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Comparison of Motor Reflex and Vocalization Thresholds Following Systemically Administered Morphine, Fentanyl, and Diazepam in the Rat: Assessment of Sensory and Performance Variables

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BORSZCZ, G. S., C. P. JOHNSON AND K. A. FAHEY. *Comparison of motor reflex and vocalization thresholds following systemically administered morphine, fentanyl, and diazepam in the rat: Assessment of sensory and performance variables.* PHARMACOL BIOCHEM BEHAV **49**(4) 827-834, 1994.—The relative influence of systemically administered morphine, fentanyl, and diazepam on the thresholds of spinal motor reflexes (SMRs), vocalizations elicited during stimulation (VDSs), and vocalization afterdischarges (VADs) was assessed. Responses were elicited by applying graded electric current to the tail. Performance (latency and amplitude) of all three responses was monitored to determine whether elevations in threshold were confounded by performance decrements. All three drugs were found to elevate VAD thresholds more readily than VDS and SMR thresholds. VADs were also most susceptible to the deleterious effects of these drugs on motor performance. Nevertheless, across the dose range of morphine and fentanyl that elevated thresholds of all three responses without disrupting the performance of any response, the order of susceptibility to threshold increases remained VAD, VDS, and SMR. Diazepam also elevated VAD thresholds more readily than VDS thresholds across a dose range that failed to disrupt performance of either response. SMR thresholds were only elevated by diazepam when administered in doses that significantly disrupted performance. Results are discussed in terms of supporting the validity of VADs as a model of the affective-motivational dimension of pain.

Nociception Analgesia Benzodiazepines Narcotics Threshold Performance Motor reflex Vocalizations Affective-motivational Sensory-discriminative

PSYCHOPHYSICAL assessment of human pain, both experimental and clinical, has revealed that it is composed of at least two dimensions (21,43). The sensory-discriminative dimension signals the location, quality, intensity, and physical properties of a noxious stimulus and evokes rapid responses designed to prevent further injury. The affective-motivational dimension ascribes to a noxious stimulus the perception of unpleasantness that ultimately motivates aversive behaviors such as avoidance and recuperation (14,36). A principal concern of investigators of the neural mechanisms of nociception is the development of animal models that accurately reflect the human pain experience. Development of such models would enable information regarding the underlying neural mechanisms of nociception and analgesia to be more readily generalized to human experimental and clinical studies. One strategy for the development of viable animal models of human pain is to identify pain behaviors of the animal that are differentially influenced by treatments that have been shown to dissociate the dimensions of human pain. Such a correspondence enables one to argue that particular animal behaviors may reflect distinct components of human pain.

Administration of the benzodiazepine diazepam (15,22)

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and the opiate narcotics morphine and fentanyl (25,44,45) has been shown to preferentially suppress the affective-motivational aspect of human pain. It has been argued that vocalizations of the rat that exceed application of noxious tail shock (i.e., vocalization afterdischarges, VAD) reflect this aspect of the pain experience. Consistent with this interpretation are reports that the systemic administration of diazepam (31) and morphine (5,6,13,31,41) raises VAD thresholds more readily than either vocalizations that occur during application of tail shock (i.e., vocalizations during stimulation, VDS) or tail shock-generated withdrawal reflexes (i.e., spinal motor reflexes, SMR). This interpretation is also supported by our recent observation that the capacity of tail shock to support avoidance conditioning in the rat covaries with the probability that VAD is generated, and is independent of the proportion of SMR that is concomitantly elicited (5).

The present study was designed to provide a fuller description of the relative influence of systemically administered morphine, fentanyl, and diazepam on VAD, VDS, and SMR thresholds in the rat. In addition to the assessment of response thresholds, the performance (i.e., latency and amplitude) of each response was monitored to determine the relative effects of these drug treatments on the capacity of animals to fully generate the responses. This analysis permits us to establish whether the differential susceptibility of responses to increases in threshold may be confounded by their differences in susceptibility to the deleterious effects of these drugs on motor performance. The results of the present study will aid in establishing whether the VAD/VDS/SMR paradigm may provide a viable animal model of the human pain experience.

METHOD

Subjects

Female Long-Evans-derived rats, 90–130 days old and weighing between 200 and 330 g at the beginning of the experiment, were used. Animals were individually housed under a 14 L : 10 D cycle and given ad lib access to food and water. Animals were handled two-three times per day for at least 1 week prior to testing. All testing was conducted during the light portion of the light/dark cycle.

