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NMDA or non-NMDA Receptor Antagonism within the Amygdaloid Central Nucleus Suppresses the Affective Dimension of Pain in Rats: Evidence for Hemispheric Synergy

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Abstract

The amygdala contributes to generation of affective behaviors to threats. The prototypical threat to an individual is exposure to a noxious stimulus and the amygdaloid central nucleus (CeA) receives nociceptive input that is mediated by glutamatergic neurotransmission. The present study evaluated the contribution of glutamate receptors in CeA to generation of the affective response to acute pain in rats. Vocalizations that occur following a brief noxious tailshock (vocalization afterdischarges) are a validated rodent model of pain affect, and were preferentially suppressed by bilateral injection into CeA of the NMDA receptor antagonist D-2-amino-5-phosphonovalerate (AP5, 1μg, 2μg, or 4μg) or the non-NMDA receptor antagonist 6-Cyano-7-nitroquinoxaline-2,3-dione disodium (CNQX, .25μg, .5μg, 1μg, or 2μg). Vocalizations that occur during tailshock were suppressed to a lesser degree, whereas, spinal motor reflexes (tail flick and hindlimb movements) were unaffected by injection of AP5 or CNQX into CeA. Unilateral administration of AP5 or CNQX into CeA of either hemisphere also selectively elevated vocalization thresholds. Bilateral administration of AP5 or CNQX produced greater increases in vocalization thresholds than the same doses of antagonists administered unilaterally into either hemisphere indicating synergistic hemispheric interactions.

Perspective—The amygdala contributes to production of emotional responses to environmental threats. Blocking glutamate neurotransmission within the central nucleus of the amygdala suppressed rats' emotional response to acute painful stimulation. Understanding the neurobiology underlying emotional responses to pain will provide insights into new treatments for pain and its associated affective disorders.

Keywords

nociception; emotion; laterality; amygdala; AP-5; CNQX; vocalization; rat; glutamate

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1. Introduction

The affective-motivational dimension of pain underlies the suffering and disability associated with the pain state.⁴² Despite this preeminent clinical significance, little is known regarding the neuronal mechanisms responsible for production or modulation of the affective component of the pain experience. The amygdaloid central nucleus (CeA) contributes to the execution of affective behaviors to environmental threats.³⁸ The prototypical threat to an individual is exposure to a noxious stimulus^{10,23} and the CeA receives direct nociceptive inputs from the spinal cord dorsal horn^{19,45} and indirect nociceptive inputs relayed via the parabrachial nucleus (PB)^{2,32} and intralaminar thalamic nuclei.^{46,57}

Neural activity within CeA increases in response to acute noxious somatic stimulation or during chronic pain states in rats.^{3,39} Neuroimaging studies implicated a role of the amygdala in the processing of pain affect in humans.^{37,52,60} Although human neuroimaging has yet to attain the resolution necessary to identify individual amygdaloid nuclei, recent electrophysiological recordings from human brain revealed noxious-evoked activity in CeA.⁴¹ Additionally, damage of the amygdala in humans^{18,30,33} and CeA in rats^{12,56} suppresses their affective responding to noxious peripheral stimulation.

The rat CeA contains NMDA and non-NMDA receptors that contribute to nociceptive processing.³⁹ Rats with experimentally induced arthritis of the knee exhibit increased spontaneous and evoked neural activity within the CeA, and this activity is mediated by NMDA and non-NMDA receptors within CeA. Additionally, non-arthritic rats show noxious-evoked CeA neural activity that is also mediated by these glutamate receptors. Thus, NMDA and non-NMDA receptors within CeA are implicated in the arthritic pain state as well as acute nociceptive transmission.

Given that the CeA is implicated in production of affective responses and contributes to nociceptive processing via glutamatergic neurotransmission, the present study evaluated the role of NMDA and non-NMDA receptors in CeA to generation of pain affect in rats to an acute noxious stimulus. Previous research in this laboratory validated vocalization afterdischarges (VADs) as a rodent model of pain affect (see Methods). VADs occur immediately after application of noxious tail shock, are organized within the forebrain, and have distinct spectrographic characteristics compared with vocalizations that occur during shock (VDSs).^{7,22} We predicted that bilateral glutamate receptor antagonism in CeA would preferentially elevate VAD threshold compared with thresholds of tail shock-elicited behaviors organized at spinal (SMR = hind limb movements and tail flexion) and medullary (VDS = vocalizations during shock) levels of the neuraxis.^{16,22} As hemispheric laterality of amygdala activation has been implicated in the processing of pain affect in both humans and rats^{21,34,37} we also evaluated hemispheric laterality of glutamate receptor antagonism in CeA in suppressing rats' affective response to pain.

