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Contribution of the Ventromedial Hypothalamus to Generation of the Affective Dimension of Pain

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Abstract

The ventromedial hypothalamus (VMH) is a core structure underlying the generation of affective behaviors to threats. The prototypical threat to an individual is exposure to a noxious stimulus and the dorsomedial division of the VMH (dmVMH) receives nociceptive input. The present study evaluated the contribution of the dmVMH to generation of the affective reaction to pain in rats. Noxious tailshock elicits from rats vocalization afterdischarges (VADs) that have distinct spectrographic characteristics and are a validated model of the affective reaction to pain. VAD-like vocalizations (vocalizations with the same spectral characteristics of VADs) were elicited by stimulation (electrical or chemical) of the dmVMH. Stimulation in the vicinity of the dmVMH was ineffective in eliciting VADs. Manipulation of GABA_A neurochemistry within the dmVMH altered the threshold for elicitation of VADs by dmVMH stimulation or tailshock. Administration of the GABA_A antagonist bicuculline or the GABA_A agonist muscimol into the dmVMH lowered and elevated VAD thresholds, respectively. These treatments did not alter thresholds of other tailshock elicited responses (vocalizations during tailshock or spinal motor reflexes). Bicuculline and muscimol administered into the dmVMH also elevated and lowered the asymptotic level of fear conditioning supported by dmVMH stimulation or tailshock. These findings demonstrate that the dmVMH contributes to the processing of pain affect and that the affective dimension of pain belongs to a broader class of sensory experience that represents threat to the individual.

Keywords

Nociception; Emotion; Hypothalamus; GABA_A receptor; Pavlovian conditioning; Vocalization

1. Introduction

The affective-motivational dimension of pain underlies the suffering and disability associated with the pain state (Bernard and Bandler, 1998; Loeser, 2000). Despite this preeminent clinical significance, little is known regarding the neuronal mechanisms responsible for production of the affective component of the pain experience. The ventromedial hypothalamus (VMH) is a forebrain site implicated in generation of pain affect. The dorsomedial division of the VMH (dmVMH) receives nociceptive afferents (Bester et al., 1995; Bernard et al., 1996; Braz et al., 2005), and neural activity is evoked in dmVMH by noxious peripheral stimulation in animals (Bullitt, 1990; Parry et al., 2002). Although current neuroimaging technology does not permit identification of individual hypothalamic nuclei the medial hypothalamus is activated in humans exposed to noxious stimulation (Hsieh et al., 1996; Zubieta et al., 2001; Petrovic et al., 2004). Contribution of dmVMH to production of pain affect is suggested by its involvement in processing stimuli that threaten the individual and in organizing the execution of innate

defensive behaviors (Siegel, 2005). As exposure to a noxious stimulus is the prototypical threat to an individual it is likely that nociceptive afferents to the dmVMH would mediate execution of innate affective reactions.

Research in this laboratory validated a rodent model of pain affect. Vocalization afterdischarges (VADs) occur immediately following application of noxious tailshock and have distinct spectrographic characteristics compared to vocalizations that occur during shock (VDSs; Borszcz, 1995; Borszcz and Leaton, 2003). Systemically administered drug treatments that preferentially suppress the affective reaction of humans to pain (Gracely et al., 1978; Price et al., 1985) also preferentially suppress production of VADs (Borszcz et al., 1994). Generation of VADs is suppressed by damage of or drug treatments into forebrain sites known to contribute to production of the affective response of humans to clinical and experimental pain (Mark et al., 1961; Hoffmeister, 1968; Sweet, 1980; Borszcz, 1999; Zubieta et al., 2001; Borszcz and Leaton, 2003; Harte et al., 2000, 2005; Nandigama and Borszcz, 2003; Greer et al., 2005). Additionally, the capacity of noxious tailshock to support fear conditioning is directly related to its production of VADs (Borszcz, 1993; Borszcz, 1995; Borszcz and Leaton, 2003).

The present study evaluated whether electrical or chemical stimulation of the dmVMH elicits VAD-like vocalizations (i.e., vocalizations with the same spectrographic characteristics as VADs). Because the dmVMH is under tonic GABA_A inhibition (Siegel, 2005) the effects of manipulating GABA_A neurochemistry in dmVMH on production of VADs elicited by dmVMH stimulation and tailshock also were evaluated. Finally, dmVMH-mediated generation of the affective state associated with noxious stimulation was validated via Pavlovian fear conditioning. Manipulations of GABA_A neurochemistry within dmVMH that altered thresholds of VADs elicited by dmVMH stimulation or tailshock were assessed for their capacity to alter the asymptotic level of fear conditioning using either dmVMH stimulation or tailshock as the unconditional stimulus. The results demonstrate the involvement of the dmVMH in production of the innate affective reaction to pain.

2. Materials and Methods

2.1. Subjects.—Eighty-three male Long-Evan rats (90-120 days old) were used. Rats were housed individually in a temperature-controlled vivarium illuminated on a 14:10-h light/dark cycle, and given ad lib access to food and water. Rats were handled 1-2 times per day for at least one week before testing. All testing was conducted during the light portion of the cycle. The experiments were approved by the Institutional Animal Care and Use Committee.

2.2 Surgery & Histology.—Unilateral intracerebral implants (cannulae, electrodes or chemitrodes) were made under aseptic conditions using sodium pentobarbital anesthesia (45 mg/kg, i.p.) supplemented with atropine sulfate (.5 mg/kg) to reduce mucous secretions. Implants were directed toward the dorsomedial division of the ventromedial hypothalamus (dmVMH), ventrolateral division of the ventromedial hypothalamus (vlVMH), lateral hypothalamus (LH), dorsomedial hypothalamus (DMH), or perifornical area. The side of implantation was counter-balanced for all experiments. The initial coordinates were obtained from the atlas of Paxinos and Watson (1998) and then adjusted from the histology of a series of preliminary surgeries. Stainless steel 26-gauge guide cannulae (Plastic One, Roanoke, VA) used for drug microinjections were implanted 2 mm dorsal of these targets. Implants were affixed to the skull with three stainless steel screws and cranioplastic cement. Guide cannulae were fitted with 33-ga dummy cannulae to maintain their patency.

