3.8 Supplementary material

Gene Forward Primer 5' - 3' Reverse Primer 5' - 3' Ccl2 ACACTGGTTCCTGACTCCTCT ACCTGAGGACTGATGGTGGT Chemokine (C- C motif) ligand CTCTGTCCTTAAAGCGGCTTA ACCTGAGGAGGTTCAAGATGTT Clifferentiation CTGAGCCAGGGGGTTTGG GTTGCGGAGGTTCAAGATGTCC Dopamine GTGAACAGGCGGAGAATGGA ACTTTTCGGGGAGGATGGCC Dopamine GTGAACAGGCGGAGAATGGA TGGGAGGGATGGGGCTATAC Dopamine GTGAACAGGCGGAGAATGGA TGGGAGGGATGGGGCTATAC Dopamine GGGAGGGGTGTGAACGGATTT GGGAGGGGATGGGGCCTATAC Dopamine GGGTCGGTGTGAACGGATTT TGTAGACCATGTAGTTGAGGTC Gapdh - ACGTCCCCCACCAACGAC TGTGAGCCATGTAGTTGAGGTC Ghrl- G ATCGTCCTCACCACCAAGAC CTTGGATTCCTTCCTGGGCTT Grehlin Transcript ATCGTCCTCACCACCAAGAC CTTTTTCGCTGCATCAGGTT Yariant 1 ATCGTCCTCACCACCAAGGACACT CCAGGCGTAGCTGTTGTACT A High mobility TGGGAGATGTCCTCAACTGC CCAGGCGTAGCTGTTGTACT TGTATGGACTGTTGGGAAGTTG Yariant 3 TGCCAACTTTTGACAGGACACT TGATGGACTGTTGGGAAGTTG TGATGGACTGTTGGGAAGTTG Hibb -			
Ccl2 - Chemokine (C- C motif) ligand ACACTGGTTCCTGACTCCTCT ACCTGAGGACTGATGGTGGT Cd14 - Cluster of differentiation CTCTGTCCTTAAAGCGGCTTA C GTTGCGGAGGTTCAAGATGTT Drd1 - Common GTTGAGTCCAGGGGTTTTGG G ACTTTCGGGGAGTGCTGCC Dopamine receptor D1 transcript variant 1 GTGAACAGGCGGAGAATGGA G ACTTTCGGGGATGGGGCTATAC Dopamine receptor D2 GTGAACAGGCGGAGAATGGA Gapdh - Ghh - Ghh - Ghh - G AGGTCGGTGTGAACGGATTT G TGTAGACCATGTAGTTGAGGTC A Ghrden variant 1 ACGTCCCTCACCACCAAGAC Ghh - Ghh - TGGGAGATGTCCTCACCACCAAGAC CTTGGATTCCTTTCTCTGGGCTT Grehlin transcript variant 1 High mobility group box 1, transcript variant 1 CCATTGGTGATGTTGCAAAGG CTTTTTCGCTGCATCAGGTT TGTATGGACTGTTGGAAGTTG CCATTGGTGATGTCCTCAACTGC Ifnb - Interferon beta 1, fibroblast TGGGAGATGTCCTCAACTGC C T CCAGGCGTAGCTGTTGGGAAGTTG C T If1b - Interleukin 10 Md2 - Lymphocyte antign 96 TGCCTTTACTGACTGCCATGTG CCTCAGTCTTATGCAGGGTTCA CCTCAGTCTTATGCAGGGTTCA	Gene	Forward Primer 5' – 3'	Reverse Primer 5' – 3'
Chemokine (C- C motif) ligand CTCTGTCCTTAAAGCGGCTTA GTTGCGGAGGTTCAAGATGTT Cd14 - CTCTGTCCTTAAAGCGGCTTA GTTGCGGAGGTTCAAGATGTT Cluster of differentiation C ACTTTCGGGGATGCTGCC Drd1 - GTTGAGTCCAGGGGTTTTGG ACTTTTCGGGGATGCTGCC Dopamine receptor D1 transcript variant 1 GTGAACAGGCGGAGAATGGA TGGGAGGGATGGGGGCTATAC Dopamine receptor D2 GTGAACAGGCGGAGACGGAATGGA TGGGAGGGATGGGGGCTATAC Oppamine receptor D2 GTGAACAGGCGGAGACGGAATGGA TGTAGACCATGTAGTTGAGGTC Glyceraldehyde -3-phosphate dehydrogenase transcript variant 1 AGGTCGGTGTGAACGGACTT TGTAGACCATGTAGTGAGGTC Ghrl - GrcACTGTCCTCACCACCAAGAC CTTGGATTCCTTTCTCTGGGCTT A Grehlin transcript variant 1 ATCGTCCTCACCACCAAGAC CTTTTTCGCTGCATCAGGTT Hmpb1 - High mobility group box 1, transcript variant 3 CCATTGGTGATGTTGCAACGC CCAGGCGTAGCTGTTGTACT Interferon beta 1, fibroblast TGCCAACATTTGACAGGACACT C T TGATGTGCTGCTGCGAGAGTTG CTATGGGAGCTGTGGGAGAGTTG Interleukin 10 beta GCCTCTTACTGACTGGCATGA CGCAGCTCTAGGAGCATGTG CCTCAGTCTTATGCAGGGTTCA Interleukin 10 beta G G GCCTCTTACTGACTGCCATGATGAG CCTCAGTCTTATGCAGGGCATGG GCTTTCTCCCATATTGA	Ccl2 –	ACACTGGTTCCTGACTCCTCT	ACCTGAGGACTGATGGTGGT
