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Main Figures: 7

Supplementary Figures: 16

Supplementary Tables: 0

Supplementary Videos: 0

Reporting Checklist for Nature Neuroscience

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. For more information, please read [Reporting Life Sciences Research](#).

Please note that in the event of publication, it is mandatory that authors include all relevant methodological and statistical information in the manuscript.

► Statistics reporting, by figure

- Please specify the following information for each panel reporting quantitative data, and where each item is reported (section, e.g. Results, & paragraph number).
- Each figure legend should ideally contain an exact sample size (n) for each experimental group/condition, where n is an exact number and not a range, a clear definition of how n is defined (for example x cells from x slices from x animals from x litters, collected over x days), a description of the statistical test used, the results of the tests, any descriptive statistics and clearly defined error bars if applicable.
- For any experiments using custom statistics, please indicate the test used and stats obtained for each experiment.
- Each figure legend should include a statement of how many times the experiment shown was replicated in the lab; the details of sample collection should be sufficiently clear so that the replicability of the experiment is obvious to the reader.
- For experiments reported in the text but not in the figures, please use the paragraph number instead of the figure number.

Note: Mean and standard deviation are not appropriate on small samples, and plotting independent data points is usually more informative. When technical replicates are reported, error and significance measures reflect the experimental variability and not the variability of the biological process; it is misleading not to state this clearly.

		TEST USED		n			DESCRIPTIVE STATS (AVERAGE, VARIANCE)		P VALUE		DEGREES OF FREEDOM & F/t/z/R/ETC VALUE	
FIGURE NUMBER	WHICH TEST?	SECTION & PARAGRAPH #	EXACT VALUE	DEFINED?	SECTION & PARAGRAPH #	REPORTED?	SECTION & PARAGRAPH #	EXACT VALUE	SECTION & PARAGRAPH #	VALUE	SECTION & PARAGRAPH #	
example 1a	one-way ANOVA	Fig. legend	9, 9, 10, 15	mice from at least 3 litters/group	Methods para 8	error bars are mean +/- SEM	Fig. legend	p = 0.044	Fig. legend	F(3, 36) = 2.97	Fig. legend	
example results, para 6	unpaired t-test	Results para 6	15	slices from 10 mice	Results para 6	error bars are mean +/- SEM	Results para 6	p = 0.0006	Results para 6	t(28) = 2.808	Results para 6	
+ -												

TEST USED		n			DESCRIPTIVE STATS (AVERAGE, VARIANCE)		P VALUE		DEGREES OF FREEDOM & F/t/z/R/ETC VALUE		
FIGURE NUMBER	WHICH TEST?	SECTION & PARAGRAPH #	EXACT VALUE	DEFINED?	SECTION & PARAGRAPH #	REPORTED?	SECTION & PARAGRAPH #	EXACT VALUE	SECTION & PARAGRAPH #	VALUE	SECTION & PARAGRAPH #
1b	unpaired t-test	Fig. 1b legend	3,3,3,3,3,3	For each group of eRNA and mRNA level post KCl stimulation, n=3. 2.5 million cultured cortical neurons were used in each of the three trials and measured at four different time-points.	Fig. 1b legend	error bars are mean +/- SEM	Fig. 1b legend				
2a	Student's unpaired t-test / unpaired t-test with Welch correction	Fig. 2a legend	3,3,3,3,3	For c-fos mRNA fold induction and mRNA level, 2.5 million cultured cortical neurons were used in each of the four stimulation groups (Un, KCl, BDNF, Forskolin).	Fig. 2a legend	error bars are mean +/- SEM	Fig. 2a legend	mRNA level: p=0.0418 t(2)=4.736 p=0.0333 t(2)=5.339 p=0.0448 t(2)=4.566 c-fos eRNA: KCl: e1 p=0.0418 t(4)=6.654 F=10.187 (sdP=0.0894) e2 p=0.008 t(4)=11.103 F=3.149 (sdP=0.3267) e5 p=0.0264 t(4)=3.436 F=26.341 (sdP=0.0566) BDNF: e1 p=0.0329 t(4)=3.200 F=16.876 (sdP=0.0559) e4 p=0.0271 t(4)=3.408 H=17.578 (sdP=0.0538) e5 p=0.0214 t(2)=6.72 Forskolin: e1 p=0.0498 t(4)=2.718 F=6.333 (sdP=0.1364) e5 p=0.0485 t(4)=2.805 F=4.048 (sdP=0.1981)	Fig. 2a legend		