At the conclusion of testing, rats were administered a lethal dose of sodium pentobarbital (120 mg/kg, i.p.). Sites that received drug injections were then marked by the injection of 0.25 μ l of safranin-O dye. To verify placements of stimulating electrodes an anodal current was passed through the electrode. Rats were then perfused intracardially with normal saline followed by

10% buffered formalin. The brains were removed from the skulls and stored in 10% buffered formalin for 24 hr and then transferred to a 20% sucrose-formalin solution for a further 24 hr. Coronal sections were cut at 40 μ m on a freezing microtome. Every third section was mounted and stained with cresyl violet. Placements were assessed by reconstructing them on diagrams derived from the Paxinos and Watson (1998) brain atlas by an experimenter unaware of the behavioral outcomes.

2.3. Apparatus—Testing was controlled by custom computer programs via a multifunction interface board (DT-2801, Data Translation, Marlboro, MA) installed in a PC. The same apparatus was used for all behavioral testing (see photograph in Borszcz, 1995). Rats were restrained in a custom-made torso suit with Velcro strapping. They were restrained to a Plexiglas pedestal by additional strapping that ran underneath their torso through loops in the suit. This design maintained rats in a crouching posture throughout training, permitted them to breath and vocalize normally, and permitted unobstructed access to the head. The apparatus was contained within a sound-attenuating anechoic isolation chamber.

2.3.1. Brain Stimulation.: A custom designed (STIMTEK: Arlington, MA) constant current stimulator provided electrical brain stimulation. The stimulator permitted computer control (via D-to-A and DIO ports) of the timing, intensity and frequency of stimulation (pulse duration was set manually). The stimulator also provided a voltage output that was proportional to the intensity of current. This voltage output was monitored by the computer (via A-to-D port, 500 Hz sampling rate) to detect current fluctuations. Electrodes were constructed of insect pins (.23 mm diameter in the shaft that narrowed to the tip) insulated (Epoxytite) except at the tip. Tips were polished to provide a tip diameter of .12 mm. Current return to the electrode was via an Amphenol plug attached to skull screws.

2.3.2. Intracerebral Drug Injections.: Intracerebral injections were administered in a constant volume of 0.25 μ l via 33-gauge injectors (Plastics One, Roanoke, VA) that extended 2 mm beyond the end of the guide cannula. Injectors were connected via polyethylene tubing to a 1 μ l Hamilton syringe. Injections began 2 min after insertion of the injector into the cannula, and were made at a constant rate over 2 min via an infusion pump (Model PHD 2000, Harvard Apparatus, Holliston, MA). Injectors were left in place for 2 min after the completion of injections to aid the diffusion of drugs into the tissue.

2.3.3. Intracerebral Drug Injection at the site of Brain Stimulation.: Chemitrodes consisted of two concentric stainless steel cannulae (20-ga and 30-ga). The inner (30-ga) cannula was insulated with Epoxytite. A removable 33-ga stimulating electrode (uninsulated) was cut flush with the inner cannula (i.e., the stimulating electrode was insulated by the inner cannula except at its tip = .12 mm diameter). For drug injections (.25 μ l volume at .175 μ l/min), the stimulating electrode was replaced with a 36-ga stainless-steel injector that was connected via polyethylene tubing to a 1 μ l Hamilton syringe. The injector did not penetrate the brain rather drug was injected into the cannula and permitted to passively diffuse into tissue. This procedure limited damage to the brain site that subsequently received electrical stimulation. Drug injections were made by an infusion pump as described above with the exception that the injector was left in place for 4 min to ensure diffusion of drug into tissue.

2.3.4. Tailshock.: Shock electrodes were constructed of two 0-ga stainless steel insect pins. The pins were held in place by the chucks of insect pin holders that were imbedded in plastic clothespins. The tension of the clothespins on the tail was adjusted to insure that circulation to the tail was not restricted. The electrodes were placed intracutaneously (.5 mm below the skin surface) on opposite sides of the tail 7.0 cm (cathode) and 8.5 cm (anode) from the base. Current was delivered to the tail via a custom made computer controlled shocker (STIMTEK,

Arlington, MA). The intensity, duration and timing of tailshocks were controlled by the computer. Current intensity was monitored by an A-to-D converter that digitized (500 Hz sampling rate) an output voltage of the shocker that was proportional to the current delivered.

2.3.5. Vocalization Recording.: Vocalizations within the audible range of frequencies (0-20 kHz) were measured by a pressure-zone microphone (Realistic Model 33-1090, Tandy, Ft. Worth, TX) placed approximately 16 cm in front of the rat. The microphone was attached to an audio amplifier (Technics Model SA-160, Tandy, Ft. Worth, TX) and a 10-band frequency equalizer. The frequency equalizer was adjusted to selectively amplify frequencies above 1500 Hz. At 80 dB, frequencies below 1500 Hz were attenuated by approximately 12 dB. The response function of the system was relatively flat (± 0.5 dB) from 1500 to 20000 Hz. The filtering of low frequencies prevented extraneous noise (i.e., animals' respiration and movement artifacts) from contaminating vocalization records. The output of the amplifier was integrated by a contour following integrator (2 ms time base, Coulbourn Instruments, Allentown, PA) and then digitized (500 Hz sampling rate) by an A-to-D converter of the computer.

The audio system was calibrated by determining the relation between the peak digitized output of the A-to-D converter and the amplitude (SPL, B Scale) of a 3.0 kHz pure tone—the approximate fundamental frequency of pain-induced vocalizations of the rat (Borszcz, 1995; Borszcz and Leaton, 2003). The derived function was used to convert A-to-D inputs to decibels (dB). Sound intensities up to 113.0 dB could be measured. The most intense vocalization measured during any sampling period was 102.7 dB. The computer recorded the peak intensity (in decibels), latency (in milliseconds), duration (in milliseconds) and number of vocalizations elicited by brain stimulation and tailshock. The ambient background noise level in the isolation chamber was 54.0 dB. Sounds above 57.0 dB were considered vocalizations.

Ultrasonic vocalizations (USV, 22 kHz) were measured with a heterodyne bat detector (Mini-2 Bat Detector, Ultra Sound Advice, London, UK). The output of the bat detector was amplified and integrated by a second contour following integrator (2 ms time base). The output of the integrator was digitized by a separate A-to-D converter. The computer recorded the latency (in milliseconds), duration (in milliseconds) and number of USV in response to brain stimulation and tailshock.