C motif) ligand CTCTGTCCTTAAAGCGGCTTA GTTGCGGAGGTTCAAGATGTT Cd14 - CTCTGTCCTTAAAGCGGCTTA GTTGCGGAGGTTCAAGATGTT L4 antigen C ACTTTCGGGGATGCTGCC Drd1 - GTTGAGTCCAGGGGTTTTGG ACTTTTCGGGGATGCTGCC Dord2 - GTGAACAGGCCGAGAATGGA TGGGAGGGATGGGGCTATAC Dord2 - GTGAACAGGCGGAGAATGGA TGGGAGGGATGGGGGCTATAC Dopamine receptor D1 TGTAGGCCGTGTGAACGGATTT TGTAGACCATGTAGTTGAGGTC Gapdh - AGGTCGGTGTGAACGGATTT GGGAGGATGCGGGCTAGGGGCTATAC GGGAGGATGCGGGCTTTAC Gopanine AGGTCGGTGTGAACGGATGT TGTAGACCATGTAGTTGAGGTC A Gapdh - AGGTCGGTGTGAACGGATGT TGTAGACCATGTAGTTGAGGTC A Gapdh - AGGTCGTCCCACCACCAAGAC CTTGGATTCCTTTCTCTGGGCTT A Gapdh - ACGTCCTCACCACCACCAAGAC CTTGGATTCCTTTCTCTGGGCATG A Variant 1 A CCATTGGTGATGTTGCAAAG CTTTTTCGCTGCATCAGGTT Hingb 1 - TGGGAGATGTCCTCAACTGC CCAGGCGTAGCTGTTGTACT CTATGGACTGTTGGGAAGTTG Interferon beta 1. C C T CTATGGGACTGTTGGGAGAGTGG 1, fibroblast Lepr - TGCC	Chemokine (C-		
2 Cd14 Cd14 CTCTGTCCTTAAAGCGGCTTA GITGAGTCCAGGGGTTTTGG GTTGCGGAGGTTCAAGATGTC Drd1 GTTGAGTCCAGGGGTTTTGG Dopamine G receptor D1 GTGAACAGGCGGAGAATGGA Dopamine GTGAACAGGCGGAGAATGGA Dopamine GTGAACAGGCGGAGAATGGA TGGGAGGGATGGGGGCTATAC Dopamine receptor D2 GTGAACAGGCGGAGAATGGA Gapdh AGGTCGGTGTGAACGGATTT Ghyceraldehyde - -3-phosphate G dehydrogenase transcript variant 1 CCATTGGTGATGTTGCAAGGA <i>Chrl</i> - ATCGTCCTCACCACCAAGAC Grehlin ATCGTCCTCACCACCAAGAC CTTTTCGCTGCATCAGGTT TGTAGGACGTGTGAACGGCTT <i>Hmgb1</i> - High mobility group box 1, transcript variant 1 TGGGAGATGTCCTCAACTGC <i>Inbrb</i> TGGGAGATGTCCTCAACTGC Interferon beta TCCAAAAGAGAACGGACACT I, fibroblast TGCCACCTTTTGACAGTGGATG <i>Lepr</i> - TGCCACCTTTTGACAGTGCATGA Interleukin 1 G <i>Md2</i> - GCCTTACTGACTGCCCATGTG Interleukin 10 G <i>Md2</i> - CGCTGCTTTCTCCCATATTGA CTTCAGTGTTACTGACTGGCATGA	C motif) ligand		
Cd14 - CTCTGTCCTTAAAGCGGCTTA GTTGCGGAGGTTCAAGATGTT Cluster of differentiation 14 antigen C ACTTTTCGGGGAGGTTCAAGATGTT Drd1 - GTTGAGTCCAGGGGTTTTGG ACTTTTCGGGGATGCTGCC Dopamine receptor D1 transcript GTGAACAGGCGGAGAATGGA TGGGAGGGATGGGGGCTATAC Dopamine receptor D2 GTGAACAGGCGGGAGAATGGA TGGGAGGGATGGGGGCTATAC Dopamine receptor D2 AGGTCGGTGTGAACGGATTT TGTAGACCATGTAGTTGAGGTC Gapdh - AGGTCGGTGTGAACGGATTT TGTAGACCATGTAGTTGAGGTC Ghrl - AGGTCGCTCACCACCAAGAC CTTTGGATTCCTTTCTCTGGGCTT Ghrl - ATCGTCCTCACCACCAAGAC CTTTGGATTCCTTTCTCTGGGCTT Yariant 1 TGGGAGATGTCCTCACCACCAAGAC CTTTTTCGCTGCATCAGGTT High mobility group box 1, transcript CCATTGGTGATGTTGCAAAG CTTTTCGCTGCATCAGGTT Iffnb - TGGGAGATGTCCTCAACTGC CCAGGCGTAGCTGTTGTACT Interferon beta 1, fibroblast C TGCAAAAGAGAACGGACACT TGATGGACTGTTGGGAAGTTG Lepr - TCCAAAAGAGAGAACGGACACT TGATGTGCTGCTGCGGAGAGT TGATGTGCTGCTGCGGAGAGTTG Interleukin 10 G GCTCTTACTGACTGGCATGA CGCAGCTCTAGGAGACTGTG Interleukin 10 G GCCTTTACTGACTGGCATGA CCT	2		
Cluster of differentiation 14 antigen C Drd1 - GTTGAGTCCAGGGGTTTTGG Gopamine receptor D1 transcript variant 1 ACTTTTCGGGGATGCTGCC Drd2 - GTGAACAGGCGGAGAATGGA Dopamine receptor D2 TGGGAGGGATGGGGGCTATAC Dogamine receptor D2 GTGAACAGGCGGAGAATGGA AGGTCGGTGTGAACGGATTT Glyceraldehyde dehydrogenase transcript variant 1 TGTAGACCATGTAGTTGAGGTC A Ghrl - ATCGTCCTCACCACCAAGAC Grehlin transcript variant 1 CTTGGATTCCTTTCTCTGGGCTT Grehlin transcript variant 1 Hmgb1 - ATCGTCCTCACCACCAAGAC CATTGGTGATGTTGCAAGG CTTTGGATTCCTTTCTCTGGGCTT GTGGGAGATGTCCTCAACTGC Interferon beta 1, fibroblast <i>Lepr</i> - Leptin receptor transcript variant 3 TGCCACCTTTGACAGTGATGT C T CCAGGCGTAGCTGTTGGGAAGTTG CT <i>IIIb</i> - Interleukin 1 beta TGCCACCTTTGACAGGGATGG GCTGTTCTCCCATGGGATGATG CGCTGCTTCTCCCATATTGA CGCTGCTTACTGACTGGCATGA Lymphocyte antign 96 transcript CGCTGCTTTCTCCCATATTGA	Cd14 –	CTCTGTCCTTAAAGCGGCTTA	GTTGCGGAGGTTCAAGATGTT
differentiation 14 antigen Drd1	Cluster of	С	
