EUROPEAN RESPIRATORY *journal*

FLAGSHIP SCIENTIFIC JOURNAL OF ERS

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Original article

Morphine alters respiratory control but not other key OSA phenotypes: A randomised trial

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Please cite this article as: Martins RT, Carberry JC, Wang D, *et al*. Morphine alters respiratory control but not other key OSA phenotypes: A randomised trial. *Eur Respir J* 2020; in press (https://doi.org/10.1183/13993003.01344-2019).

Title: Morphine alters respiratory control but not other key OSA phenotypes: A randomised trial

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Take home message: Contrary to previous concerns, 40mg of MS-Contin (morphine) does not impair pharyngeal muscle activity, airway collapsibility or alter the arousal threshold but breathing responses to CO_2 are reduced, which could place certain OSA patients at risk of harm.

ABSTRACT

Accidental opioid-related deaths are increasing. These often occur during sleep. Opioids such as morphine may worsen obstructive sleep apnoea (OSA). Thus, people with OSA may be at greater risk of harm from morphine. Possible mechanisms include respiratory depression and reductions in drive to the pharyngeal muscles to increase upper airway collapsibility. However, the effects of morphine on the 4-key phenotypic causes of OSA (upper airway collapsibility [Pcrit], pharyngeal muscle responsiveness, respiratory arousal threshold and ventilatory control [loop gain] during sleep) are unknown.

Twenty one men with OSA (AHI range=7-67 events/h) were studied on 2 nights (1-week wash-out) according to a double-blind, randomised, cross-over design (ACTRN12613000858796). Participants received 40mg of MS-Contin on one visit and placebo on the other. Brief reductions in continuous positive airway pressure (CPAP) from the therapeutic level were delivered to induce airflow limitation during non-REM sleep to quantify the 4 phenotypic traits. CO_2 was also delivered via nasal mask on therapeutic CPAP to quantify hypercapnic ventilatory responses during non-REM sleep.

Compared to placebo, 40mg of morphine did not change Pcrit (-0.1±2.4 vs. -0.4±2.2cmH_2O, p=0.58), genioglossus muscle responsiveness (-2.2[-0.87 to -5.4] vs. -1.2[-0.3 to -3.5]microV/cmH_2O, p=0.22), or arousal threshold (-16.7±6.8 vs. -15.4±6.0cmH_2O, p=0.41), but did reduce loop gain (-10.1±2.6 vs. -4.4±2.1 dimensionless, p=0.04) and hypercapnic ventilatory responses (7.3±1.2 vs. 6.1±1.5L/min, p=0.006).

Concordant with recent clinical findings, 40mg of MS-Contin does not systematically impair airway collapsibility, pharyngeal muscle responsiveness or the arousal

threshold in moderately severe OSA patients. However, consistent with blunted chemosensitivity, ventilatory control is altered.

Keywords: Sleep-disordered breathing, opioids, phenotyping, respiratory physiology, upper airway physiology.

Introduction

Opioids are commonly prescribed for acute and chronic pain management and as an adjunct to anaesthesia. Global usage rates of opioids including morphine have increased substantially [1-3]. So too have of the number of opioid-related deaths (>33,000 in the US in 2015 alone) [4-6]. More than 40% of unintentional medication poisoning deaths in the US are caused by opioid analgesics [2, 5-7] which pain management experts believe occur as a result of patients trying to manage unrelenting pain [8]. Most opioid-related deaths occur in people aged 22-54 years [5, 7]. Breathing can slow and become irregular with high doses of opioids leading to hypercapnia and hypoxia [9]. Deaths nearly always occur during sleep [10]. While the precise mechanisms are unclear, opioid-induced changes combined with sleep-related reductions in respiratory control and chemoresponsiveness are likely important contributors.

People with obstructive sleep apnoea (OSA), a common condition characterised by intermittent cessation of airflow and blood gas disturbances, may be particularly vulnerable to harm with opioids. There are at least four key causes of OSA. These include impaired anatomy/upper airway collapsibility, poor pharyngeal muscle responsiveness during sleep, heightened arousal responses to airway narrowing (low respiratory arousal threshold) and unstable respiratory control (high loop gain) [11, 12].

The effects of morphine on each of these traits in people with OSA are unknown. Animal studies indicate that opioids activate laryngeal adductors and depress abductor motoneuron pools to reduce airway patency [13]. Fentanyl can also suppress hypoglossal motoneuron output to the largest upper airway dilator muscle,

the genioglossus [14]. These findings suggest that opioids may reduce pharyngeal muscle activity which would worsen OSA.

In humans, naloxone, an opioid antagonist, reduces upper airway collapsibility [15]. This suggests opioids may increase upper airway collapsibility. Indeed, opioids can reduce upper airway reflexes when combined with anaesthesia [16, 17]. Postoperatively, sufentanil, alfentanil and remifentanil are all associated with upper airway obstruction [18-20]. During wakefulness, opioids blunt responsiveness to hypercapnia [21] and hypoxia [22]. Thus, opioids may paradoxically stabilise breathing in certain OSA patients by reducing unstable ventilatory control (high loop gain) [23, 24]. However, individuals with poor ventilatory responses to CO_2 are more vulnerable to ventilatory depression and blood gas disturbances with narcotic drugs [25]. The effects of opioids on respiratory-induced arousals in humans are unknown. However, acutely, morphine disrupts sleep and reduces N3 and REM sleep [26, 27].

Our recent double-blind, randomised clinical study indicate that neither 30mg (N=10) nor 40mg (N=60) of oral controlled released morphine systematically worsen OSA severity [23, 28]. However, there is considerable inter-individual variability which is explained, at least in part, due to differences in baseline chemosensitivity and blood morphine concentration [23, 28].Accordingly, given the absence of data on the effects of morphine on the key contributors to OSA and the potential for harm, this study aimed to determine the effects of morphine on the key phenotypic causes of OSA.

Materials and methods

The current detailed upper airway physiology/phenotyping during sleep investigation was designed as a sub-study of a larger randomised trial that focussed on

wakefulness breathing responses and the clinical effect (as measured via standard overnight polysomnography) of morphine on OSA severity (ACTRN12613000858796) [28]. All of the participants who were enrolled in the larger clinical trial, N=60 middle-aged men with untreated OSA, were approached in person by a member of the research team during their first visit or via follow-up phone call to participate in the current detailed physiology sub-study (Figure 1). Our target recruitment rate from the main clinical study was one in three.