2.3.6. Spectrographic Analysis of Vocalizations.: During testing audible vocalizations were also recorded on stereo cassette tape for subsequent spectrographic analysis. The unfiltered vocalizations were recorded on one track of the cassette tape (Optimus SCT-36 high-speed stereo cassette deck, Tandy, Ft. Worth, TX). The other track recorded different computer generated tones (not audible to the rat in the isolation chamber) that identified different testing intervals. For sonographic analysis the audio tapes were digitized (44.1 kHz sampling rate) by an Audiomedia card (Digidesign Inc., Menlo Park, CA) installed in a NuBus slot of a Macintosh IIfx computer. The Tape Deck module of Sound Designer II software (Digidesign Inc.) controlled the digital recording. This software was also used to display both channels (vocalizations and tones) of the audio tapes and select the vocalizations to be analyzed. Selected vocalizations were transferred to Soundscape/8 software (GW Instruments Inc., Sommerville, MA) for sonographic analysis. Spectrograms were produced using the Spectrogram module with the display range set at 0-20 kHz and the number of data points used by the FFT algorithm set at 2048. The Average Spectrum module with the display range and number of FFT points set as for spectrogram production generated the power spectra.

2.3.7. Spinal Motor Reflex (SMRs) Measurement.: SMRs (tailflick and hindlimb movement) were assessed in response to tailshock. Rats' tails, distal to the shock electrodes, were attached via cotton thread to a semi-isotonic displacement transducer (Lafayette

Instruments Model 76614, Lafayette, IN). The arm of the transducer was positioned behind and perpendicular to the tail such that the thread extended in a straight line directly behind the rat. Movement of the transducer arm beginning with shock onset was used to measure SMRs. The output voltage of the transducer was amplified (x50) and then digitized (500 Hz sampling rate) by an A-to-D converter. This system was calibrated by determining the relation between digital conversions of voltage outputs from the transducer/amplifier and millimeter movements of the transducer arm. The computer used this derived function to convert digitized voltages to millimeters of tail movement. SMR was defined as movement of the transducer arm by at least 1 mm. Once SMR criterion was exceeded the output voltage of the transducer was monitored for 2000 ms. The computer recorded the latency (in milliseconds), peak amplitude (in millimeters) and magnitude (integrated voltage output expressed as centimeters x milliseconds) of tail movement on each trial. Displacements up to 100 mm could be detected.

2.4. Procedures—On two consecutive days prior to the beginning of each experiment rats were preexposed to the testing apparatus for 20 min. Following each test session, the testing apparatus was cleaned with 5% ammonia hydroxide to eliminate stress odors (Fanselow, 1985).

2.4.1. Vocalizations elicited by Brain Stimulation.: During a test session, brain stimulation (monophasic rectangular cathodal current) was administered thirteen times with its intensity and duration set at 0.80 μ A (1ms pulses) and 10 sec, respectively. The frequency of stimulation ranged from 10 Hz to 640 Hz in equal log units. During testing the frequency of stimulation was randomly varied on each trial. Modulating frequency of the stimulus rather than amplitude or pulse duration maintained the area of stimulation constant while increasing the number of elicited neural impulses (Yeomans, 1990). The peak amplitude, duration, and number of vocalizations were recorded during brain stimulation and for 5 sec following stimulation offset. On three additional trials (i.e., catch trials) no stimulation was presented to assess false alarm rates.

2.4.2. Vocalizations elicited by Bicuculline.: Prior to 4 separate test sessions, separated by 5-7 days, rats were administered either saline or 3 doses of bicuculline methiodide (BIC: 10 pmol, 20 pmol and 40 pmol) unilaterally into the dmVMH or surrounding sites. Drugs were administered on a quasi-Latin square schedule with the restriction that highest dose of BIC (40 pmol) was given first or last to one-half of the rats. Comparison of responses to this dose of BIC at these times permitted determination of the effects of testing and repeated drug administration on BIC-elicited vocalizations (i.e., order effects). Following bicuculline or saline administration the peak amplitude, duration, and number of vocalizations were recorded during 20 consecutive 30 sec epochs.

2.4.3. GABA_A modulation of Vocalization Thresholds.: One group of rats received unilateral implants of chemitrodes directed toward the dmVMH. As described above, rats received electrical stimulation of dmVMH and threshold frequencies for production of VADs were determined for each rat. Prior to three separate test sessions (separated by 5-7 days) rats were administered into the dmVMH either saline, a sub-threshold dose of BIC (dose that did elicit vocalizations = 10 pmol), or the GABA_A agonist muscimol (MUS: 100 pmol free base). Pilot studies demonstrated that this dose of MUS did not elicit any vocal responses.

The effects of BIC (10 pmol) and MUS (100 pmol) administered into dmVMH on thresholds of VADs elicited by tailshock were assessed in a second group of rats. Rats received unilateral implants of a cannula directed toward the dmVMH. The experimental design was identical to that used to assess thresholds of VADs elicited by dmVMH stimulation. Rats received a randomized series of 13 tailshocks (DC, 20 ms pulses at 25 Hz for 1 sec) ranging from .02-2.5 mA in equal log steps and three catch trials. Vocalizations were recorded during tailshock

(vocalizations during shock = VDS) and for the 2 sec interval following shock offset (VADs). The effects of drug treatments of SMR thresholds also were evaluated.

Drugs were administered on a quasi-Latin square schedule with the restriction that saline treatments were given first or last to one-half of the rats. Comparison of responses to saline treatment at these times permitted determination of the effects of testing and repeated drug administration on baseline responding (i.e., order effects).

2.4.4. Pavlovian Fear Conditioning.: Fear conditioning was conducted using the Pavlovian conditional vocalization paradigm (Borszcz, 1995-see photograph; Borszcz and Leaton, 2003) in which vocalizations (VCRs) are conditioned to a conditional stimulus paired with an aversive unconditional stimulus. The conditional stimulus (CS) was light provided by a 60-W incandescent bulb in the otherwise dark isolation chamber. The lightbulb was placed approximately 4 cm in front and 12 cm above the rats' head. Either tailshock or dmVMH stimulation served as the unconditional stimulus (US). Intensity of the US was set as the mean frequency of dmVMH stimulation (112 Hz) or the mean current intensity of tailshock (.42 mA) that produced 3/4 maximal responding under control conditions. When dmVMH stimulation was the US it was presented for 5 sec and overlapped the last 5 sec of the 30 sec light CS. When tailshock was the US it was present for 1 sec and overlapped the last 1 sec of the 6 sec light CS. The latency from CS onset, duration, number, and peak amplitude of VCRs generated during the CS-period (portion of CS presentation that did not overlap with the US) was recorded on each trial. The sonographic characteristics of VCRs also were analyzed.