+ -	2b	unpaired t-test with Welch correction	Fig. 2b legend	3,3,3	For each stimulation group (KCl, BDNF, Forskolin) n=3 consisting of 2.5 million cultured cortical neurons.	Fig. 2b legend	error bars are mean +/- SEM	Fig. 2b legend	<p>KCl: e1 p=0.0242 t(3)=6.306 e2 p=0.0103 t(3)=5.784 e5 p=0.0191 t(2)=7.140 BDNF: e1 p=0.0350 t(2)=5.207 e4 p=0.0056 t(2)13.349 e5 p=0.0391 t(2)4.907 Forskolin: e1 p=0.0087 t(2)=10.63 e5 p=0.0438 t(2)=4.618</p>	Fig. 2b legend		
+ -	2c	Student's unpaired t-test / unpaired t-test with Welch correction	Fig. 2c legend	3,5,2,2,2,3	For each stimulation group (KCl, BDNF, Forskolin) n=3,5,2,2,2,3 consisting of 2.5 million cultured cortical neurons.	Fig. 2c legend	error bars are mean +/- SEM	Fig. 2c legend	<p>KCl: e1 vs. e1+e2+e4+d5 p=0.0001 t(5)=12.256 e1 vs. e1+e2+e5 p=0.0246 t(2)=6.259 e1+e2+e4+e5 vs. e1+e4+e5 p=0.0240 t(2)=6.576 e1+e2+e4+e5 vs. e1+e5 p=0.0128 t(2)=8.764 BDNF: e1 vs. e1+e2+e4+d5 p=0.0007 t(7)=5.818 e1+e2+e4+e5 vs. e1+e2+e5 p=0.0034 t(3)=8.537 F=1.411 (sdP=0.3569) e1 vs. e1+e4+e5 p=0.0004 t(3)=18.125 F=6.346 (sdP=0.1280) e1+e2+e4+e5 vs. e1+e5 p=0.0030 t(3)=2.822 F=63.970 (sdP=0.0946) Forskolin: e1 vs. e1+e2+e4+d5 p=0.0001 t(5)=12.601 e1 vs. e1+e2+e5 p=0.0189 t(2)=7.164 F=126.28 (sdP=0.0565)</p>	Fig. 2c legend		

