

Affective Analgesia Following Muscarinic Activation of the Ventral Tegmental Area in Rats

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Abstract: Cholinergic stimulation of dopamine neurons in the ventral tegmental area (VTA) underlies activation of the brain reward circuitry. Activation of this circuit is proposed to preferentially suppress the affective reaction to noxious stimulation. Vocalization afterdischarges (VADs) are a validated model of the affective response of rats to noxious tail shock. The antinociceptive action of the acetylcholine agonist carbachol microinjected into the VTA on VAD threshold was compared with its effect on the thresholds of other tail shock-elicited responses (VDS, vocalizations during shock; SMR, spinal motor reflexes). Whereas VADs are organized within the forebrain, VDSs and SMRs are organized at medullary and spinal levels of the neuraxis, respectively. Carbachol (1 μ g, 2 μ g, and 4 μ g) injected into VTA produced dose-dependent increases in VAD and VDS thresholds, although increases in VAD threshold were significantly greater than increases in VDS threshold. Administration of carbachol into VTA failed to elevate SMR threshold. Elevations in vocalization thresholds produced by intra-VTA carbachol were reversed in a dose-dependent manner by local administration of the muscarinic receptor antagonist atropine sulfate (30 μ g and 60 μ g). These results provide the first demonstration of the involvement of the VTA in muscarinic-induced suppression of pain affect.

Perspective: Cholinergic activation of the brain reward circuit produced a preferential suppression of rats' affective reaction to noxious stimulation. The neurobiology that relates reinforcement to suppression of pain affect may provide insights into new treatments for pain and its associated affective disorders.

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Key words: Ventral tegmental area, acetylcholine, carbachol, muscarinic receptors, vocalization, emotion, reinforcement.

The possibility that the neural substrates of reward and antinociception overlap has been considered for more than 6 decades.⁴³ Evidence supporting this hypothesis derives from observations that strong analgesics (eg, morphine, amphetamine) have high abuse potential and are self-administered in both animals and humans.^{21,22} The reinforcing properties of these drugs are believed to contribute to their addictive liability and antinociceptive action by reducing the level of distress that normally accompanies noxious stimulation. This phenomenon is referred to as "affective analgesia" and

reflects the preferential suppression of the emotional reaction to pain.²¹

The affective analgesia hypothesis proposes that neural substrates underlying reinforcement contribute to suppression of the affective reaction to pain. Activation of dopamine neurons in the ventral tegmental area (VTA) that project to nucleus accumbens (nAb) underlies the reinforcement produced by morphine, amphetamine, other drugs of abuse, natural reinforcers (food, water, sexual interaction), and lateral hypothalamic stimulation.^{29,56} Activation of this mesoaccumbal dopamine system also contributes to the antinociceptive action of morphine and amphetamine.²

Mesoaccumbal dopaminergic neurons in the VTA are activated via cholinergic projections from the laterodorsal tegmental (LDTg) and pedunculopontine tegmental (PPTg) nuclei.⁴⁴ Injecting muscarinic or nicotinic antagonists into the VTA attenuates the accumbal efflux of dopamine that accompanies electrical stimulation of

Received July 1, 2007; Revised January 25, 2008; Accepted January 28, 2008.

Supported by grant R01 NS045720 from the National Institute of Neurological Disorders and Stroke (NINDS) (to G.S.B.).

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doi:10.1016/j.jpain.2008.01.334

LDTg.¹⁹ Microinjecting nicotinic and muscarinic agonists into the VTA excites dopaminergic neurons in the VTA via activation of local cholinergic receptors^{15,32} and increase the release of dopamine in nucleus accumbens.^{25,41}

Cholinergic activation of mesoaccumbal dopamine neurons is critical for reinforcement. Self-administration of lateral hypothalamic brain stimulation results in the efflux of acetylcholine in VTA⁴⁸ and is attenuated by administration of muscarinic antagonists into the VTA.⁵⁷ Infusion into the VTA of the antisense oligonucleotides targeting muscarinic M₅ mRNA reduced M₅ receptor density and inhibited lateral hypothalamic self-stimulation in rats.⁵⁸ Mutant mice with deletion of the M₅ receptor exhibited reduced conditioned place preference learning with systemic morphine injections.⁴ Alternately, the rewarding effects of lateral hypothalamic stimulation are enhanced by infusion of acetylcholine into the VTA.⁴⁹ Intra-VTA administration of the nonspecific cholinergic agonist carbachol supports development of conditioned place preference learning, and rats learn to self-administer carbachol into the VTA.^{30,59} These reinforcing effects of carbachol were attenuated more effectively by pretreating rats with muscarinic versus nicotinic receptor antagonists.

