

Supplementary materials

List of antibodies used in western blot assays

Target	Phosphorylation site	Species	Company	Reference number
Rabbit IgG	N/A	Goat	Abcam	ab6721
Phospho-PDK1	Ser241	Rabbit	Cell Signalling	3061
Phospho-mTOR	Ser2448	Rabbit	Cell Signalling	5536
Phospho-p70 S6 Kinase	Thr389	Rabbit	Cell Signalling	9205
Phospho-Akt	Ser473	Rabbit	Cell Signalling	4060
Phospho-GSK-3 α/β	Ser21/9	Rabbit	Cell Signalling	9331

List of primers pairs used for the RT-qPCR assays

Gene	Primer sequences (5'-3')	
	Sense	Antisense
<i>Akt1</i>	CGTTCTTTGCCAACATCGT	TCATCTTGATCAGGCGGTGT
<i>Akt2</i>	GGCACGCTTTTATGGAGCAG	ATCTCGTACATGACCACGCC
<i>Eef1a1</i>	TTGGACACGTAGATTCCGGC	TAGTGATACCACGCTCACGC
<i>Eif4e</i>	TACAGAACAGGTGGGCACTC	CATCGTCCTCCCCGTTTGT
<i>Gapdh</i>	TCCCTGTTCTAGAGACAG	CCACTTTGTCACAAGAGA
<i>Gsk3a</i>	GCCCAACGTGTCCTACATCT	TTGGCGTCCCTAGTACCTTG
<i>Gsk3b</i>	CCGAGGAGAGCCCAATGTTT	CTTCGTCCAAGGATGTGCCT
<i>Igf1r</i>	ATCTCCGGTCTCTAAGGCCA	CCAGGTCTCTGTGGACGAAC
<i>Igf2r</i>	TCACAATCGAGGTGGACTGC	CACCCGGTGACAGACATTGA
<i>Insr</i>	GCTTCTGCCAAGACCTTCAC	TAGGACAGGGTCCCAGACAC
<i>Mtor</i>	GGTGGACGAGCTCTTTGTCA	AGGAGCCCTAACACTCGGAT
<i>Rp6kb1</i>	ACTGGAGCACCTCCATTAC	GCTTGGACTTCTCCAGCATC
<i>Pdk1</i>	GAAGCAGTTCCTGGACTTCG	GCTTTGGATATACCAACTTTGTACC
<i>Pik3ca</i>	GAGCACAGCCAAGGAAACTC	TCTCCCCAGTACCATTACGC
<i>Rptor</i>	CTTGGACTTGCTGGGACGAT	ATGAAGACAAGGAGTGGCCG
<i>Rictor</i>	CCGTCGCAGCAATCAAAGAC	CCCCCAATTCGATGAGCCAA
<i>Rps6</i>	CGTCTTGTTACTCCCCGTGT	GCCTACGTCTCTTGGCAATC
<i>Sgk1</i>	TGGTAGCAATCCTCATCGCTTT	GTGAGGGGTTGGCGTTCATA
<i>Eif4ebp2</i>	TCCTGGCGCCTTAATGGAAG	AAGATGTGGCTGGACAGAGC

Interesting relationships between morphine consumption and amygdalar gene expression

In addition to the results presented in the main body of the article, we analyzed potential correlations between behavioral and biochemical variables. None of the correlations studies achieved statistical significance after a false discovery rate control for multiple comparisons, probably due to the elevated number of comparisons (18 genes, 8 phosphoproteins, 2 behavioral variables, 3 brain areas & 2 experimental groups) that makes it difficult for any correlation between a behavioral variable and a biochemical one surpass the corrected

threshold for statistical significance. In spite of this, we decided to plot two here two scatter dot plots suggestive of interesting potential relationships that may merit further research.

In the rats of the group which self-administered morphine, the total number of infusions seemed to correlate with *Rps6* and *Eef1a1* expression in the amygdala. *Rps6* is one of the effectors of the mTORC1 complex, and the ribosomal protein S6 regulates *Eef1a1* translation, the latter being a key regulator of protein synthesis. If these results were corroborated in targeted experiments, they would support the findings in this study regarding the effects of morphine self-administration in mTORC1 function and the activation of translation machinery in the amygdala.