+ -	3b	Student's unpaired t-test / unpaired t-test with Welch correction	Fig. 3b legend	3,5	Three trials of 3C analysis for enhancer 1,2 and the promoter both before, and after KCl stimulation.	Fig. 3b legend	error bars are mean +/- SEM	Fig. 3b legend	KCl : B[e1] p=0.0452 t(2)=4.545 C[e2] p=0.0109 t(4)=4.492 F=5.459 (sdP=0.1548)			
+ -	3c	unpaired t-test with Welch correction	Fig. 3c legend	3	Three trials of 3C analysis for enhancer 1,2 and the promoter both before, and after BDNF stimulation.	Fig. 3c legend	error bars are mean +/- SEM	Fig. 3c legend	BDNF : B[e1] p=0.0482 t(2)=4.389			
+ -	3d	Student's unpaired t-test	Fig. 3d legend	3	Three trials of 3C analysis for enhancer 1,2 and the promoter both before, and after Forskolin stimulation.	Fig. 3d legend	error bars are mean +/- SEM	Fig. 3d legend	Forskolin: B[e1] p=0.0016 t(2)=7.132 F=49.091 (sdP=0.0903)			
+ -	3f	unpaired t-test with Welch correction	Fig. 3f legend	3	Three trials of 3C analysis for enhancer 5 and the promoter before and after KCl stimulation.	Fig. 3f legend	error bars are mean +/- SEM	Fig. 3f legend	KCl : F[e5] p=0.0492 t(2)=3.554			
+ -	3g	Student's unpaired t-test	Fig. 3g legend	3	Three trials of 3C analysis for enhancer 5 and the promoter before and after BDNF stimulation.	Fig. 3g legend	error bars are mean +/- SEM	Fig. 3g legend	BDNF : F[e5] p=0.0001 t(4)=14.393 F=2.872 (sdP=0.2583)			
+ -	3h	Student's unpaired t-test	Fig. 3h legend	3	Three trials of 3C analysis for enhancer 5 and the promoter before and after Forskolin stimulation.	Fig. 3h legend	error bars are mean +/- SEM	Fig. 3h legend	Forskolin : F[e5] p=0.0489 t(2)=3.481 F=8.843 (sdP=0.2065)			
+ -	4a	Student's unpaired t-test	Fig. 4a legend	3,3,3	For each TF knockdown group (CREB, MEF2A, NPAS4) n=3 consisting of 2.5 million cultured cortical neurons in each of the three trials (Scr KCl, shCREB un, shCREB KCl).	Fig. 4a legend	error bars are mean +/- SEM	Fig. 4a legend	CREB: p=0.022 t(2)=6.600 F=27.833 (sdP=0.1192) MEF2A: p=0.0417 t(2)=4.741 F=11.189 (sdP=0.1849) NPAS4: p=0.0455 t(2)=4.524 F=28.538 (sdP=0.1178)	Fig. 4a legend		

+ -	4b	Student's unpaired t-test	Fig. 4b legend	3,3,3	For each TF knockdown group (CREB, MEF2A, NPAS4) n=3 consisting of 2.5 million cultured cortical neurons in each of the trials (e1-e5).	Fig. 4b legend	error bars are mean +/- SEM	Fig. 4b legend	CREB: e2 p=0.0382 t(4)=3.046 F=1.078 (sdP=0.4813) e5 p=0.04 MEF2A: e2 p=0.0478 t(4)=2.820 F=3.517 (sdP=0.2214) e5 p=0.0339 t(4)=3.169 F=2.995 (sdP=0.2503) NPAS4: e2 p=0.0164 t(2)=7.714 F=9.324 (sdP=0.2015)	Fig. 4b legend		
+ -	4c	Student's unpaired t-test	Fig. 4c legend	3,3,3	For each BDNF-mediated c-fos mRNA induction group (CREB, MEF2A, SRF) n=3 consisting of 2.5 million cultured cortical neurons.	Fig. 4c legend	error bars are mean +/- SEM	Fig. 4c legend	c-fos mRNA: MEF2A p=0.0010 t(4)=8.656 F=2.157 (sdP=0.3168)	Fig. 4c legend		
+ -	4d	Student's unpaired t-test	Fig. 4d legend	3,3,3	For each BDNF-mediated c-fos eRNA induction group (CREB, MEF2A, SRF) n=3 consisting of 2.5 million cultured cortical neurons in each of the trials (e1-e5).	Fig. 4d legend	error bars are mean +/- SEM	Fig. 4d legend	c-fos eRNA: MEF2A e4 p=0.0439 t(3)=2.906 F=8.631 (sdP=0.1038) e5 p=0.0485 t(4)=2.807 F=5.381 (sdP=0.1567)	Fig. 4d legend		
+ -	5a	Student's unpaired t-test	Fig. 5a legend	3	For each IEG mRNA group (c-fos, Arc, Gadd45b, Egr-1) n=3 consisting of 2.5 million cultured cortical neurons in each of the trials (CTL un, CTL KCl, CRISPRi TSS un, CRISPRi TSS KCl).	Fig. 5a legend	error bars are mean +/- SEM	Fig. 5a legend	c-fos mRNA: p=0.0189 t(2)=12.637 F=4.830 (sdP=0.2718)	Fig. 5a legend		
+ -	5b	Student's unpaired t-test	Fig. 5b legend	2,3,2,2	For each e1, e2, e4 and e5 enhancer-targeted CRISPRi, n=2,3,2,2 consisting of 2.5 million cultured cortical neurons in each of the trials (CTL un, CTL KCl or BDNF).	Fig. 5b legend	error bars are mean +/- SEM	Fig. 5b legend	c-fos eRNA: e1 p=0.0151 t(2)=8.037 F=4.147 (sdP=0.2906) e2 p=0.0393 t(4)=3.015 F=3.461 (sdP=0.2242) e4 p=0.0206 t(2)=6.864 F=25.000 (sdP=0.1257) e5 p=0.0104 t(2)=9.711 F=32.398 (sdP=0.1107)	Fig. 5b legend		