14 antigen GTTGAGTCCAGGGGTTTTGG ACTTTCGGGGATGCTGCC Dopamine G G receptor D1 transcript G variant 1 Drd2 – GTGAACAGGCGGAGAATGGA TGGGAGGGATGGGGCTATAC Dopamine C GGCCGGTGTGAACGGATTT TGTAGACCATGTAGTTGAGGTC Gapdh – AGGTCGGTGTGAACGGATTT TGTAGACCATGTAGTTGAGGTC Glyceraldehyde	differentiation		
Drd1 - GTTGAGTCCAGGGGTTTTGG ACTTTTCGGGGATGCTGCC Dopamine G G receptor D1 GTGAACAGGCGGAGAATGGA TGGGAGGGATGGGGCTATAC Dopamine receptor D2 GTGAACAGGCGGAGAATGGA TGGGAGGGATGGGGCTATAC Dopamine receptor D2 AGGTCGGTGTGAACGGATTT TGTAGACCATGTAGTTGAGGTC Gapdh - AGGTCGCTGTGAACGGATTT TGTAGACCATGTAGTTGAGGTC A Synophate dehydrogenase G TGTGGATTCCTTTCTCTGGGCTT Ghrl - ATCGTCCTCACCACCAAGAC CTTTGGATTCCTTTCTCTGGGCTT Ghrl - ATCGTCCTCACCACCAAGAC CTTTGGGATTCCTTTCTCTGGGCTT Ghrl - ATCGTCCTCACCACCAAGAC CTTTGGGATTCCTTCTCTGGGCATCAGGTT Grehlin transcript ATCGTCCTCACCACCAAAGAC CTTTTTCGCTGCATCAGGTT Yariant 1 TGGGAGATGTCCTCAACTGC CCAGGCGTAGCTGTTGTACT TTTTCGGTGCATCAGGTT Ifnb - TGGGAGATGTCCTCAACTGC CCAGGCGTAGCTGTTGTACT CCAGGCGTAGCAGTTG Ifnb - TGCCAACTTTGACAGGACACT TGTATGGACTGTTGGGAAGTTG CTTTGGAGCTGTTGGGAAGTTG Ifnb - TGCCACCTTTTGACAGTGACGGACACT TGATGTGCTGCTGCGGGGAGAGTTG TGATGTGCTGCTGCGGGGAGAGTTG Inteleukin 10 G	14 antigen		
Dopamine receptor D1 transcript variant 1 G G Drd2 - ceptor D2 GTGAACAGGCGGAGAATGGA Gapdh - dehydrogenase transcript variant 1 TGGAGACCATGTAGTTGAGGTC G Ghri - Grehlin transcript variant 1 ATCGTCCTCACCACCAAGAC CTTGGATTCCTTTCTCTGGGCTT Ghri - Grehlin transcript variant 1 ATCGTCCTCACCACCAAGAC CTTGGATTCCTTTCTCTGGGCTT Hmgb1 - High mobility group box 1, transcript variant 1 CCATTGGTGATGTTGCAAAG CTTTTCGCTGCATCAGGTT Ifnb - Interfeon beta 1, fibroblast TGGGAGATGTCCTCAACTGC C CCAGGCGTAGCTGTTGGGAAGTTG CT Leptr - Leptr - Leptin receptor transcript variant 3 TGCCACCTTTTGACAGTGATG C TGATGGACTGTTGGGAAGTTG CT 110 - Interleukin 1 beta TGCCACCTTTTGACAGTGATG G TGATGTGCTGCTGCGGAGATTG C 110 - Interleukin 10 beta GCTCTTACTGACTGGCATGA G CGCTGCTTTATGCAGGGATCA CCTCAGTCTTATGCAGGGTTCA	Drd1 –	GTTGAGTCCAGGGGTTTTGG	ACTTTTCGGGGATGCTGCC
receptor D1 transcript variant 1 Drd2 - Gapdh - GTGAACAGGCGGAGAACGGA Capdh - GGapdh - GAGTCGGTGTGAACGGATTT GAAGACCATGTAGTTGAGGTC A AGGTCGGTGTGAACGGATTT Gapdh - G AGGTCGGTGTGAACGGATTT GAAGACCATGTAGTTGAGGTC A AGGTCGGTGGTGAACGGATTT Grahin transcript variant 1 Hmgb1 - High mobility group box 1, transcript variant 1 Hmgb1 - Interfeon beta 1, fibroblast Lept - Lept - Lept - CT TGCAACAGGAGAACGGACACT CT TGCAAAGAGAACGGACACT CT TGCAACTTTGGCATGTGGAGGTGTTGCAAGG CT CT TGCAAAGAGAGAACGGACACT CT TGTATGGACTGTTGGGAAGTTG CT TGCAACGGCACGTGTTGGGAAGTTG CT TGCAACGGCACGTGTTGGGAAGTTG CT TGCACCTTTTGACAGTGATG TGATGTGCTGCTGCGGAGATT Interleukin 1 beta III0 - Interleukin 10 G Md2 - Lymphocyte antign 96 transcript	Dopamine	G	
Notion D1 variant 1GTGAACAGGCGGAGAATGGATGGGAGGGATGGGGCTATACDopamine receptor D2GTGAACAGGCGGAGAATGGATGGAGACGGGATGGGGCTATAC $Gapdh -$ Gyceraldehyde -3-phosphate dehydrogenase transcript variant 1AGGTCGGTGTGAACGGATTT GTGTAGACCATGTAGTTGAGGTC A $Ghrl -$ Grehlin transcript variant 1ATCGTCCTCACCACCAAGACCTTGGATTCCTTTCTCTGGGCTT G $Hmgb1 -$ High mobility group box 1, transcript variant 1CCATTGGTGATGTTGCAAAGCTTTTTCGCTGCATCAGGTT $Ifnb -$ Interferon beta 1, fibroblastTGGGAGATGTCCTCAACTGCCCAGGCGTAGCTGTTGTACT $Ifnb -$ Leptin receptor transcript variant 3TGCCAACAGGAACGGACACT CTGATGGACTGTTGGGAAGTTG $Ifnb -$ Interleukin 1 betaTGCCACCTTTTGACAGTGATGTGATGTGCTGCTGCGGAGATT $Ifno -$ Interleukin 10 betaGCTCTTACTGACTGGCATGA GCCCAGGCTTAGGAGCATGTG $Ifno -$ Interleukin 10 betaGCTCTTACTGACTGGCATGA GCCCAGGCTCTAGGAGCATGTG $Md2 -$ Lymphocyte antign 96 transcriptCGCTGCTTTCTCCCATATTGACCTCAGTCTTATGCAGGGTTCA	recentor D1	•	