The present study evaluated whether cholinergic activation of dopamine neurons in VTA suppresses the affective reaction of rats to noxious stimulation. Research in this laboratory validated a rodent model of pain affect (see Discussion). Vocalization afterdischarges (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).^{6,9,14,16} The effects of carbachol injected into the VTA on VAD threshold were compared with its effects on tail shock-elicited behaviors organized at spinal (SMR, hind limb movements and tail flexion) and medullary (VDS) levels of the neuraxis.^{10,16} It was predicted that VAD threshold would be preferentially elevated by VTA-administered carbachol. Muscarinic receptor mediation of carbachol-induced threshold increases was assessed by pretreating the VTA with the nonspecific muscarinic receptor antagonist atropine.

Materials and Methods

Animals

Male Long-Evans rats (Charles River, Raleigh, NC) ranging from 90 to 150 days old were used. Rats were housed as pairs in plastic cages in a climate-controlled vivarium (lights on 6 AM–6 PM), and given ad libitum access to food and water. Testing occurred during the light portion of the cycle. Rats were handled 1 to 2 times per day for at least 1 week before testing to minimize possible effects of stress from human contact. All procedures were approved by the Animal Investigation Committee of Wayne State University and followed international guidelines.

Surgery and Histology

Surgeries were performed under aseptic conditions. Rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.) after pretreatment with atropine sulfate (1 mg/kg i.p.) and positioned in a Kopf small animal stereotaxic frame (Kopf, Tujunga, CA). Guide cannulas (22-gauge stainless steel hypodermic tubing) were implanted unilaterally at a 10° angle (lateral to medial) 2 mm above the VTA according to coordinates extrapolated from the rat brain atlas of Paxinos and Watson.⁴⁶ The coordinates (in mm) relative to the bregma suture and the top of the level skull were AP = −5.7, L = 2.7, DV = −7.2. Guides were affixed to the skull with 3 stainless steel bone screws and cranioplastic cement. Each guide cannula was fitted with a 28-gauge dummy cannula that extended the length of the guide to keep it clear and free of debris. Rats were given 7 to 10 days to recover before the initiation of testing.

After testing, rats were killed by carbon dioxide asphyxiation. Injection sites were marked by safran-O dye (0.5 μ L) and brains were extracted and placed in a 20% (wt/vol) sucrose formalin solution for 48 to 72 hours. Brains were sectioned at 50 μ m on a freezing microtome, and injection sites were localized with the aid of the Paxinos and Watson⁵⁶ brain atlas by an experimenter unaware of the behavioral outcomes.

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 (Velcro Inc., Manchester, NH) and restrained on a plexiglas pedestal using Velcro strapping (Velcro, Inc.) that passed through loops located on the underside of the suits (see photograph in Borszcz⁶). This design maintained the rat in a crouching posture throughout testing, enabled rats to breathe and vocalize normally, and permitted unobstructed access to the head for intracerebral injections. Testing was conducted within a sound attenuating, lighted, and ventilated chamber equipped with a small window that enabled visual monitoring of rats during testing.

Tail shock (20-ms pulses at 25 Hz for 1000 ms) was delivered by a computer-controlled constant current shocker (STIMTEK, Arlington, MA) through electrodes (0-gauge stainless steel insect pins) placed intracutaneously (0.5 mm below the skin surface) on opposite sides of the tail, 7.0 cm (cathode) and 8.5 cm (anode) from the base. The utility of this form of tail shock as a noxious stimulus has been extensively discussed.^{5,7,11} The intensity, duration, and timing of tail shocks were controlled by the computer. Current intensity was monitored by the computer via an analog-to-digital converter of the multifunction board that digitized (500 Hz sampling rate) an output voltage of the shocker that was proportional to the current delivered.

SMRs were measured with a semi-isotonic displacement transducer (Model 76614; Lafayette Instruments,

Lafayette, IN) attached to the rat's tail with cotton thread. The output voltage of the transducer was amplified ($\times 50$) and then digitized (500-Hz sampling rate) by a second analog-to-digital converter of the multifunction board/computer. SMR was defined as movement of the transducer arm by at least 1.0 mm after shock onset. Once SMR criterion was exceeded, the output voltage of the transducer was monitored for 2000 ms. The computer recorded the latency (milliseconds), peak amplitude (millimeters), and magnitude (centimeters \times milliseconds) of tail movement on each trial. Displacement up to 100 mm could be detected.

Vocalizations were recorded 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) and a 10-band frequency equalizer adjusted to selectively amplify frequencies above 1500 Hz. The filtering of low frequencies prevented extraneous noise (ie, 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 third analog-to-digital converter of the multifunction board/computer.

