Supplemental Information

Administration of Tramadol or Buprenorphine via the drinking water for post-operatively analgesia in a mouse-osteotomy model

Paulin Jirkof[1#], Mattea Durst[1,2#], Robert Klopfleisch[3], Rupert Palme[4], Christa Thöne-Reineke[5], Frank Buttgereit[2,6], Katharina Schmidt-Bleek[7,8], Annemarie Lang[2,6,8]*

[1] Division of Surgical Research, University Hospital Zurich, University of Zurich, Zurich, Switzerland

[2] Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Department of Rheumatology and Clinical Immunology, Berlin, Germany

[3] Institute of Veterinary Pathology, Freie Universität Berlin, Germany

[4] Unit of Physiology, Pathophysiology and Experimental Endocrinology, Department of Biomedical Sciences, University of Veterinary Medicine, Vienna, Austria

[5] Institute of Animal Welfare, Animal Behavior and Laboratory Animal Science, Department of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany

[6] German Rheumatism Research Centre (DRFZ) Berlin, a Leibniz Institute, Berlin, Germany

[7] Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Julius Wolff Institute and Center for Musculoskeletal Surgery, Berlin, Germany

[8] Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health Berlin Brandenburg Center for Regenerative Therapies, Berlin, Germany

[#] These authors contributed equally.

*Corresponding author: Annemarie Lang, Address: Charitéplatz 1, 10117 Berlin, Germany. Tel: (+49) 30 450 513450. Email: annemarie.lang@charite.de

Table S1: Food and water intake of the control groups (AN, DW)

	Food intake (g) Median (Min – Max)					**Water intake (ml)** Median (Min – Max)				
Groups	**0 h**	**24 h**	**48 h**	**72 h**	**96 h**	**0 h**	**24 h**	**48 h**	**72 h**	**96 h**
T_{low} AN	8.28 (7.63 – 8.93)	8.25 (2.4 – 14.1)	4.44 (3.9 – 5.0)	10.07 (5.4 – 14.7)	8.94 (4.5 – 13.4)	8.32 (8.1 – 8.6)	4.96 (1.3 – 8.6)	8.52 (7.1 – 10.0)	12.79 (11.3 – 14.3)	9.62 (9.6 – 9.6)
T_{high} AN	9.23 (8.7 – 9.8)	5.56 (5.0 – 6.0)	2.93 (0.3 – 5.5)	8.50 (7.2 – 9.8)	10.61 (10.3 – 10.9)	9.18 (8.3 – 10.1)	8.65 (6.0 – 11.3)	1.63 (0.9 – 2.4)	10.13 (9.4 – 10.8)	10.52 (9.9 – 11.2)
Bup AN	8.21 (7.3 – 9.1)	7.30 (7.0 – 7.6)	4.39 (2.4 – 6.4)	7.47 (6.4 – 8.5)	8.94 (7.3 – 10.6)	8.54 (8.2 – 8.9)	7.47 (6.4 – 8.6)	6.41 (6.0 – 6.8)	9.68 (9.5 – 9.8)	8.09 (8.1 – 8.1)
T_{low} DW	8.19 (8.0 – 8.4)	10.78 (8.6 – 13.0)	8.36 (7.8 – 8.9)	6.96 (6.5 – 7.4)	8.06 (5.2 – 10.9)	8.76 (8.3 – 9.2)	10.44 (8.4 – 12.5)	9.08 (5.8 – 12.4)	8.86 (8.8 – 8.9)	7.81 (7.8 – 7.8)
T_{high} DW	8.53 (8.2 – 8.9)	8.01 (7.6 – 8.4)	8.40 (6.5 – 10.4)	6.27 (2.7 – 9.8)	10.28 (7.2 – 13.3)	9.27 (8.2 – 10.3)	9.86 (4.7 – 15.1)	6.89 (5.3 – 8.5)	7.38 (6.9 – 7.8)	10.52 (10.8 – 10.3)
Bup DW	8.60 (7.3 – 9.9)	10.32 (9.9 – 10.7)	6.50 (6.3 – 6.7)	7.65 (7.6 – 7.8)	8.03 (7.2 – 8.8)	8.65 (8.3 – 9.0)	4.62 (2.3 – 7.0)	6.37 (4.5 – 8.3)	8.55 (7.3 – 9.8)	6.40 (6.1 – 6.7)

