3.8 Supplementary material

Table 1 Primer sequence used in qPCR

Gene	Forward Primer 5' – 3'	Reverse Primer 5' – 3'
Ccl2 – Chemokine (C-C motif) ligand 2	ACACTGGTTCCTGACTCCTCT	ACCTGAGGACTGATGGTGGT
Cd14 – Cluster of differentiation 14 antigen	CTCTGTCCTTAAAGCGGCTTAC	GTTGCGGAGGTTCAAGATGTT
Drd1 – Dopamine receptor D1 transcript variant 1	GTTGAGTCCAGGGGTTTTGGG	ACTTTTCGGGGATGCTGCC
Drd2 – Dopamine receptor D2	GTGAACAGGCGGAGAATGGA	TGGGAGGGATGGGGCTATAC
Gapdh – Glyceraldehyde-3-phosphate dehydrogenase transcript variant 1	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
Ghrl – Grehlin transcript variant 1	ATCGTCCTCACCACCAAGAC	CTTGGATTCCTTTCTCTGGGCTT
Hmgb1 – High mobility group box 1, transcript variant 1	CCATTGGTGATGTTGCAAAG	CTTTTTCGCTGCATCAGGTT
Ifnb – Interferon beta 1, fibroblast	TGGGAGATGTCCTCAACTGC	CCAGGCGTAGCTGTTGTACT
Lepr – Leptin receptor transcript variant 3	TCCAAAAGAGAACGGACACTC T	TGTATGGACTGTTGGGAAGTTG
Il1b – Interleukin 1 beta	TGCCACCTTTTGACAGTGATG	TGATGTGCTGCTGCGAGATT
Il10 – Interleukin 10	GCTCTTACTGACTGGCATGAG	CGCAGCTCTAGGAGCATGTG
Md2 – Lymphocyte antigen 96 transcript variant 1, 2	CGCTGCTTTCTCCCATATTGA	CCTCAGTCTTATGCAGGGTTCA

Gene	Forward Primer 5' – 3'	Reverse Primer 5' – 3'
Myd88 – Myeloid differentiation primary response gene 88	TCATGTTCTCCATACCCTTGGT	AAACTGCGAGTGGGGTCA
Oprm1 – Opioid receptor, mu 1, transcript variant 1C, 1M, 1U	TCCGACTCATGTTGAAAAACCC	CCTTCCCCGGATTCCTGTCT
Rxfp1 – Relaxin/insulin-like family peptide receptor 1	CGAGCTGTCCCATCAGTTTCT	AGACGCTCACGGAGTGAATC
Tlr4 – Toll-like receptor 4	GCCTTTCAGGGAATTAAGCTCC	GATCAACCGATGGACGTGTAAA
Trif – Toll-like receptor adaptor molecule 1	AACCTCCACATCCCCTGTTTT	GCCCTGGCATGGATAACCA
Th –Tyrosine Hydroxylase	CCTTCCGTGTGTTTCAGTGC	TCAGCCAACATGGGTACGTG

3.8.1 Statistics for in-text figures

Figure 2 Circadian timing affects the intake and preference of alcohol (a - b), saccharin (c - d) but not quinine (e - f) and the conditioned preference towards alcohol (g – h). All data analysed using a two-way ANOVA with Tukey post hoc.

(a) *Alcohol intake*

Effect of concentration, $F_{(8, 72)} = 68.34$, $p < 0.0001$

Effect of light-cycle, $F_{(1, 9)} = 5.21$, $p = 0.048$

Interaction: concentration x light-cycle, $F_{(8, 72)} = 2.02$, $p = 0.056$

(b) *Alcohol preference*

Effect of concentration, $F_{(8, 72)} = 2.2$, $p = 0.037$

Effect of light-cycle, $F_{(1, 9)} = 9.16$, $p = 0.014$

Interaction: concentration x light-cycle, $F_{(8, 72)} = 0.30$, $p = 0.96$

(c) *Saccharin intake*

Effect of concentration, $F_{(4, 36)} = 18.94$, $p < 0.0001$

Effect of light-cycle, $F_{(1, 9)} = 15.53$, $p = 0.0034$

Interaction: concentration x light-cycle, $F_{(4, 36)} = 2.98$, $p = 0.0318$

(d) *Saccharin preference*

Effect of concentration, $F_{(4, 36)} = 1.74$, $p = 0.16$

Effect of light-cycle, $F_{(1, 9)} = 8.32$, $p = 0.015$

Interaction: concentration x light-cycle, $F_{(4, 36)} = 0.40$, $p = 0.81$

(e) *Quinine intake*

Effect of concentration, $F_{(4, 36)} = 180.4$, $p < 0.0001$

Effect of light-cycle, $F_{(1, 9)} = 12.09$, $p = 0.0052$

Interaction: concentration x light-cycle, $F_{(4, 36)} = 6.14$, $p = 0.0005$

(f) *Quinine preference*

Effect of concentration, $F_{(4, 36)} = 2.94$, $p = 0.031$

Effect of light-cycle, $F_{(1, 9)} = 20.31$, $p = 0.0009$

Interaction: concentration x light-cycle, $F_{(4, 36)} = 0.35$, $p = 0.84$

Figure 5 Circadian timing affects the expression of genes relating to reward (a), thirst and hunger (b) and the TLR4 pathway (c – d). All data analysed using a two-way ANOVA with Tukey post hoc.

(c) *Nucleus accumbens*

Effect of light cycle, *Cd14*, t = 0.67 df = 4, $p = 0.54$

Effect of light cycle, *Md2*, t = 0.22 df =4, $p = 0.84$

Effect of light cycle, *Myd88*, t = 1.37 df = 4, $p = 0.24$

Effect of light cycle, *Trif*, t =1.62 df = 4, $p = 0.18$

Effect of light cycle, *Il1b*, t = 0.12 df = 4, $p = 0.90$

Effect of light cycle, *Il10*, t = 0.89 df=4, $p = 0.42$

Effect of light cycle, *Hmgb1*, t = 0.033 df = 4, $p = 0.98$

(d) *Hypothalamus*

Effect of light cycle, *Cd14*, t = 1.31 df = 4, $p = 0.26$

Effect of light cycle, *Myd88*, t = 0.52 df = 4, $p = 0.63$

Effect of light cycle, *Ccl2*, t = 1.16 df = 4, $p = 0.33$

Effect of light cycle, *Il10*, t = 1.78 df = 4, $p = 0.68$

Effect of light cycle, *Hmgb1*, t = 1.6 df = 4, $p = 0.24$

Figure 6 Circadian timing influences the efficacy of (+)-Naltrexone on decreasing the intake and preference for alcohol (a – b) and saccharin (c – d) but not quinine (e – f). All data analysed using a two-way ANOVA with Tukey post hoc.

