 57140, Stoelting, Wood Dale, IL), using an 18-mm liquid filled cell (.5% NaCl + 3mL/L Basile wetting compound). The plethysmometer was calibrated to a standard volumetric mass (1mL) prior to each test session

Procedure

Experiments were conducted 6-days posthatch. The groups were initially separated by three variables: the pain condition (.25% carrageenan), the anxiety treatment condition (mirror [two 20 x 20] or no-mirror) in accordance with Feltenstein et al. (2004), and the drug treatment condition. The carrageenan dose was used according to the findings of Roach and Sufka (2003). Groups of chicks (n = 12) were injected with 0.25% carrageenan 120 min before the anxiety test so that the pain and edema would achieve a

maximal effect (Roach and Sufka 2003). Chicks were injected intraperitoneally (ip) with either Chlordiazepoxide (8 mg/kg), Imiprimine (10 mg/kg) or Vehicle (.9% physiological saline) 15 min before the anxiety test. The group under the drug treatment condition of Dexamethasone (dissolved in DMSO) received a prophylactic intraperitoneal injection 120 min prior to the carageenan injection and 240 min prior to the anxiety test. The chicks were returned to their home cages during the time intervals in between carrageenan injection and drug treatment as well as drug treatment and anxiety test.

During the stress test groups of chicks (n = 6) were placed into the six isolation chambers (one in each chamber) either with a mirror (low stress) or no-mirror (high stress) condition for 180-s testing interval. The dependent measures obtained during the test session were the distress vocalizations (dVocs) and an activity score (0-5), 0 = Lying down, eyes closed, 1 = Lying down, eyes open, 2 = Sitting, 3 = Standing, 4 = Walking/ Moving, 5 = Jumping. This activity score was taken to ensure that the chicks were not incapacitated by either the carrageenan or the drug treatment.

Directly after anxiety testing, the chicks that received carrageenan injections undergwent the nociception and edema tests. The dependent measure for the analgesia test was withdrawal latency, measured in seconds, to a thermal stimulus. Roach and Sufka (2003) define withdrawal as a ballistic vertical withdrawal from the heat source, which was readily distinguishable from ambulation (i.e. slower, horizontal movement). Edema was measured with a plethysmometer, which accurately registered the paw volume of a chick. Paw volume difference was caluculated by subtracting the non-inflamed foot edema score from the carrageenan inflamed foot edema score. In order to

obtain a baseline measure for withdrawal latency and foot volume, one group of non-inflamed chicks underwent the analgesia and edema tests.

All experiments were conducted in accordance with the International Association for the Study of Pain ethical guidelines for investigations of experimental pain in conscious animals (1983) and received approval by the University of Mississippi International Animal Care and Use Committee (IACUC; Protocol No. 06-008).

Statistical Analyses

All analyses were computed using SPSS (v. 11.01). Treatment effects for paw withdrawal latency and foot volume scores were analyzed using a one-way ANOVA using the baseline scores of the non-inflamed group. Treatment effects for drug treatment across inflammation treatment on distress vocalizations were analyzed first by a two-way ANOVA followed by one-way ANOVAs of the carrageenan-inflamed groups and the non-inflamed groups. All post hoc analyses were conducted using Fischer's PLSD (using criterion $p<.05$) for appropriate comparisons.

RESULTS

Edema Measures

The mean paw volume difference scores are summarized in Figure 1. A one-way ANOVA revealed a significant treatment effect, $F(4, 55) = 27.70$, $p<.0001$. Carrageenan produced a robust inflammatory response in all chicks. Additionally, dexamethasone significantly attenuated carrageenan-induced edema. Post hoc analyses revealed that all groups receiving carrageenan incurred a significant increase in paw volume differences compared to the vehicle non-inflamed group, $p^S < .0001$. Dexamethasone significantly

attenuated paw volume differences when compared to the vehicle inflamed groups, $p<.05$, revealing a significant anti-inflammatory effect.

