Opioid microinjection into raphe magnus modulates cardiorespiratory function in mice and rats

Kevin M. Hellman¹, Scott J. Mendelson², Marco A. Mendez-Duarte¹, James L. Russell¹, Peggy Mason¹, ²*

¹Department of Neurobiology,
²Committee on Neurobiology, University of Chicago, Chicago, IL

Running Title: RM modulation of cardiorespiratory function

*To whom correspondence should be addressed:
Peggy Mason
Department of Neurobiology
University of Chicago, MC 0928
947 East 58th St.
Chicago, IL 60637
Phone: (773) 702-3144, Fax: (773) 702-1216
E-mail: p-mason@uchicago.edu

Acknowledgements:

This research was supported by NIH (R21DA022429) and an American Academy of Sleep Medicine Faculty Career Advancement Award to KMH. The authors thank the NIDA Drug Supply Program for kindly providing DAMGO.
Abstract

The raphe magnus (RM) participates in opioid analgesia and contains pain modulatory neurons with respiration-related discharge. Here we asked whether RM contributes to respiratory depression, the most prevalent lethal effect of opioids. To investigate whether opioidergic transmission in RM produces respiratory depression, we microinjected a mu-opioid receptor agonist, DAMGO or morphine, into the RM of awake rodents. In mice, opioid microinjection produced sustained decreases in respiratory rate (170 to 120 breaths per minute) as well as heart rate (520 to 400 beats per minute). Respiratory sinus arrhythmia, indicative of enhanced parasympathetic activity, was prevalent in mice receiving DAMGO microinjection. We performed similar experiments in rats but observed no changes in breathing rate or heart rate. Both rats and mice experienced significantly more episodes of bradypnea, indicative of impaired respiratory drive, after opioid microinjection. During spontaneous arousals, rats showed less tachycardia after opioid microinjection than before microinjection, suggestive of an attenuated sympathetic tone. Thus, activation of opioidergic signaling within RM produces effects beyond analgesia including the unwanted destabilization of cardiorespiratory function. These adverse effects on homeostasis consequent to opioid microinjection imply a role for RM in regulating the balance of sympathetic and parasympathetic tone.
Introduction

Increasing clinical usage of opioid analgesics has led to an escalation of accidental death due to opioid respiratory depression (39, 66, 83). It is unclear which neural loci are responsible for respiratory depression because of the vast complexity of interactions and compensatory responses in the neural networks participating in respiration and responding to opioids (31, 58). In the rat, the medullary raphe magnus (RM) is essential for opioid analgesia (4, 13, 21, 29) and contains opioid-responsive neurons so that injecting opioids directly into RM produces analgesia (15, 33, 35, 36, 55). Yet RM participates in functions beyond pain modulation (for review see 41). RM neurons project to respiratory control centers in the brainstem (18, 20, 43, 70) and to the phrenic motor nucleus (6, 9, 32, 85). Further, RM cells discharge in relation to respiration rate (26) and phase (44). These findings prompted us to ask if RM is involved in opioid respiratory depression.

While microinjection of DAMGO into RM has no effect on respiration in the anesthetized rat (88), it produces respiratory depression in the anesthetized mouse (26). Electrical stimulation of RM in either rat or cat (3, 8, 79) reduces respiration rate while producing analgesia in a naloxone-reversible manner (67), implying a role for RM opioidergic neurons in respiratory regulation concurrent with analgesia. To determine the role of RM in opioid respiratory depression, we microinjected opioid receptor agonists in awake, behaving mice and rats while measuring breathing. To examine the full range of functions for opioidergic RM modulation (17, 40, 42), we also recorded heart rate, sleep and gross motor activity. Our results show that opioids within RM alter heart rate and
breathing, complementing decades of work on RM-mediated analgesia and indicating an expanded role for RM in the maintenance of homeostasis.
**Methods:**