Inclusion criteria for the main clinical study and the current physiology sub-study were men aged between 18-65 years; BMI≤40 kg/m^2; awake SpO_2>90%; sleep SpO_2 nadir 60% and AHI≥5 events/h. Exclusion criteria included shift workers, other significant sleep disorders such as PLMS; severe medical and/or psychiatric disorders, history of drug abuse and/or positive urine drug test to narcotic or other illicit drugs, history of allergy to morphine, concurrent CPAP users and medication use that could interfere with the study objectives (e.g. sedatives). People with current acute illnesses (e.g. respiratory infections or rhinitis) were not studied until symptoms were clear for at least 4-weeks. Other than their recent participation in the main clinical study [28], participants had no or minimal prior exposure to MS-Contin or other opioids.

Participants recruited from the main clinical study completed two additional detailed overnight studies in the sleep physiology laboratory for the current physiology sub-study with a 1-week wash-out between visits according to a double-blind, randomised, placebo-controlled, cross-over design (prospectively registered: ACTRN12613000858796, Figure 1).

Participants were admitted at 5pm following informed written consent. Shortly after arrival, the allocated study intervention was given (40mg of MS-Contin or placebo).

Electroencephalograms, electrooculograms and electrocardiogram leads were then fitted. A blood sample was drawn at 9.30pm to assess blood morphine concentration at the anticipated peak concentration level. Participants were then instrumented with two intramuscular genioglossus electrodes per orally to create a bipolar EMG recording after topical anaesthesia with 1% lignocaine as described previously [29]. An epiglottic pressure catheter (MPR-500 Millar) was also inserted per nasally to measure pharyngeal pressures during sleep [29]. A nasal mask (Philips Respironics Comfort Gel) was fitted with a pressure sensor (Validyne CD19A), pneumotachograph (3700A, Hans Rudolph) and a $PETCO_2$ sensor (VacuMed 17630 CO_2 Analyser) in series. A finger pulse oximeter (Nonin 7500) was also attached.

Participants were asked to sleep supine as much as possible throughout the night. If an individual rolled onto their side for more than a few minutes, a researcher entered the room to remind them to return to their back. Body position was continuously monitored via infrared camera. Signals were acquired using a 1401 analogue to digital converter and Spike 2 software (CED, Cambridge, UK).

During the night, participants were connected to a modified continuous positive airway pressure (CPAP) device capable of delivering positive and negative pressure (Philips Pcrit-3000). Initially, the optimal holding pressure required to eliminate inspiratory flow limitation was determined. Transient CPAP reductions were then performed during stable non-REM sleep to induce upper airway narrowing or collapse. CPAP reductions lasted up to 3 minutes and were performed as many times as possible throughout the night to allow quantification of the phenotypic traits [11, 30]. Specifically, the priority was to quantify pharyngeal critical closure pressure (Pcrit), genioglossus muscle responsiveness and the respiratory arousal threshold. Where possible, loop gain data was also obtained as a secondary outcome given

that quantification of this trait typically requires an additional night of study [11]. Accordingly, given the importance of gaining knowledge of the effects of morphine on respiratory control parameters during sleep steady-state hypercapnic ventilatory responses were also acquired in a sub-sample of participants. External CO_2 was administered via the nasal mask for up to 5 minutes in each condition while on therapeutic CPAP to achieve 5 and 7.5mmHg above baseline during stable non-REM sleep. Another blood sample was drawn upon awakening the following morning.

Data analysis

Sleep staging and arousal scoring was performed by a single experienced sleep technician blinded to the study intervention. To calculate Pcrit, peak inspiratory flow was plotted against mask pressure for breaths 3-5 following each transient CPAP reduction if flow-limited. Linear regression to zero flow was then performed to calculate passive Pcrit [11, 12]. Muscle responsiveness was calculated by plotting nadir epiglottic pressure versus genioglossus peak EMG activity for every artefact-free breath during CPAP reductions and linear regression performed to calculate the slope of the relationship [11, 12]. Arousal threshold was calculated as the nadir epiglottic pressure immediately prior to the scored arousal as described previously [11].

When available, steady-state loop gain was calculated as the ventilatory disturbance (reduction in minute ventilation during the last 60s of a CPAP reduction) to ventilatory response (increase in minute ventilation upon reintroduction of CPAP) ratio as described previously [30]. During the steady-state hypercapnic ventilatory response protocol, thirty seconds of breath-by-breath ventilatory and genioglossus muscle data were analysed and averaged for the pre-CO_2 baseline period. Similarly,

the last arousal-free 30 seconds of each CO_2 increment level ($PETCO_2$ +5 and +7.5mmHg) were analysed [31, 32].

Statistical Analyses

Two-tailed, Student's paired t-tests or a Mann-Whitney rank test were used to compare the effects of morphine versus placebo on the 4 key causes of OSA as appropriate. Alpha was set at $P<0.05$. We estimated that a sample size of N=20 would be sufficient to detect a 1.2cmH_2O change in Pcrit (SD=1.8 [33], a 3cmH_2O change in the respiratory arousal threshold (SD=4.6 [34]), and a 2.3 MicroV/cmH_2O epiglottic pressure in genioglossus muscle responsiveness (SD=3.4; in house reproducibility data) between morphine and placebo conditions with a two-tailed, paired t-test with ~80% power. Linear mixed model analyses for repeated measures were used to compare ventilatory and genioglossus parameters during the CO_2 protocol (SPSS version 24). Parameters included were: condition (placebo or morphine), experiment (baseline, +5 mmHg and +7.5mmHg) and actual $PETCO_2$ level.

Results

Participant characteristics

Twenty three participants were recruited for this study. Two withdrew during their first overnight visit as they could not tolerate CPAP. The study intervention was well tolerated. Anthropomorphic, demographic, lung function and polysomnographic characteristics for the 21 study participants who completed both study nights is summarised in Table 1.

Effects of morphine on the phenotypic traits

Peak blood morphine concentration was 8.6±3.8 (range: 1.7 to 16.9) ng/ml. Morning blood morphine concentration was 2.2±0.8 (range: 0.9 to 3.8) ng/ml. 21±6 CPAP reductions were delivered on the placebo night and 22±6 on the morphine night. Artefact-free data from 14±3 CPAP reductions during non-REM sleep were used to quantify the traits during the placebo condition and 14±6 during the morphine night. Compared to placebo, 40mg of morphine did not change upper airway collapsibility as measured by Pcrit (Figure 2A, p=0.58), genioglossus muscle responsiveness to increased negative pharyngeal pressure (Figure 2B, p=0.22), or the respiratory arousal threshold (Figure 2C, p=0.41). However, while the number of participants in whom data were acquired during both conditions was small for the secondary outcome of loop gain (n=4), morphine significantly reduced loop gain (more positive value) compared to placebo (Figure 2D, p=0.04).