Methods

Animals

Forty-two male Long-Evan rats (90–120 days old) were used. Rats were housed as pairs in polycarbonate cages in a climate-controlled vivarium illuminated on a 12:12-hr light/dark cycle with lights on at 0600 hours, and given ad lib access to food and water. All testing was conducted between 0700 and 1700 hours. Rats were handled 1–2 times per day for at least one week before testing to minimize the effects of stress from human contact. The experiments were approved by the Institutional Animal Care and Use Committee of Wayne State University.

Surgery and Histology

Surgeries were performed under aseptic conditions. Rats were anesthetized with sodium pentobarbital (50mg/kg, i.p.) following pretreatment with atropine sulfate (1mg/kg, i.p.). The intra-CeA microinjection of antagonists was accomplished via chronic indwelling cannulae. Two stainless steel 26-gauge single-cannulae (Plastics One, Roanoke, VA) were bilaterally implanted for all experiments. Stereotaxic coordinates were extrapolated from the rat brain atlas of Paxinos and Watson⁴⁹ following histological analysis of preliminary surgeries. Stereotaxic coordinates were measured relative to the bregma suture and the top of the level skull. Final coordinates for CeA implants were: AP = - 2.0 mm, L = $\pm$ 4.0 mm, DV = -6.0 mm. To assess anatomical specificity of the effects of antagonist treatments, implants were also made 1.2 mm medial or lateral to CeA. Cannulae were affixed to the skull with 4 stainless steel bone screws (3/16 in) and cranioplastic cement. Each guide cannula was fit with a 33-gauge dummy cannula that extended the length of the guide to maintain its patency. Rats were given 7–10 days to recover before the initiation of testing. Sixty-two days was the maximum amount of time that a rat remained in the study prior to sacrifice.

At the conclusion of testing, rats were sacrificed by carbon dioxide asphyxiation and injection sites were marked by the injection of 0.25 μ l of safran-O dye. The brains were immediately extracted and placed in a 20% (wgt/vol) sucrose formalin solution for 24 – 48 hours. Brains were sectioned at 45 μ m on a freezing microtome and injection sites were localized with the aid of the Paxinos and Watson⁴⁹ brain atlas by an experimenter unaware of the behavioral outcomes.

Assessment of pain affect

Research in this laboratory validated vocalization afterdischarges (VADs) as a rodent model of pain affect. These vocalizations occur immediately following application of noxious tailshock, are organized within the forebrain, and have distinct spectrographic characteristics compared to vocalizations that occur during shock (VDS).^{7,10,22,31} Systemically administered drug treatments that preferentially suppress the affective reaction of humans to pain^{25,51} also preferentially suppress production of VADs.¹³ 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.^{9,12,26–28,31,43,44,55,60} Additionally, the capacity of noxious tailshock to support fear conditioning is directly related to its production of VADs.^{6,7,10,12} In the present study, the effects of experimental treatments on VAD threshold were compared with their effects on the thresholds of other tail shock-elicited responses that are organized at medullary (vocalizations during shock, VDS) and spinal (spinal motor reflexes, SMR) levels of the neuraxis.^{16,22}

Apparatus

Testing was controlled by custom computer programs via a multifunction interface board (DT-2801, Data Translation, Marlboro, MA) installed in a PC. Rats were placed into custom made Velcro body suits and restrained on a Plexiglas pedestal using Velcro strapping that passes through loops located on the underside of the suits. This design maintains the rat in a crouching posture throughout testing, permits normal respiration and vocalizing, and allows unobstructed access to the head for intracerebral injections (see photograph in Borszcz⁷). Testing was conducted within a sound attenuating, lighted, and ventilated chamber equipped with a small window that enabled visual monitoring of the animal during testing.

Tailshock (20 ms pulses at 25 Hz for 1,000 ms) was delivered by a computer controlled constant current shocker (STIMTEK, Arlington, MA) through electrodes (0-gauge stainless

steel insect pins) placed intracutaneously on opposite sides of the tail, 7.0 cm (cathode) and 8.5 cm (anode) from the base. The intensity, duration, and timing of tailshocks were controlled by the computer. Current intensity was monitored by an analog-to-digital converter that digitized (500 Hz sampling rate) an output voltage of the shocker that was proportional to the current delivered.

Spinal motor reflexes (SMRs) were measured with a semi-isotonic displacement transducer (Lafayette Instruments Model 76614, Lafayette, IN) attached to the rat's tail with cotton thread. 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. The output voltage of the transducer was amplified (x50) and then digitized (500 Hz sampling rate) by an analog-to-digital converter of the interface board. SMR was defined as movement of the transducer arm by at least 1.0 mm following shock onset. The computer recorded the latency (ms), peak amplitude (mm), and magnitude (cm × ms) of tail movement on each trial. Displacements up to 100 mm can be detected, and latencies in 2 ms increments can be measured.