Drugs

Morphine sulfate and fentanyl citrate were dissolved in physiological saline and administered IP in a constant volume of 1 ml/kg. Diazepam was dissolved in 100% dimethyl sulfoxide (DMSO) and injected IP in a constant volume of 0.5 ml/kg. Separate groups of rats received either vehicle or drugs in the following doses: morphine (1, 4, 8, 10, 12, or 16 mg/kg), fentanyl (0.02, 0.04, 0.08, 0.12, or 0.16 mg/kg), diazepam (0.30, 0.63, 1.25, 2.5, 3.75, 5, 7.5, or 10 mg/kg). Each group contained eight rats.

Apparatus

The apparatus for measuring SMR, VDS, and VAD thresholds has been described in detail elsewhere (5). Animals were tested in an adjustable Plexiglas restraining tube (Braintree model #700R) that was housed in a sound-attenuating chamber. The internal diameter of the tube was adjusted for each animal to hold it securely and ensure that its head faced the headplate. The headplate was constructed of hardware cloth framed by Plexiglas. The tailplate was constructed of Plexiglas with a 1.5 × 2.5 cm slit at its base through which the rat's tail extended.

Shock electrodes were constructed of two 0-ga stainless steel insect pins. The pins were placed intracutaneously on opposite sides of the tail 7.0 cm (cathode) and 8.5 cm (anode) from the base. The wires connecting the electrodes to the shocker were suspended behind the rat and above the electrodes, such that the tail extended in a straight line directly behind the rat. Current (20-ms pulses at 25 Hz for 1000 ms) was delivered to the tail via a computer-controlled shocker. The intensity, duration, and timing of tail shocks were controlled by a microcomputer. Different current intensities were generated by applying different input voltages to the shocker via a digital-to-analog converter. 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. It should be emphasized that this form of tail shock does not produce the sensation of vibration that is generated by the superficially applied 60 Hz AC current commonly used in pain studies. Bromm and Meier (11) reported that intracutaneously applied pulsed DC current selectively activates primary afferent nociceptors (A-delta and C fibers) and generates a sharp, stabbing, or hot pain in humans rather than the diffuse paraesthesia produced by superficially applied AC current. We have confirmed that these are the sensations generated by the stimulus used in the present study (personal observation of G.S.B.) and are confident that the elicited responses reflect nociceptive processing.

The rat's tail distal to the shock electrodes was attached via cotton thread to a semi-isotonic displacement transducer (Lafayette model #76614). 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 SMR. The output voltage of the transducer was amplified (×50) and then digitized (500 Hz sampling rate) by a second analog-to-digital 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 0.5 mm. Once SMR criterion was exceeded, the output voltage of the transducer was monitored for 1500 ms. The microcomputer recorded the latency (in milliseconds), peak amplitude (in millimeters), and magnitude (integrated voltage output expressed in arbitrary units of digitized voltage × milliseconds) of tail movement on each trial. Displacements up to 100 mm could be detected and latencies in 2-ms increments could be measured.

Vocalizations were measured by a pressure-zone microphone (Realistic model #33-1090) placed 5 cm in front of the restraining tube centered on the headplate. The microphone was attached to an audio amplifier (Technics model SA-160) 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 18,000 Hz. The filtering of low frequencies prevented extraneous noise (i.e., animals' respiration and movement within the restraining tube) from contaminating vocalization records. The output of the amplifier was integrated by a Coulbourn contour following integrator (2-ms time base) and then digitized (500 Hz sampling rate) by a third analog-to-digital converter of the microcomputer.

The audio system was calibrated by determining the relation between the peak digitized output of the converter and

the amplitude (SPL, A Scale) of a 2.5-kHz pure tone, the approximate fundamental frequency of pain-induced vocalizations of the rat (31). The derived function was used to convert analog-to-digital inputs to decibels. Sound intensities up to 95.7 dB could be measured. The microcomputer recorded the peak intensity (decibels) and latency (milliseconds) of vocalizations during the shock epoch (i.e., VDS) and for the 1000-ms interval following shock termination (i.e., VAD). The ambient background noise level in the isolation chamber was 37.5 dB. Sounds above 40.5 dB were considered to be vocalizations.

Procedure

Following drug administration, animals were returned to their home cages for either 30 min (morphine group), 20 min (fentanyl group), or 10 min (diazepam group). Animals were then placed in the restraining tube and the electrodes and transducer were attached to the tail. Ten minutes of adaptation was provided prior to testing. Timing of injections was based on preliminary studies of the time-effect relationship of these drugs and on previous reports (31,41,50).