The audio system was calibrated by determining the relation between the peak digitized output of the analog-to-digital 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.^{6,9,14} The derived function was used to convert analog-to-digital inputs to decibels (dB). Sound intensities up to 113.0 dB could be measured. The most intense vocalization measured during any sampling period was 101.3 dB. The computer recorded the peak intensity (in decibels), latency (milliseconds), and duration (milliseconds) of vocalizations during the shock epoch (VDS) and for the 2000-ms interval after shock termination (VAD).

Procedures

Pain Testing

For 2 consecutive days before testing, rats were adapted to the testing apparatus for a period of 20 min/day to minimize the effects of restraint stress. Experimenters were blind to the group assignment of rats. Testing began 10 to 12 minutes after completion of intracerebral injections. Test sessions consisted of 20 trials. On 16 trials, tail shocks between 0.01 and 2.50 mA were delivered, and on 4 trials no current was delivered so as to assess false-alarm rates. Trials were presented in a randomized order to control for the impact of any particular tail shock intensity on subsequent responding and to prevent rats from anticipating the intensity of successive tail shocks. Trials were presented with a minimum intertrial interval of 30 seconds, and each test session concluded within 20 minutes. These procedures caused no observable damage to the tail. After each test session,

the testing apparatus was cleaned with 5% ammonia hydroxide to eliminate stress odors.¹⁸

Drug Injections

Intracerebral injections were administered in a volume 0.5 μ L at a constant rate over 2 minutes via 28-gauge injectors connected to a microinfusion pump (Model PHD 2000; Harvard Apparatus, Holliston, MA). Injectors extended 2 mm beyond the guide cannulas into the VTA. Injectors were left in place for 2 minutes after the completion of injections to aid the diffusion of drugs into tissue. All drugs were dissolved in sterile saline. All drugs were purchased from Sigma-Aldrich (St. Louis, MO).

Experiment 1: Dose-Response Analyses

To quantify the dose-response relationship between carbachol administered into the VTA and SMR, VDS, and VAD thresholds, rats received unilateral microinjections of carbachol (1 μ g, 2 μ g, and 4 μ g) and saline into the VTA before four separate test sessions. Test sessions were separated by 3 to 5 days. The order of injections was counterbalanced using a quasi-Latin square design that maintained the saline injection at either the beginning or the end of the test sequence. This design permitted evaluation of the effects of repeated testing on baseline responding.

Experiment 2: Antagonism Analyses

The pharmacological specificity of carbachol was evaluated in a separate group of rats. Elevations in thresholds generated by 4 μ g carbachol were challenged with the intra-VTA administration of the muscarinic receptor antagonist atropine sulfate. Atropine (30 μ g or 60 μ g) was administered 10 minutes before carbachol treatment. Administration of these doses of atropine into VTA was effective in reducing lateral hypothalamic self-stimulation in rats.⁵⁷ Animals received unilateral injections of saline + saline, saline + 4 μ g carbachol, 30 μ g atropine + 4 μ g carbachol, and 60 μ g atropine + 4 μ g carbachol, 30 μ g atropine + saline, and 60 μ g atropine + saline. The order of injections was counterbalanced using a quasi-Latin square design that maintained the saline + saline injection at either the beginning or the end of the test sequence. Tests were separated by 3 to 5 days.

Data Analysis

Some animals did not complete all testing sessions due to illness ($n = 1$) or blocked and damaged cannulas ($n = 5$). This attrition resulted in unequal sample sizes necessitating that data be considered from independent groups and analyzed accordingly.

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 minimum current intensity from a string of at least 2 consecutive intensities that generated the response. Response thresholds in the dose-response experiment ($n = 5$ – 7 rats per group) were directly compared using repeated-measures multivariate analysis of variance (MANOVA).

The effects of dose on individual responses were analyzed by 1-way analysis of variance (ANOVA). The doses of carbachol that elevated response thresholds above baseline levels were determined by planned comparisons of thresholds after saline and carbachol treatments using Student *t* test for independent groups. The capacity of atropine to reduce carbachol-induced increases in response thresholds was analyzed for each response by 2-factor ANOVA ($n = 6-7$ rats per group). One factor was antagonist drug treatment (3 levels: 30 μg atropine, 60 μg atropine, and saline); the second factor was agonist drug treatment (2 levels: 4 μg carbachol and saline). Because atropine was predicted to reduce carbachol-induced increases in thresholds, a significant ANOVA was followed by planned comparisons using Student *t* tests for independent groups.

Data from rats ($n = 7$) were analyzed separately as anatomic controls when histological evaluation revealed that their injection site was outside the VTA.