(a) *Alcohol intake*

Effect of dose of (+)-Naltrexone, $F_{(6, 48)} = 15.72$, $p < 0.0001$

Effect of light-cycle, $F_{(1, 8)} = 15.12$, $p = 0.0046$

Interaction: dose of (+)-Naltrexone x light-cycle, $F_{(6, 48)} = 4.99$, $p = 0.0005$

(b) *Alcohol preference*

Effect of concentration, $F_{(6, 48)} = 7.57$, $p < 0.0001$

Effect of light-cycle, $F_{(1, 8)} = 40.85$, $p = 0.0002$

Interaction: dose of (+)-Naltrexone x light-cycle, $F_{(6, 48)} = 0.64$, $p = 0.70$

(c) *Saccharin intake*

Effect of dose of (+)-Naltrexone, $F_{(6, 48)} = 2.56$, $p = 0.076$

Effect of light-cycle, $F_{(1, 8)} = 64.85$, $p < 0.0001$

Interaction: dose of (+)-Naltrexone x light-cycle, $F_{(6, 48)} = 0.86$, $p = 0.53$

(d) *Saccharin preference*

Effect of dose of (+)-Naltrexone, $F_{(6, 48)} = 3.82$, $p = 0.0034$

Effect of light-cycle, $F_{(1, 8)} = 39.16$, $p = 0.0002$

Interaction: dose of (+)-Naltrexone x light-cycle, $F_{(6, 48)} = 2.68$, $p = 0.024$

(e) *Quinine intake*

Effect of dose of (+)-Naltrexone, $F_{(6, 48)} = 3.05$, $p = 0.013$

Effect of light-cycle, $F_{(1, 8)} = 11.09$, $p = 0.010$

Interaction: dose of (+)-Naltrexone x light-cycle, $F_{(6, 48)} = 1.67$, $p = 0.15$

(f) *Quinine preference*

Effect of dose of (+)-Naltrexone, $F_{(6, 48)} = 0.79$, $p = 0.58$

Effect of light-cycle, $F_{(1, 8)} = 1.08$, $p = 0.33$

Interaction: dose of (+)-Naltrexone x light-cycle, $F_{(6, 48)} = 1.29$, $p = 0.28$

Figure 7 Circadian timing influences the efficacy of (+)-Naltrexone (60 mg/kg) on decreasing and the intake and preference for alcohol (a – b) and saccharin (c – d) but not quinine (e – f) and the conditioned preference for alcohol (g – h). All data analysed using a three-way ANOVA with Tukey post hoc.

(a) *Alcohol intake*

Effect of concentration, $F_{(7, 320)} = 61.53$, $p < 0.0001$

Effect of light-cycle, $F_{(1, 320)} = 4.12$, $p = 0.043$

Effect of pretreatment, $F_{(1, 320)} = 4.95$, $p = 0.026$

Interaction: concentration x light-cycle, $F_{(7, 320)} = 3.05$, $p = 0.0040$

Interaction: concentration x pretreatment, $F_{(7, 320)} = 0.60$, $p = 0.76$

Interaction: light-cycle x pretreatment, $F_{(1, 320)} = 2.33$, $p = 0.13$

Interaction: concentration x light-cycle x pretreatment, $F_{(7, 320)} = 2.82$, $p = 0.0073$

(b) *Alcohol preference*

Effect of concentration, $F_{(7, 320)} = 3.72$, $p = 0.0007$

Effect of light-cycle, $F_{(1, 320)} = 311.2$, $p < 0.0001$

Effect of pretreatment, $F_{(1, 320)} = 25.68$, $p < 0.0001$

Interaction: concentration x light-cycle, $F_{(7, 320)} = 2.52$, $p = 0.016$

Interaction: concentration x pretreatment, $F_{(7, 320)} = 2.31$, $p = 0.026$

Interaction: light-cycle x pretreatment, $F_{(1, 320)} = 11.17$, $p = 0.0009$

Interaction: concentration x light-cycle x pretreatment, $F_{(7, 320)} = 0.79$, $p = 0.60$

(c) *Saccharin intake*

Effect of concentration, $F_{(4, 220)} = 97.07$, $p < 0.0001$

Effect of light-cycle, $F_{(1, 220)} = 75.11$, $p < 0.0001$

Effect of pretreatment, $F_{(1, 220)} = 8.95$, $p = 0.0031$

Interaction: concentration x light-cycle, $F_{(4, 220)} = 12.39$, $p < 0.0001$

Interaction: concentration x pretreatment, $F_{(4, 220)} = 1.43$, $p = 0.23$

Interaction: light-cycle x pretreatment, $F_{(1, 220)} = 9.11$, $p = 0.0028$

Interaction: concentration x light-cycle x pretreatment, $F_{(4, 220)} = 1.83$, $p = 0.13$

(d) *Saccharin preference*

Effect of concentration, $F_{(4, 220)} = 0.85$, $p = 0.49$

Effect of light-cycle, $F_{(1, 220)} = 31.38$, $p < 0.0001$

Effect of pretreatment, $F_{(1, 220)} = 0.25$, $p = 0.62$

Interaction: concentration x light-cycle, $F_{(4, 220)} = 0.68$, $p = 0.61$

Interaction: concentration x pretreatment, $F_{(4, 220)} = 0.24$, $p = 0.92$

Interaction: light-cycle x pretreatment, $F_{(1, 220)} = 1.37$, $p = 0.24$

Interaction: concentration x light-cycle x pretreatment, $F_{(4, 220)} = 0.27$, $p = 0.89$

(e) *Quinine intake*

Effect of concentration, $F_{(4, 220)} = 45.38$, $p < 0.0001$

Effect of light-cycle, $F_{(1, 220)} = 0.016$, $p = 0.90$

Effect of pretreatment, $F_{(1, 220)} = 4.32$, $p = 0.039$

Interaction: concentration x light-cycle, $F_{(4, 220)} = 0.38$, $p = 0.83$

Interaction: concentration x pretreatment, $F_{(4, 220)} = 4.27$, $p = 0.0024$

Interaction: light-cycle x pretreatment, $F_{(1, 220)} = 0.018$, $p = 0.89$

Interaction: concentration x light-cycle x pretreatment, $F_{(4, 220)} = 0.062$, $p = 0.99$

