ONLINE DATA SUPPLEMENT

USING A LABORATORY MODEL TO TEST TREATMENT:

MORPHINE REDUCES DYSPNEA AND HYPERCAPNIC VENTILATORY RESPONSE

Robert B. Banzett, Lewis Adams, Carl R. O'Donnell, Sean A. Gilman, Robert W. Lansing,

Richard M. Schwartzstein

Supplemental Methods

1. Breathing apparatus & stimuli

During the laboratory dyspnea challenge, minute ventilation was limited to 0.13 liters/min/kg by the apparatus depicted in Fig. E1. The subject's minute ventilation was limited to the flow rate of gas into the bag. During each expiration gas flowed into the bag – if the subject attempted to draw a tidal volume larger than the gas supplied to the bag, the bag collapsed (see stimulus descriptions). Because the impedance of the gas source was high, the subject could draw no more gas from the bag than that supplied by the needle valve.

During the hypercapnic ventilatory response challenge (HCVR), the volume of gas available to the subject was unrestricted. The inspiratory resistance of the system was 2.5 cm H_2O liter/sec. A previously described contrivance held $P_{ET}CO_2$ stable despite changes in minute ventilation (1). Each $P_{ET}CO_2$ level was held steady for approximately 3 min to allow the subject to achieve steady-state ventilation (2).

2. Protocol

Time-base tracings of a typical experimental runs are shown in Fig. E2. Table E1 shows an example of the timeline of an entire experiment.

Table E1: Timeline Subject 3, Morphine study day

8:40-8:55am	Place iv catheter
9:05-9:11am	Resting $P_{ET}CO_2$ measurement
9:11-9:21	Break
9:21-9:42am	Pre-drug Dyspnea Challenge (Trial 1) – see Fig E2a
9:42-9:45am	Administer MDP
9:45-9:54am	Break
9:54-10:06am	Pre-drug Hypercapnic Ventilatory Response (Trial 2)
10:06-10:12am	Break
10:12-10:17am	Slow infusion of morphine
10:17-10:26am	Resting $P_{ET}CO_2$ measurement
10:28-10:50am	Post-drug Dyspnea Challenge (Trial 3) – see Fig E2b
10:50-10:53am	Administer MDP
10:53-11:04am	Break
11:04-11:19am	Post-drug Hypercapnic Ventilatory Response (Trial 4)
11:19-11:21	Break
11:21-11:43am	Post-drug Dyspnea Challenge (Trial 5)
11:43-11:46	Administer MDP
11:46am-12n	Post-drug Hypercapnic Ventilatory Response (Trial 6)
12:00-12:05pm	Break
12:05-12:24pm	Post-drug Dyspnea Challenge (Trial 7)
12:24-12:27pm	Administer MDP
12:27	Debrief, Post Anesthesia Discharge Score, Dismiss subject

3. Dyspnea measurement

Visual Analog Scale for online ratings of Breathing Discomfort (BDVAS): The scale was implemented electronically to provide a signal for recording together with physiological variables. The subject's hand rested on a potentiometer knob, which controlled a linear array of LED lights with adjacent scale markings positioned in the subject's field of view. The subject could change the rating whenever breathing discomfort changed. Subjects were instructed as follows:

"… You should use the visual scale to tell us how unpleasant or bad your breathing feels.. The top of the scale is labelled 'Unbearable,' the bottom is labelled 'Neutral'. If your breathing is comfortable, and you could continue for a long time, you should score 'Neutral'. If the breathing discomfort becomes intolerable, that is, if you can't stand it and you want us to stop it, rate 'Unbearable'. If you do, we will change the settings right away – it may take several breaths before you feel relief…"

4. Analysis

Physiological measures: We selected an analysis period at the end of each stimulus step that used the maximum amount of steady state data available for that step. Step duration was varied to make the stimulus less predictable to the subject; thus the median duration of analysis periods was 50sec, but some periods were as short as 18sec. We did not use data immediately following a step change in $P_{ET}CO_2$; we required at least 45 sec of steady-state $P_{ET}CO_2$ (± 1 Torr) before the start of the analysis period. We used a model of the air hunger dynamic response to change in $P_{ET}CO_2$ to correct for any small dynamic response remaining at this time (3). The analysis period ended when the step period was ended (subject took 2-3 large breaths). Because the ventilatory response has similar time characteristics (2), the same model was used in analysis of HCVR data. This model calculates the effective $P_{ET}CO_2$ stimulus by applying a low pass filter and delay to the breath-by-breath $P_{ET}CO_2$ measure. Other physiological variables were simply averaged over the analysis period.