Groups received three conditioning sessions with each session separated by 48 hrs. Each session consisted of 15 trials of paired presentations of CS and US presented on a VI 2.5 min schedule. A non-signaled presentation of the US (probe trial) was presented on the 16th trial of each session. Probe trials permitted assessment of unconditional responses (URs = VADs, VDSs, SMRs) uncontaminated by the occurrence of VCRs. Performance of URs was also recorded on the first training trial of Day 1. Prior to each training session separate groups of rats were administered either saline, sub-threshold dose of BIC (10 pmol), or MUS (100 pmol) into the dmVMH. Separate control groups were administered drugs (saline, BIC = 10 pmol or MUS = 100 pmol) prior to each training session but given unpaired presentations of CS and US (Unpaired Controls).

Forty-eight hours after the last training session rats were exposed to the CS on three extinction trials and then given one probe trial. This session was not preceded by drug injections and permitted determination of whether differences in conditioning observed during training were attributable to the effects of drugs on the rats' ability to vocalize, or reflected differences in fear conditioning.

2.5. Data Analysis

2.5.1. Thresholds.: Following testing, data were re-organized in ascending order of the frequency of brain stimulation or intensity of tailshock. For each rat on each session, log frequency of brain stimulation or log current intensity of tailshock was plotted against performance (amplitude, duration, 1/latency) of vocalizations. Least-squares analysis (Prism 3.0, GraphPad Software, San Diego, CA) plotted the best-fit regression line over the dynamic range of each performance variable. Threshold frequency and threshold current intensity was calculated as the x-axis intercept. Drug-induced changes in thresholds were assessed via one-way ANOVA followed by post-hoc pair-wise comparisons using Student's t-test for correlated groups with Bonferroni correction of the alpha level (.05)

2.5.2. Bicuculline-elicited Vocalizations.: For 10 min following bicuculline or saline administered into the dmVMH, the peak amplitude, duration, and number of vocalizations were

recorded during consecutive 30 sec epochs. Dose-dependent differences in the performance of elicited vocalizations were assessed by two-factor repeated measures ANOVA. Dunnett's multiple comparisons test assessed whether vocalizations generated during each epoch differed from vocalizations recorded during the 30 sec baseline epoch that immediately preceded dmVMH injections.

2.5.3. Pavlovian Conditioning.: Differences between groups in the performance (amplitude, duration, number and 1/latency) of VCRs generated over the 45 conditioning trials were assessed by two factor repeated measures analysis of variance (ANOVA). Increases in the performance of VCRs generated by each group over the 45 conditioning trials were analyzed via one factor repeated measures ANOVA. Group differences in unconditional responses (VAD, VDS and SMR) during the first conditioning trial and probe trials were analyzed via Student's t test for independent groups.

2.5.4. Spectrograms.: The frequencies of the fundamental and harmonics of vocalizations were calculated from the peaks of the power spectra by an experimenter unaware of the type of vocalization being analyzed. Differences in the frequencies of the fundamental and harmonics of VCRs, VADs, and VDSs were analyzed via one-way ANOVA and Student's t test for independent groups. Group differences in the spectrographic characteristics of VCRs, VADs, and VDSs also were assessed by Student's t test for independent groups.

3. Results

3.1. Experiment 1: Vocalizations Elicited by Electrical Stimulation of dmVMH—

Electrical stimulation of the dmVMH elicited VAD-like vocalizations in all rats ($n = 6$, ESB in Figure 1A). The spectrographic characteristics of these vocalizations were identical to VADs elicited by tailshock (Figure 1B, Table 1). With increases in stimulus frequency the power in the fundamental frequency and harmonics increased but there was no change in frequency characteristics. The amplitude, duration and 1/latency of VADs increased with increases in the frequency of stimulation, all $F_s > 49.80$, $ps < .001$ (Figure 2B). Over their dynamic range each performance variable was significantly correlated with frequency of dmVMH stimulation, all $r^2 > .86$, all $ps < .001$. Threshold frequencies of VADs (x-axis intercept of the performance vs. log frequency function) calculated using the different performance variables did not differ ($F < 1.0$): amplitude = 33.6 ± 4.83 Hz, duration = 34.6 ± 5.72 Hz, 1/latency = 32.7 ± 5.17 Hz. Stimulation of dmVMH did not elicit VDS-like vocalizations or ultrasonic vocalizations (USVs).

Stimulation of the lateral hypothalamus (LH, $n = 5$) failed to elicit vocalizations (Figures 2A & 2B). Other areas in the vicinity of the dmVMH that were stimulated were localized in the DMH ($n = 2$), vlVMH ($n = 2$) and the perifornical area ($n = 1$). Stimulation of the DMH and vlVMH elicited VAD-like vocalizations at the highest frequencies (Figures 2A & 2B). However, these VADs had long latencies, were of low amplitude and short duration, and performance was not related to frequency of stimulation. No vocalizations were recorded during catch trials indicating that vocalizations did not occur spontaneously but were elicited by brain stimulation.

3.2. Experiment 2: VADs Elicited by Chemical Stimulation of dmVMH—

To assess whether VADs elicited from the dmVMH reflect activation of cell bodies rather than fibers of passage the capacity of chemical stimulation of dmVMH to elicit VADs was evaluated. The dmVMH is under tonic GABA_A inhibition and injection of bicuculline (BIC = GABA_A antagonist) into dmVMH elicits vocalizations in cats associated with defensive responding (Strzelczuk and Romaniuk, 1995). It was therefore predicted that BIC would produce dose-dependent increases in VADs.

Spectrographic characteristics of vocalizations elicited by injection of BIC into dmVMH were identical to that of VADs elicited by electrical stimulation of dmVMH or tailshock (Figure 1A & B). The power in the fundamental frequency and harmonics increased with increases in amplitude (i.e., dose of drug), but there were no significant differences in the frequency of the fundamental or in the number of harmonics (Table 1).

The number, peak amplitude and duration of VAD-like vocalizations recorded during each sampling epoch were directly related to the dose of BIC injected into dmVMH ($n = 6$, Figure 2C). Omnibus two-factor (dose and time) repeated measures ANOVA revealed significant main effects of dose, $F_s > 407.1$, $ps < .001$, and time, $F_s > 41.8$, $ps < .001$, and significant Dose $\times$ Time interactions, $F_s > 15.9$, $ps < .001$, on all measures of VAD generation. These interactions reflect the finding that whereas the two highest doses of BIC elicited VADs ($F_s > 16.9$, $ps < .001$) the lowest dose of BIC failed to elicit VADs ($F_s < 1.0$). Comparison of the two highest doses yielded significant main effects of dose, $F_s > 132.8$, $ps < .001$, and time, $F_s > 40.9$, $ps < .001$, and a significant Dose $\times$ Time interactions, $F_s > 3.8$, $p < .001$, indicating a higher incidence and higher level of performance of VADs following administration of the highest dose of BIC. This analysis was confirmed by comparison of VADs generated during each epoch following BIC administration with VADs generated prior to BIC administration during the baseline epoch (BL, Dunnett's test, $p < .05$). VADs were generated during a greater proportion of the testing session following administration of the highest dose of BIC.