(f) *Quinine preference*

Effect of concentration, $F_{(4, 220)} = 4.39$, $p = 0.0020$

Effect of light-cycle, $F_{(1, 220)} = 0.0058$, $p = 0.94$

Effect of pretreatment, $F_{(1, 220)} = 2.02$, $p = 0.16$

Interaction: concentration x light-cycle, $F_{(4, 220)} = 2.49$, $p = 0.0443$

Interaction: concentration x pretreatment, $F_{(4, 220)} = 0.89$, $p = 0.47$

Interaction: light-cycle x pretreatment, $F_{(1, 220)} = 14$, $p = 0.0002$

Interaction: concentration x light-cycle x pretreatment, $F_{(4, 220)} = 0.51$, $p = 0.73$

Figure 8 Circadian timing influences efficacy of (+)-Naltrexone on relative change in conditioned chamber time. All data analysed using a two-way ANOVA with Tukey post hoc.

Relative conditioned place preference

Effect of pretreatment, $F_{(1, 7)} = 20.52$, $p = 0.0027$

Effect of light-cycle, $F_{(1, 7)} = 0.0011$, $p = 0.92$

Interaction: pretreatment x light-cycle, $F_{(1, 7)} = 1.62$, $p = 0.24$)

3.8.2 Supplementary figures

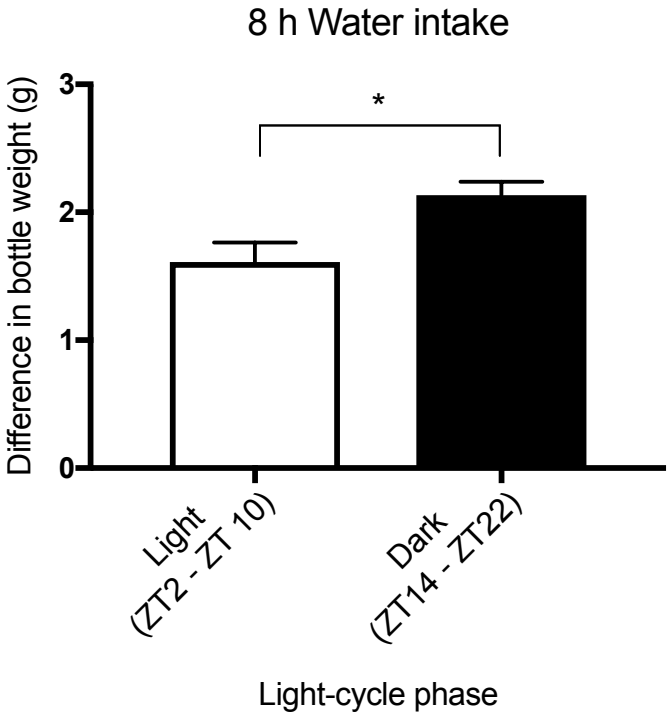

Figure s1 Circadian timing affects the intake of water. Mice in the dark cycle consumed significantly more water compared to mice in the light cycle. All data was analysed using a paired two-tail t-test. Summary values represented mean±SEM; n=49, *$p < 0.05$.

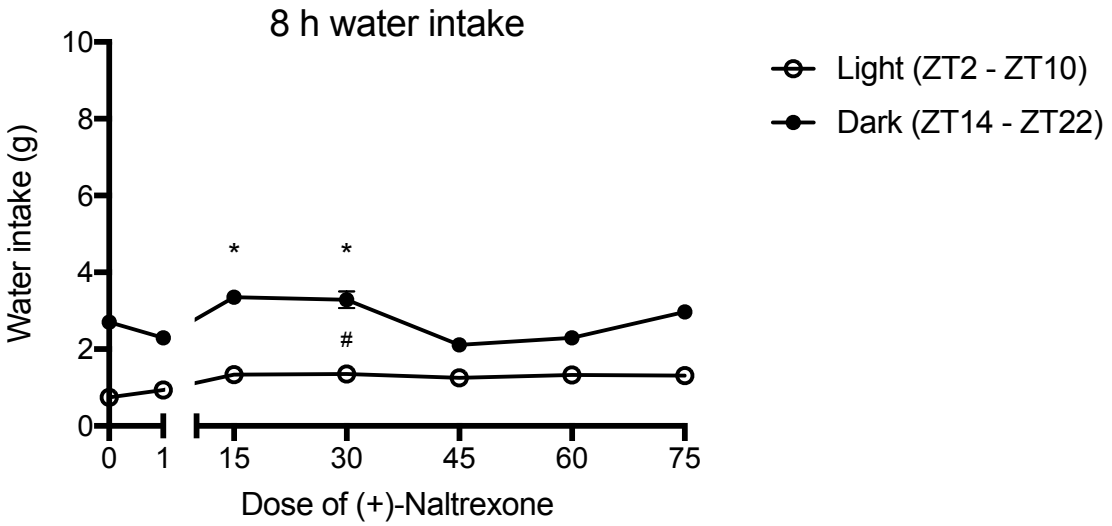

Figure s2 Circadian timing and the dose of (+)-Naltrexone significantly modify water intake. Mice receiving (+)-Naltrexone in the dark cycle consumed significantly more water compared to mice in the light cycle**.** All data was analysed using a two-way ANOVA with Tukey post hoc. Summary values represented as mean±SEM; n=9, *p < 0.05 compared to saline (dark); # p < 0.05 compared to saline (light).