Ventilatory and genioglossus muscle responses to increased CO_2 on CPAP

Effects of hypercapnia on respiratory parameters

The hypercapnia protocol was introduced after the first 2 participants completed the study. Of the remaining 19 participants, there was a technical issue (e.g. target hypercapnia levels not achieved or a major mask leak) in 5 participants. Thus, 14 participants successfully completed the hypercapnia protocol in whom data were analysed. Overall, with increasing CO_2 levels epiglottic pressure swings became more negative (p=0.04), inspiratory time tended to decrease (p=0.05), expiratory time decreased (p=0.02), whereas peak inspiratory flow (p<0.01), breathing frequency (p<0.01), tidal volume (p=0.047) and minute ventilation increased (Table 2 and Figure 3).

Effects of morphine versus placebo on respiratory parameters during hypercapnia

The targeted increases in $PETCO_2$ from baseline were achieved during both conditions (Table 2, Figure 3A). However, consistent with reduced minute ventilation with morphine (Figure 3B, $p<0.01$), absolute $PETCO_2$ levels tended to be higher during the morphine night, including at baseline (Figure 3A).

Minute ventilation increased to a similar extent from baseline during morphine and placebo nights during the +5mmHg condition. However, this was not the case during the +7.5mmHg condition in which the ventilatory response to hypercapnia was reduced during the morphine condition (Figure 3B, interaction effect: $p=0.02$). Epiglottic pressure swings were also less during morphine compared to placebo (Figure 3C, $p=0.04$). Inspiratory time was lower ($p=0.04$) while expiratory time was prolonged ($p<0.01$) and breathing frequency was less ($p<0.01$) during morphine versus placebo. Tidal volume ($p=0.37$) and peak inspiratory flow ($p=0.89$) did not differ between conditions (Table 2).

Genioglossus muscle activity

Peak (24.0±24.1 vs. 25.4±33.1 microV, $p=0.85$) and tonic (10.1±11.2 vs. 8.3±10.3 microV, $p=0.59$) genioglossus muscle activity were similar between morphine and placebo conditions on stable therapeutic CPAP. Peak genioglossus EMG activity increased with hypercapnia (Figure 4A, $p=0.03$). There was no overall increase in tonic activity with hypercapnia (Figure 4B, $p=0.24$). The pairwise comparisons indicated that increases in peak genioglossus EMG from baseline were significant at the +5mmHg ($p=0.01$) and +7.5mmHg levels ($p=0.02$) during placebo whereas there were no significant changes from baseline during the morphine condition (Figure 4A, $p>0.11$). Similarly, the pairwise comparisons indicated that tonic EMG tended to increase from baseline to the +7.5mmHg condition during placebo ($p=0.05$) but there was no change from baseline during the morphine condition ($p>0.33$).

Discussion

The main findings of this study are that a single dose of 40mg of MS-Contin does not systematically impair upper airway collapsibility, muscle responsiveness or the respiratory arousal threshold in men with predominantly moderately severe OSA. This is consistent with our recent clinical trial data in which 30 and 40 mg of morphine (pilot study and main trial, respectively) did not systematically worsen OSA severity as measured by the AHI or time spent below SaO_2 of 90% (T90) in men with mild-moderately severe OSA [23, 28]. In contrast, respiratory control as measured by loop gain and ventilatory responses to CO_2 were reduced by morphine, particularly at increased CO_2 levels, consistent with blunted chemosensitivity [28, 35]. Similarly, genioglossus activity increased with hypercapnia during the placebo condition but not with morphine. These findings provide novel insight into the effects of a moderate dose of morphine on upper airway physiology and respiratory control during sleep in OSA.

Pharyngeal critical closure pressure (Pcrit)

In contrast to the current findings, a previous study conducted in five healthy young men aged between 27-31 years found that the opioid antagonist naloxone reduces Pcrit post-sleep fragmentation by blocking the effects of endorphin [15]. This suggests that endogenous opioids increase pharyngeal collapsibility under these conditions. The reason for the apparent difference between this finding and the current study include differences in participant characteristics (5 healthy young men versus middle aged men with OSA), study intervention and experimental protocols. Consistent with the current findings during sleep, two standard doses of

hydromorphone (2 and 4 mg) did not increase upper airway resistance during wakefulness in healthy individuals [36].

Genioglossus muscle activity and responsiveness to negative pressure and hypercapnia

Experiments conducted on anesthetised rats indicate that morphine reduces hypoglossal motoneuron activity via increases in acetylcholine [37], to inhibit genioglossus muscle activity [14]. In contrast, but consistent with the wakefulness data with hydromorphone in healthy individuals [36], the current findings in men with OSA did not reveal any systematic reductions in pharyngeal muscle activity on therapeutic CPAP or during transient airway narrowing during sleep with morphine. While the reasons for the apparent discrepancy are unknown, factors such as the use of different opioid classes and doses, location of administration and species differences may be important. Nonetheless, the current findings on nasal CPAP which minimises negative pharyngeal pressure changes, suggest that 40mg of morphine does not reduce central neural drive to genioglossus during sleep. Similarly, pharyngeal reflexes to the epiglottic pressure swings that occur during airway narrowing and closure prior to arousal are also not impaired with 40mg of morphine. However, consistent with diminished reflex activation to hypercapnia, genioglossus muscle activity increased with hypercapnia during placebo but not during the morphine condition. Thus, while larger follow-up studies are required, impairment of this important protective mechanism with morphine may perpetuate blood gas disturbances in susceptible individuals.

Respiratory arousal threshold

Opioids are often associated with sedation via suppression of cortical arousal systems [38]. Thus, morphine would be expected to increase the arousal threshold

to respiratory stimuli. However, acute opioid administration is also known to cause sleep disruption and changes in the sleep architecture [39]. These contrasting effects likely explain the lack of overall change in the respiratory arousal threshold in the current study and considerable inter-participant variability in response to morphine. These finding are also consistent with recent wakefulness physiology data in which 40mg of MS-Contin did not alter upper airway tactile sensation, respiratory load detection thresholds, or respiratory load magnitude perception in people with obstructive sleep apnoea [40]. In future studies it will be important to determine if higher doses and more prolonged use result in sustained increases in the threshold to arousal from respiratory stimuli such as airway narrowing which if severe could place OSA patients at risk of harm.