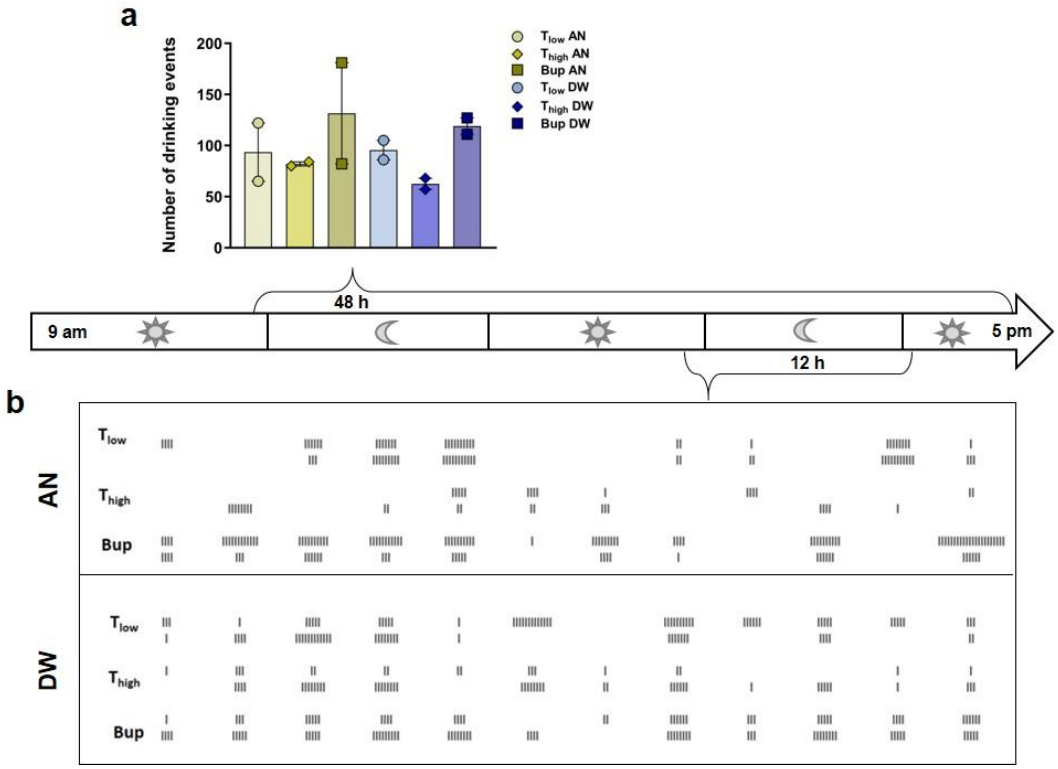

Figure S1: Drinking frequency of control (AN, DW) groups. (a) Total number of drinking events for 48 h was assessed via video recording. Data are shown for AN and DW groups as scatter dot plot and bar with median ± interquartile ranges (n = 2). (b) Depiction of the drinking events over 12 h between 36 h and 48 h post-osteotomy. Each line indicates one drinking event (n = 2).