Automp. GTGAACAGGCGGAGAATGGA TGGGAGGGATGGGGCTATAC Dopamine AGGTCGGTGTGAACGGATTT TGTAGACCATGTAGTTGAGGTC Gapdh – AGGTCGGTGTGAACGGATTT TGTAGACCATGTAGTTGAGGTC Ghrd – G AGGTCCTCACCACCAAGAC A Ghrd – ATCGTCCTCACCACCAAGAC CTTGGATTCCTTTCTCTGGGCTT Ghrd – ATCGTCCTCACCACCAAGAC CTTGGATTCCTTTCTCTGGGCTT Grehlin ATCGTCCTCACCACCAAGAC CTTGGATTCCTTTCTCTGGGCTT Yariant 1 ATCGTCCTCACCACCAAGAC CTTTTCGCTGCATCAGGTT High mobility Group box 1, transcript TGGGAGATGTCCTCAACTGC CCAGGCGTAGCTGTTGTACT Interferon beta 1, fibroblast TCCAAAAGAGAAACGGACACT TGTATGGACTGTTGGGAAGTTG Lepr – TCCAAAAGAGAAACGGACACT TGTATGGACTGTTGGGAAGTTG TGATGGACTGTTGGGAAGTTG IfIb – TGCCACCTTTTGACAGTGATG TGATGTGCTGCTGCGGGAGATT TGATGTGCTGCTGCGGGAGATT Interleukin 1 G G G G Md2 – CGCTGCTTTCTCCCATATTGA CCTCAGTCTTATGCAGGGTTCA Lymphocyte GCTGTTTCTCCCATATTGA CCTCAGTCTTATGCAGGGTTCA	transcript		
Junk 1GTGAACAGGCGGAGAATGGATGGGAGGGATGGGGCTATACDopamine receptor D2AGGTCGGTGTGAACGGATTTTGTAGACCATGTAGTTGAGGTCGapdh - Glyceraldehyde edhydrogenase transcript variant 1AGGTCGTCGCTCACCACAAGACCTTGGATTCCTTTCTCTGGGCTTGhrl - Ghrl - Grehlin transcript variant 1ATCGTCCTCACCACCAAGACCTTGGATTCCTTTCTCTGGGCTTHingb1 - Higb mobility group box 1, transcript variant 1CCATTGGTGATGTTGCAAAGCTTTTTCGCTGCATCAGGTTInterferon beta 1, fibroblastTGGGAGATGTCCTCAACTGCCCAGGCGTAGCTGTTGTACTInterferon beta 1, fibroblastTCCAAAAGAGAAACGGACACTTGATGGACTGTTGGGAAGTTGII1b - betaTGCCACCTTTTGACAGTGATGTGATGTGCTGCTGCGGAGATTII1b - hterleukin 1 betaGCTCTTACTGACTGGCATGACGCAGCTCTAGGAGCATGTGII10 - betaGCTCTTACTGACTGGCATGACCCCAGGCGTCTAGGAGCATGTGInterleukin 10 betaGCGCTGCTTTCTCCCATATTGAMd2 - Lymphocyte antign 96 transcriptCGCTGCTTTCTCCCATATTGACCTCAGTCTTATGCAGGGTTCA	variant 1		
Dopamine receptor D2 Growine Gooden and the cooden	Drd2 -	GTGAACAGGCGGAGAATGGA	TGGGAGGGATGGGGCTATAC
Dopamine AGGTCGGTGTGAACGGATTT TGTAGACCATGTAGTTGAGGTC Gapdh - AGGTCGGTGTGAACGGATTT TGTAGACCATGTAGTTGAGGTC Glyceraldehyde -3-phosphate A dehydrogenase transcript A variant 1 A CTTGGATTCCTTTCTCTGGGGCTT Ghrl - ATCGTCCTCACCACCAAGAC CTTGGATTCCTTTCTCTGGGCTT Ghrl - ATCGTCCTCACCACCAAGAC CTTGGATTCCTTTCTCTGGGGCTT Grehlin transcript	Donamine		
Gapdh – AGGTCGGTGTGAACGGATTT TGTAGACCATGTAGTTGAGGTC Glyceraldehyde G AGGTCGGTGTGAACGGATTT TGTAGACCATGTAGTTGAGGTC Glyceraldehyde G A A dehydrogenase ranscript A A yariant 1 ATCGTCCTCACCACCAAGAC CTTTGGATTCCTTTCTCTGGGCTT Ghrl – ATCGTCCTCACCACCAAGAC CTTTGGATTCCTTTCTCTGGGCTT Grehlin TGTAGACCATGTGCCAAGGAC CTTTTCGCTGCATCAGGTT High mobility G CCATTGGTGATGTTGCAAAG CTTTTTCGCTGCATCAGGGTT Yariant 1 TGGGAGATGTCCTCAACTGC CCAGGCGTAGCTGTTGTACT Interferon beta TGCCAAAAGAGAAACGGACACT TGTATGGACTGTTGGGAAGTTG I, fibroblast TCCAAAAGAGAAACGGACACT TGTATGGACTGTTGGGAAGTTG Leptin receptor C T TGCCACCTTTTGACAGTGATG I110 – TGCCACCTTTTGACAGTGATG TGATGTGCTGCTGCGGAGATT Interleukin 1 G CCCTTACTGACTGCCATGA CGCAGCTCTAGGAGCATGTG Md2 – CGCTGCTTTCTCCCATATTGA CCTCAGTCTTATGCAGGGTTCA Lymphocyte CGCTGCTTTCTCCCATATTGA CCTCAGTCTTATGCAGGGTTCA	recentor D2		