+ -	5c	Student's unpaired t-test	Fig. 5c legend	KCl: 3,5,2,2 BDNF: 2,2,2,2	For each IEG group stimulated by KCl n=5 and for each IEG group stimulated by BDNF n=2. Each trial consisted of 2.5 million cultured cortical neurons.	Fig. 5c legend	error bars are mean +/- SEM	Fig. 5c legend	KCl: CRISPRi e2 pre-mRNA p=0.0433 t(4)=2.918 F=1.388 (sdP=0.4187) CRISPRi e2 mRNA p=0.0102 t(8)=3.340 F=1.114 (sdP=0.459) CRISPRi e5 pre-mRNA p=0.0077 t(2)=11.350 F=3.550 (sdP=0.3106) CRISPRi e5 mRNA p=0.0207 t(2)=6.845 F=1.122 (sdP=0.4817) BDNF CRISPRi e4 pre-mRNA p=0.0485 t(2)=1.807 F=1.388 (sdP=0.4868) CRISPRi e4 mRNA p=0.0322 t(2)=5.440 F=137.97 (sdP=0.0541) CRISPRi e5 pre-mRNA p=0.0489 t(2)=4.353 F=17.288 (sdP=0.1503) CRISPRi e5 mRNA p=0.0223 t(2)=6.582 F=26.669 (sdP=0.1218)	Fig. 5c legend	
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+ -	6a	unpaired t-test with Welch correction	Fig. 6a legend	5	For c-fos mRNA and eRNA level in each brain region group (Cortex, Hippocampus, Cerebellum) n=5 mice.	Fig. 6a legend	error bars are mean +/- SEM	Fig. 6a legend	c-fos mRNA Cortex: p=0.0458 t(2)=4.511 Hippocampus: p=0.0067 t(5)=4.455 Cerebellum: p=0.0133 t(2)=47.767 c-fos eRNA Cortex e4 p=0.0150 t(4)=4.084 e5 p=0.034 t(4)=3.154 Hippocampus e1 p=0.0386 t(4)=3.036 e2 p=0.0419 t(4)=2.717 e4 p=0.0211 t(4)=3.686 e5 p=0.0028 t(4)=5.454	Fig. 6a legend		
+ -	6b	unpaired t-test with Welch correction	Fig. 6b legend	3	For c-fos mRNA fold induction and eRNA level in the visual cortex, n=3 mice.	Fig. 6b legend	error bars are mean +/- SEM	Fig. 6b legend	Visual cortex c-fos mRNA p=0.0069 t(2)=11.966 c-fos eRNA e5 p=0.0485 t(4)=2.806	Fig. 6c legend		
+ -	7b	Student's unpaired t-test / unpaired t-test with Welch correction	Fig. 7b legend	4,4	For c-fos mRNA and eRNA induction in the hippocampus following BDNF injection n=4 per group (mice)	Fig. 7b legend	error bars are mean +/- SEM	Fig. 7b legend	c-fos mRNA - Hippocampus: BDNF p=0.0236 t(3)=4.267 c-fos eRNA Hippocampus: e4 p=0.0001 t(6)=10.373 F=5.082 (sdP=0.1074) e5 p=0.0402 t(3)=3.475	Fig. 7b legend		
+ -	7c	Student's unpaired t-test	Fig. 7c legend	3,3,3,3	For c-fos mRNA and eRNA induction in the hippocampus following KA induced seizure n=3 mice per group	Fig. 7c legend	error bars are mean +/- SEM	Fig. 7c legend	c-fos mRNA - Hippocampus: WT-KA vs. BDNF KO-KA p=0.0216 t(4)=3.660 F=3.756 (sdP=0.2103) c-fos eRNA - Hippocampus: e4 p=0.0476 t(4)=2.825 F=10.113 (sdP=0.0900)	Fig. 7c legend		