Testing consisted of presenting 40 tail shocks each at a different current intensity between 0.01 and 1.50 mA. The particular current intensities employed were calculated using: $mA = 0.05 \times [\exp(i \times 0.0784) - 1] + 0.01 \times i$, where i = tail shock number 1-40. These current intensities were chosen on the basis of preliminary studies that were designed to determine the minimum number of tail shocks that could be administered while maintaining the capacity to accurately assess all three response thresholds. On one additional trial no current was administered (i.e., catch trial) so as to assess false alarm rates. Tail shocks were presented in a randomized order rather than in an ascending series. Randomization was designed to control for the impact of any particular tail shock on subsequent response generation, and to prevent animals from anticipating the intensity of successive tail shocks. Tail shocks were present with a minimum 30-s interstimulus interval. To ensure that animals were not moving or vocalizing immediately prior to shock presentation, the outputs of the transducer and microphone were monitored on an oscilloscope by the experimenter. If these traces indicated that the animal was moving or vocalizing, then tail shock was delayed until movement or vocalization abated. Testing was concluded within 32 min. These parameters caused no observable damage to the tail.

Data Analysis

Data were reorganized in ascending order according to tail shock intensity. SMR, VDS, and VAD thresholds for each animal were calculated as the minimum current intensity of a string of at least three consecutive intensities that generated the response. Thresholds that exceeded 1.50 mA were arbitrarily assigned this value. Dose-effect curves for each response were analyzed via one-way ANOVA over doses of drug that did not disrupt performance. Doses of drug that significantly elevated response thresholds were tested via planned comparisons of drug-treated groups with vehicle-treated control groups. The influence of performance decrements on response thresholds was assessed via regression-discontinuity analysis (12). This analysis contrasted the observed thresholds of each response that were associated with performance decrements with those predicted from the best-fit regression equations generated over thresholds not associated with performance decrements. Performance decrements for each response were analyzed by contrasting vehicle-treated control groups with drug-treated groups via planned comparisons that followed significant one-way ANOVA.

RESULTS AND DISCUSSION

Response Profile

Oscilloscope traces at SMR, VDS, and VAD thresholds generated by a saline-treated animal are depicted in Fig. 1. The relation of performance variables to current intensity was evaluated in vehicle-treated control groups. Consistent with our previous report (5), latency, amplitude, and magnitude of SMR, latency and amplitude of VDS, and amplitude of VAD were observed to be linearly related to current intensity when plotted on double-log coordinates. For all animals each performance variable was significantly correlated with the intensity of tail shock that was administered (all Pearson $r \geq 0.81$, $p < 0.001$). As current intensity increased, the latency of SMR and VDS decreased, the amplitude of all three responses increased, and the magnitude of SMR increased. In contrast, latency of VAD was not clearly related to current intensity. This lack of correspondence occurred because at least two different types of VADs were generated and the type generated was not related to current intensity. One type of VAD was a distinct vocalization that was initiated following termination of tail shock (Fig. 1C), and a second type was a continuation of VDS into the postshock period. This second type of VAD was observed when multiple VDSs were generated during the shock epoch (Fig. 1C) and represent the extension of the late VDS into the postshock period. Because this form of vocalization is eliminated following brain lesions that selectively abolish VADs (13,23), it is assumed they are a type of VAD rather than a type of VDS.

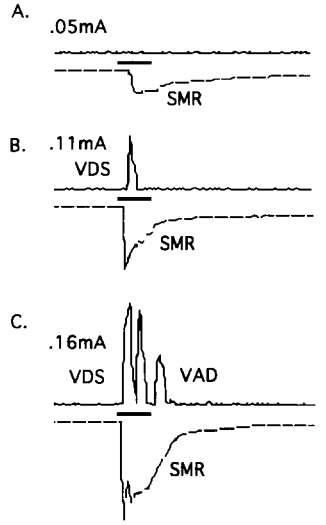

FIG. 1. Oscilloscope (MacScope) traces at threshold current intensities for (A) SMR (spinal motor reflex), (B) VDS (vocalization during stimulation), and (C) VAD (vocalization afterdischarge) of a saline-treated animal. Integrated vocalizations are represented on the upper trace and SMR on the bottom trace of each record. The line between the traces indicates the 1-s tail shock epoch. VDS was measured during tail shock, SMR was measured for 1500 ms after criterion was exceeded, and VAD was measured during the 1000-ms interval that followed shock offset.

Carroll and Lim (13) demonstrated via serial transections of the neuraxis that SMR, VDS, and VAD are organized at spinal, hindbrain, and forebrain levels, respectively [also see (7)]. In the present study, the more rostrally organized responses were rarely generated without those integrated more caudally within the CNS. On all trials in which VAD was elicited, VDS and SMR were not concomitantly generated on only 1.8% of the trials. Similarly, VDS was generated without SMR on 3.7% of trials in which VDS was the most rostrally elicited response. In addition, the overall response probabilities on trials in which no tail shock was presented (i.e., false alarms) were found to be very low (SMR = 2.6%, VDS = 2.2%, VAD = 0.8%), indicating that responses did not occur spontaneously but were generated by application of current to the tail.