Ventilatory response to hypercapnia and loop gain

Reduced ventilatory responses to hypercapnia with morphine during sleep as detected in the current study are consistent with prior wakefulness data in healthy individuals [22, 36] and our recent findings in men with OSA [28]. Indeed, several studies have shown that acute opioid use reduces central respiratory responses to increased CO_2 levels [35, 41], the slope of the hypercapnic [22] and hypoxic [22, 42] ventilatory responses during wakefulness. Interestingly, it was only at the higher level of hypercapnia (+7.5mmHg above baseline) that diminished ventilatory responses during morphine were observed. Thus, these protective mechanisms seem to be preserved with this dose of morphine up to +5mmHg but not beyond. Accordingly, patients who experience more severe changes in blood gases (e.g. severe OSA in the morbidly obese) may be especially at risk of harm with opioids.

Methodological considerations

While these detailed physiological experiments are the first to quantify changes in the key phenotypic causes of OSA with morphine during sleep, there are several limitations that need to be acknowledged. Firstly, only untreated men with OSA were studied. Given the relatively small sample size for this complex repeated measures study, this design was felt necessary to avoid the confounding influence of fluctuating hormonal levels throughout menstrual cycle in women which are known to alter respiratory control [43]. Thus, it will be crucial to include women in larger, appropriately designed future trials. Indeed, to detect differences in Pcrit, arousal threshold and muscle responsiveness between morphine and placebo of the magnitude observed in the current study which, are arguably not of physiological/clinical importance, a sample in the order of 100 or more would be required. Also, the number of participants in whom loop gain data were available during both conditions was small. This reflects the complexity of the measurement techniques and the primary objective of the current study to quantify the other three traits. Indeed, quantification of all four traits typically requires two nights of data collection [11] which was not feasible for this study. Nonetheless, despite the small sample size we did detect important changes in loop gain which were further complimented by the data obtained during the hypercapnia protocol. However, these findings would benefit from replication in a larger cohort. Finally, we studied people who predominantly had moderately severe OSA who were untreated and largely MS-Contin/opioid naive, during a standard single dose of oral morphine. Thus, the current findings may not be generalisable beyond these conditions. Accordingly, it remains a priority to investigate the effects of different doses of morphine including higher doses beyond the moderate 40mg dose used in the current study. In addition,

it will be important for future, appropriately designed studies on sleep, breathing and upper airway physiology to investigate different durations of exposure, types of opioids and patient characteristics that include both men and women with a wide range of OSA severities.

Clinical implications and conclusions

In the light of the current opioid epidemic and its widespread adverse consequences, the current study provides important, novel insights into the mechanisms of morphine on upper airway physiology and respiratory control during sleep in OSA. Specifically, while caution is warranted, the lack of systematic impairment in upper airway collapsibility, pharyngeal muscle responsiveness and respiratory arousal threshold using gold standard methodology is important from a safety perspective, at least at the acute dose of morphine tested and in this patient population. In the absence of changes in these key traits with 40mg of morphine, the detected changes in respiratory control may have differential effects on OSA severity. Indeed, consistent with pilot data with 30mg of acute morphine [23], reductions in loop gain with 40mg of morphine may stabilise breathing and improve blood gases for certain OSA patients (i.e. those with mild to moderate OSA with unstable respiratory control). Conversely, it may put others at risk, due to centrally mediated hypoventilation effects of morphine and diminished ability to respond to high levels of CO_2 via impairment of protective respiratory/upper airway reflexes during obstructive events (i.e. morbidly obese patients with severe disease or with co-administration of other central nervous system depressants). Similar to our previous report [23] and clinical findings from the larger cohort [28], there was substantial inter-individual variability in blood morphine concentrations. This may explain, at least in part, some of the variability in responses with morphine to some of the traits detected. Ultimately,

accurate detection tools are required to determine which patients are most at risk of harm versus those in whom they can be used safely. Given the scope of the opioid problem, there is an urgent need for further research on this important topic including investigation into the effects on sleep and breathing using different classes of opioids, different patient populations and longer durations of use.

Acknowledgements

The authors would like to thank our collaborators at the Woolcock Institute of Medical Research (Professors Keith Wong and Brendon Yee) and the Prince of Wales Hospital (Associate Professor David McKenzie) who assisted with participant recruitment and/or medical oversight for this study. We would also like to thank the Prince of Wales Hospital Clinical Trials Pharmacy team for preparing the study interventions and coordinating the randomisation process. Finally, we would like to thank Professor Andrew Somogyi and his team from the University of Adelaide for performing the blood morphine concentration analyses.

Author contributions

DJE, DW and RRG developed the study concepts. DJE designed the study. RTM, JCC, and DJE collected and analysed the data. All authors provided important insight on data interpretation and contributed to the final version of the manuscript.

Grant support

This work was supported by the National Health and Medical Research Council (NHMRC) of Australia (1043633, 1060992 and 1049814). DJE is supported by a NHMRC Senior Research Fellowship (1116942). RRG is supported by a NHMRC Senior Principal Research Fellowship (1106974).

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Figure legends

Figure 1. CONSORT diagram highlighting the recruitment and enrolment approach and participant flow through the protocol and analysis steps for this double-blind, randomised, placebo-controlled, cross-over, physiology sleep study. Participants were recruited and studied in the current protocol between August 2013 and August 2015. Refer to the text and other Figure Legends for further details.

Figure 2. A. Pharyngeal critical closure pressure (Pcrit) on morphine versus placebo during non-REM sleep. Individual data in N=13 in whom Pcrit data was available during both conditions is displayed (Pcrit data was obtained for only one night in 4 participants and no Pcrit data was obtained during either night in 4 additional participants). Adjacent data indicate the group mean±SD for each condition. **B.** Slope of the relationship between peak genioglossus muscle activity to increasing negative epiglottic pressure (muscle responsiveness) on morphine versus placebo during non-REM sleep. Individual data in N=20 in whom muscle responsiveness data was available during both conditions is displayed (data was not obtained in 1 participant as he was unable to obtain sufficient sleep on continuous positive airway pressure therapy). Adjacent data indicate the group median and interquartile range for each condition. **C.** Respiratory arousal threshold on morphine versus placebo during non-REM sleep. Individual data in N=19 in whom arousal threshold data was available during both conditions is displayed (inadequate data for arousal threshold quantification in two participants). Adjacent data indicate the group mean±SD for each condition. **D.** Loop gain on morphine versus placebo during non-REM sleep. Individual data in N=4 in whom loop gain data was available during both conditions is displayed (data was obtained in a single night for 6 participants and in 11

participants there were insufficient replicate trials to quantify loop gain). Adjacent data indicate the group mean±SD for each condition.* indicates significant difference between conditions.