Results

Behavioral Profile

As demonstrated by Carroll and Lim,¹⁶ SMR, VDS, and VAD reflect nociceptive processing at progressively higher levels of the neuraxis. Their analysis of rats that received transections of the neuraxis revealed that SMRs are organized at the spinal level,¹⁰ VDSs within the medulla below the pontomedullary border, and VADs within the forebrain. Consistent with our previous reports, responses organized rostrally within the central nervous system (CNS) were rarely generated without those integrated more caudally within the CNS.^{5,6,11,40} VAD generation, without concomitant elicitation of VDS and SMR, occurred on 0.005% of all trials. VDS were elicited without SMR on 0.002% of the trials in which VDS was the most rostrally elicited response.

The effects of carbachol treatment on performance of each response at threshold were also analyzed. The capacity of monitored performance variables to detect decrements in performance that confound threshold measurement was established by previous studies.^{5,11} Performance variables at threshold attained after saline treatment were compared with performance variables at threshold attained after treatment with each dose of carbachol. In experiment 1, latency, amplitude, and magnitude of SMRs, and latency, amplitude, and duration of VDSs and VADs did not differ after saline and carbachol (1 μg , 2 μg , or 4 μg) administration except for VDS amplitude after 2 μg and 4 μg . Compared with performance after saline administration, VDS amplitude was reduced after both 2 μg and 4 μg administration of carbachol into VTA ($t_s > 2.21$, $P_s < .05$). The reductions in VDS amplitude were modest, and the resulting amplitudes fell within the normal range observed in past experiments conducted in our laboratory [mean amplitudes (\pm SEM): saline = 78.3 dB \pm 2.24, 2 μg carbachol = 71.1 dB \pm 2.28, and 4 μg carbachol 69.8 dB \pm 1.49]. In experiment 2, comparison of performance variables after saline + saline and saline + 4 μg carbachol treatments revealed no

differences in performance at threshold for any response.

False-alarm rates for each response were low (SMR = 0.05%, VDS = 0.007%, VAD = 0.01%). The low incidence of false alarms indicates that responses were not induced by drug treatments, were not occurring spontaneously, and were not conditioned responses to the context, but instead were generated by tail shock.

Experiment 1: Dose-Response Analyses

The dose-dependent effects of carbachol (1 μg , 2 μg , and 4 μg) administration into the VTA on SMR, VDS, and VAD thresholds are shown in Fig 1. Comparison of response thresholds after saline administration revealed no differences in baseline thresholds ($F < 1$). Repeated drug administration into the VTA did not alter baseline thresholds. No differences in response thresholds after saline treatments were observed in subgroups that were administered saline first or last in the testing sequence ($t_s < 1.0$).

Comparison of response thresholds across saline and carbachol treatments (repeated-measures MANOVA, Wilk's λ) revealed significant main effects of dose [$F(3, 21) = 21.15$, $P < .001$] and response [$F(2, 20) = 44.72$, $P < .001$] and a significant dose \times response interaction [$F(6, 40) = 8.62$, $P < .001$]. This interaction reflects the finding that carbachol preferentially increased VAD threshold. Analysis of each response across saline and carbachol treatments revealed that VDS and VAD thresholds were increased in a dose-dependent manner [VDS, $F(3, 24) = 3.92$, $P < .05$; VAD, $F(3, 24) = 27.27$, $P < .001$], whereas SMR threshold was not elevated after carbachol administration ($F < 1$). Planned comparisons of VDS and VAD thresholds after saline treatment and each dose of carbachol revealed that the minimum effective doses of carbachol that elevated thresholds above baseline were 2 μg for VDS [$t(11) = 3.10$, $P < .01$] and 1 μg for VAD [$t(11) = 3.59$, $P < .01$]. Direct comparison of VAD and VDS

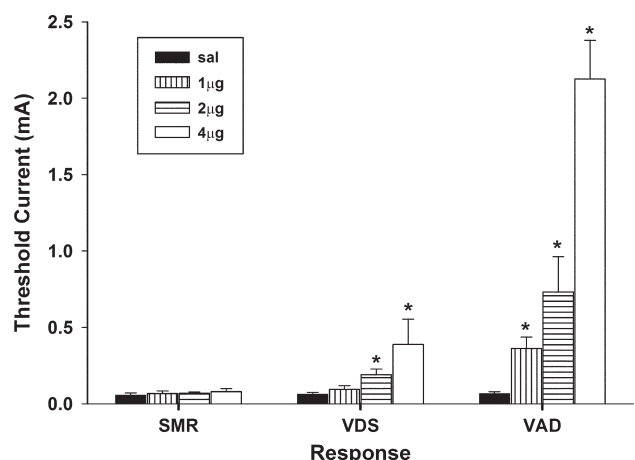


Figure 1. Effects of unilateral administration of carbachol (1, 2, or 4 μg) into the ventral tegmental area on the mean (\pm SEM) thresholds of spinal motor reflexes (SMRs), vocalizations during shock (VDSs), and vocalization afterdischarges (VADs). *Significantly elevated above saline treatment.

thresholds revealed that VAD threshold was significantly elevated above VDS threshold after administration of 1 μ g, 2 μ g, and 4 μ g carbachol (all t s > 2.50, all P s < .05).