Dyspnea treatment effect response feature (see Fig E3): We averaged BDVAS ratings over the same analysis period used for physiological variables, thus providing paired $P_{ET}CO_2$ and BDVAS measures for regression. The response feature was calculated as follows: For each subject we fitted a regression line to the BDVAS vs $P_{ET}CO_2$ data before treatment, truncating any points below the $P_{ET}CO_2$ threshold for discomfort. We selected the point on the before-treatment regression at 60% BDVAS as the 'reference point'; the $P_{ET}CO_2$ at this point was used as the reference $P_{ET}CO_2$ for that individual. We then determined BDVAS rating following morphine (or placebo) at the reference $P_{ET}CO_2$. The difference in BDVAS at the reference point $P_{ET}CO_2$ after treatment is defined as the treatment effect. (The median reference $P_{ET}CO_2$ identified under baseline conditions for response feature analysis was 8.5 Torr above resting value; range 5 to 16 Torr). This analysis provided a single measure of treatment response for each subject, while at the same time utilizing multiple data points to reduce the effect of noise on a single measurement. In addition it obviates the need to exactly match $P_{ET}CO_2$ before and after drug.

HCVR effect response feature: Using paired $P_{ET}CO_2$ and minute ventilation ($\dot{V}_E$) data from HCVR trials we calculated regression lines for $\dot{V}_E$ vs $P_{ET}CO_2$. Because we wished to compare

treatment effect on ventilatory drive with treatment effect on BDVAS, we selected the $\dot{V}_E$ measures at the same P_{ETCO_2} as used for the dyspnea response feature in that subject.

Supplemental Results

1. Respiratory sensations during Dyspnea Challenge

Detailed MDP responses

To look at possible differential effects of morphine on the components of dyspnea trials were selected to match A_1 ratings within each subject before and after morphine. The operator increased P_{ETCO_2} to achieve similar discomfort during the focus period despite the treatment effect of morphine. Ventilation was held constant at 0.13L/m/kg. One matched MDP pair was available in each of the 6 subjects. Values shown are mean of 6 subjects.

Table E2 MDP responses following matched trials before and after iv morphine .07mg/kg. The rating scales contained in the MDP are presented with mean values expressed as percent of Full Scale (%FS). For immediate unpleasantness the scale maximum was "unbearable", for sensory qualities the scale maximum was "as intense as I can imagine", and for emotion the scale maximum was "most I can imagine". P values are from paired T-test, 2 tail, uncorrected for multiple comparison.

Variables measured during MDP focus period	pre morphine rating % FS	post-morphine rating % FS	*P value*
$PETCO_2$	49.4 torr	54.7 torr	
Unpleasantness, how bad breathing feels (A_1)	58%	58%	
Sensory qualities (SQ)			
Not getting enough air, hunger for air, smothering	65%	60%	*0.46*
Breathing requires mental effort, concentration	48%	45%	*0.75*
Breathing requires muscle work or effort	27%	33%	*0.24*
Chest and lungs feel tight or constricted	33%	32%	*0.74*
Breathing a lot (rapidly, deeply or heavily)	18%	8%	*0.04*
Subject's choice for best descriptor	air hunger 5/6	air hunger 5/6	
Emotions (A_2)			
Depressed	5%	3%	*0.36*
Anxious	28%	12%	*0.01*
Frustrated	15%	13%	*0.36*
Angry	2%	0%	*0.36*
Afraid	3%	0%	*0.36*

Alternate Statistical Test of dyspnea treatment effect.

On the advice of a reviewer, we performed an alternate analysis *post-hoc* using the individual differences in response to placebo and morphine (i.e., subtracting each subjects' placebo response from that individual's morphine response). The statistical summary is very similar to our *a priori* analysis: the median treatment effect was 34%FS, $p < 0.01$.

2. Respiratory sensations during HCVR

We performed HCVR trials to assess the change in respiratory drive following morphine, not to assess sensation. Dyspnea during HCVR was not an *a priori* measurement objective because it is difficult to evoke much discomfort at reasonable levels of $P_{ET}CO_2$ (4). We did not attempt to match level of sensation during HCVR, and have fewer data points. Nonetheless, at the urging of the reviewers we present here descriptive data on sensation during HCVR.

BDVAS: When subjects were allowed to breathe spontaneously, BDVAS ratings were much less through the range of $P_{ET}CO_2$ examined, as expected. Morphine reduced breathing discomfort during HCVR. The mean regressions for BDVAS before and after morphine are shown in Fig. E4. At 10 torr above resting $P_{ET}CO_2$, BDVAS before morphine was 27%FS, and fell to 12%FS following drug. Placebo had no effect.

Multidimensional Dyspnea Profile: In the 33 MDP responses following HCVR (before and after treatment), the overall average immediate unpleasantness rating was 41%FS. (The average $P_{ET}CO_2$ during the focus period is determined by the operator. During the HCVR focus period it was 17 Torr above resting, while in the limited ventilation dyspnea challenge it was 10 Torr above resting). The average rating for 'breathing requires muscle work or effort' was 40%FS (during limited ventilation dyspnea challenge the leading descriptor was air hunger.). The second most highly rated descriptor groups were 'breathing requires mental effort or concentration' and 'air hunger', both averaging 29%FS.