**Habituation**

Male C57/B6 mice (12-16 weeks, 25-30 g) and Sprague-Dawley rats (8-12 weeks, 300-400g, Charles River, Portage MI) were individually housed under standard colony conditions. Only male mice and rats were used because of previously reported differences in descending opioidergic modulation across sex and estrus cycle (reviewed in 11, 23). Food and water were provided *ad libitum*. Animals were maintained on a 12 hour light/12 hour dark cycle with lights on at 0600. All handling and experiments began at 1000. Animals were habituated to handling (30 min/day) and were individually placed in recording chambers (3 hr/day) for 5 days prior to surgery. After allowing 1 week of recovery from surgery, habituation to the handling and recording chambers resumed for an additional 5 days prior to drug administration and recording. All animal care and experiments were in accordance with the National Institute of Health guidelines and approved by the Institutional Animal Care and Use Committee of the University of Chicago.

**Surgery**

Anesthesia was induced with 5% isoflurane and maintained by 1.2–1.6% isoflurane. Core temperature was maintained with a water-perfused heating pad. EEG, EKG and intercostal EMG electrodes were inserted and routed to a plastic connector pedestal (Plastics One, Roanoke, VA) that was subsequently cemented to the skull. A 1-mm diameter craniotomy was drilled caudal to lambda (1.5 mm caudal in rats and 1.0 mm caudal in mice) to allow for placement of a cannula guide through which RM could be
accessed. Immediately and 12 hours after surgery, animals were given buprenorphine (0.1 mg/kg) as an analgesic.

**Drugs**

Morphine sulfate (Malinckrodt, St. Louis, MO) and d-Ala², N-Me-Phe⁴-Gly⁵-ol-enkephalin (DAMGO; Multiple Peptide Systems, San Diego, CA) were dissolved in phosphate-buffered saline (PBS). Each animal received a drug injection and a control PBS injection, in a randomized, counter-balanced order, separated by 48 hours. Mice received DAMGO at a concentration of 1 ng/nl (2 mM) with the following volumes: 20 nl (40 pmol, n=5), 100 nl (200 pmol, n=5), or 250 nl (500 pmol, n=4). Initially, an injection of 100 ng (200 pmol) of DAMGO in 100 nl into the RM of a rat had no observable effect. Upon escalating the dosage and volume to 250 ng/250 nl and 500 ng/500 nl (1 nmol) in additional rats, we still did not see dramatic alterations in cardiorespiratory function. In the remaining rats, we microinjected 25 μg/500 nl (130 mM, 65 nmol) of the more stable, but less potent, opioid receptor agonist morphine HCl (n=8).

**Recording Sessions**

Physiological signals were recorded via a 6-channel commutator for one hour of baseline activity prior to microinjection of drug. A piezoelectric microphone sensitive to mechanical energy from movement, including breathing, was affixed to the base of the recording chamber and used to assess breathing and movement activity. After acquisition of baseline data, animals were removed from the recording chamber and an injection cannula replaced the dummy guide. Microinjection of drugs was performed over a 30 s
period with a Hamilton syringe connected to polyurethane tubing attached to a 31 gauge injection cannula. After microinjection, the injection guide was removed and the dummy guide was reinserted. Animals were returned to the recording chamber for an additional 3 hours. At the conclusion of the final experiment, the injector was filled with Pontamine Sky blue. The dye-filled injector was placed back into the guide cannula and a 500 nl injection was made to mark the microinjection site.

Histology

Animals were overdosed with 5% isoflurane and perfused with a fixative containing 4% paraformaldehyde and 7% sucrose in 0.1 M PBS. The brain stem was removed, postfixied for 24 hours, and then immersed in 30% sucrose in 0.1 M PBS. Coronal sections (25 µm) were cut on a cryostat. Sections were mounted on gelatin-coated slides and then stained with cresyl violet. Microinjection sites were identified and assigned an anterior-posterior location and then plotted on standard sections adapted from Figure 2 of VanderHorst and Ulfhake (2006). In the mouse, RM included a region 600 µm wide centered on the midline that stretched from the medullary ventrum to a point 1,000 µm dorsal to the base of the brain. The anterior and posterior borders of RM were the first section caudal to the nucleus of the trapezoid body and the first section rostral to nucleus ambiguus, respectively. Microinjection into sites superficial to RM located in nucleus reticularis gigantocellularis (n=3) were not included in this report. Rat RM included a region 600 µm wide centered on the midline and extending from the base of the brain to a point 1,500 µm dorsal, at levels from -11.6 mm to -10.0 mm relative to bregma.
**Digital Signal Processing**