No order effects of BIC treatments were observed. No differences in VADs elicited by 40 pmol BIC were observed in subgroups that were administered this dose first or last in the testing sequence. Comparison of these groups revealed significant main effects of time, $F_s > 22.5$, but the main effect of order and the Order $\times$ Time interactions were not significant, $F_s < 1.0$.

No VDS-like vocalizations were recorded following injection of BIC into dmVMH. Bouts of 22 kHz USV were occasionally recorded following administration of the highest dose of BIC. These vocalizations were never the initial vocal response but emerged toward the end of the testing session as VADs began to dissipate. All rats generated VADs following administration the two highest doses of BIC, but only a subset of rats (2 of 6 rats) generated USVs following injection of the highest dose of BIC.

Injection of the highest dose of BIC into the vicinity of the dmVMH was ineffective in eliciting any type of vocalization (Figure 1A & 2C). Prior to two test sessions a separate group of rats received unilateral injections of 40 pmol of BIC or saline into the LH ($n = 2$), perifornical area ($n = 2$), DMH ($n = 2$) or vlVMH ($n = 2$). No differences were observed in the generation of vocalizations from these sites and the data from these animals were combined for the purpose of statistical analysis. Comparisons of VAD performance following saline and BIC treatment revealed no significant differences ($F_s < 1.0$, Figure 2C).

3.3. Experiment 3: dmVMH Contributes to Generation of VADs elicited by

Noxious Stimulation—The previous experiments demonstrated that stimulation of the dmVMH elicits vocalizations with the same spectral characteristics as VADs elicited by noxious tailshock. However, it has yet to be determined whether the dmVMH contributes to the generation of pain-elicited VADs. It is possible that VADs elicited by stimulation of the dmVMH or tailshock are mediated by different integrative circuits within the limbic forebrain that converge upon a common set of brainstem neurons that coordinates the execution of the same vocal response (Jürgens, 2002). The present study evaluated this possibility by examining whether manipulations of GABA_A neurochemistry within the dmVMH that modify VADs elicited by stimulation of the dmVMH also modify VADs elicited by tailshock. Corresponding changes in the generation of these vocalizations will provide evidence that the dmVMH contributes to pain-elicited VADs. VAD thresholds were assessed following administration

into dmVMH of a subthreshold dose of BIC (dose that did not directly elicit VADs = 10 pmol), muscimol (MUS: GABA_A agonist = 100 pmol) or saline.

Drug treatments within the dmVMH produced corresponding changes in the thresholds of VADs elicited by dmVMH stimulation ($n = 6$) or tailshock ($n = 6$). Thresholds calculated using the different performance variables did not differ (all $F_s < 1$, $p_s > .95$). Thresholds of both types of VADs were raised by MUS and lowered by BIC treatments (Figure 3A & B). Comparisons of thresholds following saline with thresholds following MUS and BIC revealed significant differences ($t_s > 3.5$, $p_s < .025$). Changes in thresholds reflect the finding that the stimulus-response functions for both types of VADs were shifted to the right by MUS and shifted to the left by BIC. Although VAD thresholds were altered by drug treatments, performance of VADs was not affected as indicated by the parallel stimulus-response functions, and the equivalent maximum responding exhibited under the different drug treatments (Table 2). Therefore, changes in VAD thresholds do not reflect the effects of drugs on motor performance. Thresholds of VDS and SMR were not altered by BIC or MUS injections into the dmVMH when compared to following injection of saline into dmVMH ($t_s < 1.0$, Figure 3C & D).

Comparisons of response thresholds following saline treatments from subgroups administered saline first or last in the testing sequence revealed no order effects ($t_s < 1.0$). False alarm rates during catch trials were low (VAD - dmVMH stimulation = 0.2%, VAD - tailshock = 0.17%, VDS = 0.2%, SMR = 0.24%) indicating that responses did not occur spontaneously but were elicited by dmVMH stimulation or tailshock.

The spectrographic characteristics of VADs elicited by dmVMH stimulation were identical to that observed in the previous experiment and the administration of BIC and MUS did not alter the spectral characteristics (Table 1). Spectrographic characteristics of VADs elicited by tailshock were identical to those elicited by dmVMH stimulation (Table 1) and those we have previously reported (Borszcz, 1995; Borszcz and Leaton, 2003). VDSs elicited by tailshock also exhibited spectral characteristics identical to those we have previously reported (Table 1). Administration of BIC and MUS did not alter the spectral characteristics of tailshock elicited VADs or VDSs.

3.4. Experiment 4: dmVMH Contributes to Generation of the Affective Dimension of Pain—Innate defensive reactions elicited by noxious or threatening stimuli are accompanied by a negative affective state (i.e., unconditional fear). If a response to a noxious or threatening stimulus is a direct reflection of this affective state then its elicitation should predict fear conditioning. We provided evidence that the capacity of tailshock to support fear conditioning correlates with its capacity to elicit VADs (Borszcz, 1993; Borszcz, 1995; Borszcz and Leaton, 2003). If the dmVMH contributes to processing the affective dimension of pain then manipulations of GABA_A neurochemistry in the dmVMH that altered thresholds of VADs elicited by tailshock or dmVMH stimulation will also alter the capacity of these eliciting stimuli to support fear conditioning.

Figure 4 depicts the effects of training and drug treatments on the peak amplitude of VCRs. Identical results were observed with the other measures of VCR performance (latency, duration, number). None of the unpaired control groups demonstrated a significant increase in VCRs over trials nor did they differ from one another and therefore were combined (within US type). Both dmVMH stimulation and tailshock were effective in supporting the development of fear conditioning in all groups that received paired presentations of CS and US, $F_s > 9.48$, $p_s < .001$. However, the asymptotic level of conditioning was modulated by manipulations of GABA_A neurochemistry within the dmVMH. For both dmVMH stimulation and tailshock, comparisons of paired groups that received saline with paired groups that

received BIC or MUS revealed significant effects of groups ($F_s > 51.60$, $p_s < .001$), trials ($F_s > 25.22$, $p_s < .001$) and Group $\times$ Trials interactions ($F_s > 2.71$, $p_s < .001$). The interactions reflect the finding that the asymptotic level of fear conditioning was elevated by BIC treatment and reduced by MUS treatment. Comparison of VCR performance across groups on the last trial of training on Day 3 revealed significant differences between the saline treated groups and the groups treated with BIC or MUS ($F_s > 211.7$, $p_s < .001$).