Experiment 2: Antagonism Analyses

Fig 2 depicts the effects of atropine on increases in response thresholds generated by carbachol administered into the VTA. Unilateral administration of 4 μ g carbachol into the VTA produced results consistent with those from experiment 1. VDS and VAD thresholds were significantly elevated after carbachol treatment, but there was no significant change in SMR threshold. An overall 2-factor ANOVA (carbachol treatment and atropine treatment), revealed significant main effects of car-

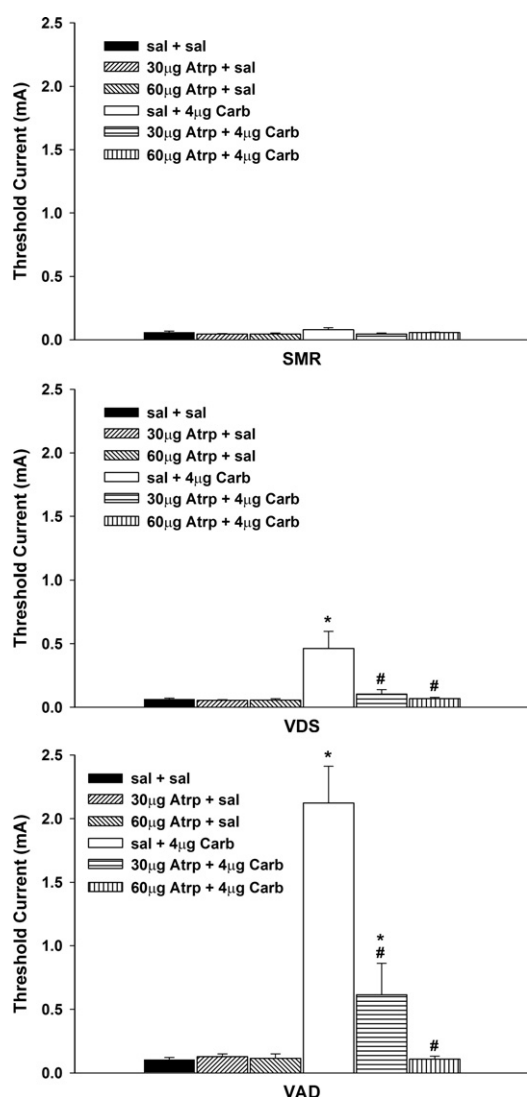


Figure 2. Effects of unilateral administration of atropine (Atrp: 30 μ g and 60 μ g) into the ventral tegmental area (VTA) on increases in vocalization thresholds produced by intra-VTA injections of 4 μ g carbachol (Carb). Data are plotted as the mean (\pm SEM) threshold of spinal motor reflexes (SMRs), vocalizations during shock (VDSs), and vocalization afterdischarges (VADs). Thresholds significantly elevated above saline (sal) + saline treatment. *,# Thresholds significantly reduced compared with sal + 4 μ g Carb treatment.

bachol treatment for both VDS [$F(1, 37) = 8.38, P < .01$] and VAD [$F(1, 37) = 36.88, P < .001$] thresholds but not SMR thresholds [$F(1, 37) = 2.02, P > .15$]. Planned comparisons of response thresholds after administration of saline + saline and saline + carbachol treatments revealed significant increases in VDS [$t(11) = 2.73, P < .05$] and VAD thresholds [$t(11) = 5.39, P < .001$] but not SMR threshold [$t(11) = 1.18, P > .25$].