EEG, EKG, intercostal EMG, and microphone activity were amplified and acquired by a Power 1401 data acquisition system (CED, Cambridge, UK). Intercostal EMG and microphone activity were integrated and filtered with Spike2 version 6.08. Proprietary band pass filters included within the software (infinite impulse response) optimized the signal in the desired frequency range (0.25 Hz to 5 Hz). In general, microphone activity was more sensitive to respiratory activity than were intercostal electrodes and therefore microphone activity was used to identify breathing patterns except in a few situations in which intercostal activity proved more reliable. Individual breaths and beats were isolated from the signals and peaks above an amplitude threshold were detected. Resultant data were visually inspected to identify and remove artifacts which were recognized by their characteristic high variance.

Sleep/wakefulness activity was scored using classical criteria (22). The presence of high amplitude, low frequency EEG activity in the absence of movement marked non-rapid eye movement sleep (NREM). Highly coherent EEG theta (6-8 Hz) activity in the absence of EMG activity indicative of movement defined rapid eye movement sleep (REM). Waking was identified by fast, desynchronized EEG waves. To detect movement, non-respiratory EMG and microphone activity were integrated over continuously running 30-s bins (similar to 57, 82). Motor activity was calculated as the percentage of activity above a set threshold. The median value of the parameters described above was determined sequentially in 30-minute bins using Spike2 (CED,
Cambridge, UK). Analysis of Variance (ANOVA) with Newman-Keuls post hoc tests were used to identify significant effects.

*Heart Rate Variability Analysis*

Artifact free EKG segments, 500 s in duration, were processed and analyzed with Kubios Heart Rate Variability (HRV), a publicly available analysis software package (54). HRV analysis determines the different frequency components of variance that contribute towards beat-to-beat variability. Whereas consecutive beat-to-beat variance is most influenced by parasympathetic inhibition (high frequency variance), variation over longer periods of time (low frequency variance) is influenced by a combination of parasympathetic and sympathetic activity (7, 56). We used a standard technique to assess variability: Poincaré plots that graph each heart beat interval, HR$_n$, as a function of the subsequent heart beat interval, HR$_{n+1}$ (see Figure 4). Using the default Kubios software settings, SD$_1$ was computed as the standard deviation of the points perpendicular to the line-of identity, representative of the high frequency parasympathetic component. SD$_2$ was computed as the standard deviation along the line of identity, representative of a mixture of low frequency sympathetic and parasympathetic components.
Results:

*In mice, microinjection of DAMGO within RM produces bradycardia, bradypnea and respiratory depression.*

PBS microinjections into RM (Figure 1) had no effect on either heart rate (p = 0.85) or respiration rate (P = 0. 98) during the 2 hour period after injection compared to the baseline period prior to injection. In contrast, DAMGO microinjection (20-250 ng, Figure 1) within the murine RM resulted in an immediate 25-30% drop in median heart rate (p ≤ 0.05) (Figures 2-3). Bradycardia was short-lived (< 1 hour) in the mice receiving 20 ng DAMGO, but persisted for 2 hours or more in mice receiving 100 or 250 ng. Bradycardia occurred during both wakefulness and sleep. DAMGO microinjection had no effect on locomotor activity (P = 0.75) or time spent asleep (P = 0.54) during the 2 hour period after microinjection (data not shown). Hence, changes in locomotion and/or sleep are unlikely to be responsible for the decrease in heart rate observed.