Vocalizations were measured by a pressure-zone microphone (Realistic model 33-1090, Tandy, Ft. Worth, TX) located on the wall of the testing chamber 15 cm from the rat's head. The microphone was connected to an audio amplifier (Technics model SA-160, Tandy, Ft. Worth, TX) and a 10-band frequency equalizer adjusted to selectively amplify frequencies above 1500 Hz. The filtering of low frequencies prevented extraneous noise (i.e., rats' respiration and movement artifacts) from contaminating vocalization records. The output of the amplifier was integrated by a Coulbourn Instruments (Allentown, PA) contour following integrator (2 ms time base) and digitized (500 Hz sampling rate) by a separate analog-to-digital converter of the interface board. The peak intensity (in decibels: SPL, B scale), latency (ms), and duration (ms) of vocalizations during the shock epoch (VDS) and for the 2,000 ms interval following shock termination (VAD), were recorded by the computer.

Pain Testing

For 2 consecutive days prior to testing, rats were adapted to the testing apparatus for a period of 20 minutes each day to minimize the effects of restraint stress. In all experiments, testing began 7–10 minutes following completion intracerebral injections. Test sessions consisted of 20 randomly presented trials. On 16 of the trials, tailshocks of various current intensities between 0.02 mA and 2.50 mA were delivered, and on 4 trials no current was delivered so as to assess false alarm rates. Trials were presented with a minimum intertrial interval of 30 seconds and each test session concluded within 20 minutes. These procedures cause no observable damage to the tail. Following each test session, the testing apparatus was cleaned with 5% ammonia hydroxide to eliminate stress odors.²⁴

Drug Injections

Intracerebral injections were administered in a constant volume of 0.25 µl via 33-gauge injectors that extended beyond the end of the cannulae. All injections were made at a constant rate over 1 min via an infusion pump (Harvard Model PHD 2000), and injectors were left in place for 2 min after the completion of injections to aid the diffusion of drugs into tissue. The NMDA receptor antagonist D-2-amino-5-phosphonovalerate (AP5; Tocris Bioscience, Ellisville, MO) was dissolved in normal sterile saline and the non-NMDA (i.e., AMPA/kainite) receptor antagonist 6-Cyano-7-nitroquinoxaline-2,3-dione disodium salt (CNQX; Sigma-Aldrich, St. Louis, MO) was dissolved in sterile water.

Procedures

Experiment 1: AP5 and CNQX dose-response analysis—The dose-response relationship between CeA-administered AP5 or CNQX and SMR, VDS, and VAD

thresholds was assessed. Antagonists were bilaterally administered via injectors that extended 3.0 mm beyond the end of the guide cannula. Only rats that completed all test sessions were included in the analysis. One rat from the AP5 treatment group and 2 rats from the CNQX treatment group were excluded due to cannula implant failure leaving 7 rats in each group. The AP5 treatment group received bilateral intra-CeA microinjections of AP5 (1 μ g, 2 μ g, and 4 μ g) and vehicle prior to four separate testing sessions. The CNQX treatment group received bilateral intra-CeA microinjections of CNQX (.25 μ g, .5 μ g, 1 μ g, or 2 μ g) and vehicle prior to five separate testing sessions. Sessions were counterbalanced using a quasi-Latin Square design that maintained the vehicle injection at either the beginning or the end of the testing sequence. Comparison of these vehicle treatments permitted evaluation of multiple test sessions on baseline thresholds. Test sessions were separated by 5 – 10 days.

Experiment 2: Anatomical specificity of the AP5 and CNQX effect—To assess the anatomical specificity of intra-CeA AP5 and CNQX treatments, rats received bilateral microinjections of the high dose of antagonists used in the dose response experiment (4 μ g AP5 or 2 μ g CNQX) into sites within and surrounding CeA. Only rats that completed all test sessions were included in the analysis. One rat from the CNQX treatment group was excluded due to cannula implant failure. The AP5 ($n = 8$) and CNQX ($n = 7$) treatment groups each comprised three groups: central, medial, and lateral. Groups were named based on the medial-lateral plane of guide cannulae implants relative to CeA stereotaxic coordinates. The central group (AP5, $n = 3$; CNQX, $n = 3$) received injections 1.2 mm dorsal to, 1.2 mm ventral to, and within CeA. The medial group (AP5, $n = 3$; CNQX, $n = 2$) received injections 1.2 mm medial to CeA, and also 1.2mm dorsal to and 1.2mm ventral to this vicinity. The lateral group (AP5, $n = 2$; CNQX, $n = 2$) received injections 1.2mm lateral to CeA, and also 1.2 mm dorsal to and 1.2 mm ventral to this vicinity.