Administration of atropine abolished carbachol-induced threshold elevations for VDS and VAD in a dose-dependent manner. The overall 2-factor ANOVA also revealed significant main effects of atropine treatment for both VDS and VAD thresholds [VDS, $F(2, 37) = 6.09, P < .01$, and VAD, $F(2, 37) = 19.53, P < .001$]. The carbachol \times atropine interaction was also significant for VDS and VAD thresholds [all F s(2,37) > 5.54, all P s < .01]. This interaction reflects the finding that atropine reduced increases in VDS and VAD thresholds generated by injection of carbachol but did not alter baseline thresholds. Planned comparisons revealed that both doses of atropine significantly reduced carbachol-induced increases in VDS threshold [all t s(11) > 2.40, all P s < .05] and returned threshold to baseline levels [all t s(10) < 1.11, all P s > .25]. Carbachol-induced increase in VAD threshold was reduced after administration of 30 μ g atropine [$t(11) = 3.90, P < .01$] but remained marginally elevated relative to baseline [$t(10) = 2.08, P = .065$]. Administration of 60 μ g atropine also reduced carbachol-induced increases in VAD threshold [$t(10) = 6.40, P < .001$] and returned threshold to baseline levels [$t(10) < 1$].

Atropine alone did not alter baseline response thresholds. One-way ANOVA comparing thresholds of each response after saline + saline, 30 μ g atropine + saline, and 60 μ g atropine + saline revealed no significant changes in thresholds [all F s < 1]. Repeated drug administration into the VTA did not alter baseline thresholds. No differences in response thresholds after saline + saline treatments were observed in subgroups that were administered saline first or last in the testing sequence [t s < 1.0].

Anatomic Specificity

No systematic differences were observed in the distribution of sites within the VTA that received different doses of carbachol or atropine. The effectiveness of any specific dose of carbachol to elevate response thresholds did not differ across sites. The effectiveness of atropine in antagonizing carbachol-induced increases in VAD and VDS thresholds also did not differ across injection sites. Anatomical specificity of carbachol-induced antinociception within the VTA was determined by evaluating the effects on response thresholds of 4 μ g carbachol administered into sites dorsal, ventral, lateral, and medial to the VTA (Fig 3).

The effects of carbachol on response thresholds when administered within the VTA versus outside the VTA are summarized in Table 1. SMR and VDS thresholds after administration of 4 μ g carbachol into sites outside the VTA were not different from those observed after the administration of saline into VTA [all t s < 1]. On the other hand, VAD threshold after 4 μ g carbachol admin-

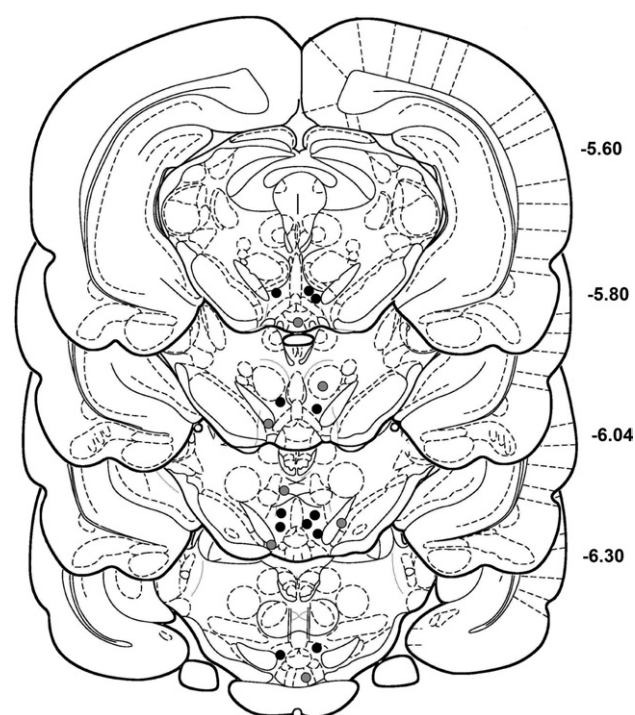


Figure 3. Histological reconstruction of sites that received unilateral injections of 4 μ g carbachol. Black circles indicate injection sites within the ventral tegmental area (VTA) that were effective in elevating response thresholds. Gray circles indicate injection sites dorsal, ventral or medial to VTA that produced no change in response thresholds. Coordinates are in millimeters posterior to bregma. Plates are derived from the rat brain atlas of Paxinos and Watson.⁵⁶ Right side of the midline was targeted for all injections, but for the sake of clarity of presentation, injection sites are plotted on both sides of the midline.

istration into extra-VTA sites was elevated above baseline thresholds [$t(17) = 2.65$, $P < .05$]. However, the elevation in VAD threshold observed after carbachol administration into extra-VTA sites was significantly lower than the elevation of VAD thresholds observed after carbachol administered into the VTA [$t(17) = 7.80$, $P < .001$].