Thresholds

Dose-effect curves relating drug treatments to increases in VAD, VDS, and SMR thresholds are depicted in Figs. 2–4. Best-fit regression lines are plotted for each response across drug doses that did not result in performance deficits (see Table 1). All three drugs were shown to elevate VAD thresholds more readily than either VDS or SMR thresholds. One-way ANOVA revealed significant increases in thresholds for all three responses across these doses of morphine [VAD, $F(3, 28) = 13.60, p < 0.001$; VDS, $F(4, 35) = 12.65, p < 0.001$; SMR, $F(5, 42) = 10.66, p < 0.001$] and fentanyl [VAD, $F(3, 28) = 22.71, p < 0.001$; VDS, $F(5, 42) = 14.20, p < 0.001$; SMR, $F(5, 42) = 10.23, p < 0.001$]. Planned comparisons of individual drug groups with saline-treated controls revealed that the minimum doses of morphine and fentanyl that significantly elevated response thresholds were: morphine-VAD = 1 mg/kg, $F(1, 28) = 4.83, p < 0.05$; VDS = 4 mg/kg, $F(1, 35) = 8.88, p < 0.01$; SMR = 8 mg/kg, $F(1, 42) = 13.24, p < 0.001$; fentanyl-VAD = 0.02 mg/kg, $F(1, 28) = 4.55, p < 0.05$; VDS = 0.08 mg/kg, $F(1, 42) = 13.60, p < 0.001$; SMR = 0.08 mg/kg, $F(1, 42) = 6.82, p < 0.05$. On the other hand, whereas diazepam significantly elevated VAD, $F(4, 35) = 12.33, p < 0.001$, and VDS, $F(6, 49) = 12.43, p < 0.001$, thresholds across doses that did not disrupt performance, SMR thresholds were not increased over this dose range, $F(7, 56) = 1.81, p > 0.10$. The minimum doses of diazepam that significantly elevated thresholds were: VAD = 0.63 mg/kg, $F(1, 35) = 8.63, p < 0.01$; VDS = 2.5 mg/kg, $F(1, 49) = 8.52, p < 0.01$; SMR = 10 mg/kg, $F(1, 63) = 84.02, p < 0.001$.

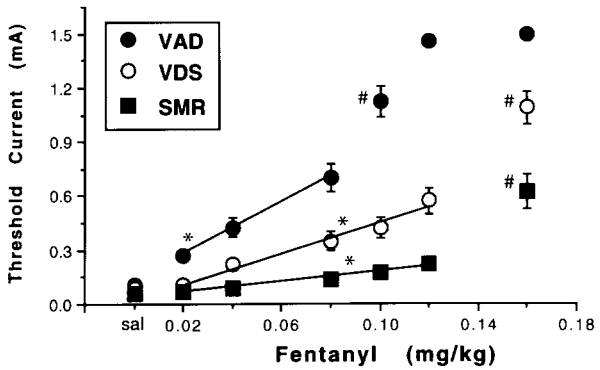

FIG. 3. Mean ± SE threshold currents for SMR, VDS, and VAD following systemic administration of saline (sal) or fentanyl citrate. *Minimum dose of fentanyl that significantly elevated response thresholds over thresholds measured following saline treatment. #Minimum dose of fentanyl that resulted in a significant decrement in performance (i.e., latency, amplitude, and/or magnitude) when compared to performance following saline treatment. Best-fit regression lines are fitted across doses of fentanyl that did not disrupt performance.

Performance

The order of susceptibility of VAD, VDS, and SMR to increases in threshold was mirrored by the sensitivity of these responses to the disrupting effects of morphine, fentanyl, and diazepam on performance at threshold (Table 1). For all three

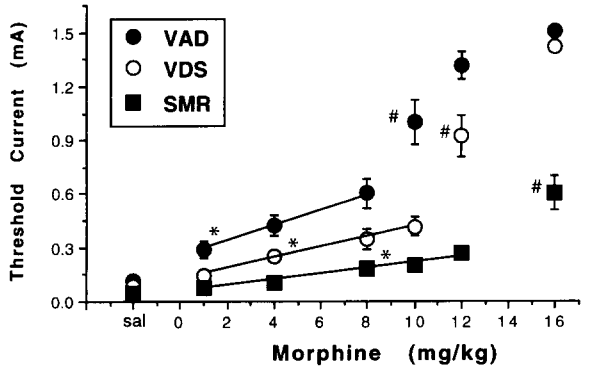

FIG. 2. Mean ± SE threshold currents for SMR, VDS, and VAD following systemic administration of saline (sal) or morphine sulfate. *Minimum dose of morphine that significantly elevated response thresholds over thresholds measured following saline treatment. #Minimum dose of morphine that resulted in a significant decrement in performance (i.e., latency, amplitude, and/or magnitude) when compared to performance following saline treatment. Best-fit regression lines are fitted across doses of morphine that did not disrupt performance.