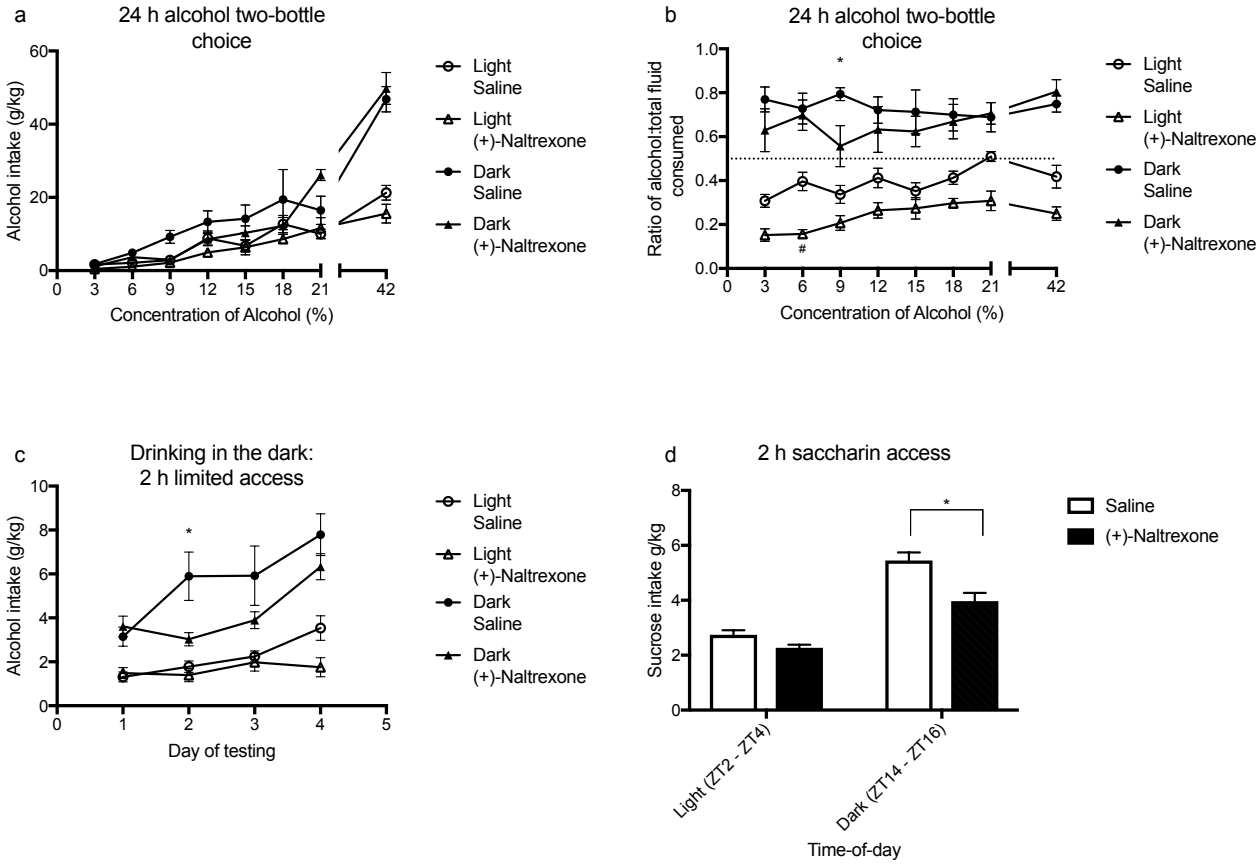

Figure s3 The efficacy of (+)-Naltrexone (60 mg/kg) on decreasing 24 h intake and preference (a –b) of alcohol (20%), 2-4 h intake of alcohol (c) and saccharin (15mM) (d) is greatest during the dark cycle. All data was analysed using a three-way ANOVA with Tukey HSD post hoc (a – c) and a two-way ANOVA with Tukey post hoc (d). There was a significant effect of pretreatment for the intake and preference of alcohol during the 24 h two-bottle choice tests. Similarly, the drinking in the dark and 2 h saccharin access tests exhibited a significant effect of pretreatment. Post hoc analysis determined (+)-Naltrexone significantly attenuated intake compared to saline during the dark but not light cycle in both paradigms. Summary values represented as mean±SEM; n=11–12, $p < 0.05$ compared to saline (dark); # $p < 0.05$ compared to saline (light).

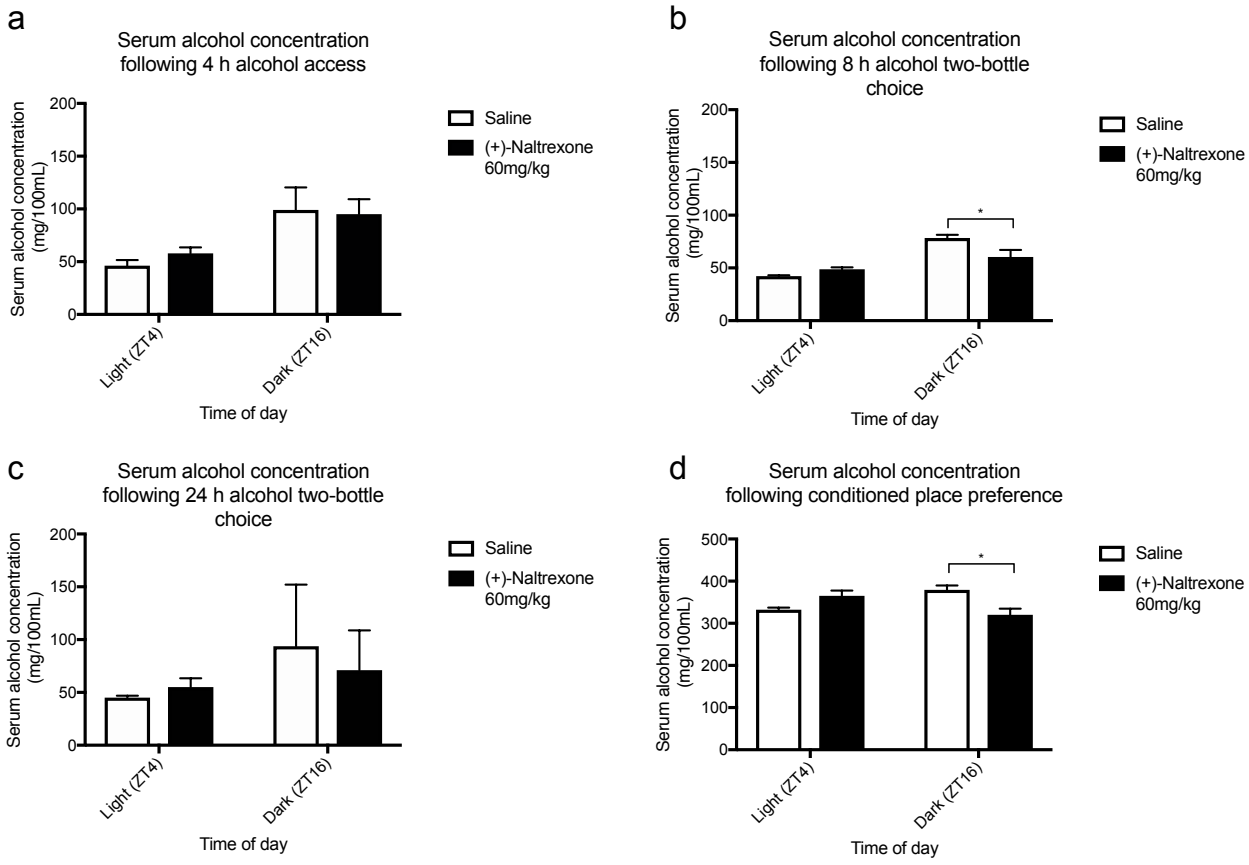

Figure s4 Serum alcohol concentration from saline and (+)-Naltrexone-treated mice (60 mg/kg) following 2 h (a), 8 h (b) and 24 h (c) alcohol drinking tests and conditioned place preference (d). All data was analysed using a two-way ANOVA with Tukey post hoc (a – d). Summary values represented as mean±SEM; n=6.