Nociception Test

The effects of three drug probes on carrageenan induced hyperalgesia are summarized in Figure 2. Carrageenan produced a hyperalgesic effect by significantly attenuating chick paw withdrawal latency. Both dexamethasone and imiprimine significantly reversed the carrageenan-induced hyperalgesia shown by the increase in paw withdrawal latency. A one-way ANOVA revealed a significant treatment effect, $F(4,55) = 12.05$, $p<.0001$. Post hoc analyses revealed a significant hyperalgesic effect, represented by the significant attenuation in paw withdrawal latency, produced by carrageenan inflammation, $p<.0001$. Additionally, the hyperalgesic effect produced by carrageenan was significantly attenuated, represented by an increase in paw withdrawal latency, by both imiprimine, $p<.05$, and dexamethasone, $p<.05$, resulting in significantly higher mean paw withdrawal latencies compared to the vehicle inflamed group.

Treatment Effects on Distress Vocalizations

In order to illustrate the effects of social separation stress and carrageenan inflammation on distress vocalization, data analyses and descriptive data of isolated inflamed, isolated non-inflamed, and social non-inflamed groups are summarized in figure 3a. Isolation produced a stress effect by robustly increasing distress vocalizations. The mean distress vocalization of the isolated, carrageenan-inflamed group was lower than the isolated non-inflamed group. A one-way ANOVA of vehicle treated chicks receiving social mirror or isolation treatments revealed a significant treatment effect, $F(2,32) = 44.46$, $p<.0001$. Post hoc analyses revealed a significant increase in distress

vocalization in both isolated groups compared to the social mirror group, p^s<.0001. Additionally, the mean distress vocalization of the isolated carrageenan inflamed group was significantly lower than the isolated non-inflamed group, p<.01.

The effect of drug treatment (chlordiazepoxide, imipramine, dexamethasone) on distress vocalizations across inflammation treatment groups is summarized in figure 3b. A two-way ANOVA of these data revealed a significant main effect for the drug treatment $F(3, 84) = 7.00$, $p<.0005$. The main effects of the drug and inflammation interaction terms were not significant. In order to make the appropriate statistical comparisons, it was necessary to perform a one-way ANOVA of the carageenan inflamed groups and the non-inflamed groups.

In the non-inflamed groups, chlordiazepoxide, imipramine and dexamethasone attenuated distress vocalizations. A one-way ANOVA of the non-inflamed groups revealed a significant treatment effect, $F(3, 43) = 5.28$, $p<.005$. Post hoc analyses showed significant attenuation of distress vocalization by all three drug manipulations, $p<.05$, with chlordiazepoxide showing a slightly more potent anxiolytic effect.

In the carrageenan-inflamed groups, only chlordiazepoxide was able to effectively attenuate distress vocalizations. A one-way ANOVA revealed a significant treatment effect, $F(3,41) = 3.35$, $p<.05$. Post hoc analyses revealed that chlordiazepoxide was the only drug manipulation able to significantly attenuate distress vocalizations, $p<.05$. Both imiprimine and dexamethasone produced no significant difference in distress vocalizations compared to the saline vehicle.

DISCUSSION

Predictions were made based on the research conducted by Fernandez-Guasti et al. (2005) in which they found that inflammatory nociception produced by uric acid actually brought about less anxiety-like behavior in rats as observed both in the elevated plus maze and in cumulative burying behavior. These researchers also discovered that the inflammatory nociception produced by uric acid weakened the anxiolytic effects of two potent anxiolytics, buspirone and diazepam. The purpose of this project was to gain a more complete understanding of the relationship between chronic pain and anxiety, specifically the effects chronic pain might produce on stress measures and how this pain manipulation might alter the responsivity of different classes of drugs, chlordiazepoxide and imipramine, both with anxiolytic potential, and dexamethasone, a potent antinociceptive drug. The present study addressed these research questions using a chick model that combines the carrageenan inflammatory and nociception model characterized by Roach and Sufka (2003) and the chick separation stress paradigm characterized by Sufka and Weed (1994), Feltenstein et al (2004), Warnick, McCurdy and Sufka (2005). Intra-plantar injections of carrageenan not only produced robust edema and hyperalgesia measures in accordance with Roach and Sufka (2003) but also the inflammatory nociception effectively reduced distress vocalizations in the chick separation stress paradigm. Additionally, the carrageenan inflammation altered the responsiveness of the two anxiolytics chlordiazepoxide and imipramine. Dexamethasone, the anti-inflammatory drug, was administered to ensure that carrageenan inflammation alone caused the changes in distress vocalizations. The results of the experiment were generally consistent with the findings of Fernandez-Guasti (2005) which are detailed below.