+ -	S2a	Student's unpaired t-test	SFig. 2a legend	3,3,3,3	For each group of NIH 3T3 cell eRNA and mRNA level, n=3 and was measured at 4 different time-points.	SFig. 2a legend	error bars are mean +/- SEM	SFig. 2a legend		SFig. 2a legend		
+ -	S2b	unpaired t-test with Welch correction	SFig. 2b legend	3,3	For each individual enhancer activity measured by luciferase activity in NIH 3T3 cells, n=3.	SFig. 2b legend	error bars are mean +/- SEM	SFig. 2b legend	Serum: e5 p=0.0075 t(2)=11.513	SFig. 2b legend		
+ -	S4	unpaired t-test with Welch correction	SFig. 4 legend	3,3,3,3,3,3,3	For fold induction of mRNA, n=3 in each group (SRF, MEF2C, CREB, MEF2A, NPAS4 and MEF2D). Each trial consisted of 2.5 million cultured cortical neurons.	SFig. 4 legend	error bars are mean +/- SEM	SFig. 4 legend	CREB: shCREB p=0.0031 t(2)=18.040 MEF2A: shMEF2A p=0.0029 t(2)=18.616 MEF2C: shMEF2C p=0.0019 t(2)=22.975 MEF2D: shMEF2D p=0.0012 t(2)=28.633 NPAS4: shNPAS4 p=0.0019 t(2)22.709 SRF: shSRF p=0.0014 t(2)=26.942	SFig. 4 legend		
+ -	S5	Student's unpaired t-test	SFig. 5 legend	3,3,3,3,3,3	For c-fos eRNA and mRNA level in each knockdown group (SRF, MEF2C and MEF2D) and stimulation condition, n=3. Each trial consisted of 2.5 million cultured cortical neurons.	SFig. 5 legend	error bars are mean +/- SEM	SFig. 5 legend				
+ -	S6	Student's unpaired t-test	SFig. 6 legend	3	For each group of (MEF2A, MEF2D and MEF2C) mRNAs, n=3. Each trial consisted of 2.5 million cultured cortical neurons.	SFig. 6 legend	error bars are mean +/- SEM	SFig. 6 legend				
+ -	S7b	unpaired t-test with Welch correction	SFig. 7b legend	2,2	For each the cortical neuron culture and glial cell culture the trials consisted of 2.5 million cultured cells (n=2).	SFig. 7b legend	error bars are mean +/- SEM	SFig. 7b legend	Cortical neuron culture: p=0.0001 t(3)=37.044 Glial cell culture: p=0.0089 t(3)=6.084	SFig. 7b legend		