Each animal in these groups received vehicle and antagonist treatments using an injector that extended 1.8 mm, 3.0 mm, and 4.2 mm past the end of the guide cannulae. Thus, each animal received a total of six treatments on six separate test sessions in three different brain regions (test sessions separated by 5 – 10 days). Vehicle was administered on the first test, and either AP5 or CNQX was given on the second test at each site.

Experiment 3: AP5 and CNQX laterality analysis—The middle and high doses of AP5 (2 μ g and 4 μ g) or CNQX (1 μ g and 2 μ g) from the dose response analysis were chosen to assess effects of hemispheric laterality of receptor antagonism in CeA. Only rats that completed all test sessions were included in the analysis. One rat each from the AP5 and CNQX treatment groups were excluded due to cannula implant failure and one additional rat from the CNQX group was eliminated due to misplacement of the cannula. The AP5 ($n = 7$) and CNQX ($n = 6$) treatment groups each received five treatments: (i) vehicle in the right CeA and high dose antagonist in the left CeA, (ii) high dose antagonist in the right CeA and vehicle in the left CeA, (iii) middle dose antagonist bilaterally, (iv) high dose antagonist bilaterally, and (v) vehicle bilaterally. Injections were counterbalanced using a quasi-Latin Square design that maintained the bilateral vehicle injection at either the beginning or the end of the testing sequence. Comparison of these vehicle treatments permitted evaluation of multiple test sessions on baseline thresholds. Test sessions were separated by 5 – 10 days.

Data Analysis

After each test session, data were reorganized in ascending order according to tail shock intensity. SMR, VDS, and VAD thresholds for each rat were calculated as the lesser current intensity from a string of at least two consecutive intensities that generated the response. For *Experiment 1*, dose-dependent effects of AP5 and CNQX on response thresholds were directly compared using repeated-measures multivariate analysis of variance (MANOVA).

The effects of dose on individual responses were analyzed by oneway analysis of variance (ANOVA). The doses of AP5 and CNQX that elevated response thresholds above baseline levels were determined by post-hoc comparisons of thresholds after vehicle and drug treatments using Dunnett's test.

For *Experiment 2*, comparisons of response thresholds generated following the bilateral administration of vehicle and either 4 μ g AP5 or 2 μ g CNQX into the CeA and into sites surrounding the CeA were made using oneway ANOVA for each response. Post-hoc pairwise comparisons of the effects of treatments on response thresholds were made using Tukey HSD test.

For *Experiment 3*, the effects on thresholds of unilateral and bilateral administration of AP5 and CNQX were evaluated for each response by oneway ANOVA. Post-hoc pairwise comparisons of the effects of treatments on response thresholds were made using Tukey HSD test.

Results

Behavioral Profile

The SMR, VDS, and VAD responses reflect nociceptive processing at progressively higher levels of the neuraxis.^{16,22} Analysis of rats that received transections of the neuraxis revealed that SMRs are organized at the spinal level, VDSs within the medulla below the pontomedullary border, and VADs within the forebrain. Responses were rarely generated without those integrated more caudally within the CNS. VAD generation, without concomitant elicitation of VDS and SMR, occurred on 0.27% of all trials. VDSs were elicited without SMRs on 0.05% of the trials in which only these responses were generated. False alarm rates were low (SMR = 2.3%, VDS = 0.2%, VAD = 0.4%), indicating that responses were not induced by drug administration, were not occurring spontaneously, and were not conditioned responses to the context, but instead were generated by tailshock.

Experiment 1: AP5 and CNQX dose-response analysis

Injections were administered bilaterally into CeA in all rats. Figure 1 is a photomicrograph of a representative injection site in CeA. The effects of the bilateral administration of AP5 or CNQX into CeA on SMR, VDS, and VAD thresholds are depicted in Figure 2. Comparison of response thresholds across doses of AP5 and CNQX (repeated measures MANOVA, Wilk's Lambda) revealed significant main effects of dose, $F_s > 11.16$, $p_s < .001$, and response, $F_s > 53.62$, $p_s < .001$, and a significant Dose x Response interactions, $F_s > 4.45$, $p_s < .001$. This interaction reflects the finding that AP5 and CNQX preferentially increased VAD threshold. Pair-wise comparisons of VAD threshold with VDS and SMR thresholds yielded significant main effects of response, $F_s > 24.51$, $p_s < .001$, and significant Dose x Response interactions, $F_s > 2.72$, $p_s < .05$. Both antagonists dose-dependently increased thresholds of VAD, $F_s > 14.73$, $p_s < .001$, and VDS, $F_s > 3.26$, $p_s < .05$, but failed to elevate SMR threshold, $F_s < 0.15$, $p_s > .05$. Lower doses of AP5 and CNQX were able to elevate VAD versus VDS thresholds. Compared to vehicle treatment, VAD threshold was significantly elevated following bilateral administration of 2 μ g AP5 or 0.5 μ g CNQX, whereas VDS threshold was significantly elevated following bilateral administration of 4 μ g AP5 or 2 μ g CNQX, Dunnett, $p_s < .05$. Direct comparisons of VAD and VDS thresholds revealed that VAD and VDS thresholds did not differ following vehicle treatments, $t_s(6) < 1.01$, $p_s > .05$, but VAD threshold was significantly elevated above VDS threshold following bilateral intra-CeA administration of 2 μ g and 4 μ g AP5, $t_s(6) > 3.60$, $p_s < .05$, and .25 μ g, 0.50 μ g and 2 μ g CNQX, $t_s(6) > 2.46$, $p_s < .05$.