Carrageenan showed robust effects in both the nociception test and the edema test. All subjects that received carrageenan displayed large edema measures as well as significantly low paw withdrawal latencies, effectively displaying the carrageenan induced edema and hyperalgesia. These findings are consistent with the results of Roach and Sufka (2003) that showed intraplantar injections of .25% carrageenan significantly increased edema and significantly decreased paw withdrawal latencies, and further demonstrate carrageenan as an effective inflammatory agent.

There has been significant research showing that separation from conspecifics causes a measurable state of stress in the chick (Sufka and Weed 1994; Feltenstein et al 2004; Warnick, McCurdy and Sufka 2005). Chicks that underwent the 180s separation stress test in isolation had significantly elevated distress vocalizations when compared to those tested in the social mirror condition. These results are consistent with the research of Sufka and Weed (1994), Feltenstein et al. (2004) in which they showed that isolation from conspecifics causes a heightened sense of stress in a chick.

Carrageenan induced inflammation produced less anxiety-like behavior for chicks in the separation stress paradigm by significantly attenuating distress vocalizations. Fernandez-Guasti (2005) found that inflammatory nociception in rats caused less anxiety-like behavior in two anxiety tests. Rats that received uric acid inflammation showed more exploration of open arms in the elevated plus maze as well as decreased cumulative burying behavior. The present findings can be interpreted and explained in three different ways. The chicks that are both isolated and carrgeenan inflamed are in either a less stressful state or a more stressful state than the chicks that are isolated and not carrageenan inflamed. A literal interpretation of the fact that carrageenan inflammation

results in fewer distress vocalizations might suggest that fewer distress vocalizations directly correlates to a less stressful state. However, this interpretation seems incomplete and perhaps inherently flawed because it assumes that a chick experiencing a combination of carrageenan inflammation and separation stress is somehow in a reduced stress state compared to a chick that experiences only the separation stress engendered by isolation.

If the carrageenan inflamed and isolated chicks are experiencing more stress due to the additive effects of inflammation and separation stress, then higher distress vocalizations would be expected. However, there are a few explanations that could account for the observed less anxiety-like behavior in the chicks. If the stress level in a chick is translated onto an inverted "U" curve for distress vocalizations, a higher stress level (measured in distress vocalizations) would be shown by fewer distress vocalizations on the descending curve of the inverted parabola. Under this explanation, the separation stress brought on by isolation is represented at the peak of the inverted parabolic curve. The additive stress created by carrageenan inflammation compounds the already high level of stress and pushes the distress vocalizations down the descending arm of the inverted parabola. This theory fits our vehicle data in that the carrageenan-inflamed chicks produced fewer distress vocalizations which might suggest that the stress level has reached the descending arm of the inverted U-curve. However, the inconsistency comes in our anxiolytic drug data. If the chicks are in an intensified stress state then an anxiolytic drug should attenuate the stress level and, in the inverted U-curve theory, the distress vocalizations should actually increase in order to show a decrease in stress level.

Our data is inconsistent with this theory since chlordiazepoxide significantly attenuates distress vocalizations in the carrageenan-inflamed, isolated chicks.

Another theory perhaps best explains how an isolated and carrageenan-inflamed chick could be in a greater state of stress but exhibits less anxiety-like behavior. The reduction in distress vocalizations occurred because of a competing response between the separation distress and the carrageenan inflammation. In this way the chick is unable to allocate its total attention to either of the two stressful manipulations, resulting in decreased distress vocalizations. Explanations two and three are plausible but not incompatible. Because inflammation causes a competing response with isolation-induced separation stress, distress vocalizations may not be the best indicator of stress level when combining multiple stressors. A novel marker, such as corticosterone level (Feltenstein et al. 2003), that is not subject to the confounds of a competing response is necessary to obtain a better overall stress measure.