Figure 3. End-tidal carbon dioxide (**A.**), minute ventilation (**B.**) and epiglottic pressure (**C.**) values with morphine versus placebo during the hypercapnia protocol (baseline on therapeutic continuous positive airway pressure [CPAP] during non-REM sleep and with 5 and 7.5mmHg increases in end-tidal carbon dioxide above baseline (+5mmHg and +7.5mmHg, respectively). Values are estimated group mean and 95% confidence intervals from the mixed model analyses (N=14 individuals successfully completed this sub-protocol). * indicates a statistically significant difference from baseline within the placebo condition. † indicates a statistically significant difference from baseline within the morphine condition.

Figure 4. Peak (**A.**) and tonic (**B.**) genioglossus muscle responsiveness on morphine versus placebo during the hypercapnia protocol (baseline on therapeutic continuous positive airway pressure [CPAP] during non-REM sleep and with 5 and 7.5mmHg increases in end-tidal carbon dioxide above baseline (+5mmHg and +7.5mmHg, respectively). Values are estimated group mean and 95% confidence intervals from the mixed model analyses (N=14 individuals successfully completed this sub-protocol). * indicates a statistically significant difference from baseline within the placebo condition. EMG=electromyography.

Table 1. Anthropometric, sleep and lung function parameters

	N = 21
Age (years)	51 ± 10
BMI (kg/m^2)	28 ± 4
AHI (events/h)	26 ± 17
Nadir SpO_2 (%)	85 ± 6
ESS	8 ± 3
Sleep efficiency (%)	84 ± 9
Neck circumference (cm)	40 ± 4
FEV1 (% predicted)	106 ± 11
FVC (% predicted)	110 ± 13
FEV1/FVC	0.8 ± 0.1

BMI=body mass index, AHI= apnea-hypopnea index, SpO_2= peripheral capillary oxygen saturation, ESS=Epworth sleepiness score, FEV_1= forced expiratory volume in the first second, FVC=forced vital capacity. Data are mean±SD.

Table 2. Respiratory parameters during the hypercapnic protocol on continuous positive airway pressure

	Placebo			Morphine		
	Baseline	**+5 mmHg**	**+7.5 mmHg**	**Baseline**	**+5 mmHg**	**+7.5 mmHg**
Δ CO_2 (mmHg)	-	4.87 ± 0.53	8.03 ± 0.80	-	5.33 ± 0.29	7.95 ± 0.51
PIF (L/s)	0.57 ± 0.04	0.68 ± 0.04	0.76 ± 0.05	0.62 ± 0.03	0.68 ± 0.03	0.70 ± 0.04
Vt (L)	0.50 ± 0.02	0.55 ± 0.02	0.56 ± 0.03	0.50 ± 0.01	0.53 ± 0.02	0.51 ± 0.03
Fb (#/min)	13.0 ± 0.6	14.1 ± 0.4	15.2 ± 0.6	11.1 ± 0.5*	12.3 ± 0.5*	13.0 ± 0.7*
Ti (s)	2.08 ± 0.10	1.94 ± 0.07	1.84 ±0.09	1.94 ± 0.08*	1.85 ± 0.06*	1.77 ± 0.08*
Te (s)	2.69 ±0.14	2.39 ± 0.06	2.20 ± 0.10	3.57 ± 0.18*	3.26 ± 0.15*	3.09 ± 0.17*

* indicates significant difference compared to the equivalent condition during placebo. Refer to the text for overall changes in respiratory parameters with increasing CO_2 levels. ΔCO_2= change in carbon dioxide from the baseline level asleep, PIF= peak

inspiratory flow, Vt= tidal volume, Fb= breathing frequency, Ti= inspiratory time, Te=expiratory time, Baseline= therapeutic continuous positive airway pressure [CPAP] during non-REM sleep, +5mmHg= 5mmHg increase in end-tidal carbon dioxide above baseline condition and +7.5mmHg= 7.5mmHg increase in end-tidal carbon dioxide above baseline condition. Data are means±SEM.

Figure 1.