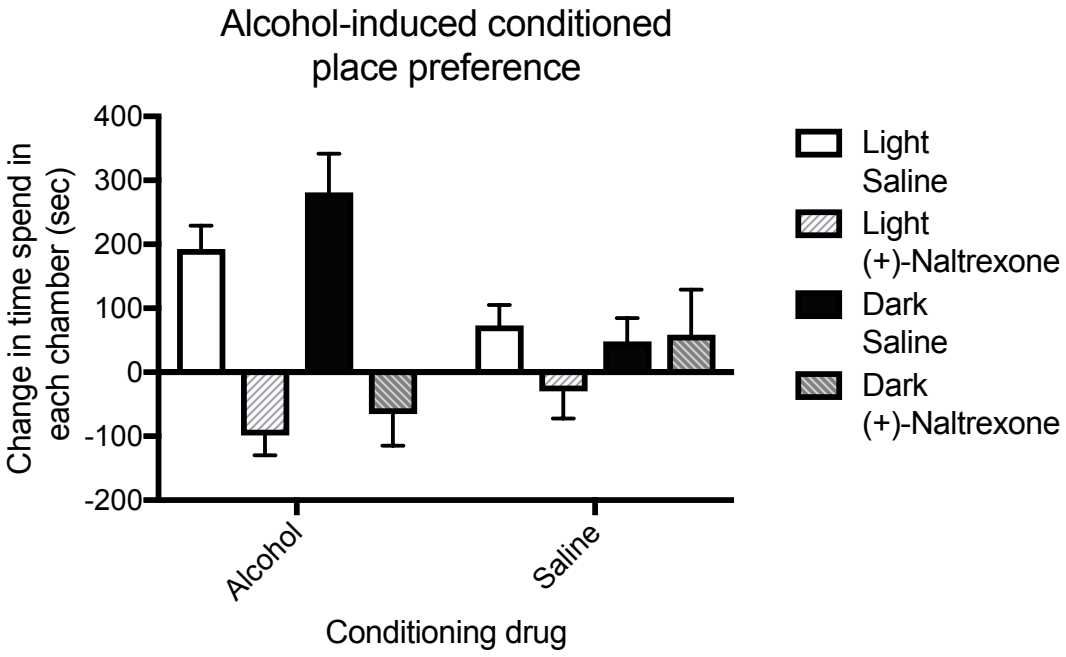

Figure s5. Circadian timing influences efficacy of (+)-Naltrexone (60 mg/kg) on change in conditioned chamber time. All data was analysed using a three-way ANOVA with Tukey post hoc (a – d). Summary values represented as mean±SEM; n=8, *$p < 0.05$.

