

## Supplementary Material

# Morphine Accumulates in the Retina Following Chronic Systemic Administration

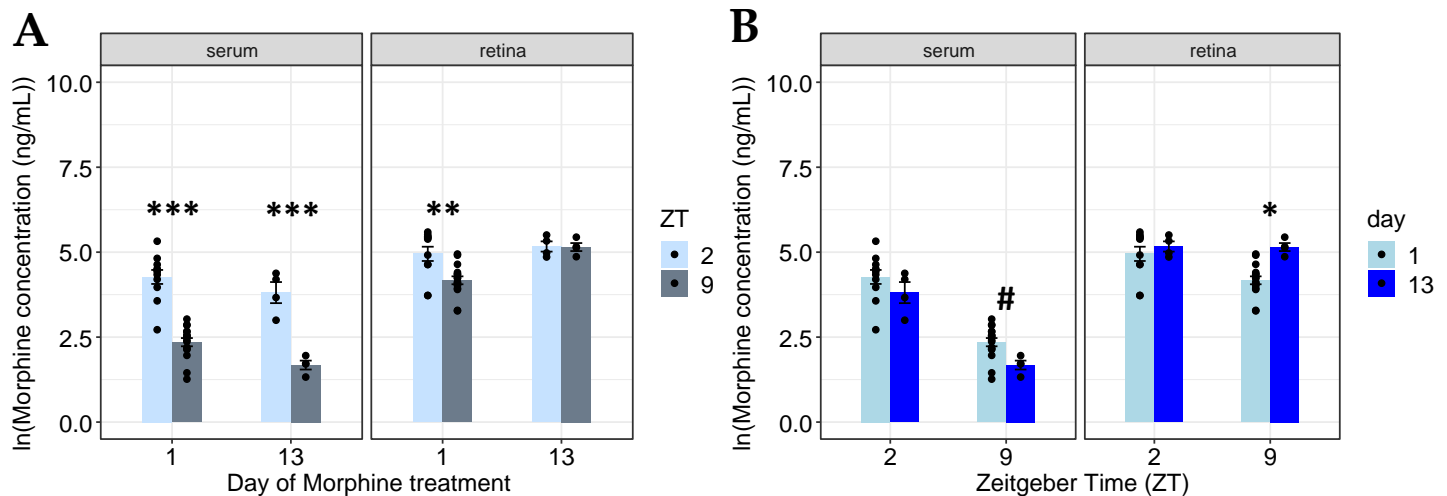
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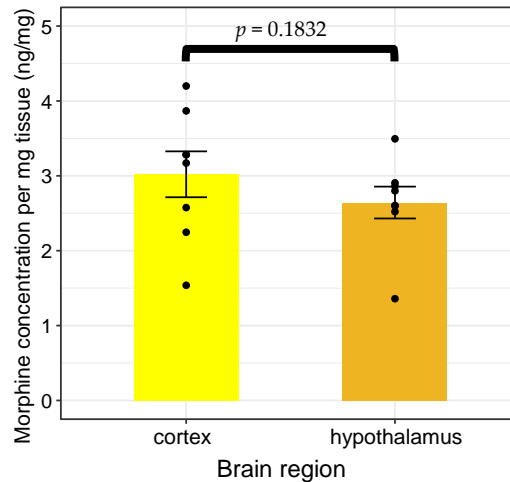
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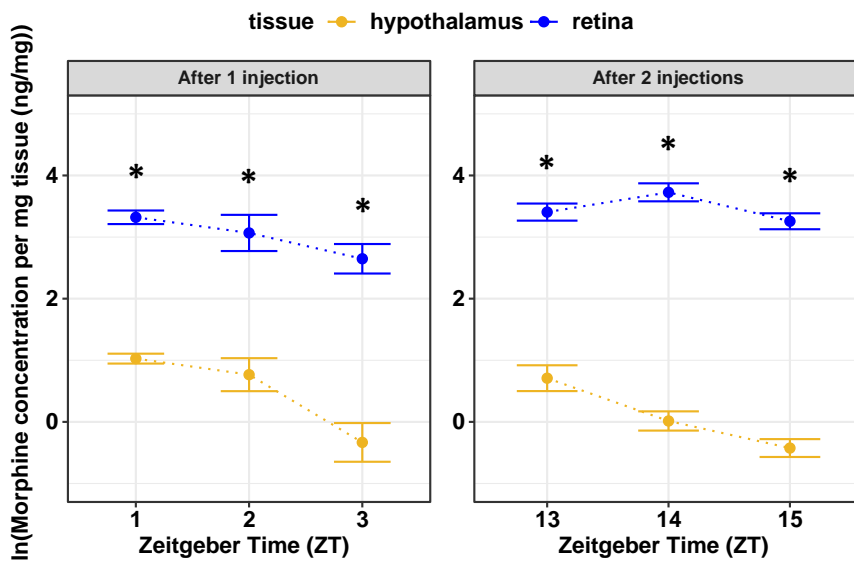
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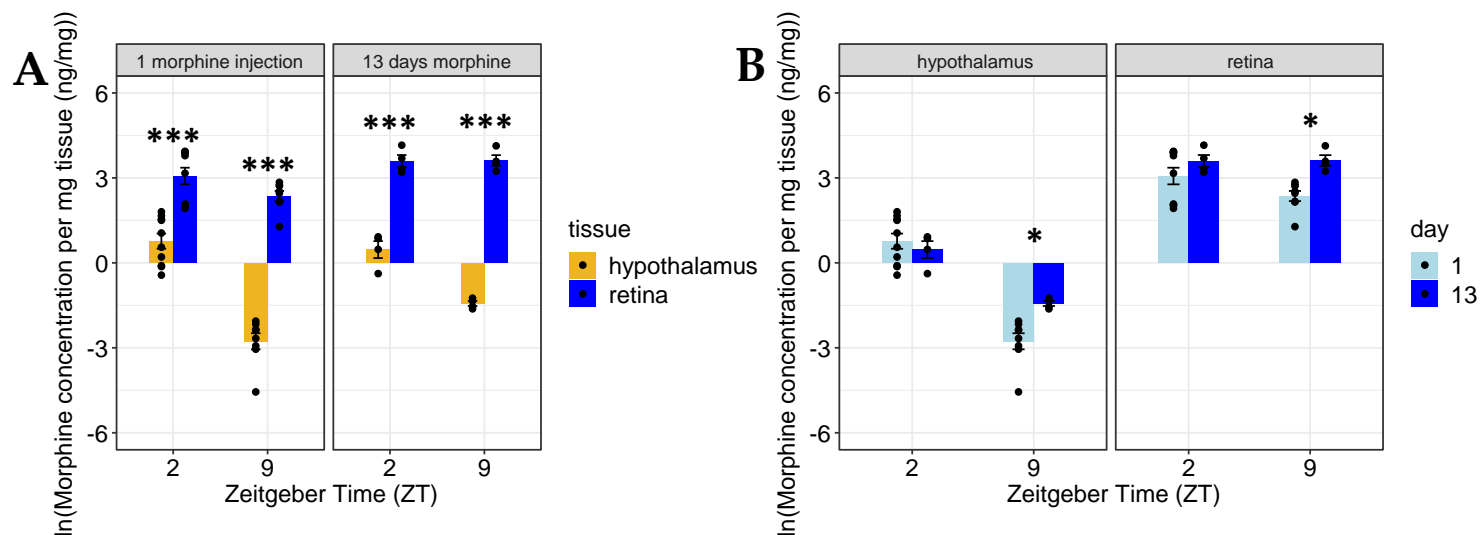
**Figure S1.** Morphine accumulates in the retina following chronic systemic exposure. (A, B) Morphine appears accumulate in the retina following 13 days of chronic morphine treatment, while the serum pharmacokinetics remain similar for samples collected at ZT 2 and ZT 9. Three-Way ANOVA with a Tukey post-hoc adjustment natural logarithmic (ln) scale. (#  $p < 0.05$ , \*  $p < 0.001$ , \*\*  $p < 0.001$ , \*\*\*  $p < 0.0001$ ). Data presented as the mean  $\pm$  SEM.