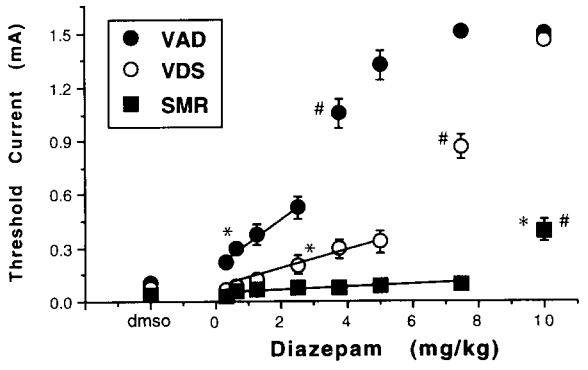

FIG. 4. Mean ± SE threshold currents for SMR, VDS, and VAD following systemic administration of dimethyl sulfoxide (dmso) or diazepam. *Minimum dose of diazepam that significantly elevated response thresholds over thresholds measured following dmso treatment. #Minimum dose of diazepam that resulted in a significant decrement in performance (i.e., latency, amplitude, and/or magnitude) when compared to performance following dmso treatment. Best-fit regression lines are fitted across doses of diazepam that did not disrupt performance.

TABLE 1

PERFORMANCE OF SMR, VDS, AND VAD AT THRESHOLD FOLLOWING SYSTEMICALLY ADMINISTERED MORPHINE, FENTANYL, OR DIAZEPAM

	SMR			VDS		VAD	
Drugs	Latency (ms)	Amplitude (mm)	Magnitude (a.u.)	Latency (ms)	Amplitude (dB)	Latency* (ms)	Amplitude (dB)
Morphine							
Saline	217.6 ± 18.5	4.06 ± 0.37	14.1 ± 1.7	348.1 ± 32.6	66.1 ± 0.82	1079.8 ± 44.3	60.4 ± 1.24
1 mg/kg	231.0 ± 23.5	3.69 ± 0.57	13.8 ± 1.6	340.8 ± 33.2	66.4 ± 1.12	1100.2 ± 56.3	60.7 ± 1.03
4 mg/kg	224.0 ± 13.6	3.85 ± 0.56	13.2 ± 1.6	328.8 ± 20.3	65.2 ± 1.10	1049.0 ± 34.4	59.2 ± 1.42
8 mg/kg	240.8 ± 23.6	3.91 ± 0.45	14.3 ± 1.4	336.5 ± 25.6	66.2 ± 1.04	1180.8 ± 57.5	57.2 ± 1.32
10 mg/kg	216.0 ± 14.8	3.90 ± 0.61	11.7 ± 0.7	350.8 ± 19.8	65.6 ± 1.17	1171.7 ± 37.2	53.6 ± 1.32†
12 mg/kg	233.2 ± 14.9	3.81 ± 0.53	13.3 ± 2.3	350.6 ± 25.0	58.5 ± 1.17‡	1459.0 ± 152.8‡	49.3 ± 1.06‡
16 mg/kg	250.2 ± 15.2	1.65 ± 0.23†	4.6 ± 0.4‡	NR	NR	NR	NR
Fentanyl							
Saline	234.8 ± 19.1	3.92 ± 0.45	13.3 ± 1.6	344.0 ± 22.0	68.0 ± 1.44	1088.0 ± 59.3	61.1 ± 1.50
0.02 mg/kg	223.0 ± 13.0	3.95 ± 0.54	14.6 ± 2.1	322.8 ± 12.1	67.6 ± 0.84	1054.2 ± 28.0	60.2 ± 1.17
0.04 mg/kg	221.5 ± 24.6	4.24 ± 0.41	14.9 ± 2.2	351.0 ± 16.6	67.8 ± 1.44	1070.8 ± 36.1	63.2 ± 1.58
0.08 mg/kg	238.5 ± 20.8	3.81 ± 0.49	14.1 ± 2.0	319.2 ± 20.3	66.7 ± 0.81	1103.5 ± 42.8	61.0 ± 1.39
0.10 mg/kg	233.2 ± 16.7	4.04 ± 0.58	14.6 ± 2.1	322.8 ± 15.3	67.2 ± 1.05	1182.0 ± 48.5	54.3 ± 1.22†
0.12 mg/kg	238.8 ± 25.2	3.75 ± 0.68	15.3 ± 2.5	357.5 ± 15.8	66.2 ± 0.67	NR	NR
0.16 mg/kg	252.8 ± 27.4	1.71 ± 0.24†	4.8 ± 0.5†	380.6 ± 31.3	58.4 ± 1.16‡	NR	NR
Diazepam							