Performance of VADs on the first trial of training and on probe trials at the end of each conditioning session confirmed the previous findings (Figure 5). For groups given paired presentations of CS and US, VADs elicited by tailshock and dmVMH stimulation were enhanced in the group administered BIC and suppressed in the group that received MUS ($F_s > 114.70$, $p_s < .001$). No differences were observed in the performance of VADs across probe trials ($F_s < 1.0$), or when VAD performance on probe trials was compared to VAD performance on the first conditioning trial of Day 1 ($t_s < 1.0$). These findings indicate that VAD performance was not affected by training. VDSs and SMRs elicited by tailshock were not altered by drug treatments (data not shown). Identical results were observed in the unpaired control groups; however, the number of rats in each subgroup precluded statistical comparisons.

Differences in conditioning could not be attributed to the effects of drugs on expression of VCRs (ability of rats to vocalize). Expression of VCRs during the Extinction session (no significant decrease in VCRs was observed over the three extinction trials) did not differ from that observed during the last day of training despite the fact that drugs were not administered prior to this session (Figure 4). Comparison of VCR performance on the last trial of Day 3 of training and the first trial of the Extinction session revealed no significant differences ($t_s < 1.0$, $p_s > .35$). During the Extinction session the groups administered BIC and MUS respectively exhibited enhanced and reduced levels of fear conditioning when compared to saline treated groups ($F_s > 222.5$, $p_s < .001$). Evidence that drugs were not active during the Extinction session was confirmed on the US probe trial that revealed a convergence of VAD performance across groups (Figure 5: $F_s < 1.0$). Unpaired control groups also exhibited convergence of VAD performance although the number of rats in each subgroup precluded statistical comparisons (data not shown). Furthermore, among groups trained with tailshock no differences were observed in performance of VDSs during training. Therefore, differences in VCR performance during training cannot be the result of drug-induced changes in the ability of rats to vocalize.

Consistent with our previous reports (Borszcz, 1995; Borszcz and Leaton, 2003), VCRs and VADs shared spectral characteristics (Table 1). The administration of MUS and BIC did not alter these spectral characteristics. Spectrographic characteristics of VDSs were identical to those in the previous experiment and were not affected by drug treatments.

4. Discussion

Results of the present study demonstrate that the dmVMH is critically involved in generating the innate affective reaction to pain. Electrical or chemical stimulation of the dmVMH elicited a distinctive type of vocalization from the rat. These vocalizations were identical in their spectrographic characteristics to vocalizations that occur following application of noxious tailshock (VADs). That the dmVMH contributes to the processing of pain affect is demonstrated by the findings that manipulation of GABA_A neurochemistry within the dmVMH altered the thresholds of VADs elicited by dmVMH stimulation or tailshock. Microinjection of a subthreshold dose (one that did not directly elicit VADs) of the GABA_A antagonist bicuculline lowered VAD thresholds, whereas, injection of the GABA_A agonist muscimol raised VAD thresholds. These differences in VAD thresholds cannot be attributed to drug-induced effects on rats' ability to vocalize. Changes in thresholds were not accompanied by changes in performance of VADs. Furthermore, manipulation of GABA_A neurochemistry

within the dmVMH did not alter threshold or performance of vocalizations that occurred during tailshock (VDSs).

The observed elicitation of VADs and the effects of GABA_A manipulation on VAD thresholds were restricted to the dmVMH. Frequency dependent increase in VAD performance was only observed following electrical stimulation of dmVMH. Stimulation of the DMH and vlVMH produced intermittent VADs with performance not related to frequency of stimulation. The long latencies of these VADs may indicate that they were elicited by current spread to the dmVMH. Microinjection of bicuculline into the DMH and vlVMH did not elicit VADs. This anatomical specificity is consistent with reported estimates of functional spread of bicuculline following its intracerebral administration in the doses and volume used in the present study (Shekhar and Katner 1995; Smith and Berridge, 2005).

We previously demonstrated that tailshock elicited VADs are a valid rodent model of the innate affective reaction to pain because their generation is critical for tailshock to support fear conditioning (Borszcz, 1993; Borszcz, 1995; Borszcz and Leaton, 2003). Consistent with these findings is the current observation that the asymptotic level of fear conditioning supported by either tailshock or dmVMH stimulation was directly related to the magnitude of VADs generated by these USs. Administration of bicuculline or muscimol into the dmVMH prior to each conditioning session respectively raised or lowered the level of VCR conditioning. These differences in VCR conditioning were directly related to the amplitude, duration and 1/latency of VADs elicited by either tailshock or dmVMH stimulation. Differences in VCR conditioning observed during training cannot be attributed to the effects of drugs on the ability of rats to vocalize. Group differences in conditioning were observed during extinction training that was not preceded by drug treatments and during which the groups no longer differed in the generation of VADs.

Contribution of the dmVMH to the processing of pain affect is also supported by the earlier findings of Adams and Flynn (1966). They reported that cats trained to avoid noxious tailshock exhibited transfer when dmVMH stimulation was substituted for tailshock. That is, dmVMH stimulation was able to sustain avoidance responding that was originally conditioned using tailshock. Consistent with the present findings, dmVMH stimulation was only effective if it elicited defensive vocalizations from the cats. These findings and those of the present study provide the behavioral instantiation of the reported nociceptive projections to the dmVMH (Bester et al., 1995; Bernard et al., 1996; Braz et al., 2005) by demonstrating the critical involvement of this site in generating the innate affective reaction to pain.

The present findings are consistent with our earlier report that lesions of the central nucleus of the amygdala (CeA) prior to training prevented VCR conditioning using tailshock (Borszcz and Leaton, 2003). This conditioning deficit was also related to a selective increase in VAD threshold and performance of VADs at threshold was not altered by CeA lesions. Similarly, inactivation of the basolateral amygdaloid complex (BLA) prior to training blocked acquisition of a variety of Pavlovian fear CRs (freezing, ultrasonic vocalizations, and defecation), and suppressed the elicitation of these URs during training with footshock (Lee et al., 2001). The CeA and BLA also receive nociceptive afferents (Bernard and Besson, 1990; Newman et al., 1996) and modulate the processing of threatening stimuli by the dmVMH (Siegel, 2005). These structures may form part of a core limbic circuit that processes the affective dimension of noxious stimuli that underlies the conditioning of fear (see below).