Table E3 Side effects following placebo and morphine. Subjects were asked about the following possible side effects: nausea or indigestion; itching; lightheadedness or faintness; difficulty concentrating; sleepiness. Responses following after placebo and .07 mg/kg morphine are listed in the table below. Responses in the 2 subjects following high morphine dose: Subject 1 noted nausea, sense of being disconnected and spacey during infusion. Difficulty focusing eyes. transient muscle aching and subject number 13 reported no side effects. *Subjects 7 and 12 were given morphine, but data were not included in the analysis for reasons given in the text

Subject	Placebo	Morphine
1	none noted	wave of fatigue after injection
3	mild sleepiness, felt "slow"	tingling over body, dry mouth
6	tired	transient mild itching and fatigue after injection. mild nausea, mild difficulty concentrating, mild sleepiness and mild lightheadedness.
7*	Not done	strong nausea, study had to be stopped.
11	sleepiness	a little sleepy
12*	slightly sleepy	slight nausea during injection, sleepy,
13	none noted	slightly sleepy, no difficulty concentrating
14	"floaty", not dizzy. a little out of it. spacey	whole body heaviness, disconnected spacey

3. Dropped Subjects

One subject was dropped during data analysis because we were not able to induce the prescribed pre-drug BDVAS rating within the IRB-agreed limit for P_{ETCO_2}, we examined the response feature at 33%FS BDVAS before drug; morphine caused a drop of 21%FS – thus inclusion of this subject would not have reduced the effect size and would have increased the statistical significance of the group results. We only obtained two data points in the subject who experienced strong nausea, but these two points indicated that she, too, experienced a pronounced decrease in BDVAS after morphine. Although a lower morphine dose or a different narcotic might have produced a clinically important treatment effect with less nausea, the subject elected not to return for further study.

References, Online Supplement

1. Banzett RB, Garcia RT, Moosavi SH. Simple contrivance "Clamps" End-tidal P_{CO_2} and P_{O_2} despite rapid changes in ventilation. *J Appl Physiol* 2000;88:1597-1600.

2. Cunningham D, Robbins P, Wolff C. Integration of respiratory responses to changes in alveolar partial pressures of CO_2 and O_2 and in arterial pH. In: Cherniack NS, Widdicombe JG, editors. Handbook of Physiology, section 3: The Respiratory System. Washington, D.C.: American Physiological Society; 1986. p. 475-528.

3. Banzett RB. Dynamic response characteristics of co_2-induced air hunger. *Respir Physiol* 1996;105:47-55.

4. Banzett RB, Pedersen SH, Schwartzstein RM, Lansing RW. The affective dimension of laboratory dyspnea: Air hunger is more unpleasant than work/effort. *Am J Respir Crit Care Med* 2008;177:1384-1390.

Figure Legends, Online Supplement

Figure E1. Breathing Apparatus

During Dyspnea Challenge administration, subjects breathed via a mouthpiece connected to a non-rebreathing valve system via a viral filter/re-humidifier (Airlife HEPA, Cardinal Health, McGaw Park IL); inspired gas was supplied from a 5-liter rubber anesthesia bag and expired gas exited to the room via a one-way valve. Subjects inspired through a one way valve connected to a 5 liter anesthesia bag. Gas was supplied to the anesthesia bag via a high output impedance source (a needle valve with upstream pressure at 50psi), thus the subject could not inspire more gas than supplied by the source. Flow could be finely adjusted and was metered. Inspired P_{CO_2} was adjusted by the operator to target desired levels of P_{ETCO_2}. Flow rate was adjusted to meet the needs of the stimulus. Inspired oxygen concentration remained high, at 30%. Gas entering the bag was mixed from sources containing 30% O_2 and either zero CO_2 or 10% CO_2. P_{ETCO_2} was manipulated by altering inspired P_{CO_2}.

Figure E2. Time tracings depicting dyspnea challenge assessments

Physiological measures and continuous BDVAS ratings in Subject 3 before (panel a) and after (panel b) administration of i.v. morphine. Data reported were obtained from several steady-state measurement periods during each trial as indicated in the figure - the median length of measurement periods was 50 sec, but a few periods were as short as 18 sec. Measurement epochs are indicated by dark horizontal bars. Tidal P_{CO_2} trace shows manipulations of inspired P_{CO_2} to achieve various levels of P_{ETCO_2}. BDVAS: Online ratings. P_{AO}: airway opening pressure (despite instructions not to 'pull' against the collapsed bag, this subject did not entirely suppress reflex respiratory drive). VOL: Proportioned sum of thoracic and abdominal motion showing tidal volume and FRC change. After morphine, BDVAS ratings are substantially lower at any given P_{ETCO_2}. (This particular post-drug trial did not achieve matched BDVAS ratings despite substantially higher P_{ETCO_2} after morphine.). Three large breaths permitted at the end of each measurement period precede a brief fall in BDVAS.

Figure E3. Response Feature Example

Baseline measurements are shown as hollow circles, post-morphine as filled circles. The dotted arrow and hollow square denote the reference P_{CO_2} determination, the solid arrow shows the treatment effect.

Figure E4. Breathing discomfort during spontaneous ventilation (HCVR).

Regression lines showing average perceptual responses during unrestrained breathing. Dashed line depicts same-day baseline values before drug or placebo. Average regression was obtained by averaging the slopes and intercepts for individual subjects' regressions.