Significant decreases in median respiration rate were observed at 30 and 60 minutes in mice receiving DAMGO microinjection at all three doses. Additionally, brief disruptions in the respiratory rhythm, typically a missed breath, frequently occurred after DAMGO microinjection (Figure 2). To quantify episodes of bradypnea we used an algorithm that detected breath-to-breath intervals exceeding the average interval over the past 10 s by 5 times the standard deviation. Events were then visually examined to exclude sighs, arousals, and periods of gross movement. Within the 30 minute period after microinjection of DAMGO, mice had nearly twice as many episodes of bradypnea compared to the same period after saline or to the preceding baseline period (P’s < 0.05, Figure 4). It is interesting to note, that depressions in heart rate were often coincident
with incidents of bradypnea. Thus, DAMGO microinjection depressed cardiopulmonary activity.

The utilization of pontamine sky blue allowed us to identify the center of our injection sites and to roughly estimate the extent of the injectate’s diffusion. Injections of 20 nl of pontamine sky blue marked a spherical region with a 0.2 mm radius. Injections of 100 nl marked a sphere with a 0.4 mm radius and injections of 250 nl produced a 0.5 mm radius spread. The smaller injection volumes (20 and 100 nl) labeled a region immediately surrounding the center and never spread into either the dorsal reticular formation or the ventrolateral medulla, including the retrotrapezoid nucleus. The largest injections of pontamine sky blue (250 nl) marked a region that sometimes included territory in the dorsal reticular formation or ventrolateral medulla. It is unclear how differently DAMGO diffuses compared to the dye.

Microinjections of 100 ng DAMGO into the reticular area located 0.5-1.0 mm lateral to RM (n=3, data not shown) had effects on heart rate and respiration comparable to those observed after microinjections in RM. Accordingly, the murine reticular region adjacent to RM appears to participate in the DAMGO-elicited reduction of heart rate and respiration rate. These results indicate that microinjection of DAMGO within the ventral medial medullary region in mice is sufficient to produce bradycardia and respiratory depression in unrestrained, naturally sleeping and awake mice.

*In mice, microinjection of DAMGO within RM produces respiratory sinus arrhythmia and increases heart rate variability.*

During respiratory sinus arrhythmia, heart beat intervals are shortened during inspiration and lengthened during expiration (84). Such arrhythmia was never observed during the
baseline period. However, respiratory sinus arrhythmia occurred in mice that received \( \geq 100 \, \text{ng} \) of DAMGO (Figure 2). Bradycardia and respiratory depression always preceded respiratory sinus arrhythmia. Mice never displayed arrhythmia without previously entering this depressed state. The consistency of the pattern of bradycardia and respiratory depression prior to respiratory sinus arrhythmia suggests that a depressed state is a requisite stage in the evolution of arrhythmia consequent to opioid administration.

We performed a Poincaré analysis to quantify parasympathetic and sympathetic contributions to heart rate variability (Figure 5). \( S_{D_1} \), the solely parasympathetic component, and \( S_{D_2} \), the mixed sympathetic and parasympathetic component, were measured 30 minutes before and 30 minutes after microinjection. Both \( S_{D_1} \) and \( S_{D_2} \) were significantly increased in mice receiving DAMGO microinjection (\( P < 0.05 \)) consistent with increased parasympathetic tone (7, 56).

**Opioid microinjection into RM has limited effects on respiratory rate in rats.**

Rats receiving PBS microinjected into RM showed a 15 ± 2 % increase in heart rate (\( P < 0.01 \)) (Figures 3, 6). This effect was likely due to handling stress, because similar effects were observed in rats handled but not receiving a microinjection during habituation. Within 30 minutes of PBS microinjection, heart rate returned to baseline levels and never deviated significantly again.

