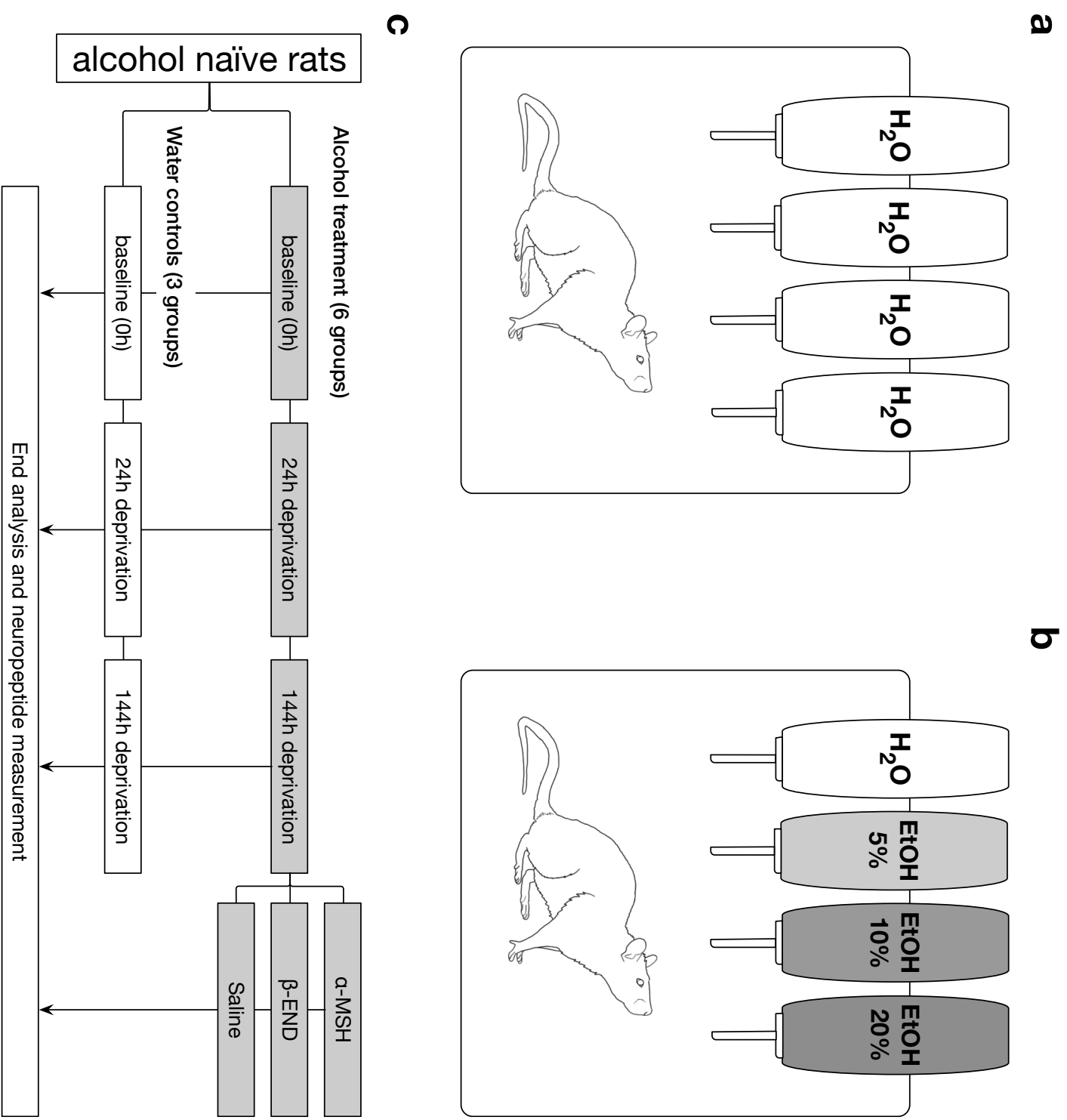


Figure S1



DNA methylation of the Leptin-gene-promoter is altered by chronic alcohol exposure in an animal model for alcohol dependence.

Supplementary Figure Legend

Supplementary Figure S1:

a and b) Pictogram illustrating the four-bottle-paradigm for alcohol dependence in rodents. Control animals (water-drinking) (a) and alcohol-consuming animals (b).

c) Pictogram illustrating the scheduled timepoints of sample collection of the present rodent study.

DNA methylation of the Leptin-gene-promoter is altered by chronic alcohol exposure in an animal model for alcohol dependence.

a) ANOVA

Leptin levels of treated groups (Alpha-MSH, Beta-Endorphin, Saline)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	70145900,690	2	35072950,345	0,514	0,604
Within Groups	1774701520,000	26	68257750,769		
Total	1844847420,690	28			

b) ANOVA

Leptin levels of water, 0h, 24h and 6 days group

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	193448318,750	5	38689663,750	0,988	0,436
Within Groups	1802089075,000	46	39175849,457		
Total	1995537393,750	51			

c) ANOVA

Weight of all groups

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	120260,562	8	15032,570	1,347	0,235
Within Groups	814949,694	73	11163,694		
Total	935210,256	81			

d) ANOVA

EtOH of treated groups (Alpha-MSH, Beta-Endorphin, Saline)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1,102	2	0,551	0,595	0,560
Within Groups	21,307	23	0,926		
Total	22,410	25			

DNA methylation of the Leptin-gene-promoter is altered by chronic alcohol exposure in an animal model for alcohol dependence.

Supplementary ANOVA Legend:

- a) ANOVA: Comparison of group-means of leptin levels between treated animal groups (Alpha-MSH, Beta-Endorphin, Saline)
- b) ANOVA: Comparison of group-means of leptin levels between water groups (pooled to one control groups as described in the results section) and alcohol groups (0h, 24h and 6d)
- c) ANOVA: Comparison of group-means of weight between all animal groups (water, 0h, 24h, 6d, Alpha-MSH, Beta-Endorphin and Saline)
- d) ANOVA: Comparison of group-means of ethanol consumption of all treated animal groups (Alpha-MSH, Beta-Endorphin, Saline)

Supplementary Table 1

DNA methylation of the Leptin-gene-promoter is altered by chro

Name	Sequence
rLepF1	TTT TTG GTT GGT TTT GAG GG
rLepR2	GGA GAG TAG GTG GTT GTT AGG TGA AT
rLepF2	TAA GTT TTT GTT ATT TGT TTT TGG A

ENSEMBL# ENSRNOG00000045797
Chromosome: Rnor_6.0 4 56334695:56352418

nic alcohol exposure in an animal model for alcohol dependence

Gene	fragment size
Leptin - 1. PCR	725bp
Leptin - First PCR / second (seminested) PCR	
Leptin - Second (seminested) PCR / Seq PCR	

Leptin found in Ensembl Genome Browser / www.ensembl.org

Leptin position on chromosome 4

DNA methylation of the Leptin-gene-promoter is altered by chronic alcohol exposure in an animal model for alcohol dependence.

Supplementary Table Legend

Supplementary Table 1:

Supplementary Table shows the oligonucleotide sequences used for PCR and as sequencing primer as indicated. Leptin genomic sequence can be found in Ensembl Genome Browser using the indicated accession number: ENSRNOG00000045797. There is additional information about Leptin's position on Chromosome 4: 56334695:56352418.