Dexamethasone, a potent anti-inflammatory was administered as a control measure to verify that inflammatory nociception was the cause of any distress vocalization change in the separation stress paradigm. Dexamethasone significantly attenuated both carrageenan-induced edema and hyperalgesia. These results are consistent with the research of Roach and Sufka (2003) that showed all doses of prophylactically administered dexamethasone significantly attenuated chick edema scores and significantly attenuated carrageenan-induced hyperalgesia by increasing paw withdrawal latencies, and reveal the efficacy of dexamethasone both as an anti-inflammatory and anti-nociceptive agent. In an unexpected finding, dexamethasone produced an anxiolytic response in the non-inflamed chicks by significantly attenuating

distress vocalizations. These findings are consistent with the recent work of De Medeiros, Reis and Mello (2005) in which dexamethasone successfully attenuated C-Fos expression in certain parts of the rat brain. Since C-Fos expression is a valid indicator of stress, dexamethasone shows anxiolytic action by significantly attenuating C-Fos expression. Such results reveal modest anxiolytic activity in dexamethasone and are consistent with the anxiolytic effects dexamethasone displayed in the chick separation stress paradigm for isolated, non carrageenan-inflamed birds. When the stress level of the chicks was affected by carrageen-induced inflammatory nociception, dexamethasone produced no effect on the distress vocalizations. The competing response caused by the multiple stress stimuli, inflammation and isolation, eliminated the anxiolytic effectiveness of dexamethasone. Since dexamethasone has both anxiolytic and anti-inflammatory capablities, its effectiveness in both capacities was in competition. As an anti-inflammatory agent it counteracted the stress effect produced by inflammatory nociception. Inflammatory nociception significantly attenuated distress vocalizations in the vehicle inflamed chicks; therefore, if dexamethasone counteracted the effect of inflammatory nociception, an increase in distress vocalizations would be expected. This did not occur because the anxiolytic activity of dexamethasone—the ability to attenuate distress vocalizations—was in competition with the anti-inflammatory activity of dexamethason—the ability to increase distress vocalizations. With the anxiolytic action of dexamethasone attempting to decrease distress vocalizations and the anti-inflammatory action of dexamethasone attempting to increase distress vocalizations, almost no change in mean distress vocalizations was measured.

Imipramine significantly attenuated carrageenan-induced hyperalgesia. These findings are consistent with the work of Feuerstein (1997) in a human model in which imipramine was modestly effective anti-nociceptive agent. Additionally, Bhargava and Saha (2001) found that imipramine showed dose dependent anti-nociception in the rat tail-flick test. Furthermore, imipramine, like dexamethasone, showed a significant anxiolytic effect by attenuating distress vocalizations in the non carrageenan inflamed birds. This is consistent with previous studies by Feltenstein et al. (2004) in which imipramine produced the same type of anxiolytic effect in the chick separation stress paradigm. Imipramine, again like dexamethasone, failed to produce any anxiolytic effect in isolated, carrageenan-inflamed birds. Because imipramine has both anti-nociceptive and anxiolytic properties, its effectiveness as an anxiolytic alone competed with its anti-nociceptive effectiveness. In a similar way to dexamethasone, the anti-nociceptive action of imipramine would cause an increase in distress vocalizations, as the effects of inflammatory nociception in the separation stress paradigm are negated by imipramine. Additionally, the anxiolytic potential of imipramine to attenuate distress vocalizations would exist. These two actions competed with one another resulting in no effect on distress vocalizations.