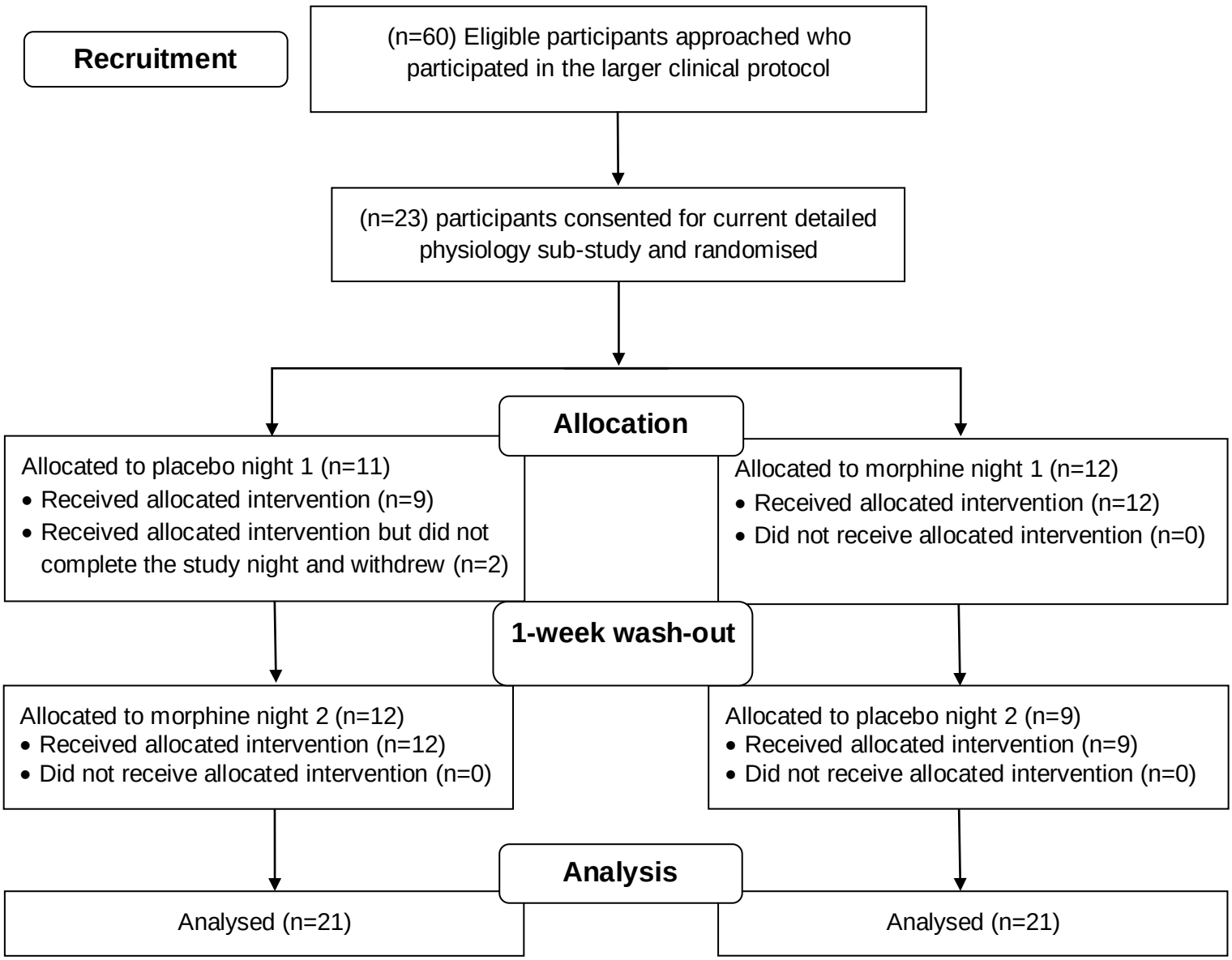
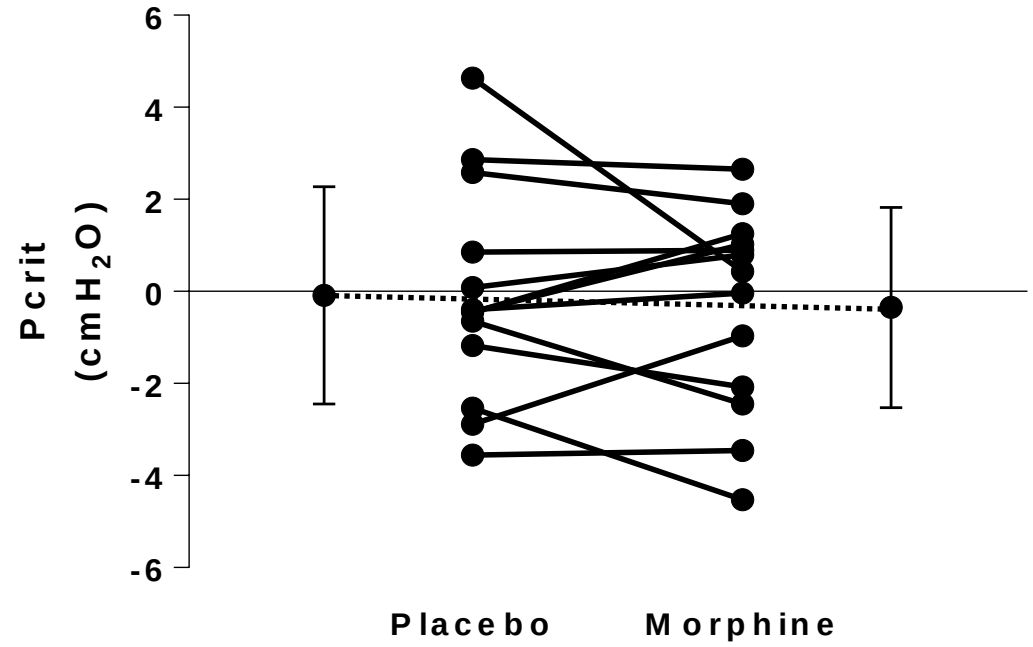

Recruitment
(n=60) Eligible participants approached who participated in the larger clinical protocol
(n=23) participants consented for current detailed physiology sub-study and randomised
Allocation
Allocated to placebo night 1 (n=11)
• Received allocated intervention (n=9)
• Received allocated intervention but did not complete the study night and withdrew (n=2)
Allocated to morphine night 1 (n=12)
• Received allocated intervention (n=12)
• Did not receive allocated intervention (n=0)
1-week wash-out
Allocated to morphine night 2 (n=12)
• Received allocated intervention (n=12)
• Did not receive allocated intervention (n=0)
Allocated to placebo night 2 (n=9)
• Received allocated intervention (n=9)
• Did not receive allocated intervention (n=0)
Analysis
Analysed (n=21)
Analysed (n=21)

Figure 2.

A.


6
4
2
0
-2
-4
-6
Pcrit
(cmH2O)
Placebo
Morphine

B.

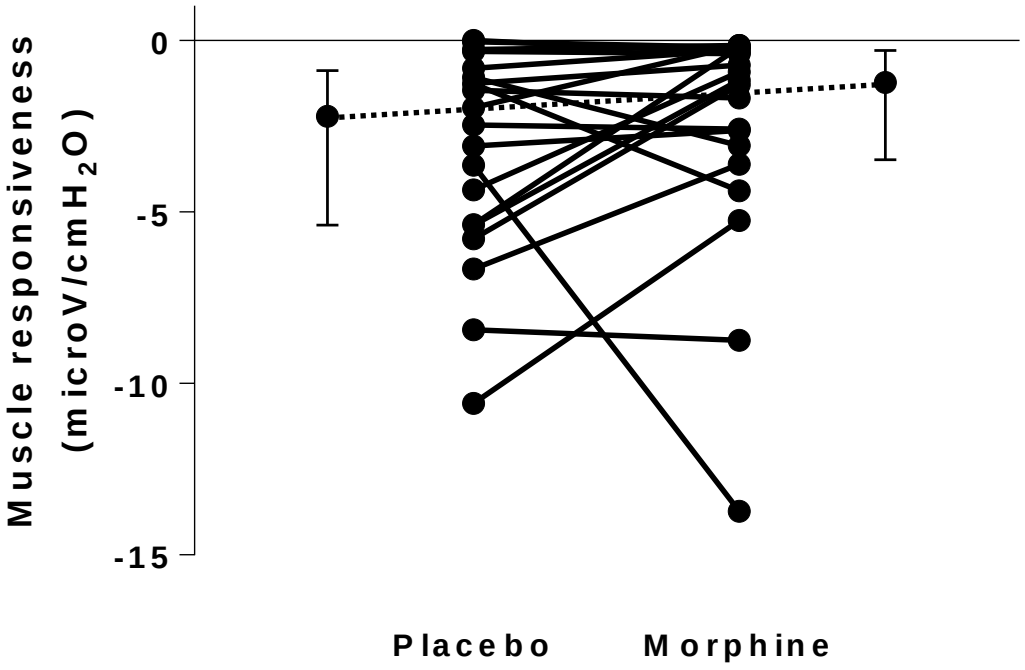
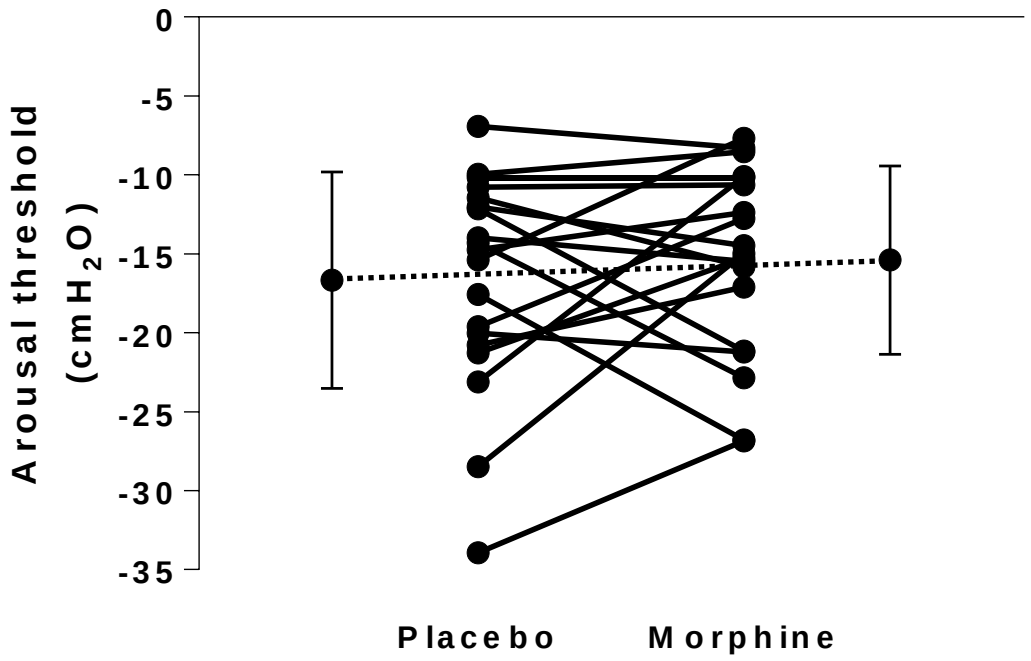