Table S2: Exact *p*-values for differences between measured and initial body weight (one sample t test; hypothetical mean = 100)

	OT					AN					DW				
Groups	**24 h**	**48 h**	**72 h**	**96 h**	**120 h**	**24 h**	**48 h**	**72 h**	**96 h**	**120 h**	**24 h**	**48 h**	**72 h**	**96 h**	**120 h**
T_{low}	***< 0.001***	***< 0.001***	***< 0.001***	*0.70*	*0.120*	***0.020***	***0.020***	***0.031***	*0.270*	*0.870*	***0.001***	***0.031***	*0.970*	***0.020***	***0.040***
T_{high}	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	*0.310*	***0.01***	***0.005***	***<0.001***	***0.009***	*0.680*	***<0.001***	***0.002***	***0.005***	*0.230*	*0.560*
Bup	***0.005***	***<0.001***	***0.003***	*0.550*	*0.560*	***0.009***	***0.031***	*0.190*	*0.860*	*0.980*	*0.070*	*0.590*	*0.050*	*0.720*	***0.020***

Table S3: Adjusted *p*-values - Two-way ANOVA fold change body weight (Tukey's multiple comparisons test)

	Hours post-osteotomy	T_{low}	T_{high}	Bup
OT	**24 vs. 48**	*0.8*	*>0.99*	*0.94*
	24 vs. 72	***<0.001***	*0.23*	***0.02***
	24 vs. 96	***<0.001***	***<0.001***	***<0.001***
	24 vs. 120	***<0.001***	***<0.001***	***<0.001***
	48 vs. 72	***0.002***	*0.13*	***0.001***
	48 vs. 96	***<0.001***	***<0.001***	***<0.001***
	48 vs. 120	***<0.001***	***<0.001***	***<0.001***
	72 vs. 96	***<0.001***	***0.01***	*0.05*
	72 vs. 120	***<0.001***	***<0.001***	*0.09*
	96 vs. 120	*0.46*	***<0.001***	*>0.99*
AN	**24 vs. 48**	*0.89*	***0.01***	*0.07*
	24 vs. 72	***<0.001***	*0.79*	*0.98*
	24 vs. 96	***<0.001***	*0.05*	*0.19*
	24 vs. 120	***<0.001***	***<0.001***	*0.35*
	48 vs. 72	***0.003***	*0.22*	*0.24*
	48 vs. 96	***<0.001***	***<0.001***	***<0.001***
	48 vs. 120	***<0.001***	***<0.001***	***<0.001***
	72 vs. 96	***0.001***	***0.001***	*0.05*
	72 vs. 120	***0.009***	***<0.001***	*0.12*
	96 vs. 120	*0.97*	*0.07*	*>0.99*
DW	**24 vs. 48**	*0.17*	*0.99*	*>0.99*
	24 vs. 72	***<0.001***	*0.26*	*0.84*
	24 vs. 96	***<0.001***	***<0.001***	*0.12*
	24 vs. 120	***<0.001***	***<0.001***	*0.17*
	48 vs. 72	*0.13*	*0.53*	*0.79*
	48 vs. 96	***<0.001***	***<0.001***	*0.1*
	48 vs. 120	***<0.001***	***<0.001***	*0.14*
	72 vs. 96	*0.11*	*0.07*	*0.66*
	72 vs. 120	*0.27*	***<0.001***	*0.75*

Table S4: Exact *p*-values for differences between measured and initial facial expression and body composite score (Wilcoxon signed rank test; hypothetical median = 0)

	OT						AN					
Groups	**1 h**	**6 h**	**12 h**	**24 h**	**48 h**	**72 h**	**1 h**	**6 h**	**12 h**	**24 h**	**48 h**	**72 h**
T_{low}	***0.008***	***0.008***	***0.031***	***0.016***	*0.125*	*0.125*	*0.125*	*0.125*	*0.250*	*0.250*	*0.500*	*0.500*
T_{high}	***0.016***	***0.031***	*0.063*	*0.063*	*0.5*	*0.25*	*0.125*	*0.125*	*0.250*	*0.250*	*0.500*	-
Bup	*0.008*	*0.008*	*0.063*	***0.031***	*0.063*	*0.500*	*0.125*	*0.250*	-	*0.500*	*> 0.99*	-