A double competing response caused the mean distress vocalization differences first for the vehicle isolated, carrageenan-inflamed chicks, and then the isolated, carrageenan-inflamed, imipramine or dexamethasone receiving chicks. For the vehicle chicks, inflammatory nociception was in competition with isolation-induced stress, inhibiting the chick from focusing on either stressful manipulation. This competing response also existed for isolated, inflamed chicks that received a drug treatment of either

dexamethasone or imipramine. However, the additional competition of anti-inflammatory or anti-nociception action with anxiolytic action in these two drugs added another level of competing response and altered the efficacy of these drugs on distress vocalizations.

Because chlordiazepoxide is strictly an anxiolytic, it did not show any analgesic or anti-inflammatory activity in either the paw withdrawal latency test or the edema test. In the chick separation stress paradigm chlordiazepoxide produced a robust anxiolytic effect, attenuating distress vocalizations in isolated, non carrageenan-inflamed birds. This is consistent with the studies of Feltenstein et al. (2004) and Feltenstein et al. (2003) in which chlordiazepoxide exhibited anxiolytic activity both behaviorally by attenuation of distress vocalizations, and biologically with corticosterone levels. Chlordiazepoxide, unlike dexamethasone and imipramine, significantly attenuated distress vocalizations of isolated, carrageenan-inflamed birds. Because it is such a strong anxiolytic, without analgesic properties, there was no competition between the effect of anxiolytic activity and anti-inflammatory or anti-nociception activity on distress vocalizations as observed in dexamethasone and imipramine. Without a competitive force to counter it, the strong anxiolytic action of chlordiazepoxide was able to attenuate the distress vocalizations of isolated, carrageenan-inflamed chicks.

This study produced data that further highlights the complex relationship between pain and stress. Just as the inflammatory and separation stress stimuli compete for the attention or responsiveness of the chick, so do these stimuli enact the same competition for the responsiveness of drugs, such as dexamethasone and imipramine, that have both anxiolytic and analgesic capabilities. Drugs such as chlordiazepoxide, that are focused on only one of the stressful stimuli, separation stress, remain effective agents of anxiolysis,

even in the presence of inflammatory nociception. More research should be done, perhaps using a massive dose response study combined with a few biological end points, such as corticosterone level or body temperature, and another behavioral end point, such as the elevated six-arm maze. Follow-up studies will allow us to better understand the relationship between chronic pain and stress level in the chick model.