0
-5
-10
-15
Muscle responsiveness
(microV/cmH2O)
Placebo
Morphine

Figure 2.

C.


0
-5
-10
-15
-20
-25
-30
-35
Arousal threshold
(cmH2O)
Placebo
Morphine

D.

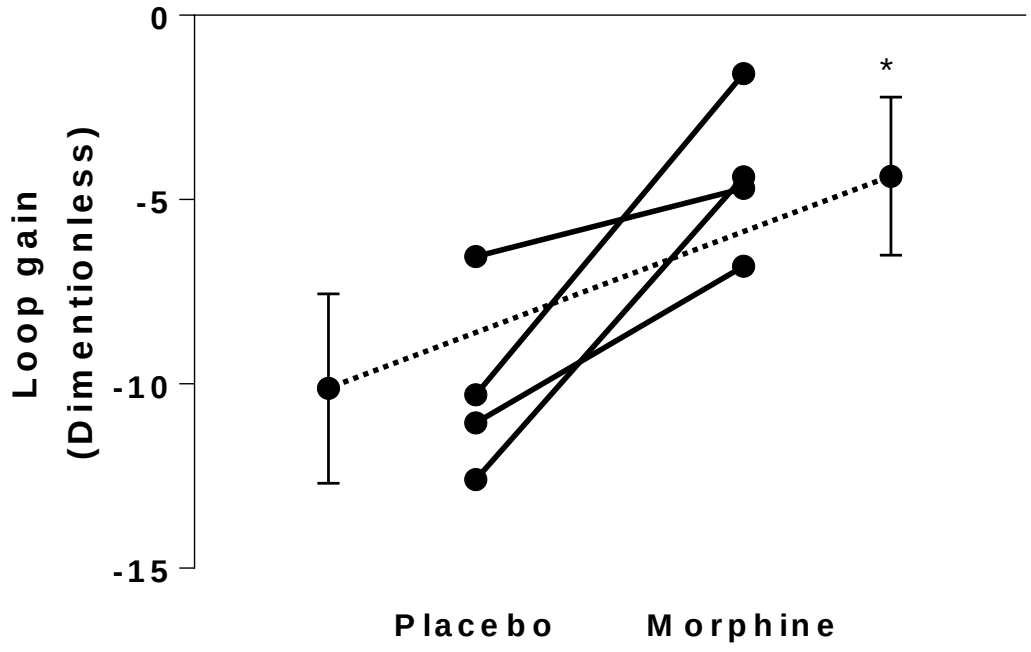

0
-5
-10
-15
Loop gain
(Dimentionless)
*
Placebo
Morphine

Figure 3.