Glyceraldehyde -3-phosphate dehydrogenase transcript variant 1 ATCGTCCTCACCACCAAGAC CTTGGATTCCTTTCTCTGGGCTT Ghrl - ATCGTCCTCACCACCAAGAC CTTGGATTCCTTTCTCTGGGCTT Ghrl - ATCGTCCTCACCACCAAGAC CTTGGATTCCTTTCTCTGGGCTT Grehlin transcript variant 1 ATCGTCCTCACCACCAAGAC CTTGGATTCCTTTCTCTGGGCTT High mobility group box 1, transcript variant 1 CCATTGGTGATGTTGCAAAG CTTTTTCGCTGCATCAGGTT Ifnb - TGGGAGATGTCCTCAACTGC CCAGGCGTAGCTGTTGTACT Interferon beta 1, fibroblast TGCCAAAAGAGAACGGACACT TGTATGGACTGTTGGGAAGTTG Lepr - TCCAAAAGAGAACGGACACT TGTATGGACTGTTGGGAAGTTG Interleukin 1 Beta G TGCCACCTTTTGACAGTGATG Interleukin 10 G G G Md2 - CGCTGCTTTCTCCCATATTGA CCTCAGTCTTATGCAGGGGTTCA Lymphocyte antigen 96 CGCTGCTTTCTCCCATATTGA CCTCAGTCTTATGCAGGGTTCA	Gandh	ACCTOCCTOTOAACCCATTT	TGTAGACCATGTAGTTGAGGTC
A -3-phosphate dehydrogenase transcript variant 1 <i>Ghrl</i> – ATCGTCCTCACCACCAAGAC <i>Ghrl</i> – ATCGTCCTCACCACCAAGAC <i>Ghrl</i> – ATCGTCCTCACCACCAAGAC <i>Ghrl</i> – ATCGTCCTCACCACCAAGAC <i>Ghrl</i> – CCATTGGTGATGTTGCAAAG <i>High</i> mobility group box 1, transcript variant 1 <i>Hmgb</i> – High mobility group box 1, transcript variant 1 <i>Ifnb</i> – Interferon beta 1, fibroblast <i>Lepr</i> – <i>Leptr</i> – <i>TCCAAAAGAGAACGGACACT</i> <i>Iterferon beta</i> 1, fibroblast <i>Lepr</i> – <i>TCCAAAAGAGAACGGACACT</i> <i>TGTATGGACTGTTGGGAAGTTG</i> <i>CT</i> <i>TGCCACCTTTTGACAGTGATG</i> <i>ITGATGTGCTGCTGCGGAGATT</i> <i>Interleukin</i> 1 <i>beta</i> <i>II10</i> – <i>ITGCCACCTTTTGACAGTGATG</i> <i>ITGCAGCTGTTGGGAAGTTG</i> <i>ITGCCACCTTTTGACAGTGATG</i> <i>ITGCAGCTGTTGCGCATGAGAGCACTGTG</i> <i>ITGATGTGCTGCTGCTGCGAGAGTTG</i> <i>ITGCACCTTTTGACAGTGATG</i> <i>ITGATGTGCTGCTGCTGCGAGAGTTG</i> <i>ITGATGTGCTGCTGCGGAGATT</i> <i>ITGCAGCTTTTGACAGTGATG</i> <i>ITGATGTGCTGCTGCTGCGGAGATT</i> <i>ITGCAGCTTTTGCCACTGGCATGA</i> <i>ITGATGTGCTGCTGCGGGAGATTG</i> <i>ITGATGTGCTGCTGCGGGAGATGG</i> <i>ITGATGTGCTGCTGCTGCGGAGATTG</i> <i>ITGCCACCTTTTGACAGTGGTG</i> <i>ITGATGTGCTGCTGCTGCGGAGATTG</i> <i>ITGATGTGCTGCTGCTGCGGAGATTG</i> <i>ITGATGTGCTGCTGCTGCGGAGATTG</i> <i>ITGCCACCTTTTGACAGTGGTG</i> <i>ITGATGTGCTGCTGCTGCGGAGATTG</i> <i>ITGCCACCTTTTGCCATATTGA</i> <i>ITGCCACCTTATGCAGGGGTTCA</i> <i>ITGCGCTGCTTCTCCCATATTGA</i> <i>ITGCAGTCTTATGCAGGGGTTCA</i> <i>ITGCAGTCTTATGCAGGGGTTCA</i> <i>ITGCAGTCTTATGCAGGGTTCA</i> <i>ITGCAGTCTTATGCAGGGTTCA</i> <i>ITGCAGTCTTATGCAGGGTTCA</i> <i>ITGCAGTCTTATGCAGGGTTCA</i> <i>ITGCAGTCTTATGCAGGGTTCA</i> <i>ITGCAGTCTTATGCAGGGTTCA</i> <i>ITGCAGTCTTATGCAGGGTTCA</i> <i>ITGCAGTCTTATGCAGGGTTCA</i> <i>ITGCAGTCTTATGCAGGGTTCA</i> <i>ITGCAGTCTTATGCAGGGTTCA</i> <i>ITGCAGTCTTATGCAGGGTTCA</i> <i>ITGCAGTCTTATGCAGGGTTCA</i> <i>ITGCAGTCTTATGCAGGGTTCA</i> <i>ITGCAGTCTTATGCAGGGTTCA</i> <i>ITGCAGTCTTATGCAGGGTTCA</i> <i>ITGCAGTCTTATGCAGGGTTCA</i> <i>ITGCAGTCTTATGCAGGGTTCA</i> <i>ITGCAGTCTTATGCAGGGTTCA</i> <i>ITGCAGTCTTATGCAGGGTTCA</i> <i>ITGCAGTCTTATGCAGGGTTCA</i> <i>ITGCAGTCTTATGCAGGGTTCA</i> <i>ITGCAGTCTTATGCAGGGTTCA</i> <i>ITGCAGTCTTATGCAGGGTTCA</i> <i>ITGCAGTCTTATGCAGGGTTCA</i> <i>ITGCAGTCTTATGCAGGTGTGTGTGTGTGTGTGTGTGTGTG</i>	Gapun –	AGGICGGIGIGAACGGAIII	
-s-phosphrate dehydrogenase transcript variant 1 <i>Ghrl</i> – ATCGTCCTCACCACCAAGAC <i>Ghrl</i> – ATCGTCCTCACCACCAAGAC <i>Ghrl</i> – ATCGTCCTCACCACCAAGAC <i>Ghrl</i> – ATCGTCCTCACCACCAAGAC <i>Ghrl</i> – CCATTGGTGATGTTGCAAAG <i>Hingb</i> 1 – CCATTGGTGATGTTGCAAAG <i>Hingb</i> 1 – CCATTGGTGATGTTGCAAAG <i>Hingb</i> 1 – CCATTGGTGATGTTGCAAAG <i>Hingb</i> 1 – TGGGAGATGTCCTCAACTGC <i>Interferon</i> beta <i>1,</i> fibroblast <i>Lepr</i> – TCCAAAAGAGAACGGACACT <i>Leptin</i> receptor <i>transcript</i> <i>variant</i> 3 <i>II1b</i> – ITGCCACCTTTTGACAGTGATG <i>II1b</i> – TGCCACCTTTTGACAGTGATG <i>II1b</i> – TGCCACCTTTTGACAGTGATG <i>II1b</i> – GCTCTTACTGACTGGCATGA <i>II1b</i> – GCTCTTACTGACTGGCATGA <i>II10</i> – GCTCTTACTGACTGGCATGA <i>II10</i> – CGCTGCTTCTCCCATATTGA <i>Jymphocyte</i> <i>antigen</i> 96 <i>transcript</i>		6	A