Whereas, increases VAD threshold were not attributable drug-induced performance decrements, performance decrements may have contributed to increases in VDS threshold. Latency, amplitude, and duration of VADs and VDSs were compared at threshold following vehicle and drug treatments, and no decrements in VAD performance were observed following AP5 or CNQX treatments, $F_s < 0.85$, $p_s > .05$. Latency and amplitude of VDS were also not altered following AP5 or CNQX administration, $F_s < 1.26$, $p_s > .05$; however, duration of VDS was reduced by both drugs. One-way ANOVA of VDS duration at threshold across doses of antagonists revealed a change in this performance variable for AP5 and CNQX, $F_s > 4.96$, $p_s < .001$. Compared to following vehicle treatment, VDS duration at threshold was significantly reduced following 2 μ g or 4 μ g of AP5 and 0.25 μ g, 1 μ g, or 2 μ g of CNQX, Dunnett, $p_s < .05$. Latency, amplitude, and magnitude of SMRs at threshold were not influenced by antagonist treatments, $F_s < 1.04$, $p_s > .05$.

Multiple testing sessions did not alter baseline response thresholds. Comparison of thresholds following vehicle treatment from the beginning and the end of the testing sequence revealed no differences in baseline responding, $t_s < 1.12$, $p_s > 0.05$.

Experiment 2: Anatomical Specificity of the AP5 and CNQX effect

Anatomical specificity of the effects of antagonists was evaluated by comparing the response thresholds following bilateral 4 μ g AP5 and 2 μ g CNQX treatments within the CeA with the effects produced by their administration into sites surrounding the CeA. Figure 3 depicts the distribution of injection sites of AP5 within and surrounding the CeA. The distribution of CNQX injections was nearly identical, but not depicted for the sake of clarity. The effects on response thresholds of antagonists administered into CeA did not differ from that observed in the dose-response experiment, $t_s < 1.0$, $p_s > .05$, and these data were combined for comparison with the effects of antagonists administered outside of CeA. No systematic differences were observed on SMR, VDS, or VAD thresholds following bilateral administration of 4 μ g AP5 or 2 μ g CNQX into sites surrounding the CeA, $t_s < 1.02$, $p_s > .05$, and thus these data were combined as a single group ('Other Sites') for each antagonist. For each antagonist group, bilateral administration of vehicle into CeA and all sites surrounding CeA did not produce a significant difference in response thresholds, $t_s < 1.38$, $p_s > .05$; thus these data were combined.

The effects on response thresholds of administering AP5 or CNQX into sites surrounding CeA are depicted in Figure 4. Comparison of response thresholds following administration of antagonists or vehicle into CeA or sites surrounding CeA revealed significant differences for VAD and VDS thresholds, $F_s > 21.72$, $p_s < .001$, but not for SMR threshold, $F_s < 1.92$, $p_s > .05$. Administration of AP5 or CNQX into CeA produced significant elevations in VAD and VDS thresholds, Tukey HSD, $p_s < .001$, but did not alter SMR threshold, Tukey HSD, $p_s > .25$. Injection of AP5 into sites surrounding CeA also elevated VAD threshold, Tukey HSD, $p < .05$, but these increases in thresholds were significantly less than observed following injection of AP5 into CeA, Tukey HSD, $p < .001$. Injections of CNQX into sites outside of the CeA were ineffective in elevating any response threshold, Tukey HSD, $p > .25$.