Involvement of the dmVMH in production of the innate affective reaction to pain provides insight into the concept of pain affect. The dmVMH along with other interconnected medial hypothalamic nuclei (dorsal premammillary nucleus and anteriomedial hypothalamus-medial preoptic area) constitute a hypothalamic behavioral control system that governs the execution

of innate defensive responses to environmental threats (Petrovich et al., 2001; Canteras, 2002). These hypothalamic nuclei exhibit c-Fos activation following exposure to either noxious or non-noxious threatening stimuli (Bullitt, 1990; Sandner, et al., 1993; Canteras et al., 1997; Liu et al., 1998; Rodella et al., 1998; Beckett et al., 1999; Dielenberg, et al., 2001; Parry et al., 2002), and inactivation or damage of these sites blocks naturally occurring defensive behaviors (Canteras et al., 1997; Cheu and Siegel, 1998; Markham et al., 2004). Stimulation of these medial hypothalamic nuclei elicits defensive responding in rats, cats and monkeys (Fernandez de Molina and Hunsperger, 1962; Lipp and Hunsperger, 1978; Milani and Graeff, 1987), and in humans generates reports of fear, anxiety and horror (Ervin et al., 1969; Heath, 1975; Iacono and Nashold, 1982; Tasker, 1982). In humans, activation of the medial hypothalamus was observed during exposure to a traumatic painful stimulus that elicited an intense emotional experience (Hsieh et al., 1996). For all these species, vocalizations are part of their defensive reaction to imminent threat (Fernandez De Molina and Hunsperger, 1962; Jürgens, 1979; Blanchard et al., 1986, 2001), and consistent with the present findings bicuculline and muscimol administered into the medial hypothalamus facilitates and suppresses defensive responding, respectively (Silveira and Graeff, 1992; Roeling et al., 1993; Silveira et al., 1995; Strzelczuk and Romaniuk, 1995; Cheu and Siegel, 1998; Zagrodzka et al., 2000). As exposure to a noxious stimulus is the prototypical imminent threat to an individual it is not surprising that noxious stimulation would engage neural circuits that govern execution of innate defensive responses. Within this context, the primary affective dimension of pain belongs to a broader class of sensory experience that represents threat to the individual and engages neuronal circuits that govern the execution of innate defensive reactions that enable the individual to cope with the threat.

The dmVMH and interconnected medial hypothalamic nuclei are part of a mesolimbic circuit that controls execution of defensive responding to threats. These hypothalamic sites send glutaminergic projections to the dorsolateral column of the periaqueductal gray (dIPAG; Beart et al., 1988) that interact with NMDA receptors to generate defensive responding (Schubert et al., 1996). These projections are activated by nociceptive input to the dmVMH. Neurons within dmVMH that exhibit c-Fos expression following presentation of a noxious cutaneous stimulus are double-labeled by administration of a retrograde tracer into the dIPAG (Parry et al., 2002). The dIPAG serves as the interface between limbic forebrain sites that process stimuli that threaten the individual and execution of innate defensive responses. Descending projections from the dIPAG to the brainstem coordinate the execution of the behavioral and autonomic responses that constitute defensive responding. Projections from the dIPAG to the rostral ventrolateral medulla initiate the autonomic reactions associated with defensive responding (Wang et al., 2002). Projections from the dIPAG to the nucleus retroambiguus initiate activity in the laryngeal, articulatory and respiratory motor neurons that generate vocalizations (Jürgens and Pratt, 1979; Jürgens, 2002).

The amygdala is the best characterized modulator of defensive responding generated from the medial hypothalamus and dIPAG. Stimulation of the BLA and medial amygdaloid nucleus generates defensive responding, and subthreshold stimulation potentiates defensive responding elicited by medial hypothalamic or dIPAG stimulation (Fernandez de Molina and Hunsperger, 1962; Egger and Flynn, 1963; Shaikh et al., 1994). Moreover, partial kindling of the CeA and BLA results in long-term increases in defensiveness that is mediated by induction of long-term potentiation in amygdaloid projections to the dmVMH and dIPAG (Adamec and Young, 2000; Adamec and Shallow, 2000). These amygdaloid nuclei receive nociceptive afferents and tonic noxious peripheral stimulation can produce long-term increases in the responsiveness of these nuclei to acute noxious stimulation (Neugebauer et al., 2004). Pain-induced plasticity in amygdaloid projections to the dmVMH and dIPAG could account for long-term increases in pain sensitivity and defensiveness that accompany the pain state. Alterations in the circuitry that controls defensive responding are implicated in conditions such as fear, anxiety, depression

and anger (Dixon, 1998; Adamec and Young, 2000). These secondary emotional reactions are components of the human pain experience, and contribute to the suffering and disability associated with pain (Crombez et al., 1999; Strahl et al., 2000; Ericsson et al., 2002). Gaining an understanding of the neural circuitry that controls the innate affective reaction to pain and how changes in this circuitry produces enduring effects on the individual is of obvious clinical importance and warrants additional study.

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Figure 1.

(A) Spectrograms of vocalization afterdischarges (VADs) elicited by electrical brain stimulation (EBS) of the dorsomedial division of the ventromedial hypothalamus (dmVMH), microinjection of 40 pmol of bicuculline (BIC) into the dmVMH or tailshock (TS). Spectrogram of vocalization recorded during tailshock (vocalization during shock = VDS). (B) Power spectra of VAD elicited by EBS of dmVMH. Identical spectra were obtained for VADs elicited by BIC and TS. (C) Power spectra of VDS.

Figure 2.

(A) Histological reconstruction of sites that received electrical stimulation or chemical stimulation with bicuculline. Stimulation was administered unilaterally with side of stimulation counterbalanced for each type of stimulation. For the sake of clarity all electrical stimulation sites are indicated on the left side of the diagrams and chemical stimulation sites are indicated on the right side of the diagrams. Black squares and circles indicate sites within the dorsomedial division of the ventromedial hypothalamus (dmVMH) where stimulation was effective in eliciting vocalization afterdischarges (VADs). Gray squares and circles indicate sites outside the dmVMH where stimulation was ineffective in eliciting VADs. Coordinates are in millimeters posterior to bregma. Plates are from the rat brain atlas of Paxinos and Watson (1998). (B) Mean psychophysical functions relating log frequency of brain stimulation to mean peak amplitude ($\pm$ SE) of VADs. Functions are from groups of rats that received stimulation of the dmVMH ($n = 6$), lateral hypothalamus (LH, $n = 5$), or other sites (Other, $n = 5$) in the vicinity of the dmVMH. Identical results were obtained when duration and 1/latency of VADs were analyzed. (C) Mean ($\pm$ SE) number of VADs elicited during each 30 sec epoch following administration of bicuculline into the dmVMH or sites in the vicinity of dmVMH (Other). Asterisks indicated significantly greater number of VADs when compared to the 30 sec epoch immediately prior to bicuculline administration (BL = baseline, Dunnett's multiple comparison test). Identical results were obtained when peak amplitude or durations of VADs during each epoch were analyzed.