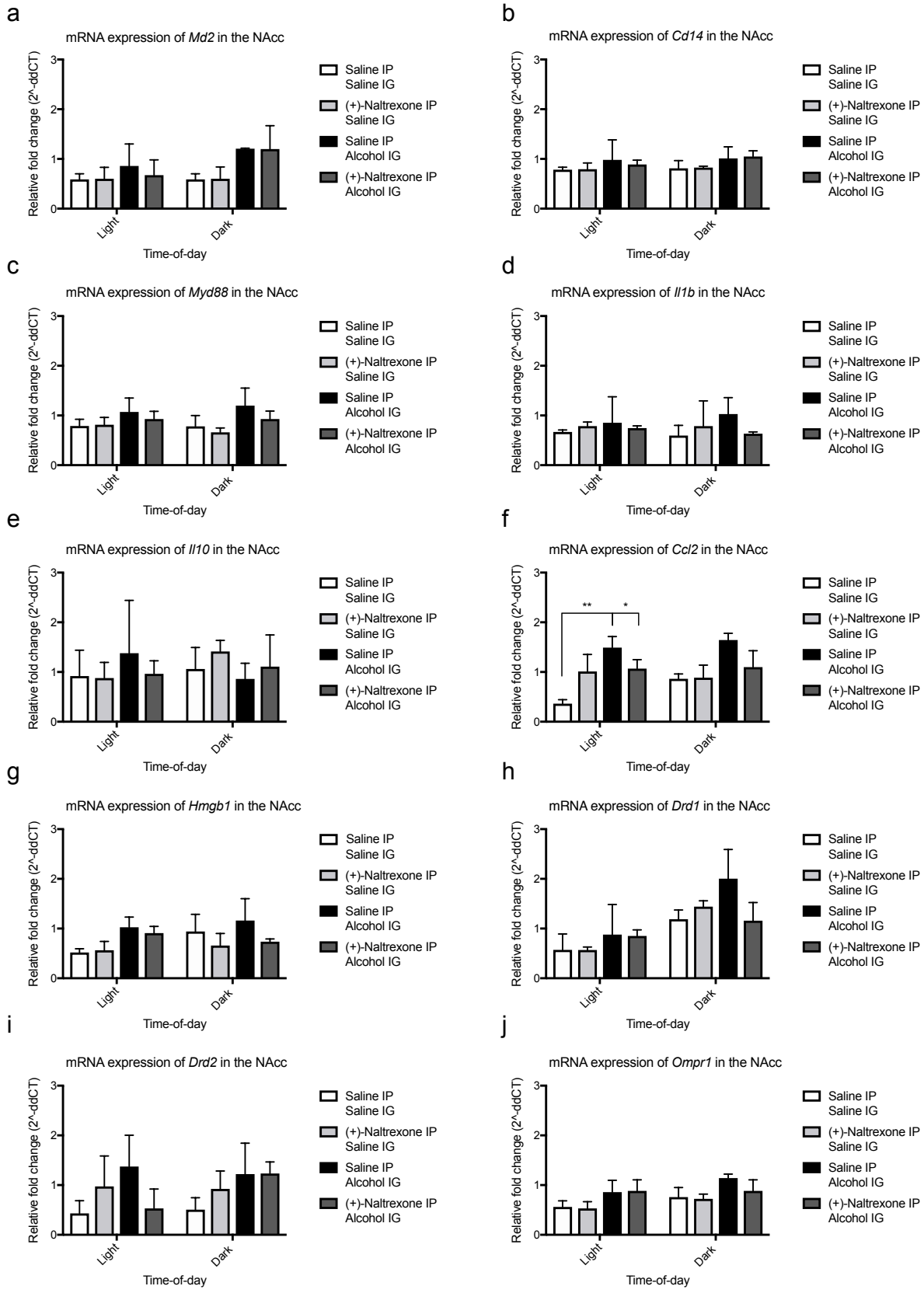

Figure s6 Effect of alcohol, saline (I.G), (+)-Naltrexone (60 mg/kg) on the expression of TLR4 and reward-related genes in the Nucleus Accumbens. All data was analysed using a two-way ANOVA with Bonferonni post hoc. Summary values represented as mean±SEM; n=3, *$p < 0.05$; **$p < 0.01$.

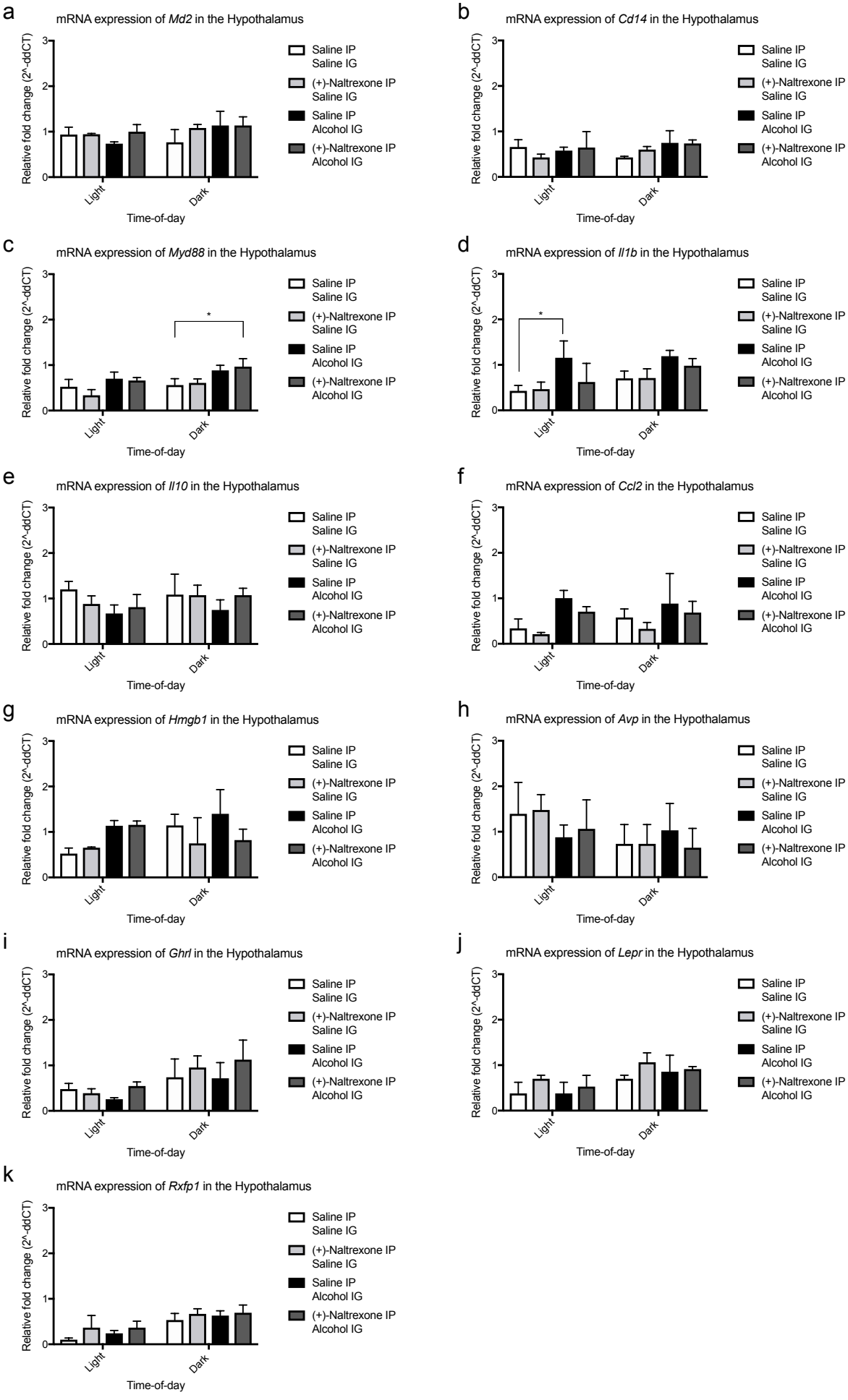
a
mRNA expression of *Md2* in the Hypothalamus
b
mRNA expression of *Cd14* in the Hypothalamus
c
mRNA expression of *Myd88* in the Hypothalamus
d
mRNA expression of *Il1b* in the Hypothalamus
e
mRNA expression of *Il10* in the Hypothalamus
f
mRNA expression of *Ccl2* in the Hypothalamus
g
mRNA expression of *Hmgb1* in the Hypothalamus
h
mRNA expression of *Avp* in the Hypothalamus
i
mRNA expression of *Ghrl* in the Hypothalamus
j
mRNA expression of *Lepr* in the Hypothalamus
k
mRNA expression of *Rxfp1* in the Hypothalamus
Relative fold change (2^-ddCT)
Time-of-day
Light
Dark
Saline IP
Saline IG
(+)-Naltrexone IP
Saline IG
Saline IP
Alcohol IG
(+)-Naltrexone IP
Alcohol IG
*

Figure s7 Effect of alcohol, saline and (+)-Naltrexone (60 mg/kg) on the expression of TLR4 and hunger/thirst-related genes in the hypothalamus. All data was analysed using a two-way ANOVA with Bonferonni post hoc. Summary values represented as mean±SEM; n=3, *$p < 0.05$; **$p < 0.01$.

3.8.3 Supplementary material statistics

Figure s1. Light-cycle dependent water intake.