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APPENDIX

FIGURE 1

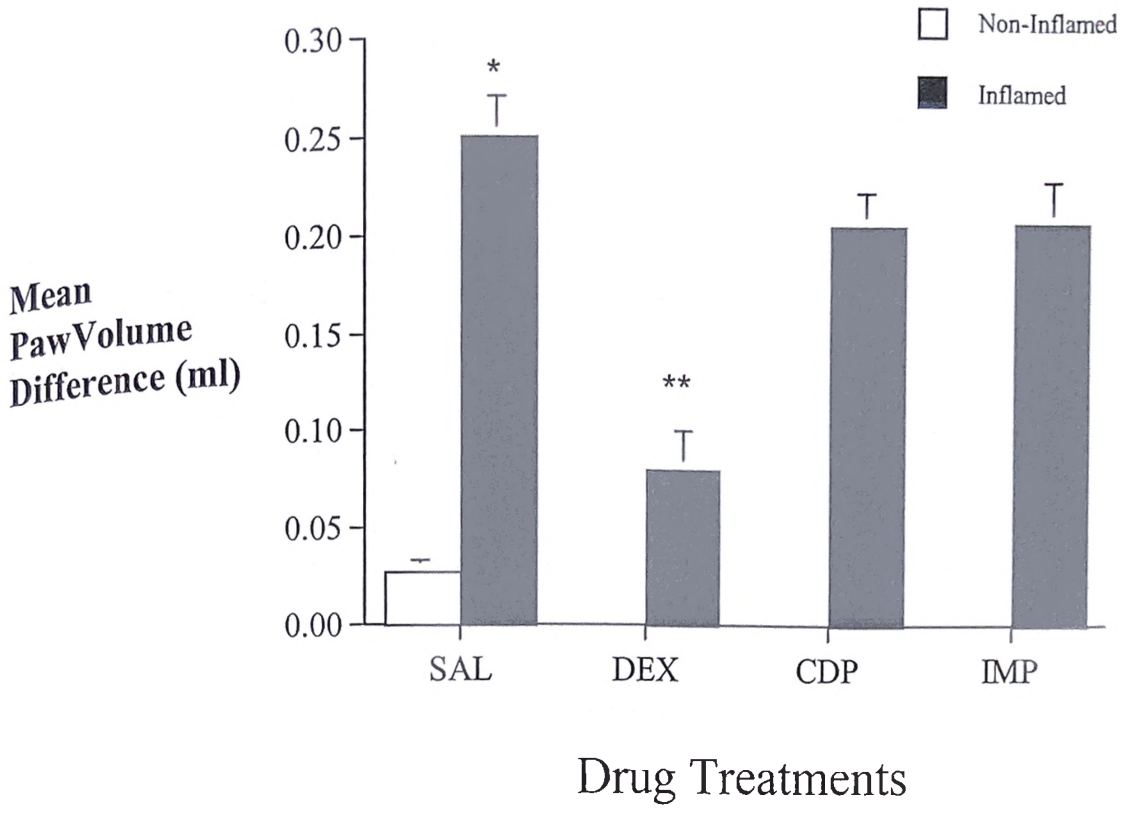

Non-Inflamed
Inflamed
Mean PawVolume Difference (ml)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
*
**
SAL
DEX
CDP
IMP
Drug Treatments