DMSO	232.8 ± 22.3	4.15 ± 0.41	15.3 ± 2.3	368.5 ± 27.0	67.1 ± 0.65	1094.8 ± 61.7	61.9 ± 0.82
0.30 mg/kg	252.8 ± 22.0	4.11 ± 0.47	15.3 ± 2.5	365.8 ± 14.4	68.0 ± 1.02	1060.2 ± 36.5	61.0 ± 1.15
0.63 mg/kg	235.8 ± 18.3	3.92 ± 0.60	13.8 ± 3.1	361.0 ± 29.1	67.7 ± 1.32	1036.2 ± 16.9	61.8 ± 1.53
1.25 mg/kg	212.2 ± 23.5	3.65 ± 0.44	12.9 ± 1.7	330.2 ± 19.6	66.8 ± 1.70	1123.0 ± 32.9	60.0 ± 1.03
2.50 mg/kg	224.8 ± 20.6	3.71 ± 0.44	13.2 ± 1.8	335.0 ± 19.5	65.5 ± 1.15	1182.0 ± 36.4	59.9 ± 1.12
3.75 mg/kg	240.2 ± 14.3	3.84 ± 0.40	14.3 ± 1.2	354.8 ± 26.0	67.7 ± 0.87	1264.8 ± 50.9§	52.7 ± 1.55‡
5.00 mg/kg	239.5 ± 21.2	4.11 ± 0.48	15.2 ± 2.6	367.2 ± 23.9	66.0 ± 1.20	1520.5 ± 83.0‡	47.9 ± 1.60‡
7.50 mg/kg	260.0 ± 26.6	3.71 ± 0.65	14.0 ± 2.0	382.0 ± 25.7	61.2 ± 1.11‡	NR	NR
10.00 mg/kg	290.8 ± 24.4	1.66 ± 0.30‡	4.6 ± 0.6‡	NR	NR	NR	NR

Values are mean ± SE. *Measured from onset of tailshock. a.u. = arbitrary units = digitized voltage × milliseconds (see text for details). †‡§ Significantly different from vehicle-treated control groups as assessed by planned comparisons: †$p < 0.01$, ‡$p < 0.001$, §$p < 0.05$. NR = no response: fewer than four of eight animals responded and performance versus control groups was not tested.

drugs performance of VAD was most easily disrupted with progressively higher doses needed to disrupt performance of VDS and SMR. When compared to vehicle-treated control groups, the minimum drug doses that generated decrements on any performance variable were: morphine-VAD (amplitude) = 10 mg/kg, $F(1, 37) = 12.69, p < 0.01$; VDS (amplitude) = 12 mg/kg, $F(1, 41) = 20.79, p < 0.001$; SMR (amplitude and magnitude) = 16 mg/kg, $F(1, 49) = 9.41, p < 0.01$ and $F(1, 49) = 16.82, p < 0.001$; fentanyl-VAD (amplitude) = 0.10 mg/kg, $F(1, 34) = 10.78, p < 0.01$; VDS (amplitude) = 0.16 mg/kg, $F(1, 48) = 36.86, p < 0.001$; SMR (amplitude and magnitude) = 0.16 mg/kg, $F(1, 49) = 8.84, p < 0.01$ and $F(1, 49) = 9.21, p < 0.01$; diazepam-VAD (latency and amplitude) = 3.75 mg/kg, $F(1, 45) = 6.69, p < 0.05$ and $F(1, 45) = 24.34, p < 0.001$; VDS (amplitude) = 7.5 mg/kg, $F(1, 56) = 11.35, p < 0.01$; SMR (amplitude and magnitude) = 10 mg/kg, $F(1, 63) = 12.33, p < 0.001$ and $F(1, 63) = 14.3, p < 0.001$.