Paired two-tail t-test

Effect of light cycle, t=2.83 df=96, $p = 0.0057$

Figure s2 Circadian timing and the dose of (+)-Naltrexone significantly modify water intake. All data analysed using a two-way ANOVA with Tukey post hoc.

Effect of dose of (+)-Naltrexone, $F_{(6, 48)} = 12.01$, $p < 0.0001$

Effect of light-cycle, $F_{(1, 8)} = 99.62$, $p < 0.0001$

Interaction: dose x light-cycle, $F_{(6, 48)} = 5.72$, $p = 0.0002$

Figure s3 Circadian timing influences the efficacy of (+)-Naltrexone on decreasing 24 h intake and preference (a –b) of alcohol, 2-4 h intake of alcohol (c) and saccharin (d). All data analysed using a two-way three-way ANOVA with Tukey post hoc (a – c) and two-way ANOVA with Tukey post hoc (d).

(a) *24 h intake*

Effect of concentration, $F_{(7, 288)} = 69.58$, $p < 0.0001$

Effect of light-cycle, $F_{(1, 288)} = 78.51$, $p < 0.0001$

Effect of pretreatment, $F_{(1, 288)} = 3.66$, $p = 0.050$

Interaction: concentration x light-cycle, $F_{(7, 288} = 14.53$, $p <0.0001$

Interaction: concentration x pretreatment, $F_{(7, 288} = 1.82$, $p = 0.071$

Interaction: light-cycle x pretreatment, $F_{(1, 288)} = 0.089$, $p = 0.77$

Interaction: concentration x light-cycle x pretreatment, $F_{(7, 288} = 1.13$, $p = 0.34$

(b) *24 h preference*

Effect of concentration, $F_{(7, 288)} = 1.37$, $p = 0.22$

Effect of light-cycle, $F_{(1, 288)} = 356.1$, $p < 0.0001$

Effect of pretreatment, $F_{(1, 288)} = 29.93$, $p < 0.0001$

Interaction: concentration x light-cycle, $F_{(7, 288)} = 1.25$, $p = 0.27$

Interaction: concentration x pretreatment, $F_{(7, 288)} = 0.55$, $p = 0.79$

Interaction: light-cycle x pretreatment, $F_{(1, 288)} = 4.60$, $p = 0.033$

Interaction: concentration x light-cycle x pretreatment, $F_{(7, 288)} = 1.13$, $p = 0.35$

(c) *2 – 4 h limited access to alcohol*

Effect of day of testing, $F_{(3, 144)} = 11.77$, $p < 0.0001$

Effect of light-cycle, $F_{(1, 144)} = 97.97$, $p < 0.0001$

Effect of pretreatment, $F_{(1, 144)} = 11.19$, $p = 0.0011$

Interaction: day of testing x light-cycle, $F_{(3, 144)} = 2.77$, $p = 0.044$

Interaction: day of testing x treatment, $F_{(3, 144)} = 2.29$, $p = 0.08$

Interaction: light-cycle x pretreatment, $F_{(1, 144)} = 2.23$, $p = 0.14$

Interaction: day of testing x light-cycle x pretreatment, $F_{(3, 144)} = 1.39$, $p = 0.25$

(d) *2 h saccharin intake*

Effect of light-cycle, $F_{(1, 9)} = 68.31$, $p < 0.0001$

Effect of treatment, $F_{(1, 9)} = 21.31$, $p = 0.0013$

Interaction: light-cycle x treatment, $F_{(1, 9)} = 5.34$, $p = 0.046$

Figure s4 Serum ethanol concentration following 2 h (a), 8 h (b) and 24 h (c) alcohol drinking tests and conditioned place preference (d). Summary values represented as mean±SEM; n=6, *$p < 0.05$; **$p < 0.01$. All data analysed using a two-way ANOVA with Tukey post hoc.

(a) *2 h*

Effect of light-cycle, $F_{(1, 5)} = 35.06$, $p = 0.0004$

Effect of pretreatment,, $F_{(1, 5)} = 0.070$, $p = 0.80$

Interaction (light-cycle x pretreatment), $F_{(1, 5)} = 0.33$, $p = 0.58$

(b) *8 h*

Effect of light-cycle, $F_{(1, 5)} = 95.86$, $p < 0.0001$

Effect of pretreatment,, $F_{(1, 5)} = 1.59$, $p = 0.24$

Interaction (light-cycle x pretreatment), $F_{(1, 5)} = 0.039$, $p = 0.85$

(c) *24 h*

Effect of light-cycle, $F_{(1, 5)} = 0.42$, $p = 0.54$

Effect of pretreatment,, $F_{(1, 5)} = 3.76$, $p = 0.088$

Interaction (light-cycle x pretreatment), $F_{(1, 5)} = 16.27$, $p = 0.0038$

(d) *Conditioned place preference*

Effect of light-cycle, $F_{(1, 5)} = 356.1$, $p < 0.0001$

Effect of pretreatment,, $F_{(1, 5)} = 29.93$, $p < 0.0001$

Interaction (light-cycle x pretreatment), $F_{(1, 5)} = 29.93$, $p < 0.0001$

Figure s5 Circadian timing influences efficacy of (+)-Naltrexone on change in conditioned chamber time. All data analysed using a two-way three-way ANOVA with Tukey post hoc.

Conditioned place preference

Effect of pretreatment, $F_{(1, 56)} = 26.65$, $p < 0.0001$

Effect of conditioning drug, $F_{(1, 56)} = 2.15$, $p = 0.15$

Effect of light-cycle, $F_{(1, 56)} = 2.74$, $p = 0.10$

Interaction: conditioning drug x pretreatment, $F_{(1, 56)} = 14.26$ $p = 0.0004$

Interaction: conditioning drug x light-cycle, $F_{(1, 56)} = 0.51$, $p = 0.48$

Interaction: pretreatment x light-cycle, $F_{(1, 56)} = 0.51$, $p = 0.48$

Interaction: conditioning drug x pretreatment x light-cycle, $F_{(1, 56)} = 0.954$, $p = 0.33$

Figure s6 Effect of alcohol and (+)-Naltrexone on the expression of TLR4 and reward-related genes in the Nucleus Accumbens. All data analysed using **t**wo-way ANOVA with Bonferonni post hoc.

(a) *Md2* light-cycle ($F_{(1, 24)} = 3.51$, $p = 0.08$), drug ($F_{(1, 24)} = 11.3$, $p = 0.04$), pretreatment ($F_{(1, 24)} = 0.133$, $p = 0.32$). No significant interactions.