3.3 Experiment 3: AP5 and CNQX laterality analysis

The effects on response thresholds of unilateral administration of 4 μ g AP5 into the left or right CeA, bilateral administration of 2 μ g AP5 into CeA, bilateral administration of 4 μ g AP5 into CeA, and bilateral administration of vehicle into CeA are depicted in Fig. 5A. Comparison of SMR threshold across treatments revealed no differences, $F(4,34) = .22$, $p > .95$. No AP5 treatment elevated SMR threshold above that observed following vehicle treatment, Tukey HSD, $p_s > .90$. Comparison of VDS and VAD thresholds revealed a

significant difference among AP5 treatments, $F_{s(4,34)} > 9.94$, $ps < .001$. All AP5 treatments elevated VAD threshold compared to following vehicle treatment, Tukey HSD, $ps < .05$. Additionally, bilateral administration of 2 μ g and 4 μ g AP5 elevated VAD threshold above that observed following unilateral administration of 4 μ g AP5 into either the left or right CeA, Tukey HSD, $ps < .05$. Compared to VDS threshold following vehicle treatment, the unilateral administration of 4 μ g AP5 into the right or left CeA failed to elevate VDS threshold, but VDS threshold was significantly elevated following bilateral administration of either 2 μ g or 4 μ g AP5, Tukey HSD, $ps < .01$. VDS threshold following bilateral administration of 2 μ g or 4 μ g AP5 was higher than that observed following administration of 4 μ g AP5 into the CeA of either hemisphere, Tukey HSD, $ps < .05$.

Laterality analysis of the effects on response thresholds of CNQX administered into CeA is depicted in Figure 5B. SMR threshold did not differ across CNQX treatments, $F(4,29) < 1.0$, $p > 0.50$; whereas, both VAD and VDS thresholds were affected by CNQX treatments, $F_{s(4,29)} > 3.60$, $ps < .05$. Unilateral administration of 2 μ g CNQX into either the right or left CeA did not elevate VAD or VDS thresholds above that observed following vehicle administration, Tukey HSDs, $ps > 0.45$. VAD and VDS thresholds were elevated following bilateral administration of 2 μ g CNQX, Tukey HSDs, $ps < 0.05$, and VAD threshold was also significantly elevated following bilateral injection of 1 μ g CNQX, Tukey HSDs, $ps < 0.05$. Moreover, VAD threshold following bilateral administration of 1 μ g and 2 μ g CNQX was elevated above that observed following unilateral administration of 2 μ g CNQX into either CeA, Tukey HSDs, $ps < 0.05$. VDS threshold following bilateral injection of 2 μ g CNQX was elevated compared to that observed following injection of 2 μ g CNQX into either hemisphere, Tukey HSDs, $p < 0.05$.

Baseline response thresholds were not altered by multiple testing sessions. Comparison of thresholds of each response in subgroups administered vehicle treatment at the beginning and the end of the testing sequence revealed no differences in baseline responding, $ts < 1.0$, $ps > 0.05$.

Discussion

The present study provides the first demonstration that affective responding of normal rats (not in a persistent pain state) to an acute noxious stimulus is suppressed following administration of NMDA or non-NMDA receptor antagonists into CeA. As described earlier, VADs are a validated model of pain affect and administration of AP5 or CNQX into CeA preferentially elevated the threshold of noxious tailshock to elicit VADs. AP5 or CNQX injected into CeA produced dose-dependent increases in VAD and VDS thresholds, but direct comparisons of thresholds revealed that VAD threshold was elevated to a greater extent by the intra-CeA injection of the glutamate receptor antagonists. Also, the minimum effective dose of AP5 and CNQX that elevated VAD threshold was lower than the dose that raised VDS threshold. The preferential increase in VAD threshold cannot be attributed to drug-induced motor deficits as increases in VAD threshold were not accompanied by performance decrements. However, a performance decrement may contribute to the observed increase in VDS threshold as VDS duration at threshold was reduced by both AP5 and CNQX. The capacity of the monitored performance variables to detect drug-induced motor deficits that confound threshold measurement was previously demonstrated.^{6,13}

The capacity of CeA-administered AP5 or CNQX to elevate vocalization thresholds is limited to its action within CeA. Systematic bilateral injections of the highest dose of CNQX (2 μ g) into sites surrounding CeA failed to elevate VAD or VDS thresholds. Similarly, bilateral injection of the highest dose of AP5 (4 μ g) into sites surrounding CeA produced greatly attenuated increases in vocalization thresholds. Therefore, glutamate receptor

activation within CeA contributes to the expression of pain affect in rats to an acute noxious stimulus observed in the present study.

Similar to the present results, administration of carbachol, 5-HT, 8-OH-DPAT (5-HT_{1A/7} agonist), morphine or NMDA into the basolateral amygdala, nucleus parafascicularis thalami (PF), ventral tegmental area, or anterior cingulate cortex produced increases in VAD and VDS thresholds without an accompanying increase in SMR threshold.^{26–29,35,44} The failure to observe an increase in SMR threshold does not reflect the resistance of this response to antinociceptive treatments. In previous studies, administration of morphine into the rostral ventromedial medulla or ventrolateral periaqueductal gray (vlPAG) produced significant increases in SMR, VDS, and VAD thresholds^{8,9,11,14} and the intrathecal administration of morphine, serotonin, or norepinephrine was equally effective in raising SMR, VDS, and VAD thresholds.¹⁵ The capacity of these central treatments to elevate SMR threshold also demonstrates that SMRs are not generated by direct stimulation of the tail musculature. These findings indicate that elevation of SMR threshold depends on the site within the CNS at which antinociceptive treatments are administered (see Borszcz⁹ for a more complete description of how nociceptive transmission is inhibited at different levels of the neuraxis).