All three drugs were found to disrupt response amplitude and magnitude more readily than response latency. Across the doses of drugs that were tested only amplitude and magnitude of SMR and amplitude of VDS were disrupted. Although both latency and amplitude of VAD were affected by morphine and diazepam, the effects on amplitude were either generated at lower doses (morphine) or more pronounced (diazepam). Although not systemically examined, the increase in VAD latency was apparently related to an increase in the proportion of VADs that were initiated following shock termination.

Thresholds and Performance

The relationship between the influence of morphine, fentanyl, and diazepam on performance and thresholds is also summarized in Figs. 2-4. These dose-effect curves reveal that across doses of morphine (1-8 mg/kg) and fentanyl (0.02-0.08 mg/kg), which elevated all three responses without disrupting performance of any response, VAD thresholds were more readily elevated than VDS or SMR thresholds. These results indicate that morphine and fentanyl differentially inhibit nociceptive processing as reflected by VAD, VDS, and SMR thresholds independent of any confounding influence on motor performance. Similarly, diazepam was also shown to elevate VAD thresholds more readily than VDS thresholds over a range of doses (0.30-2.5 mg/kg) that did not alter performance of either response. Alternately, SMR thresholds were elevated only following administration of a dose of diazepam (10 mg/kg) that generated significant performance decrements for all three responses.

The confounding influence of performance decrements to threshold measurements is further indicated by the best-fit regression lines plotted in Figs. 2–4. These regression lines highlight the observation that thresholds that were accompanied by performance deficits were elevated to a greater degree than would have been predicted from thresholds not associated with performance deficits. This observation was confirmed statistically ($ps < 0.05$) for all three responses and all three drugs by regression-discontinuity analyses. These results are interpreted as indicating that response thresholds that are confounded with performance deficits provide an overestimate of the antinociceptive action of morphine, fentanyl, or diazepam. These thresholds presumably reflect not only the effects of these drugs on nociceptive processing but also on the ability of animals to fully generate the response.

GENERAL DISCUSSION

The present study has demonstrated that systemically administered morphine, fentanyl, and diazepam elevated VAD thresholds more readily than VDS and SMR thresholds. This sensitivity of VAD to increases in threshold cannot be attributed to its greater susceptibility to the deleterious influence of these drugs on motor performance. We conclude, therefore, that these drugs preferentially inhibit nociceptive transmission in pathways responsible for generating VAD. The failure of diazepam to raise SMR thresholds without concomitantly interfering with performance is consistent with previous studies that have failed to observe analgesia with this drug using reflex tests (10,47,48). These results indicate that systemically administered benzodiazepines do not significantly suppress at spinal levels the transmission in nociceptive pathways responsible for reflex generation.

The sensitivity of VAD to the antinociceptive influence of opiates and benzodiazepines supports its use as a model of the affective-motivational component of human pain because these drugs have also been shown to preferentially suppress this dimension of the human pain experience. Consequently, an understanding of the neural circuit underlying VAD generation may provide important insight to the neural substrates responsible for generating the affective-motivational aspect of pain. Carroll and Lim (13) demonstrated that transection of the neuraxis at the level of the thalamus abolished tail shock-elicited VAD but left VDS and SMR intact. A rhinencephalic-diencephalic circuit was proposed by Hoffmeister (23) based on the observation that large stereotaxic lesions of the amygdala, midline thalamus, hypothalamus, or septal area selectively abolished VADs. Subsequently, a hierarchically organized mammalian vocalization control system has been outlined in considerable detail (20,49,50). The initiation of a vocalization requires facilitatory input from the dorsolateral periaqueductal gray (dlPAG) to the brain stem nuclei that contain the phonotory motor neurons (nucleus ambiguus and trigeminal nucleus) and expiratory premotor neurons (nucleus retroambiguus) that control the laryngeal, articulatory, and respiratory activity that constitutes a vocalization (20,24). The dlPAG, in turn, has reciprocal connections with a number of rhinencephalic-diencephalic structures, including the amygdala (basolateral and central nuclei), lateral and medial hypothalamus, septal area, and midline thalamus (29,34,46). The electrical or chemical stimulation of these structures has been shown to produce vocalizations that are accompanied by emotional reactions (17,26,27,49,52).