**Figure S2.** Morphine deposits similarly in different brain regions. Hypothalamus and cerebral cortex samples collected from the same mice show no difference in morphine deposition an hour (ZT 1) after a single 20 mg/kg i.p. morphine injection. Data presented as the mean  $\pm$  SEM and assessed using a paired samples t-test ( $n = 8$ ).



**Figure S3.** Morphine deposits more in the retina than the hypothalamus following repeat systemic administration. Morphine concentration retina and hypothalamus normalized for weight of each tissue after one and two morphine injections. Two-Way ANOVA with a Tukey post-hoc adjustment performed on natural logarithmic (ln) scale. Data presented as the mean  $\pm$  SEM (\*  $p < 0.0001$ ).



**Figure S4.** Morphine accumulates in the retina, but not the hypothalamus at ZT 2 and ZT 9. (A) Morphine concentrations exceed hypothalamic morphine concentrations at ZT 2 and ZT 9 following chronic morphine exposure (13 days morphine). (B) Morphine accumulates in the retina, but not the hypothalamus following chronic morphine exposure. Two-Way ANOVA with a Tukey post-hoc adjustment performed on natural logarithmic (ln) scale. Data presented as the mean  $\pm$  SEM (\*  $p < 0.01$ , \*\*\*  $p < 0.0001$ ).

**Table S1.** Primer and probe sequences for qRT-PCR experiments.

Target, GenBank accession number	Forward Primer (5'-3')	Reverse Primer (5'-3')	Probe Sequence (5'-3')
P-glycoprotein (Abcb1a), NM_011076.3	CAGCCAGCATTCTCCGT AATA	CCCAAGGATCAGAAACAACA	/FAM/CAGCGGCAG/ZEN/AACA GCAACTTGTTT/IABkFQ/
$\beta$ -actin (Actb), NM_007393.5	GTCATCCATGGCGAAC TGG	ACTGTGCGAGTCGCGTCC	/HEX/CGTTGCCGG/ZEN/ TCCACACCCGCCA/IABkFQ/
TATA box binding protein (Tbp), NM_013684.3	CCATGAAATAGTGATG CTGGGC	GGGTATCTGCTGGCGGTTT	/HEX/TGCGGTCGC/ZEN/ GTCATTTTCTCCGCAGT/IABkFQ/

**Table S2.** Matrix validation results for morphine LC-MS/MS.

Matrix	Accuracy	Precision	Range ng/ml	Calibration Model	Carryover	Interference	LOQ ng/ml	Stability
Retina	94-98 %	4.6-6.2%	20-5000	Linear	None	None	20	72h
Serum	87-105%	0.84-5.9%	0.5-1000	Linear	None	None	0.5	72h
Hypothalamus	94.2-108%	2.9-13.9%	0.2-50	Linear	None	None	0.2	72h

**Table S3.** Parameters and acceptance criteria for morphine LC-MS/MS matrix validation.

Parameter	Acceptance Criteria
<b>Accuracy</b>	Must not exceed +/- 20% at 3 concentrations
<b>Precision</b>	% CV must not exceed 20% at 3 concentrations
<b>Calibration Model</b>	Linear Range with R <sup>2</sup> greater than 0.99 (linear mode with 1/x weighting if appropriate fit)
<b>Carryover</b>	Carryover from highest calibrator to subsequent blank samples does not exceed 20% of signal of lowest calibrator
<b>Interference Studies</b>	No interfering signal from matrix, internal standards, standard, or other common drugs of abuse, OTC drugs, and prescription medications
<b>Limit of Quantitation (LOQ)</b>	Lowest concentration that meets accuracy and precision acceptance criteria
<b>Processed Sample Stability</b>	Length of time that analyte in extracted sample can be stored at room temperature on autosampler rack and meet accuracy and precision criteria