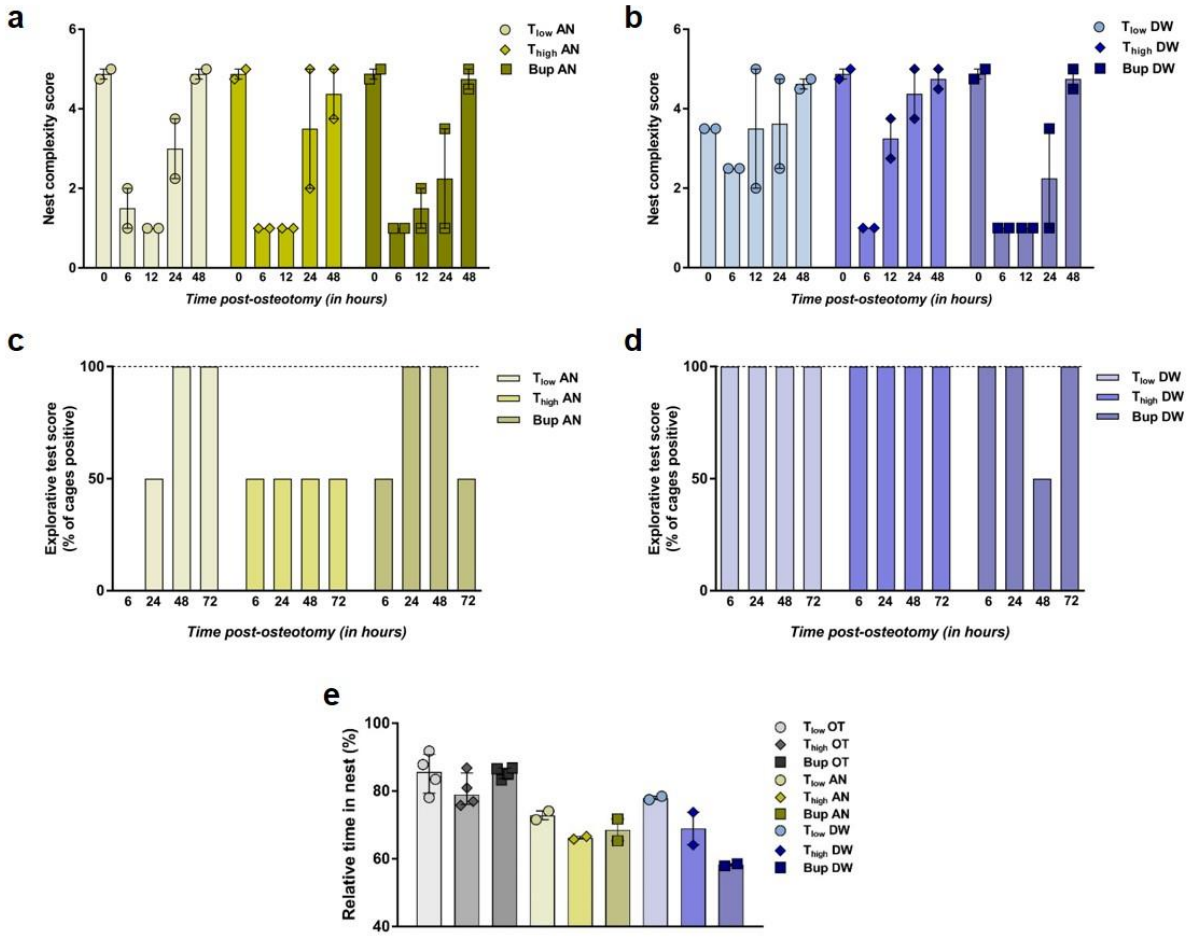

Figure S2: Nest complexity and explorative test scores of control (AN, DW) groups. (a, b) Nest complexity score are depicted for 0, 6, 12, 24 and 48 h post-osteotomy. Data are shown as scatter dot plot and bar with median ± interquartile range for n = 2 (cages). (c, d) The explorative test score is depicted for the OT groups as mean – percentage of cages that were determined positive for n = 2 (cages). (e) Relative time spent in nest for 48 h as assessed via video analysis. Data are shown as scatter dot plot and bar with median ± interquartile ranges (n = 2-4).