Discussion

The present study provides the first demonstration of behavioral antinociception generated by administration of the acetylcholine agonist carbachol into the VTA. Administration of carbachol into VTA produced dose-dependent increases in VAD and VDS thresholds but failed to elevate SMR threshold. Direct comparisons of response thresholds revealed that VAD threshold was preferentially elevated by the intra-VTA injection of carbachol. The minimum effective dose of carbachol that elevated VAD threshold was lower than the dose that raised VDS threshold. Also, baseline thresholds of VAD and VDS did not differ but VAD threshold was elevated to a greater extent after administration of each dose of carbachol. The preferential increase in VAD threshold cannot be attributed to drug-induced motor deficits as increases in VAD thresholds were not accompanied by

performance decrements. It is unlikely that carbachol-induced increases in VDS threshold results from the observed reduction in amplitude at threshold. The reduction in amplitude was small, and it was only observed in experiment 1. In experiment 2, administration of 4 μ g carbachol produced a comparable increase in VDS threshold without any decrement in performance.

Similar to the present results, administration of carbachol, 5-HT, 8-OH-DPAT (5-HT_{1A/7} agonist), or morphine into either the amygdala or nucleus parafascicularis thalami produced selective increases in VAD and VDS thresholds without an accompanying increase in SMR threshold.^{8,26,27,40} The failure to observe increases 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 produced significant increases in VAD, VDS, and SMR thresholds,^{8,12} and the intrathecal administration of morphine, serotonin, or norepinephrine was equally effective in raising VAD, VDS, and SMR thresholds.¹³ 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 the capacity to elevate SMR threshold depends on the site within the CNS at which antinociceptive treatments are administered.

The selective increase in vocalization thresholds observed in the present study presumably reflects carbachol-induced activation of dopamine neurons within the VTA that project to nAb. This interpretation is supported by findings that morphine-induced increases in the accumbal efflux of dopamine are modulated by muscarinic

Table 1. Anatomic Specificity of Carbachol (4 μ g) Injected Into the Ventral Tegmental Area on Increases in Response Thresholds

INJECTION SITE	RESPONSE		
	SMR MEAN \pm SEM	VDS MEAN \pm SEM	VAD MEAN \pm SEM
VTA-carb (12)*	0.080 \pm 0.011	0.432 \pm 0.100¶	2.124 \pm 0.190¶
Other-carb (7)†	0.064 \pm 0.013	0.069 \pm 0.011§	0.151 \pm 0.025§¶
VTA-sal (12)‡	0.058 \pm 0.008	0.063 \pm 0.007§	0.085 \pm 0.012§

Abbreviations: SMR, spinal motor reflexes; VDS, vocalizations during shock; VAD, vocalizations after discharges; VTA, ventral tegmental area; other, injection sites dorsal, ventral, medial, or lateral to VTA (Fig 3); carb, carbachol; sal, saline.

*Mean response thresholds from experiment 1 after the administration of 4 μ g carbachol plus experiment 2 after administration of saline + 4 μ g carbachol into VTA.

†Mean response thresholds after misplaced injections of 4 μ g carbachol (experiment 1) plus saline + 4 μ g carbachol (experiment 2).

‡Mean response thresholds from experiment 1 after the administration of saline plus experiment 2 after administration of saline + saline into VTA.

§Values are significantly lower than those observed after carbachol treatment in VTA (Student t test; $P < .05$).

¶Values are significantly higher than those observed after saline treatment (Student t test; $P < .05$).

NOTE. Values are mean \pm SEM of threshold current (in milliamperes). Values in parentheses represent the number of injections at each site.

receptors in VTA.³⁸ Additionally, infusion of morphine into the VTA or amphetamine into nAb (induces release from dopamine axon terminals and blocks reuptake of dopamine) suppresses paw-licking in the formalin test but does not alter withdrawal latencies in the tail flick test.^{2,34} These antinociceptive effects in the formalin test were blocked by pretreatment of nAb with the dopamine receptor antagonist raclopride.¹ Correspondingly, neurotoxic lesions of dopamine neurons in VTA blocked the suppression of paw-licking in the formalin test produced by systemic administration of morphine or amphetamine but did not alter the increase in tail flick latencies generated by these drug treatments.³⁹ These findings indicated that activation of mesoaccumbal dopamine projections selectively suppresses nociceptive processing at supraspinal levels of the neuraxis.

The capacity of VTA-administered carbachol to elevate vocalization thresholds is limited to its action within VTA. Unilateral injections of the highest dose of carbachol (4 μ g) into sites surrounding the VTA failed to elevate VDS threshold and produced a greatly attenuated increase in VAD threshold. Muscarinic mediation of the antinociceptive actions of carbachol was revealed by its dose-dependent antagonism with the intra-VTA administration of atropine. Atropine is a nonselective competitive muscarinic receptor antagonist with very limited affinity for nicotinic receptors.⁵⁵ As atropine has nearly equivalent binding affinity for the 5 known muscarinic receptor subtypes,¹⁷ the receptor subtype that mediates the increase in vocalization thresholds after carbachol treatment cannot be ascertained from results of the present study. However, only M5 mRNA is localized to cell bodies of dopamine neurons in the VTA,⁵³ and this receptor subtype mediates the sustained increase in accumbal dopamine levels generated by stimulation of LDTg or intra-VTA administration of carbachol.²⁰ We therefore speculate that activation of VTA dopamine neurons via M5 muscarinic receptors underlies the antinociceptive action of carbachol observed in the present study.