Also consistent with the results of the present study, administration of NMDA and group I metabotropic glutamate receptor (mGluR₁ or mGluR₅) antagonists into CeA blocked affective responses (as assessed by conditioned place aversion) of nerve injured rats; whereas, the intra-CeA injection of an mGluR_{1/5} agonist enhanced affective pain responses.¹ Injection of mGluR_{1/5} or group III mGluR₇ agonists into CeA also increased the duration of pain-induced vocalizations in normal rats.^{40,47} Unlike the results of the present study, these studies reported that administration of NMDA or mGluR₁ antagonists into CeA normalized withdrawal reflex thresholds in neuropathic rats; whereas, injection of mGluR_{1/5} or mGluR₇ agonist into CeA lowered the thresholds of nocifensive withdrawal reflexes in normal rats. The conditions and mechanisms through which glutamate within CeA modulates generation of nociceptive withdrawal reflexes require further study.

Previous reports from this laboratory also implicated CeA in the production of affective responses to acute noxious tailshock. The selective increase in VAD threshold following injection of a low dose of morphine (1µg) into the vlPAG was reversed by injection of the broad spectrum serotonin receptor antagonist methysergide into CeA.⁹ This increase in VAD threshold may reflect serotonin inhibition of glutamate-mediated excitation of nociceptively responsive neurons in the lateral capsular division of CeA (CeLC).³⁹ The effect of serotonin on nociceptive transmission within CeLC has not been evaluated; however, serotonin suppressed glutamate-mediated sensory evoked neuronal activity within the lateral amygdaloid nucleus.^{53,54} Additionally, this morphine-induced increase in VAD threshold involves the interaction of CeA with PF. Unilateral administration of methysergide into either CeA or PF is ineffective in reducing morphine-induced increases in VAD threshold; however, the combined unilateral administration of methysergide into CeA and PF produced inhibition of morphine-induced affective analgesia that was identical to that observed after its bilateral administration into either site.¹¹ We also observed that bilateral lesion of CeA selectively elevated VAD threshold, and prevented Pavlovian fear conditioning supported by tailshock.¹²

The results of the present study are broadly consistent with those reported by Neugebauer and colleagues in their analysis of hyperalgesia associated with arthritis in rats. CeA receives nociceptive afferents via the spino-amygdaloid pathway and indirectly via the spino-ponto-amygdaloid pathway through which nociceptive transmission is relayed to CeA via the parabrachial nucleus (PB)^{2,32,45} and noxious tailshock elicits neural activity in CeA.³

Li & Neugebauer³⁹ reported that administration of NMDA and non-NMDA receptor antagonists into CeA reduced noxious evoked neural activity in CeLC in both normal and arthritic rats. In normal rats, nociceptive transmission to CeLC involves glutamatergic afferents from PB that interact with non-NMDA receptors (but not NMDA receptors), and glutamatergic afferents from extra-PB sources that interact with NMDA receptors.^{5,39} The source of nociceptive glutamatergic afferents to CeLC that interact with NMDA receptors is not known, but they may be provided by the direct spino-amygdaloid pathway or indirectly via relays in the intralaminar thalamus.^{46,57} For example, glutamatergic projections from PF that interact with NMDA receptors in the anterior cingulate cortex contribute to the modulation of pain affect.^{26,59} The suppression of pain affect observed in the present study presumably reflects inhibition of these glutamatergic nociceptive projections that interact with NMDA and non-NMDA receptors in CeLC.

Arthritis recruits additional glutamatergic afferents from PB that interact with NMDA receptors in CeLC that promote enhanced noxious-evoked neural activity in CeLC to an acute noxious stimulus and behavioral hyperalgesia.⁵ Other persistent pain states may also recruit glutamatergic projections to CeA that interact with NMDA receptors to promote pain affect. Administration of the NMDA receptor antagonist MK-801 into CeA produced suppression of affective responding (conditioned place aversion) associated with neuropathic pain in rats.¹