Table S5: Exact *p*-values for differences between measured and initial limp score (Wilcoxon signed rank test; hypothetical median = 0)

	OT				
Groups	**1 h**	**6 h**	**24 h**	**48 h**	**72 h**
T_{low}	***0.031***	*0.250*	*0.250*	*0.500*	*0.500*
T_{high}	***0.031***	*0.063*	*0.250*	*0.500*	*0.500*
Bup	*> 0.99*	*0.500*	*> 0.99*	*> 0.99*	*> 0.99*

Table S6: Overview on the different groups enrolled in the study

Group name and colour coding	Osteotomy groups (OT)			Control groups (AN, DW)					
	T_{low}	T_{high}	Bup	T_{low} AN	T_{low} DW	T_{high} AN	T_{high} DW	Bup AN	Bup DW
Medication	Tramadol		Buprenorphine	Tramadol				Buprenorphine	
Dosage via drinking water 3d post-OP	0.1 mg/ml	1 mg/ml	0.009 mg/ml	0.1 mg/ml		1 mg/ml		0.009 mg/ml	
Mice number (n)	8	8	8	4	4	4	4	4	4
Medication pre-OP	0.03 ml Clindamycin s.c. and Buprenorphine 0.1 ml s.c. (1h prä-OP)								
Isoflurane anaesthesia	yes			yes	no	yes	no	yes	no
Euthansia after 2 weeks	yes			no - reuse					

Table S7: Facial expression and body condition composite score adapted from Jirkof et al. [1]

Parameter	Specification	Scores
Facial expression		
orbital tightening	narrowing of the orbital area, a tightly closed eyelid, or an eye squeeze (orbital muscles around the eyes contracted)	**not present = 0** **moderately = 1** **severe = 2**
ear position	ears pulled back or rotate outwards and/or back, away from the face, space between the ears may appear wider	
Body condition		
spontaneous behavior	sudden movements, backwards movements, transient involuntary muscular contraction of any body part, kicking with hind paws, licking/biting the wound, highly aggressive, vocalization	**not present = 0** **present = 1**
posture	hunched, arched back, crouched	
coat condition	ruffled, dirty, unkempt, piloerection, hair loss (alopecia)	
eyes	discharge	
body condition	sunken flanks, swollen areas, ascites	
wound	dirty, bloody, uncleaned, signs of self-injury, signs of inflammation or necrosis, i.e., unusual color (e.g., red, pale) or swollen	
movement	apathetic, sedated, decelerated, crawling, immobile, lameness, tiptoe gait	

Table S8: Limp score adapted from [2-4]

	Parameter	Specification	Scores
Limp Score	**Limping**	• normal use	**0**
		• sporadic limping/hopping, complete ground contact	**1**
		• limping, constant hopping	**2**
		• partial non-use of limb	**3**
		• complete lack of use	**4**
	Dragging	• normal use	**0**
		• sporadic dragging of toes	**1**
		• constant dragging of toes	**2**
		• sporadic dragging of complete leg	**3**
		• constant dragging of complete leg	**4**

References

1 Jirkof, P., Tourvieille, A., Cinelli, P. & Arras, M. Buprenorphine for pain relief in mice: repeated injections vs sustained-release depot formulation. *Laboratory animals*, doi:10.1177/0023677214562849 (2014).

2 Jimenez-Andrade, J. M. *et al.* Nerve growth factor sequestering therapy attenuates non-malignant skeletal pain following fracture. *Pain* **133**, 183-196, doi:10.1016/j.pain.2007.06.016 (2007).

3 Koewler, N. J. *et al.* Effects of a monoclonal antibody raised against nerve growth factor on skeletal pain and bone healing after fracture of the C57BL/6J mouse femur. *J Bone Miner Res* **22**, 1732-1742, doi:10.1359/jbmr.070711 (2007).

4 Minville, V., Laffosse, J. M., Fourcade, O., Girolami, J. P. & Tack, I. Mouse model of fracture pain. *Anesthesiology* **108**, 467-472 (2008).