(b) *Cd14*, light-cycle ($F_{(1, 24)} = 0.66$ $p = 0.43$), drug ($F_{(1, 24)} = 5.4$, $p = 0.033$), pretreatment ($F_{(1, 24)} = 0.92$, $p = 0.48$). No significant interactions.

(c) *Myd88*, light-cycle ($F_{(1, 24)} = 0.0072$ $p = 0.93$), drug ($F_{(1, 24)} = 10.11$, $p = 0.0058$), pretreatment ($F_{(1, 24)} = 2.21$, $p = 0.16$). No significant interactions.

(d) *Il1b*, light-cycle ($F_{(1, 24)}$ = 0.0006 p = 0.98), drug ($F_{(1, 24)}$ = 0.79, p = 0.37), pretreatment ($F_{(1, 24)}$ = 0.16, p = 0.69). No significant interactions.

(e) *Il10*, light-cycle ($F_{(1, 24)}$ = 0.12 p = 0.73), pretreatment ($F_{(1, 24)}$ = 0.027, p = 0.87), drug ($F_{(1, 24)}$ = 0.0024, p = 0.96). No significant interactions.

(f) *Ccl2*, light-cycle ($F_{(1, 24)}$ = 0.433 p = 0.51), pretreatment ($F_{(1, 24)}$ = 0.12, p = 0.91), drug ($F_{(1, 24)}$ = 29.7, p < 0.0001). There were significant interactions between light-cycle and preatreatment ($F_{(1, 24)}$ = 10.17, p = 0.0057) light-cycle, pretreatment and drug ($F_{(1, 24)}$ = 6.07, p = 0.025). No other significant interactions.

(g) *Hmgb1*, light-cycle ($F_{(1, 24)}$ = 1.47 p = 0.24), pretreatment ($F_{(1, 24)}$ = 3.88, p = 0.066), drug ($F_{(1, 24)}$ = 8.49, p = 0.01). No significant interactions.

(h) *Drd1*, light-cycle ($F_{(1, 24)}$ = 25.22 p = 0.001), pretreatment ($F_{(1, 24)}$ = 1.14, p = 0.30), drug ($F_{(1, 24)}$ = 3.7, p = 0.072). No significant interactions.

(i) *Drd2*, light-cycle ($F_{(1, 24)}$ = 0.62 p = 0.44), pretreatment ($F_{(1, 24)}$ = 0.032, p = 0.86), drug ($F_{(1, 24)}$ = 4.27, p = 0.55). There was a significant interactions between drug and pretreatment ($F_{(1, 24)}$ = 05.92 p = 0.027). No other significant interactions.

(j) *Oprm1*, light-cycle ($F_{(1, 24)}$ = 5.63 p = 0.031), drug ($F_{(1, 24)}$ = 17.78, p = 0.0007), pretreatment ($F_{(1, 24)}$ = 1.09, p = 0.31). No significant interactions.

Figure s7 Effect of alcohol and (+)-Naltrexone on the expression of TLR4 and hunger/thirst-related genes in the hypothalamus. All data analysed using **t**wo-way ANOVA with Bonferonni post hoc.

(a) *Md2*, light-cycle ($F_{(1, 24)} = 2.79$, $p = 0.11$), drug ($F_{(1, 24)} = 0.89$, $p = 0.36$), pretreatment ($F_{(1, 24)} = 3.82$, $p = 0.069$). No significant interactions.

(b) *Cd14*, light-cycle ($F_{(1, 24)} = 0.52$, $p = 0.48$), drug ($F_{(1, 24)} = 4.29$, $p = 0.055$), pretreatment ($F_{(1, 24)} = 0.0001$, $p = 0.99$). No significant interactions.

(c) *Myd88*, light-cycle ($F_{(1, 24)} = 13.94$, $p = 0.0018$), drug ($F_{(1, 24)} = 30.61$, $p = 0 < 0.001$), pretreatment ($F_{(1, 24)} = 0.21$, $p = 0.65$). No significant interactions.

(d) *Il1b*, light-cycle ($F_{(1, 24)} = 5.59$, $p = 0.031$), drug ($F_{(1, 24)} = 17.92$, $p = 0.006$), pretreatment ($F_{(1, 24)} = 3.22$, $p = 0.092$). No significant interactions.

(e) *Il10*, light-cycle ($F_{(1, 24)} = 1.06$, $p = 0.32$), drug ($F_{(1, 24)} = 5.27$, $p = 0.035$), pretreatment ($F_{(1, 24)} = 0.11$, $p = 0.74$). No significant interactions.

(f) *Ccl2*, light-cycle ($F_{(1, 24)} = 0.22$, $p = 0.64$), drug ($F_{(1, 24)} = 3.58$, $p = 0.077$), pretreatment ($F_{(1, 24)} = 15.65$, $p = 0.0011$). No significant interactions.

(g) *Hmgb1*, light-cycle ($F_{(1, 24)} = 1.65$, $p = 0.22$), drug ($F_{(1, 24)} = 8.29$, $p = 0.011$), pretreatment ($F_{(1, 24)} = 2.68$, $p = 0.12$). There was a significant interactions between light-cycle and pretreatment ($F_{(1, 24)} = 5.04$, $p = 0.039$). No other significant interactionss.

(h) *Avp*, light-cycle ($F_{(1, 24)}$ = 4.23, p = 0.056), drug ($F_{(1, 24)}$ = 041, p = 0.84), pretreatment ($F_{(1, 24)}$ = 3.61, p = 0.076). No significant interactions.

(i) *Grhl*, light-cycle ($F_{(1, 24)}$ = 18.36, p = 0.006), drug ($F_{(1, 24)}$ = 1.33, p = 0.27), pretreatment ($F_{(1, 24)}$ = 2.28, p = 0.15). No significant interactions.

(j) *Lepr*, light-cycle ($F_{(1, 24)}$ = 19.38, p = 0.004), drug ($F_{(1, 24)}$ = 0.22, p = 0.64), pretreatment ($F_{(1, 24)}$ = 6.437, p = 0.022). No significant interactions.

(k) *Rxfp1*, light-cycle ($F_{(1, 24)}$ = 36.89, p < 0.0001), drug ($F_{(1, 24)}$ = 11.20, p = 0.29), pretreatment ($F_{(1, 24)}$ = 6.01, p = 0.026). No significant interactions.