The dlPAG is viewed as integrating specific motivational states (via amygdala, septal, midline thalamic, and hypothalamic inputs) with their corresponding vocal expressions (via outputs to nucleus ambiguus and nucleus retroambiguus) by coordinating the internal and external stimuli that induce an animal to generate a vocalization (29). With regard to pain-elicited vocalizations, noxious stimulation reaches the PAG via collaterals of the spinothalamic tract and direct spinomesencephalic projections (37), the hypothalamus and septal area via its connections with the PAG (33), the amygdala via the spinopontoamygdoloid pathway (4,32), and projections from midline thalamic nuclei (40) that receive spinothalamic input (55). Processing of noxious stimulation by this rhinencephalic-diencephalic circuit presumably ascribes to it its emotional tone, which is reflected in production of pain-elicited vocalizations. Pain-elicited vocalizations (particularly VAD, which relies on the rhinencephalon-diencephalon) may therefore be viewed as providing a "readout" of the neural activity within the limbic-midbrain circuit responsible for processing the affective-motivational attributes of a noxious stimulus. With respect to the results of the present study, it is important to note that these structures contain high concentrations of receptors for opiates (3,39,53) and benzodiazepines (9,38,57). The relative influence of opiates and benzodiazepines microinjected into these areas on VAD, VDS, and SMR thresholds is currently being tested.

The VAD has a number of advantages over other nociceptive behaviors of the rat that have been proposed as model systems for the analysis of the affective-motivational dimension of pain. Most notable is the behavioral syndrome that is generated by the SC injection of dilute formalin into the plantar surface of the paw (18,19). Unlike VAD, the formalin-induced syndrome is *less* readily inhibited by μ-opiate agonists than tail withdrawal reflexes (2) and is not suppressed by benzodiazepines (1). Also in contrast to VAD, the formalin syndrome does not rely on the rhinencephalon-diencephalon because transections at rostral pontine levels do not significantly alter the response (35). Formalin behavior, therefore, does not reflect activation of the rhinencephalic-diencephalic structures that are believed to underlie processing of the affective-motivational aspect of pain (14,36). Additionally, unlike the anatomically well-defined vocalization circuit, the neural circuit responsible for generating the relatively diffuse formalin syndrome (i.e., paw licking, leg lifting, paw shaking) has not been described. Consequently, a detailed analysis of the neural pathways responsible for processing and modulating the affective-motivational aspect of pain is more likely using VAD as the behavioral marker.

We have previously described the advantages of the procedures used in the present study when comparing nociceptive behaviors organized at different levels of the neuraxis (7,8). A principle advantage is that the thresholds of behaviors organized at spinal vs. supraspinal levels can be simultaneously measured in response to the same noxious stimulus (i.e., shock) applied to the same locus (tail) of the animal. Another advantage concerns assessing the performance of the nociceptive behaviors being studied. This analysis permits direct determination of the possible confounding influence of motor impairments to changes in response thresholds. The capacity of this analysis to identify drug-induced motor impairments that confound threshold assessment is indicated in the present study by the deviations from the dose-response functions of response thresholds that were shown to be associated with performance deficits. For other paradigms (i.e., radiant heat/tail flick, hot plate/paw lick, formalin test), the confounding influence of motor impairments is presumably assessed by examining behaviors (i.e., catalepsy, catatonia, rotarod per-

formance) other than those serving as indices of nociception. However, the relevance of motor impairments detected by these adjunct tests on the capacity of animals to fully generate the nociceptive behaviors being tested has not been established. For example, the administration of atropine increased the catatonia generated by systemic morphine treatment but did not elevate morphine-induced increases in response thresholds (30). On the other hand, lesions of raphé nuclei did not alter the catatonia generated by systemic morphine but they reduced morphine-induced increases response thresholds (54).

The similarities and differences between tail shock-elicited SMRs and heat-elicited tail flick responses has also been discussed previously (7). Despite the differences in stimuli and procedures used in these tests, there is a striking similarity in the sensitivity of these responses to systemic morphine treatment. In the intact rat, doses of 5–8 mg/kg morphine have been shown to elevate SMR thresholds [(7,23), present study]. The dose of systemic morphine that has been observed to significantly elevate heat-elicited tail flick latencies ranges from 1.2–10.0 mg/kg depending on the route of drug administration (42), the intensity of the stimulus (50) and the location on the tail that heat is applied (56). Indeed, D'Amour and Smith (16), in their report on the radiant heat/tail flick procedure, reported that the minimum effective dose of morphine was 8 mg/kg—the same dose that elevated SMR thresholds in the present study. Consequently, information concerning the neural and neuropharmacological mechanisms governing the effects of morphine on heat-induced tail flick may be generalizable to the SMR.

In conclusion, the results of the present study support the use of the VAD/VDS/SMR paradigm to study the affective-motivational and sensory-discriminative dimensions of the pain experience. The VAD is especially well suited as a model system for analysis of the neural circuits underlying generation and modulation of the affective-motivational attributes of the pain experience. This paradigm may therefore provide a useful tool by which information garnered from animal studies examining the neural basis of pain and analgesia is more readily generalized to human experimental and clinical studies.

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