We initially injected 100 ng of DAMGO in 100 nl into rat RM and did not observe any apparent effect on heart rate or respiration rate. After escalating the dosage to 250 ng and
then to 500 ng in additional rats, we still did not see the dramatic alterations typically observed in mice. We then injected a high dose of morphine in a large volume (25 μg / 500 nl) into RM in 8 rats (Figure 6). Rats receiving morphine showed significant increases in heart rate 30 minutes and 90 minutes after morphine microinjection (P <0.001). There was no significant effect on overall respiration rate, but rats receiving morphine microinjection had more episodes of bradypnea (P <0.001, Figure 4). There were no significant effects on duration of sleep from microinjection of either DAMGO (P = 0.69) or morphine (P = 0.54). As in mice, rats receiving DAMGO microinjections had an elevated SD1 (P < 0.05, Figure 5). No significant changes in SD1 (P = 0.28) or SD2 (P = 0.10) were observed after morphine microinjection in rats.

*Species differences in baseline heart rate regulation were observed that could contribute to differences in reactions to opioid administration.*

Even prior to microinjection during the baseline period, differences in heart rate regulation between mice and rats were apparent. During baseline wakefulness, mice had a steady 660 ± 10 beats/minute (CV = 0.018 ± 0.001), but during baseline sleep, heart rate was slower (410 ± 40 beats/minute, p < 0.001) and more variable (CV = 0.102 ± 0.010, p < 0.001) (Figure 7). Transitions from sleep to wakefulness were often manifest by an increased heart rate, consistent with an elevated sympathetic drive. However, heart rate frequently increased even in the absence of a microarousal. Thus, the high beat-to-beat variability may have masked increases in sympathetic tone associated with microarousals so that tachycardia was not predictive of microarousals in mice. After
opioid microinjection, tachycardia continued to occur both in accompaniment to microarousals and independent of them (data not shown).

Rats had relatively steady heart rates during both wake and sleep (wake: 400 ± 10 beats/minute; CV = 0.030 ± 0.001; sleep: 330 ± 10 beats/minute; CV = 0.018 ± 0.001) (Figure 7). Nonetheless both the rate and variability of the heart rate were significantly less during sleep than during wake (rate, P < 0.001; CV, P < 0.001). When short arousals (5-10 s) occurred after sleep bouts of ≥3 min duration, heart rate increased by 30-60 beats/minute over a period of 5 s (Figures 7, 8). However, within 60 min of morphine microinjection, heart rate elevation was blunted in 9 out of 12 visually identified arousals (Figure 8). The reduced arousal-associated tachycardia in rats suggests a reduction in sympathetic drive after opioid administration.
Discussion

*RM has the capacity to contribute towards opioid-induced respiratory disturbances.*

The disruption of cardiorespiratory activity produced by opioids microinjected into RM is evidence that RM can contribute to the adverse conditions commonly viewed as side-effects to opioid analgesia. RM’s convincing role in analgesia may have allowed RM to evade suspicion as a protagonist in respiratory depression despite a series of findings supporting the latter possibility. Supporting the present results, there are published reports of RM manipulations including electrical or chemical stimulation (3, 8, 38, 53, 79) as well as lesioning or inactivation (38, 45) that alter breathing.

*RM projects to regions that modulate and control breathing.*

RM could affect respiration via any of several anatomical pathways. RM influences phrenic motoneurons and thus diaphragmatic activity via a nonserotonergic, GABAergic projection to the phrenic motor nucleus (6, 9, 32, 85). Both serotonergic and nonserotonergic RM neurons project to the ventral respiratory group (18, 20, 43, 70). RM’s neuromodulation of central pattern generator neurons within the ventral respiratory group may play an important role in determining respiration rate (14). RM cells also project to putative respiratory-related neurons in the nucleus of the solitary tract (51, 69) and when activated inhibit breathing-related neuronal activity while producing a naloxone-reversible apnea (67). Further, significant reciprocal connections exist between RM and chemosensory nuclei such as the retrotrapezoidal nucleus (12, 65). In sum, RM’s anatomical connections to spinal and brainstem respiratory nuclei are sufficient to support RM modulation of breathing.
**RM is a mediator of sympathovagal balance.**