+	S7c	Student's unpaired t-test	SFig. 7c legend	2,2,2,2,2,2	Expression of c-fos eRNA and mRNA in glial cells induced by KCl, BDNF, and Forskolin consisting of 2.5 million glial cells (n=2).	SFig. 7c legend	error bars are mean +/- SEM	SFig. 7c legend	<p>c-fos mRNA: KCl p=0.0239 t(2)=6.346 F=1.356 (sdP=0.4517) BDNF p=0.0286 t(2)=5.787 F=28.667 (sdP=0.1175) Forskolin p=0.0165 t(2)=7.690 F=50.055 (sdP=0.0894) c-fos eRNA: BDNF: e1 p=0.0283 t(2)=5.817 F=36.979 (sdP=0.1038) e2 p=0.0345 t(2)=5.240 F=11.137 (sdP=0.1853) e3 p=0.0195 t(2)=7.063 F=3.634 (sdP=0.3076) e4 p=0.0064 t(2)=12.464 F=17.529 (sdP=0.1493) e5 p=0.0025 t(2)=19.845 F=961.36 (sdP=0.0571) .Forskolin: e2 p=0.0312 t(2)=5.527 F=26.532 (sdP=0.1221) e3 p=0.0092 t(2)=10.368 F=2.869 (sdP=0.3395) e4 p=0.0042 t(2)=15.928 F=10.024 (sdP=0.1948) e5 p=0.0096 t(2)=10.109 F=1.775 (sdP=0.4099)</p>
+	S8	Student's unpaired t-test	SFig. 8 legend	3,3,3,3	For each group of (Arc, Egr-1) mRNAs and KCl stimulation condition, n=3.	SFig. 8 legend	error bars are mean +/- SEM	SFig. 8 legend	<p>Arc mRNA: p=0.0481 t(4)=2.814 F=6.896 (sdP=0.1267)</p>

+ -	S9	Student's unpaired t-test / unpaired t-test with Welch correction	SFig. 9 legend	2,2,2	For each stimulation condition (KCl, BDNF, Forskolin) cultures consisted of 2.5 million cortical neurons (n=2)	SFig. 9 legend	error bars are mean +/- SEM	SFig. 9 legend	MEF2A Binding: KCl: e2 p=0.0466 t(1)=13.632 e5 p=0.0180 t(2)=8.355 F=6.548 (sdP=0.2372) P p=0.0228 t(2)=6.514 F=150.94 (sdP=0.0517) BDNF: e1 p=0.0135 t(2)=8.507 F=10.418 (sdP=0.1913) e5 p=0.0419 t(2)=4.733 F=19.313 (sdP=0.1432) P p=0.0121 t(2)=8.997 F=136.89 (sdP=0.0543) Forskolin: P p=0.0182 t(2)=7.313 F=90.055 (sdP=0.0668)	SFig. 9 legend		
+ -	S10	Student's unpaired t-test	SFig. 10 legend	3	For e2 enhancer-targeted CRISPRi effect on other c-fos eRNA, n=3 consisting of 2.5 million cultured cortical neurons in each trial (CTL un, CTL KCl, CRISPRi e2 un, CRISPRi e2 KCl).	SFig. 10 legend	error bars are mean +/- SEM	SFig. 10 legend	c-fos e2 eRNA: p=0.0393 t(4)=3.015 F=3.461 (sdP=0.2242)	SFig. 10 legend		
+ -	S11	Student's unpaired t-test	SFig. 11 legend	2	For cross-linking frequency of the c-fos enhancer 5 and promoter cultures consisted of 2.5 million cortical neurons for each condition CTL un, CTL KCl, CRISPRi e5 un, CRISPRi e5 KCl) (n=2)	SFig. 11 legend	error bars are mean +/- SEM	SFig. 11 legend	e5: un vs. KCl p=0.0117 t(2)=9.144 F=2.420 (sdP=0.3637) KCl vs. CRISPRi e5 KCl p=0.0453 t(2)=4.539 F=1.398 (sdP=0.4470)	SFig. 11 legend		
+ -	S12a	Student's unpaired t-test	SFig. 12a legend	2,2	For e1 enhancer-targeted CRISPRi effect on various IEGs, n=2 consisting of 2.5 million cultured cortical neurons in each trial.	SFig. 12a legend	error bars are mean +/- SEM	SFig. 12a legend				