denydrogenase transcript variant 1 ATCGTCCTCACCACCAAGAC CTTGGATTCCTTTCTCTGGGCTT Grehlin transcript variant 1 ATCGTCCTCACCACCAAGAC CTTGGATTCCTTTCTCTGGGCTT Hmgb1 - High mobility group box 1, transcript variant 1 CCATTGGTGATGTTGCAAAG CTTTTCGCTGCATCAGGTT Ifnb - Interferon beta 1, fibroblast TGGGAGATGTCCTCAACTGC CCAGGCGTAGCTGTTGTACT Lepr - Lepr - variant 3 TCCAAAAGAGAACGGACACT TGTATGGACTGTTGGGAAGTTG Il1b - Interleukin 1 TGCCACCTTTTGACAGTGATG TGATGTGCTGCTGCGGAGATT Il10 - Interleukin 10 GCTCTTACTGACTGGCATGA CGCAGCTCTAGGAGCATGTG Il10 - Interleukin 10 GCTCTTACTGACTGGCATGA CGCAGCTCTAGGAGCATGTG Interleukin 10 G CCTCTAGTGCATGA CCTCAGTCTTAGCAGGGTTCA Jymphocyte antigen 96 transcript CGCTGCTTTCTCCCATATTGA CCTCAGTCTTATGCAGGGTTCA			
Transcript ATCGTCCTCACCACCAAGAC CTTGGATTCCTTTCTCTGGGCTT Ghrl - ATCGTCCTCACCACCAAGAC CTTGGATTCCTTTCTCTGGGCTT Grehlin transcript CCATTGGTGATGTTGCAAAG CTTTTTCGCTGCATCAGGTT High mobility group box 1, CCATTGGTGATGTTGCAAAG CTTTTTCGCTGCATCAGGTT Ifnb - TGGGAGATGTCCTCAACTGC CCAGGCGTAGCTGTTGTACT Ifnb - TGGGAGATGTCCTCAACTGC CCAGGCGTAGCTGTTGTACT Interferon beta - - 1, fibroblast - - Lepr - TCCAAAAGAGAACGGACACT TGTATGGACTGTTGGGAAGTTG Variant 3 - - 1/1b - TGCCACCTTTTGACAGTGATG TGATGTGCTGCTGCGGAGAGATT Interleukin 1 - - - beta - - - 1/10 - GCTCTTACTGACTGGCATGA CGCAGCTCTAGGAGCATGTG Interleukin 10 G - - Md2 - CGCTGCTTTCTCCCATATTGA CCTAGTCTTATGCAGGGTTCA Lymphocyte - CGCTGCTTTCTCCCATATTGA CCTAGTCTTATGCAGGGTTCA	denydrogenase		
Variant 1 ATCGTCCTCACCACCAAGAC CTTGGATTCCTTTCTCTGGGCTT Ghrl - ATCGTCCTCACCACCAAGAC CTTGGATTCCTTTCTCTGGGCTT Grehlin transcript CCATTGGTGATGTTGCAAAG CTTTTCGCTGCATCAGGTT High mobility group box 1, CCATTGGTGATGTCCTCAACTGC CCAGGCGTAGCTGTTGTACT Ifnb - TGGGAGATGTCCTCAACTGC CCAGGCGTAGCTGTTGTACT Interferon beta TCCAAAAGAGAAACGGACACT TGTATGGACTGTTGGGAAGTTG Lepr - TCCAAAAGAGAAACGGACACT TGTATGGACTGTTGGGAAGTTG Leptin receptor C T variant 3 TGCCACCTTTTGACAGTGATG TGATGTGCTGCTGCGGAGAGTT Il1b - IGCCACCTTTTGACAGTGATG CGCAGCTCTAGGAGCATGTG Il1b - GCTCTTACTGACTGGCATGA CGCAGCTCTAGGAGCATGTG Il10 - GCTCTTACTGACTGGCATGA CGCAGCTCTAGGAGCATGTG Il10 - GCTGCTTTCTCCCATATTGA CCTCAGTCTTATGCAGGGTTCA Jymphocyte G CCTCAGTCTTATGCAGGGTTCA Jymphocyte G CCTCAGTCTTATGCAGGGTTCA			
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Lymphocyte antigen 96 transcript	Md2 –	CGCTGCTTTCTCCCATATTGA	CCTCAGTCTTATGCAGGGTTCA
antigen 96 transcript	Lymphocyte		
transcript	antigen 96		
	transcript		
variant 1, 2	variant 1, 2		

Table 1 Primer sequence used in gPCR

Gene	Forward Primer 5' – 3'	Reverse Primer 5' – 3'
<i>Myd88</i> – Myeloid differentiation primary response gene 88	TCATGTTCTCCATACCCTTGG T	AAACTGCGAGTGGGGTCA
<i>Oprm1 –</i> Opioid receptor, mu 1, transcript variant 1C, 1M, 1U	TCCGACTCATGTTGAAAAACC C	CCTTCCCCGGATTCCTGTCT
Rxfp1 – Relaxin/insulin- like family peptide receptor 1	CGAGCTGTCCCATCAGTTTCT	AGACGCTCACGGAGTGAATC
<i>TIr4 –</i> Toll-like receptor 4	GCCTTTCAGGGAATTAAGCT CC	GATCAACCGATGGACGTGTAAA
<i>Trif</i> – Toll-like receptor adaptor molecule 1	AACCTCCACATCCCCTGTTTT	GCCCTGGCATGGATAACCA
<i>Th</i> –Tyrosine Hydroxylase	CCTTCCGTGTGTTTCAGTGC	TCAGCCAACATGGGTACGTG

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 < 0.0001Effect of light-cycle, $F_{(1, 9)} = 15.53$, p = 0.0034Interaction: concentration x light-cycle, $F_{(4, 36)} = 2.98$, p = 0.0318