In the present study, we focused on breathing and to a limited extent, cardiovascular function. Yet the biology of RM function is unlikely to fit within this artificially constrained domain. Consistent with a broader role for opioid modulation in RM than just analgesia, we observed that opioid microinjection increased heart rate variability. While other explanations for the decrease in heart rate observed exist - e.g. a reflex bradycardia secondary to an increase in blood pressure – the most parsimonious explanation for the bradycardia and respiratory sinus arrhythmia is that RM shifts the parasympathetic / sympathetic balance to favor parasympathetic tone. In mice, we frequently observed respiratory sinus arrhythmia exaggerated to such an extent that the heart did not beat during expiration, leaving little doubt to the underlying mechanism (84). Respiratory sinus arrhythmia is elicited during expiration by pulmonary stretch receptors evoking parasympathetic inhibition of the heart sinoatrial node (84). When central parasympathetic activity is increased, respiratory sinus arrhythmia becomes more apparent (24, 64, 68). Enhanced parasympathetic activity is a well known consequence of opioid administration and is clinically prevented by atropine pre-medication (80, 81). Vagotomy attenuates systemic morphine-induced bradycardia and microinjection of ibotenic acid into RM attenuates systemic morphine-induced bradycardia in anesthetized rats (61). While direct projections from RM to nucleus ambiguus have not been reported, tract-tracing studies with pseudorabies virus indicate that RM projects oligosynaptically to parasympathetic cardiac ganglia (72, 74).

Evidence of altered sympathetic activity after opioid microinjection in rats is manifest by a blunting of the increase in heart rate normally observed during arousal. In the absence
of opioids, a barrage of sympathetic activity during arousal increases heart rate (30, 49, 71). Although the central source for initiating sympathetic activity during arousal is not known, we speculate that RM may play a role in increasing sympathetic activity during arousal. In support of this idea, the discharge rate of one population of RM cells increases just before arousals from sleep (34) and RM activation increases blood pressure and heart rate (1, 50, 53).

RM contains more neurons than any other brainstem nucleus that projects to the intermediolateral cell column of the thoracic cord, a region responsible for sympathetic hypertensive effects (10). In resting animals, RM has the second highest c-fos expression out of all intermediolateral cell column-projecting nuclei, suggesting that it contains >20% of all brain stem neurons that modulate basal sympathetic activity. Muscimol within RM is sufficient to abolish sympathetic effects such as fear conditioning-induced vasoconstriction and tachycardia (77) and periqueductal gray stimulation-evoked cardiovascular fear reactions (5). In agreement with the above findings, microinjection of opioids into RM may disrupt sympathetic activity via direct projections from RM to sympathetic neurons within the spinal cord, a mechanism that could contribute to the disruptive effects of analgesics on sympathetic function.

*Species differences inform us about RM’s roles in physiological homeostasis.*

Larger, more consistent adverse cardiorespiratory effects were observed after opioid microinjection into mouse RM than into rat RM. It is possible that the small dimensions of the murine brainstem allowed for greater diffusion into neighboring nuclei and the consequent engagement of additional neuronal populations. However, we find this unlikely as the cardiorespiratory effects observed in mice after a 20 nl opioid
microinjection were greater than those observed in rats injected with 500 nl. While the
effects of opioid microinjection into RM were consistently larger in the mouse than the
rat, it should be noted that opioid microinjection into RM did increase the incidence of
bradypnea in rat.

Overall brainstem anatomy in rats and mice appear similar and no major differences in
the anatomy of RM between the two species have been reported. The only relevant
difference described to date is that the serotonergic RM projection to the dorsal horn is
more substantial in rats compared to mice (76). However, given that opioid analgesia
depends primarily on non-serotonergic cells (19, 25), a relative increase in the descending
serotonergic projection in rats would not explain why rat RM plays a more substantial
role in analgesia than in homeostasis.