+ -	S12b	Student's unpaired t-test	SFig. 12b legend	2,2	For e2 enhancer-targeted CRISPRi effect on various IEGs, n=2 consisting of 2.5 million cultured cortical neurons in each trial.	SFig. 12b legend	error bars are mean +/- SEM	SFig. 12b legend				
+ -	S12c	Student's unpaired t-test	SFig. 12c legend	2,2	For e4 enhancer-targeted CRISPRi effect on various IEGs, n=2 consisting of 2.5 million cultured cortical neurons in each trial.	SFig. 12c legend	error bars are mean +/- SEM	SFig. 12c legend				
+ -	S12d	Student's unpaired t-test	SFig. 12d legend	2,2	For e5 enhancer-targeted CRISPRi effect on various IEGs, n=2 consisting of 2.5 million cultured cortical neurons in each trial.	SFig. 12d legend	error bars are mean +/- SEM	SFig. 12d legend				
+ -	S13b	Student's unpaired t-test	SFig. 13b legend	2,2,2	For expression of PELL1 mRNA and eRNA in cortical neurons 2.5 million cultured cortical neurons were used in each trial (un, KCL, BDNF) (n=2).	SFig. 13b legend	error bars are mean +/- SEM	SFig. 13b legend	PELL1 mRNA: KCL p=0.0076 t(2)=11.430 F=2.346 (sdP=0.3682) BDNF p=0.0388 t(4)=4.930 F=13.471 (sdP=0.1693) PELL1 eRNA KCL p=0.0318 t(2)=5.477 F=1.154 (sdP=0.4773) BDNF p=0.0392 t(2)=4.900 F=63.601 (sdP=0.080)	SFig. 13b legend		

+ -	S14b	Student's unpaired t-test	SFig. 14b legend	2,2,2	For expression of IGF-1 mRNA and eRNA in cortical neurons 2.5 million cultured cortical neurons were used in each trial (un, KCL, BDNF) (n=2).	SFig. 14b legend	error bars are mean +/- SEM	SFig. 14b legend	IGF-1 mRNA: un vs. KCl 6h p=0.0317 t(2)=5.486 F=3.435 (sdP=0.3150) un vs. BDNF 6h p=0.005 t(2)=14.092 F=1.686 (sdP=0.4178) IGF-1 eRNA: KCl: e2: un vs. KCl 6h p=0.0280 t(2)=5.852 F=2.903 (sdP=0.3379) e3: un vs KCl 6h p=0.0465 t(2)=4.520 F=1.161 (sdP=0.4762) BDNF: e1: un vs. BDNF 6h p=0.0224 t(2)=6.570 F=3.354 (sdP=0.3182)	SFig. 14b legend			
+ -	S15	Model	Sfig. 15 legend										
+ -	S16	Full blots of the western blots shown the figure	Sfig. 16 legend										

► Representative figures

1. Are any representative images shown (including Western blots and immunohistochemistry/staining) in the paper?

If so, what figure(s)?

Yes, supplementary figure 6 and 16

2. For each representative image, is there a clear statement of how many times this experiment was successfully repeated and a discussion of any limitations in repeatability?

If so, where is this reported (section, paragraph #)?

Yes, in the legend for supplementary figure 6.

► Statistics and general methods

1. Is there a justification of the sample size?

If so, how was it justified?

Where (section, paragraph #)?

Even if no sample size calculation was performed, authors should report why the sample size is adequate to measure their effect size.

We used sample sizes that are similar to those used in the field, and this is stated under Statistical analyses in the methods section.

2. Are statistical tests justified as appropriate for every figure?

Where (section, paragraph #)?

Yes.

Figures 1, 2, 3, 5, 6, 7, and Supplementary Figures 2,4,5,6,7,8,9,10,11,12,13,14.

- a. If there is a section summarizing the statistical methods in the methods, is the statistical test for each experiment clearly defined?

Yes.

- b. Do the data meet the assumptions of the specific statistical test you chose (e.g. normality for a parametric test)?

Where is this described (section, paragraph #)?

Data distribution was assumed to be normal but this was not formally tested. This is stated under Statistical analyses in the methods section, paragraph 12.