The preferential increase in VAD threshold after intra-VTA carbachol administration reflects suppression of the affective reaction to noxious stimulation. As noted earlier, previous research in this laboratory validated VADs as a rodent model of pain affect. Systemically administered drug treatments that preferentially suppress the affective reaction of humans to pain^{24,47} also preferentially suppress production of VADs.¹¹ 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.^{8,14,26,27,36,40,51} Additionally, the capacity of noxious tail shock to support fear conditioning is directly related to its production of VADs.^{5,6,9,14}

The present findings, therefore, support the hypothesis of an overlap between the neuronal circuits that underlie reinforcement and suppression of the affective reaction to pain.²¹ Although we did not evaluate the reinforcing properties of carbachol administered into the VTA, previous studies demonstrated that unilateral

administration of similar doses of carbachol (1 μ g or 3 μ g) supported development of conditioned place preference.⁵⁹ Additionally, rats learn to self-administer carbachol into the VTA.³⁰ As noted earlier, the reinforcing properties of intra-VTA administered carbachol are mediated by activation of mesoaccumbal dopamine projections.

Dopamine neurons in the VTA also project to forebrain sites (amygdala, anterior cingulate cortex) that receive nociceptive afferents and contribute to elaboration of affective behaviors.^{3,42,45} For example, stimulation of VTA suppresses neural activity in the anterior cingulate cortex elicited by noxious peripheral stimulation,³⁵ intra-VTA administration of carbachol increases extracellular dopamine in the anterior cingulate cortex,⁵⁴ and infusion of dopamine into the anterior cingulate cortex suppresses neuropathic pain in rats.³³ Future studies will evaluate the contribution of these dopaminergic projections to the forebrain in supporting the affective analgesia elicited from the VTA.

The VTA does not project directly to midbrain or medullary sites that contribute descending antinociceptive projections. However, the forebrain sites (nAb, anterior cingulate cortex, amygdala) that receive dopaminergic input from VTA project to the ventrolateral periaqueductal gray (vPAG) and rostral ventromedial medulla (RVM), and these projections contribute to the antinociceptive effects mediated by these forebrain sites.^{23,28,31} As generation of VDSs reflects nociceptive processing within the RVM (ie, nucleus reticularis gigantocellularis) suppression of this processing (either directly or via the vPAG) may contribute to the increases in VDS threshold in the present study. These descending projections to the medulla may also inhibit nociceptive throughput to the forebrain sites responsible for production of VADs. The dual inhibition of nociceptive processing at medullary and forebrain levels could account for the greater maximum effect of intra-VTA carbachol on VAD threshold.⁸

Altier and Stewart² proposed that the relation between the rewarding and antinociceptive actions of analgesic drugs such as morphine and amphetamine can be understood from the perspective of a motivational continuum. This motivational continuum has poles of extreme negative and positive affects with normal affect located in the middle. When noxious stimulation generates a negative affective state, opiates and psychostimulants suppress pain effect by enhancing transmission in mesocorticolimbic dopamine neurons that originate in the VTA. This activation shifts the negative affective state to the middle of the motivational continuum producing affective analgesia. However, when opiates and psychostimulants are taken in the absence of pain-induced negative affect, then normal affect is shifted to the extreme positive pole of the motivational continuum. The shift to the positive pole of the continuum is speculated to underlie the addictive liability of opiates or psychostimulants.

The conceptualization of a motivational continuum may account for clinical observations that chronic pain patients who are given access to opiates for the relief of

pain rarely become addicted or tolerant.³⁷ When opioids are used for the management of clinical pain, they may help the patient to achieve an affective state normally experienced when free of pain but not the extreme positive affect that might support addiction. Supporting this view are reports that the capacity of morphine to serve as a reinforcer is attenuated when given to rats that are in a chronic/tonic pain state⁵⁰ and that morphine tolerance and dependence fails to develop in rats when adminis-

tered during a pain state.⁵² The neurobiology that relates reinforcement with analgesia is of obvious clinical importance and warrants further study.

Acknowledgments

The authors thank Laura A. Bolter for assistance in data collection.

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