The species differences observed fit with previously observed species differences in the
physiological characteristics of RM neurons (26). Whereas background activity of
opioid-inhibited RM cells in the rat is indicative of nociceptive responsiveness, the
background activity of these cells in the mouse is indicative of respiratory rate (26).
Further, opioids administered to mice did not have consistent excitatory or inhibitory
effects on any RM cell population but instead blocked the responses of RM cells to
noxious stimulation (26). Such physiological properties may allow mice to preserve
homeostasis while restricting analgesia to periods following noxious stimulation. Since
the effects of opioids on murine RM cells are only apparent as a reduction in responses to
noxious stimuli, the unaffected tonic activity of murine RM cells may allow for
maintenance of physiological control. In contrast, the physiology of RM cells in the
anesthetized rat predicts that opioids can engage homeostatic modulation only tonically and in accord with analgesic output.

Rats require ten times less morphine than mice for analgesia after correcting for size differences (46, 47, 73) even though mice have a higher density of opioid receptor ligand binding sites (86). Yet the opioid dose injected into RM that evokes respiratory changes in mice is comparable to that which evokes analgesia in rat. Therefore, we propose that mouse RM has a predominant role in homeostatic regulation while rat RM is more dedicated to modulating pain transmission. Mice may need greater dynamic control of functions such as heart rate than rats. Resting heart rate variability is far greater in mice than rats and mice transition between sleep and wakefulness more rapidly and frequently than do rats. Whereas pain represents a threat to behavioral function in both species, the greater demand in mice for homeostatic regulation may require more functional circuitry dedicated to sympathovagal balance.

The homeostatic functions of RM appear to be present in mouse (this study), rat (this study and above citations), cat (63) and even in human where fMRI imaging has indicated a role for the ventral medullary midline in respiration (75) and heart rate variability (52). While rats are a good model for normal cardiorespiratory function, mice, which have a much higher tolerance to morphine than rats, may be a better representative model of the chronic opioid tolerant condition, the condition that is most clinically relevant to the dangers of opioid respiratory depression. In support of a role for the medullary raphe in mediating opioid effects in humans, opioid receptors are found in the human raphe nuclei (59).

*Perspectives and Significance*
Selective or complete lesions of RM eliminate neuropathic pain (27, 60). Effectively eliminating neuropathic pain is rightly heralded as a breakthrough, but the clinical utilization of a technique that potentially eliminates cells involved in cardiorespiratory regulation has obvious risks. Currently, surgical implantation of electrodes for stimulation of the descending opioid pathway is used as a last-resort tactic for patients with chronic pain, and this stimulation paradigm has effects on blood pressure in humans (62, 87), cats (28) and rats (37). Of greater relevance to current clinical practice, opioid usage impairs respiratory drive. Patients that chronically utilize opioids are likely to develop central sleep apnea (2, 16, 48, 78). During central sleep apnea, all muscle related respiratory behavior spontaneously ceases potentially due to decreased respiratory drive from central pattern generators. RM should be considered a candidate locus for the opioid modulation of respiratory drive given its sensitivity to sleep/wake regulation and its modulation of respiratory control centers. The ability to increase the incidence of bradypnea with opioid microinjection into a single site provides direction for further investigation. As the same circuitry that mediates analgesia participates in homeostatic regulation, a better understanding of RM will not only yield therapeutic benefits to the treatment of chronic pain, but to a broad spectrum of physiological disorders.


73. **Szekely JI, Miglecz E, and Bajusz S.** Species differences in the relative analgesic potencies of some classical opiates and opioid peptides. *Psychopharmacology (Berl)* 82: 400-402, 1984.


Figure legends

Figure 1. Comparison of mouse and rat RM and microinjection sites. Distance from bregma (mm) is indicated in the margin adjacent to the figure panel. Representative nissl stains of mouse and rat RM with microinjection sites arranged from rostral (top) to caudal (bottom). Note that all animals received one microinjection of drug as well as one of PBS, with both administered at the same location. Locations of morphine microinjection sites are indicated by squares and of DAMGO by circles. NTB = nucleus of the trapezoid body, 7n = 7th nerve, VII = facial nucleus, stt = spinal trigeminal nerve.

