

## **Online Data Supplement**

### **The Effect of Acute Morphine on Obstructive Sleep Apnoea: A Randomised Double-Blind Placebo-Controlled Trial**

Luke Rowsell<sup>1</sup>, Keith K H Wong<sup>1,2</sup>, Brendon J Yee<sup>1,2</sup>, Danny J. Eckert<sup>3</sup>, Andrew A. Somogyi<sup>4</sup>, James Duffin<sup>5</sup>, Ronald R Grunstein<sup>1,2</sup>, David Wang<sup>1,2\*</sup>

<sup>1</sup>NHMRC Centre for Sleep and Chronobiology (CIRUS) and NHMRC CRE NeuroSleep, Woolcock Institute of Medical Research, Sydney Medical School, The University of Sydney, NSW 2006, Australia; <sup>2</sup>Department of Respiratory and Sleep Medicine, Royal Prince Alfred Hospital, Missenden Rd, Camperdown, NSW 2050, Australia; <sup>3</sup>Neuroscience Research Australia, School of Medical Sciences, University of New South Wales, Barker St, Randwick, NSW 2031, Australia; <sup>4</sup>Discipline of Pharmacology, Adelaide Medical School, The University of Adelaide, North Terrace, Adelaide, SA 5005, Australia; <sup>5</sup>Departments of Physiology and Anaesthesia, University of Toronto, 1 King's College Cir, Toronto, ON M5S1A8, Canada

### **Supplemental Methods**

***Genotyping Analysis:*** Briefly, upstream and downstream primers were designed for polymorphism rs7103572. PCR products were obtained through amplification of the products from the primer fragment. Following purification the PCR product was Sanger sequenced using an ABI 3730XL sequencer. The results were then compared to a database using a known sequence.

***Awake Ventilatory Chemoreflex Test:*** Briefly, patients hyperventilate room air for 5 minutes whilst being coached to maintain end-tidal PCO<sub>2</sub> at a partial pressure of 19-25mmHg. After hyperventilation, patients breathe from a rebreathing circuit filled with a pre-mixed mixture of 94% O<sub>2</sub> and 6% CO<sub>2</sub>. During rebreathing, the PO<sub>2</sub> in the circuit is held constant at 150 mmHg (hyperoxia). The testing system continuously analyses O<sub>2</sub> consumption over the preceding 3 breaths. A prediction model is used to determine how much O<sub>2</sub> to add to the circuit. Rebreathing continues for 5 minutes or until a clear linear increase in minute ventilation (V<sub>E</sub>) occurs. Results are automatically stored and analysed using software provided by Prof James Duffin from Toronto University (National Instruments, LabVIEW, Texas, USA).

### **Supplemental Results**

***Adverse Events:*** One patient (administered placebo) complained of abdominal pain and had trouble breathing at approximately 22:00 hours. The study physician was notified and the patient underwent a chest X-ray to exclude a pneumothorax. Symptoms subsided by 23:00 hours and the study was completed without further event. This patient was evaluated by a study physician during and post study and was without further complication. Two patients (administered real morphine) reported mild nausea in the post study period and were evaluated by a study physician. The nausea subsequently subsided without incident.

## Supplemental Tables

**Table E1** The effect on morphine on awake ventilatory chemoreflexes and resting minute ventilation

	Placebo mean (SD)	Morphine mean (SD)	Delta mean (95%CI)	Morphine effect (p)
<b>VRT</b> (mmHg)	54.1 (7.1)	55.7 (7.8)	<b>1.6 (0.3 to 2.9)</b>	<b>0.017</b>
<b>HCVR</b> (litres/min/mmHg)	1.7 (0.8)	1.3 (0.7)	<b>-0.4 (-0.6 to -0.1)</b>	<b>0.006</b>
<b>Minute ventilation</b> (litres/min)	15.4 (7.0)	14.2 (7.3)	<b>-1.2 (-2.3 to -0.1)</b>	<b>0.04</b>

**Annotation:** HCVR= Slope response to hypercapnia (L/min/mmHg); VRT = CO<sub>2</sub> ventilatory recruitment threshold (mmHg); Tested by mixed model of repeated measures controlling for treatment order age and BMI if appropriate.

**Table E2.** Plasma morphine and metabolite concentration

	Evening measure (9:30pm)			Morning measure (7:00am)		
	Plasma morphine (ng/mL)	M3G (ng/mL)	M6G (ng/mL)	Plasma morphine (ng/mL)	M3G (ng/mL)	M6G (ng/mL)
<b>n</b>	59	59	59	57	57	57
<b>Mean (SD)</b>	7.0 (2.6)	563.4 (186.3)	101.7 (36.6)	1.8 (0.7)	128.8 (46.9)	17.7 (8.0)
<b>Minimum</b>	2.3	160.1	21.1	0.8	45.8	5.1
<b>Maximum</b>	16.3	932.4	186.6	3.9	244.6	36.9

**Annotation:** Plasma morphine = Plasma morphine concentration; M3G = Morphine 3-glucuronide metabolite concentration; M6G = Morphine-6-glucuronide metabolite concentration. N=60.

### **Supplemental Figure Legend**

**Figure E1.** No correlations between placebo VRT and  $\Delta$  respiratory polysomnographic variables in 45 mild OSA patients with placebo AHI < 20 /hr (all  $p > 0.05$ )