(d) Saccharin preference

Effect of concentration, $F_{(4, 36)} = 1.74$, p = 0.16Effect of light-cycle, $F_{(1, 9)} = 8.32$, p = 0.015Interaction: 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 = 0.031Effect of light-cycle, $F_{(1, 9)} = 20.31$, p = 0.0009Interaction: 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 twoway 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, II1b, t = 0.12 df = 4, p = 0.90 Effect of light cycle, II10, 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, *ll10*, 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 = 0.013Effect of light-cycle, $F_{(1, 8)} = 11.09$, p = 0.010Interaction: 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 = 0.58Effect of light-cycle, $F_{(1, 8)} = 1.08$, p = 0.33Interaction: 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 < 0.0001Effect of light-cycle, $F_{(1, 320)} = 4.12$, p = 0.043Effect of pretreatment, $F_{(1, 320)} = 4.95$, p = 0.026Interaction: concentration x light-cycle, $F_{(7, 320)} = 3.05$, p = 0.0040Interaction: concentration x pretreatment, $F_{(7, 320)} = 0.60$, p = 0.76Interaction: light-cycle x pretreatment, $F_{(1, 320)} = 2.33$, p = 0.13Interaction: 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 = 0.0007Effect of light-cycle, $F_{(1, 320)} = 311.2$, p < 0.0001 Effect of pretreatment, $F_{(1, 320)} = 25.68$, p < 0.0001Interaction: concentration x light-cycle, $F_{(7, 320)} = 2.52$, p = 0.016Interaction: concentration x pretreatment, $F_{(7, 320)} = 2.31$, p = 0.026Interaction: light-cycle x pretreatment, $F_{(1, 320)} = 11.17$, p = 0.0009Interaction: 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 < 0.0001Effect of light-cycle, $F_{(1, 220)} = 75.11$, p < 0.0001Effect of pretreatment, $F_{(1, 220)} = 8.95$, p = 0.0031Interaction: concentration x light-cycle, $F_{(4, 220)} = 12.39$, p < 0.0001Interaction: concentration x pretreatment, $F_{(4, 220)} = 1.43$, p = 0.23Interaction: light-cycle x pretreatment, $F_{(1, 220)} = 9.11$, p = 0.0028Interaction: 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 = 0.49Effect of light-cycle, $F_{(1, 220)} = 31.38$, p < 0.0001Effect of pretreatment, $F_{(1, 220)} = 0.25$, p = 0.62Interaction: concentration x light-cycle, $F_{(4, 220)} = 0.68$, p = 0.61Interaction: concentration x pretreatment, $F_{(4, 220)} = 0.24$, p = 0.92Interaction: light-cycle x pretreatment, $F_{(1, 220)} = 1.37$, p = 0.24Interaction: 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 = 0.039Interaction: concentration x light-cycle, $F_{(4, 220)} = 0.38$, p = 0.83Interaction: concentration x pretreatment, $F_{(4, 220)} = 4.27$, p = 0.0024Interaction: light-cycle x pretreatment, $F_{(1, 220)} = 0.018$, p = 0.89Interaction: 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 = 0.0020Effect of light-cycle, $F_{(1, 220)} = 0.0058$, p = 0.94Effect of pretreatment, $F_{(1, 220)} = 2.02$, p = 0.16Interaction: concentration x light-cycle, $F_{(4, 220)} = 2.49$, p = 0.0443Interaction: concentration x pretreatment, $F_{(4, 220)} = 0.89$, p = 0.47Interaction: light-cycle x pretreatment, $F_{(1, 220)} = 14$, p = 0.0002Interaction: 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 = 0.0027Effect of light-cycle, $F_{(1, 7)} = 0.0011$, p = 0.92Interaction: pretreatment x light-cycle, $F_{(1, 7)} = 1.62$, p = 0.24)