FIGURE 2

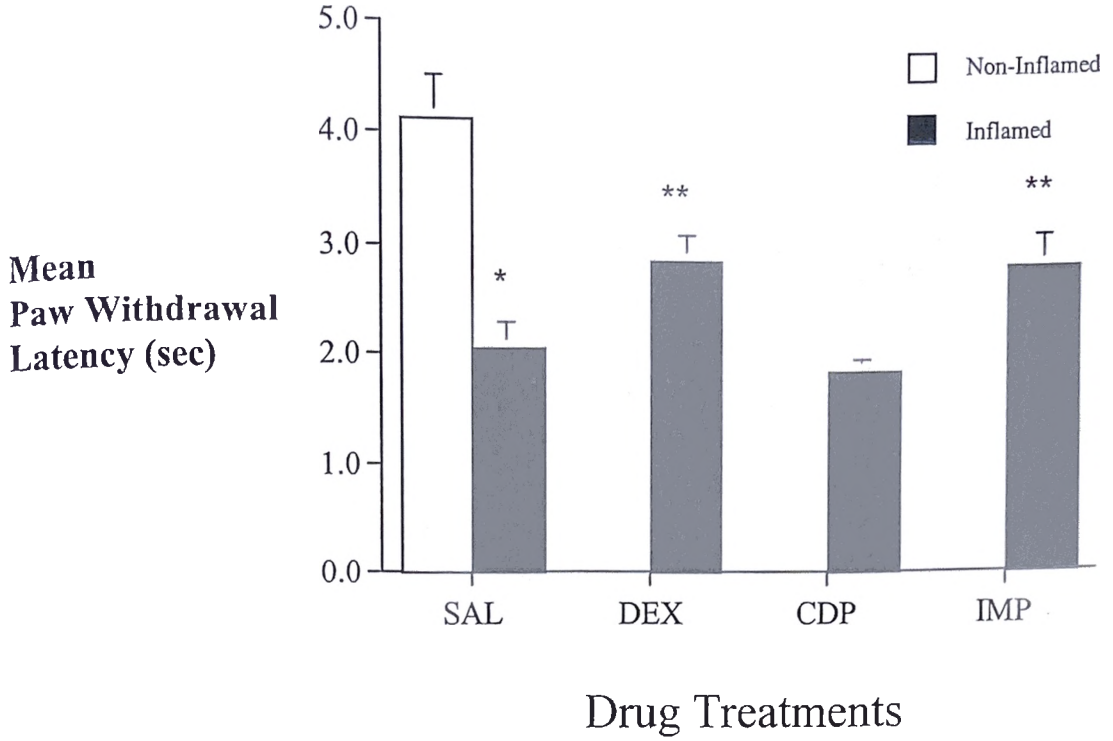
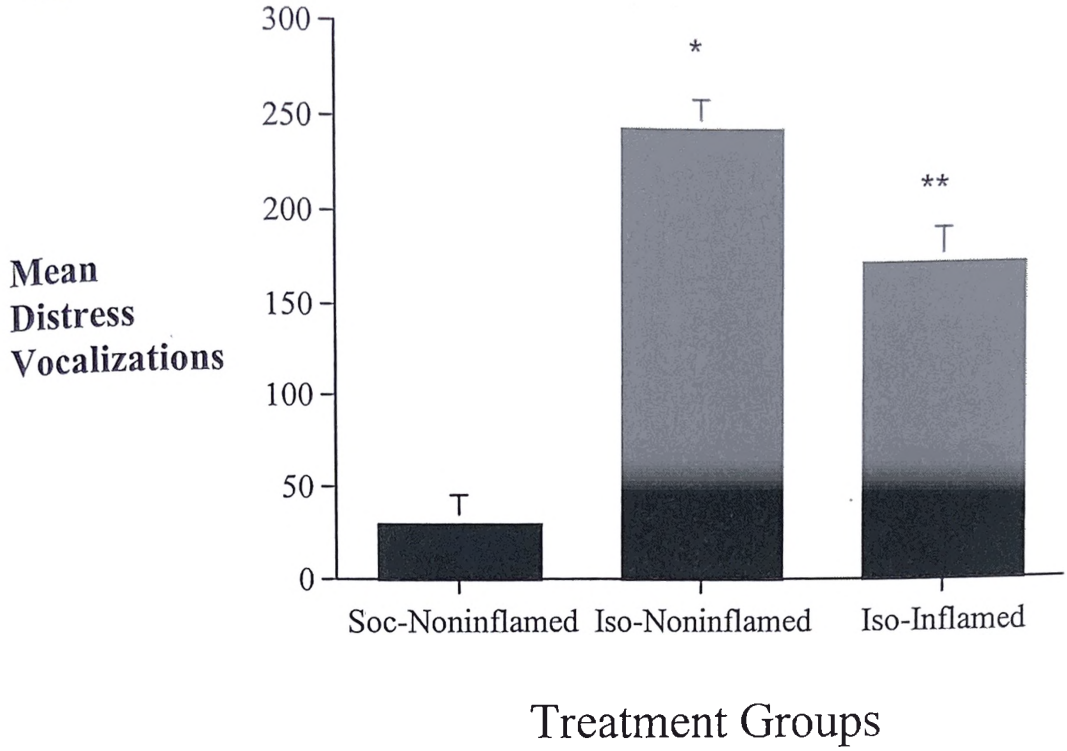

Mean
Paw Withdrawal
Latency (sec)
5.0
4.0
3.0
2.0
1.0
0.0
Non-Inflamed
Inflamed
*
**
**
SAL
DEX
CDP
IMP
Drug Treatments

FIGURE 3

A


Mean Distress Vocalizations
300
250
200
150
100
50
0
*
**
Soc-Noninflamed
Iso-Noninflamed
Iso-Inflamed
Treatment Groups