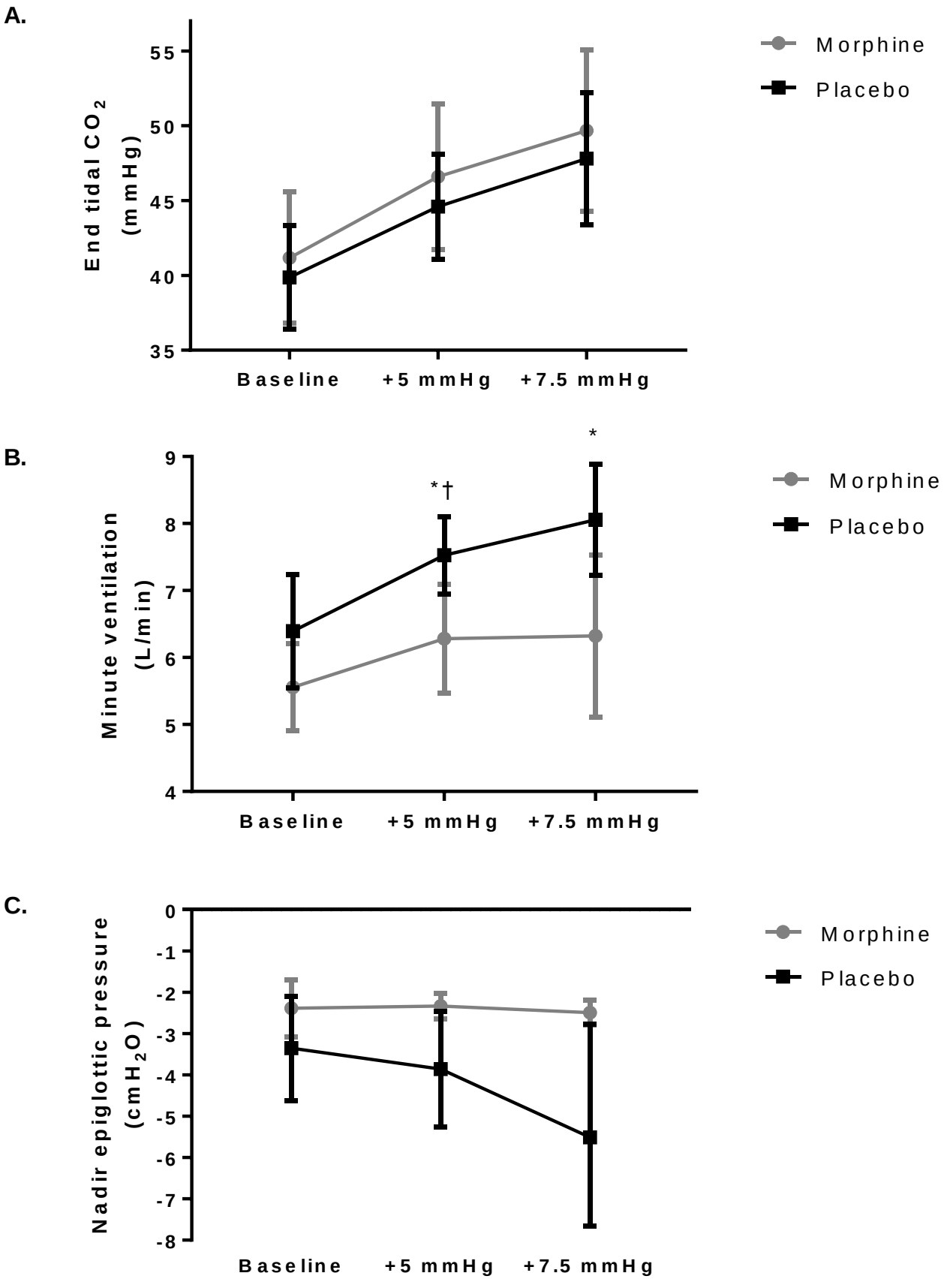
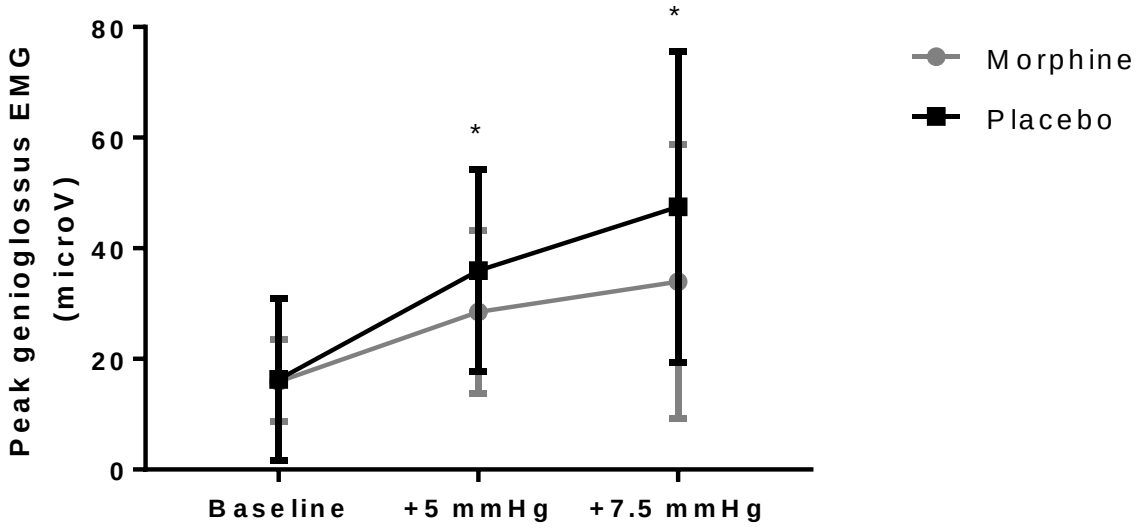
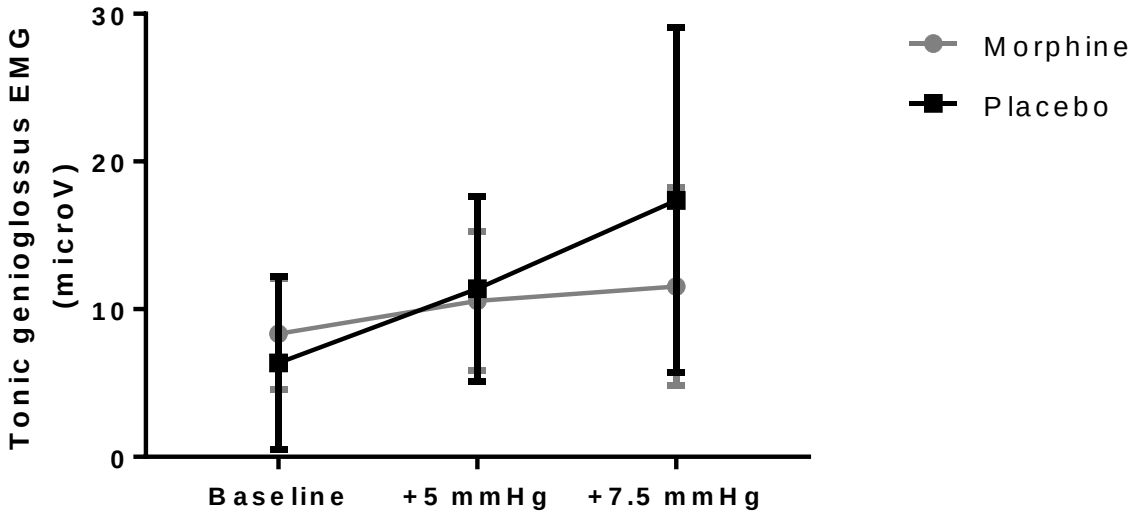

A.
End tidal CO_2 (mmHg)
55
50
45
40
35
Baseline
+5 mmHg
+7.5 mmHg
Morphine
Placebo
B.
Minute ventilation (L/min)
9
8
7
6
5
4
*†
*
Baseline
+5 mmHg
+7.5 mmHg
Morphine
Placebo
C.
Nadir epiglottic pressure (cmH_2O)
0
-1
-2
-3
-4
-5
-6
-7
-8
Baseline
+5 mmHg
+7.5 mmHg
Morphine
Placebo

Figure 4.

A.


Peak genioglossus EMG (microV)
80
60
40
20
0
*
*
Baseline
+5 mmHg
+7.5 mmHg
Morphine
Placebo

B.


Tonic genioglossus EMG (microV)
30
20
10
0
Baseline
+5 mmHg
+7.5 mmHg
Morphine
Placebo