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 threeway 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 \pm 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 \pm 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 \pm SEM; n=3, **p* < 0.05; ***p* < 0.01.



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 \pm 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 < 0.0001Effect of light-cycle, $F_{(1, 8)} = 99.62$, p < 0.0001Interaction: 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 < 0.0001Effect of light-cycle, $F_{(1, 288)} = 78.51$, p < 0.0001Effect of pretreatment, $F_{(1, 288)} = 3.66$, p = 0.050Interaction: concentration x light-cycle, $F_{(7, 288)} = 14.53$, p < 0.0001Interaction: concentration x pretreatment, $F_{(7, 288)} = 1.82$, p = 0.071Interaction: light-cycle x pretreatment, $F_{(1, 288)} = 0.089$, p = 0.77Interaction: 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 = 0.22Effect of light-cycle, $F_{(1, 288)} = 356.1$, p < 0.0001Effect of pretreatment, $F_{(1, 288)} = 29.93$, p < 0.0001Interaction: concentration x light-cycle, $F_{(7, 288)} = 1.25$, p = 0.27Interaction: concentration x pretreatment, $F_{(7, 288)} = 0.55$, p = 0.79Interaction: light-cycle x pretreatment, $F_{(1, 288)} = 4.60$, p = 0.033Interaction: 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 < 0.0001Effect of light-cycle, $F_{(1, 144)} = 97.97$, p < 0.0001Effect of pretreatment, $F_{(1, 144)} = 11.19$, p = 0.0011Interaction: day of testing x light-cycle, $F_{(3, 144)} = 2.77$, p = 0.044Interaction: day of testing x treatment, $F_{(3, 144)} = 2.29$, p = 0.08Interaction: light-cycle x pretreatment, $F_{(1, 144)} = 2.23$, p = 0.14Interaction: 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 < 0.0001Effect of treatment, $F_{(1, 9)} = 21.31$, p = 0.0013Interaction: 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 \pm 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 = 0.0004Effect of pretreatment,, $F_{(1, 5)} = 0.070$, p = 0.80Interaction (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 < 0.0001Effect of pretreatment,, $F_{(1, 5)} = 1.59$, p = 0.24Interaction (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 = 0.54Effect of pretreatment,, $F_{(1, 5)} = 3.76$, p = 0.088Interaction (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 < 0.0001Effect of pretreatment,, $F_{(1, 5)} = 29.93$, p < 0.0001Interaction (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 < 0.0001Effect of conditioning drug, $F_{(1, 56)} = 2.15$, p = 0.15Effect of light-cycle, $F_{(1, 56)} = 2.74$, p = 0.10Interaction: conditioning drug x pretreatment, $F_{(1, 56)} = 14.26 p = 0.0004$ Interaction: conditioning drug x light-cycle, $F_{(1, 56)} = 0.51$, p = 0.48Interaction: pretreatment x light-cycle, $F_{(1, 56)} = 0.51$, p = 0.48Interaction: conditioning drug x pretreatment x light-cycle, $F_{(1, 56)} = 0.51$, p = 0.48

Figure s6 Effect of alcohol and (+)-Naltrexone on the expression of TLR4 and reward-related genes in the Nucleus Accumbens. All data analysed using two-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) *II1b*, 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) *II10*, 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 two-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) *II1b*, 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) *II10*, 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.