B

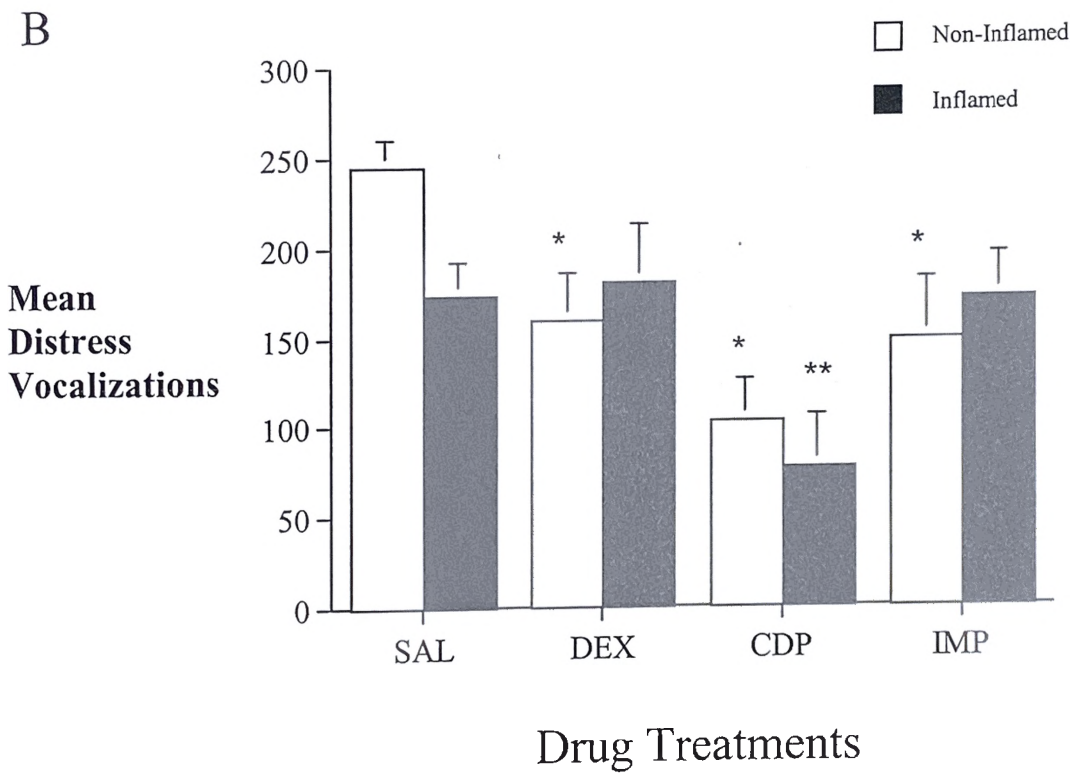

Non-Inflamed
Inflamed
Mean Distress Vocalizations
300
250
200
150
100
50
0
*
*
**
*
SAL
DEX
CDP
IMP
Drug Treatments

FIGURE CAPTIONS

Figure 1

The effects of carrageenan inflammation and drug treatment on edema. Edema data are presented as the mean (± S.E.M.) paw volume difference between inflamed and non-inflamed feet. Open bars represent non-inflamed groups while closed bars represent carrageenan inflamed groups. The abbreviations SAL, DEX, CDP, and IMP represent the drug treatments of vehicle saline, dexamethasone, chlordiazepoxide, and imipramine, respectively. * Indicates a significant increase in paw volume compared to the non-inflamed saline group. ** Indicates a significant attenuation of the carrageenan-induced inflammation. All *P* values <.0001. (n= 12 per group).

Figure 2

The effects of carrageenan inflammation and drug treatment on thermal nociception. Nociceptive data are presented as the mean (± S.E.M.) paw withdrawal latency to a noxious thermal stimulus. Open bars represent non-inflamed groups while closed bars represent carrageenan inflamed groups. The abbreviations SAL, DEX, CDP, and IMP represent the drug treatments of vehicle saline, dexamethasone, chlordiazepoxide, and imipramine, respectively. * Indicates a significant carrageenan-induced hyperalgesic response. ** Indicates a significant attenuation of the carrageenan-induced hyperalgesia. All *P* values <.05. (n= 12 per group).

Figure 3

The effects of isolation and carrageenan inflammation (panel A) and carrageenan inflammation and drug treatments (panel B) on distress vocalizations. Values represent mean (± S.E.M.). Open bars represent non-inflamed groups while closed bars represent carrageenan inflamed groups. For Panel A* indicates a significant isolation stress effect and ** indicates a significant attenuation of distress vocalizations. For Panel B * indicates a significant attenuation of distress vocalizations in the non-inflamed groups and ** indicates a significant attenuation of distress vocalizations in the carrageenan inflamed groups. All *P* values <.05. (n= 12 per group).