Figure 3.

(A) Mean psychophysical functions relating log frequency of dmVMH stimulation to mean ($\pm$ SE) peak amplitude of vocalization afterdischarges (VADs). (B,C & D) Mean psychophysical functions relating log current intensity of tailshock to mean ($\pm$ SE) peak amplitude of vocalization afterdischarges (VADs), vocalizations during shock (VDSs) and spinal motor reflexes (SMRs). Responses were assessed following unilateral administration of saline, bicuculline (BIC) or muscimol (MUS) into the dorsomedial division of the ventromedial hypothalamus. Response thresholds (arrows) were calculated as the x-axis intercept. Identical results were obtained with the analysis of other performance variables (i.e., duration, 1/latency, magnitude).

Figure 4.

Mean peak amplitude of conditional vocalizations (VCRs) during three days of training using either tailshock (A) or dmVMH (B) as the unconditional stimulus (US). Each experimental group received 15 trials of paired presentations of the light CS with the US during each of three training days. For the sake of clarity data are presented as the means of blocks of three consecutive trials. Peak amplitude on the first trial (FT) of each training day is also represented. Prior to each day of training separate groups received injections of saline (A, $n = 5$; B, $n = 6$), bicuculline (A, $n = 6$; B, $n = 7$) or muscimol (A, $n = 6$; B, $n = 6$) into the dmVMH. Control groups received explicitly unpaired presentations of the CS and US (15 each) for three days. Separate control groups received injections of saline (A, $n = 3$; B, $n = 3$), bicuculline (A, $n = 2$; B, $n = 2$) or muscimol (A, $n = 2$; B, $n = 2$) into the dmVMH prior to each training day. As the data from control groups (within each US type) did not differ they were combined (Unpaired Controls). Prior to Extinction (Ext) training (Day 4) no drugs were administered and rats received three presentations of the CS alone. Asterisks indicate significant difference from groups that received saline prior to paired conditioning trials.

Figure 5.

Mean ($\pm$ SE) peak amplitude of vocalization afterdischarges (VADs) during Pavlovian conditioning using either tailshock (A) or dmVMH stimulation (B) as the unconditional stimulus (US). VADs were assessed on the first trial (FT) of training on Day 1 and on probe trials (US presented alone) that followed conditioning trials during the three days of training. VADs were also measured following the three trials of extinction training (Extinction). Separate groups were administered saline, bicuculline (BIC) or muscimol (MUS) prior to the three days of conditioning. No drugs were administered prior to extinction training. Asterisks indicate significant difference from saline treated groups.

Mean(±SE) Frequencies (in Kilohertz) of the Fundamental and the Harmonics of Vocalizations During Shock (VDS), Vocalization Afterdischarges (VAD), and Vocalization Conditional Responses (VCR).

Harmonics	Vocalizations			
	VDS Tailshock ¹	VAD Tailshock ¹	VAD dmVMH(EBS) ¹	VCR Pavlovian ²
Fundamental				
1	2.52 ± .011	3.49 ± .041	3.49 ± .022	3.50 ± .009
2	5.05 ± .023	7.01 ± .078	6.99 ± .042	7.00 ± .019
3	7.58 ± .036	10.49 ± .115	10.49 ± .065	10.49 ± .028
4	10.10 ± .045			
5	12.62 ± .056			
6	15.14 ± .067			
	17.57 ± .067			

Note. EBS = electrical brain stimulation of the dmVMH. BIC = bicuculline microinjection in dmVMH.

¹Vocalizations from Experiment 3 collapsed across drug treatments.

²Vocalizations from Experiment 4 collapsed across drug treatments and unconditional stimulus.

Table 2
Mean($\pm$ SE) Performance of Vocalization Afterdischarges elicited by dmVMH Stimulation or Tailshock

	dmVMH stimulation		Tailshock	
	Slope ¹	Maximum	Slope	Maximum
Amplitude (dB) ²				
Sal	71.5 $\pm$ 4.3	42.6 $\pm$ 1.2	62.4 $\pm$ 8.1	43.1 $\pm$ 1.1
Mus	71.2 $\pm$ 3.0	41.8 $\pm$ 1.3	61.3 $\pm$ 3.9	42.7 $\pm$ 2.5
Bic	72.5 $\pm$ 3.6	42.3 $\pm$ 1.1	58.8 $\pm$ 9.4	42.3 $\pm$ 0.9
Duration (ms)				
Sal	6020.4 $\pm$ 780.4	4012.0 $\pm$ 277.5	2611.0 $\pm$ 147.5	1803.7 $\pm$ 43.8
Mus	6171.2 $\pm$ 968.7	4094.2 $\pm$ 165.5	2693.8 $\pm$ 85.2	1806.7 $\pm$ 39.7
Bic	5958.0 $\pm$ 818.8	4005.2 $\pm$ 273.9	2666.5 $\pm$ 84.2	1800.3 $\pm$ 36.4
Latency (ms) ^{3,4}				
Sal	.971 $\pm$.026	1620.4 $\pm$ 49.9	14.1 $\pm$.23	102.6 $\pm$ 0.4
Mus	.939 $\pm$.072	1660.8 $\pm$ 18.9	13.8 $\pm$.24	104.1 $\pm$ 0.8
Bic	.916 $\pm$.082	1638.0 $\pm$ 34.3	13.7 $\pm$.57	103.5 $\pm$ 0.7

Note: Sal = saline, Mus = muscimol (100 pmol), Bic = bicuculline (10 pmol)

¹ Slope of the function relating performance (amplitude, duration or latency) to frequency of dmVMH stimulation or mA of tailshock.

² Maximum = decibels above criterion.

³ Slope calculated with 1/latency.

⁴ Maximum = shortest latency from stimulus onset (dmVMH stimulation) or stimulus offset (Tailshock)