No laterality in the contribution of CeA in the production of pain affect was observed in the present study. Administration of AP5 into either the left or right CeA produced significant increases in vocalization thresholds, and no difference in threshold evaluations were observed following administration into the left versus right CeA. Similar effects were observed with the unilateral administration of CNQX although the increases in thresholds did not reach statistical significance. These findings are consistent with anatomical, electrophysiological and neuroimaging findings that nociceptive input to the amygdala is bilateral in rats and humans.^{4,17,32,34,41,58} Alternately, lateralized CeA responsiveness was reported following induction of persistent pain states. Following the induction of arthritis into either hindlimb of rat, nociceptively responsive neurons in the right, but not left, CeLC exhibited elevated spontaneous activity, enhanced evoked activity to innocuous and noxious acute peripheral stimulation, and increased PKA expression.³⁴ Similarly, increased ERK expression was limited to the right CeLC following induction of formalin pain in either hindpaw, and blockade of this ERK activation in the right CeLC attenuated formalin-induced mechanical hypersensitivity of either hindpaw.^{20,21} This induction of ERK activation is mediated by recruitment during formalin pain of glutamatergic projections that interact with mGluR₅ receptors in CeA.³⁶ Therefore, the lateralized contribution of CeA to pain appears to emerge with development of a persistent pain state. However, no laterality of CeA contribution to pain affect was observed in the neuropathic pain state. Administration of a mGluR₁ receptor antagonist either ipsilateral or contralateral to the nerve injured hindpaw produce equivalent dose-dependent reductions in conditioned place aversion that was supported by stimulation of the injured paw.¹ On the other hand, our failure to observe lateralized CeA effects may be due to our administration of noxious stimulation to a midline structure (tail) compared to a lateralized structure (hindlimb). Clearly, the hemispheric contribution of CeA to the generation of pain affect elicited by acute noxious stimuli in the non-pain state versus persistent pain states requires additional study.

Rather than the left and right CeA contributing differentially to production of pain affect, synergism between hemispheres was observed in the present study. Distribution of doses of AP5 or CNQX into both right and left CeA was more effective in elevating vocalization thresholds than administration of the same doses into either CeA. The synergy observed following bilateral administration indicates a functional interaction between the left and

right CeA in the production of pain affect. The right and left CeA are not directly interconnected⁵⁰, and we know of no other evidence that they interact in the production of pain affect. However, synergistic interactions between CeA and vlPAG in the production of opiate analgesia were reported⁴⁸, and as noted above CeA interacts with PF in mediating affective analgesia produced by injection of morphine into vlPAG.¹¹ Additional assessment of the hemispheric interactions of CeA in the production of pain affect and its interaction with endogenous antinociceptive circuits in the modulation of pain affect is also warranted.

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Figure 1. Schematic and photomicrograph of a coronal section of the rat brain depicting a typical cannula tract and injection site with dye spread plume within the central nucleus of the amygdala (shaded in right side of schematic). Schematic was modified from the rat brain atlas of Paxinos and Watson.⁴⁷

Figure 2.

The effects of bilateral administrations of (A) AP5 or (B) CNQX into the central nucleus of the amygdala on the mean ($\pm$ SEM) thresholds of spinal motor reflexes (SMRs), vocalizations during shock (VDSs), and vocalization afterdischarges (VADs). Asterisk (*) indicates thresholds significantly elevated above vehicle treatment ($\alpha = 0.05$).

Figure 3.

Histological reconstruction of sites that received injections of AP5 (4 μ g/site). The distribution of sites that received CNQX was nearly identical. Black circles indicate injection sites within the central nucleus of the amygdala (CeA) that were effective in elevating VAD and VDS thresholds. Gray circles indicate injections sites outside of CeA that as a group produced significantly smaller increase in VAD and VDS thresholds. Coordinates are in millimeters posterior to bregma. Schematics were derived from the rat brain atlas of Paxinos and Watson.⁴⁷

Figure 4.

Anatomical specificity of the antinociceptive action of (A) AP5 or (B) CNQX injected into the central nucleus of the amygdala (CeA). Comparison of the effects on response thresholds produced by AP5 (4 μ g/side) or CNQX (2 μ g/side) when administered into CeA or sites surrounding CeA. Data are plotted as the mean ($\pm$ SEM) thresholds of spinal motor reflexes (SMRs), vocalizations during shock (VDSs), and vocalization afterdischarges (VADs). Asterisk (*) indicates thresholds significantly elevated above those observed following vehicle treatment. Pound sign (#) indicates thresholds significantly reduced compared to following antagonist treatment. For all comparisons, $\alpha = .05$.

Figure 5.

The effects of unilateral or bilateral administration of (A) AP5 or (B) CNQX into the central nucleus of the amygdala (CeA) on the mean ($\pm$ SEM) thresholds of spinal motor reflexes (SMRs), vocalizations during shock (VDSs), and vocalization afterdischarges (VADs). Asterisk (*) indicates thresholds significantly elevated compared to following vehicle treatment and pound sign (#) indicates thresholds significantly elevated compared to unilateral administration of antagonist into left or right hemisphere, ($\alpha = .05$).