Figure 2. DAMGO (100 ng) microinjected into murine RM altered cardiorespiratory function. Top: Heart rate and respiratory rate decreased after DAMGO microinjection (arrowhead). The dots show the instantaneous values of heart rate and respiration rate, expressed in units of beats or breaths/minute. Superimposed lines indicate the running median values for each measure. No data are shown during the period immediately prior to injection because of handling-induced artifacts. Bottom: DAMGO increased the incidence of episodes of respiratory sinus arrhythmia (left) and bradypnea (right).

Figure 3. Bradycardia and respiratory depression were observed in mice but not rats after opioid microinjection. Heart rate and respiration are expressed in units of beats or breaths/minute. The median heart and breathing rate are shown for the baseline period and for sequential 30 minute periods after microinjection, starting at the time listed above the graph. For mice, samples included DAMGO microinjection of 20 nl (n=5), 100 nl (n=5), or 250 nl (n=4). For rats, samples included DAMGO (n=3) and morphine microinjection (n=8). Within-animal-controls were used for PBS microinjection. Significant differences between PBS and drug microinjections are indicated as follows: *
P < 0.05, ** P < 0.01, and *** P < 0.001. Significant differences between baseline and PBS is indicated as ## P < 0.01.

Figure 4. An increased frequency of episodes of bradypnea was observed in mice and rats after opioid microinjection. The number of bradypneic episodes increased during the 30 minute period after drug microinjection relative to the 30 minute baseline period and relative to after PBS administration. Significant differences between PBS and drug groups are indicated as follows: * P < 0.05, ** P < 0.01, and *** P < 0.001.

Figure 5. Opioid microinjection increased heart rate variability in mice and rats. First and third rows: Poincaré parameters SD1, indicative of parasympathetic activity, and SD2, indicative of both parasympathetic and sympathetic activity, are plotted for the baseline period and for the 30 minute period immediately after microinjection in mice and rats. Second and fourth row: example Poincaré plots from an individual mouse (top) and rat (bottom). Significant differences between PBS and drug groups are indicated as * P < 0.05.

Figure 6. Morphine microinjected into rat RM had no effect on heart rate or breathing rate. Heart rate and respiration rate are expressed in units of beats or breaths/minute. This example from a single rat shows the instantaneous heart rate and respiratory rate (dots) before and after morphine microinjection (arrowhead). Superimposed lines indicate the running median values. Individual episodes of bradypnea are shown at the bottom and occurred at the time points indicated by the arrows.

Figure 7. The heart rate of mice during sleep is far more variable than that of rat. Heart rate is expressed in units of beats/minute. The traces show instantaneous heart rate during a 300-s period prior to drug microinjection for one mouse and one rat. Each heart
beat is color coded to indicate whether it occurred during sleep (blue) or wakefulness (red). The graphs at the right show the average coefficient of variance (CV) for all animals in each species during wakefulness and sleep prior to drug microinjection. Significant differences from the CV of wakefulness are indicated as *** P < 0.001.

Figure 8. In rat, microarousals are accompanied by an increase in heart rate before but not consistently after morphine administration. Heart rate is expressed in units of beats/minute. In each panel, instantaneous heart rate (top trace), high frequency EEG activity (40-100 Hz; labeled EEGhi) indicative of arousal, low frequency EEG activity (1-4 Hz; labeled EEGlow) indicative of sleep, and breathing pattern are shown. An increase in heart rate accompanies arousal before (top) but not after (bottom) morphine microinjection.
100 ng of DAMGO

- Heart Rate
- Resp. Rate

Respiratory Sinus Arrhythmia

EKG

Respiration

0.5 s

Bradypnea

2 s

30 min.