- c. Is there any estimate of variance within each group of data?

Is the variance similar between groups that are being statistically compared?

Where is this described (section, paragraph #)?

Yes, the variance within each group was calculated. If the variance between the groups being statistically compared was not similar, then an unpaired t-test with a Welch correction was used in place of a standard unpaired student's t-test. This is stated under Statistical analyses in the methods section, paragraph 12.

- d. Are tests specified as one- or two-sided?

Two-sided

- e. Are there adjustments for multiple comparisons?

Yes

3. Are criteria for excluding data points reported?

Was this criterion established prior to data collection?

Where is this described (section, paragraph #)?

No, there were no data points excluded.

4. Define the method of randomization used to assign subjects (or samples) to the experimental groups and to collect and process data.

If no randomization was used, state so.

Where does this appear (section, paragraph #)?

Randomization was not used. This is stated under Statistical analyses in the methods section, paragraph 12.

5. Is a statement of the extent to which investigator knew the group allocation during the experiment and in assessing outcome included?

If no blinding was done, state so.

Where (section, paragraph #)?

Blinding was not done. This is stated under Statistical analyses in the methods section, paragraph 12.

6. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included?
Where (section, paragraph #)?
- All animal experiments performed in this study were reviewed and approved by the IACUC committee at UT Southwestern Medical Center. It is stated at the beginning of the methods section.
7. Is the species of the animals used reported?
Where (section, paragraph #)?
- Yes. Reported under the Methods section, paragraph 6.
8. Is the strain of the animals (including background strains of KO/transgenic animals used) reported?
Where (section, paragraph #)?
- Yes. Reported under the Methods section, paragraph 6.
9. Is the sex of the animals/subjects used reported?
Where (section, paragraph #)?
- Yes. Reported under the Methods section, paragraph 6.
10. Is the age of the animals/subjects reported?
Where (section, paragraph #)?
- Yes. Reported under the Methods section, paragraph 6.
11. For animals housed in a vivarium, is the light/dark cycle reported?
Where (section, paragraph #)?
- Yes. Reported under the Methods section, paragraph 6.
12. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported?
Where (section, paragraph #)?
- No.
13. For behavioral experiments, is the time of day reported (e.g. light or dark cycle)?
Where (section, paragraph #)?
- Yes. Reported under the Methods section, paragraph 6.
14. Is the previous history of the animals/subjects (e.g. prior drug administration, surgery, behavioral testing) reported?
Where (section, paragraph #)?
- Yes. Reported under the Methods section, paragraph 6.
- a. If multiple behavioral tests were conducted in the same group of animals, is this reported?
Where (section, paragraph #)?
- No.
15. If any animals/subjects were excluded from analysis, is this reported?
Where (section, paragraph #)?
- No animals were excluded from analysis.
- a. How were the criteria for exclusion defined?
Where is this described (section, paragraph #)?
- N/A

- b. Specify reasons for any discrepancy between the number of animals at the beginning and end of the study.

N/A

Where is this described (section, paragraph #)?

► Reagents

1. Have antibodies been validated for use in the system under study (assay and species)?

Yes, the antibodies have been validated.

- a. Is antibody catalog number given?

Where does this appear (section, paragraph #)?

The antibody catalog numbers are not mentioned in the paper. However, they are:

Tuj-1 covance #801201
 GFAP Sigma #G3893
 alexa 488 Invitrogen #A21202
 alexa 555 Invitrogen #A31570
 b-actin Sigma #5441
 Mef2c Proteintech #10056-AP

- b. Where were the validation data reported (citation, supplementary information, Antibodypedia)?

Where does this appear (section, paragraph #)?

The validation data of each antibody is listed in corresponding vendor's webpage. For MEF2A/D antibodies, see Flavell et al. Neuron 60, 1022-1038 (2008).

2. Cell line identity

- a. Are any cell lines used in this paper listed in the database of commonly misidentified cell lines maintained by [ICLAC](#) and [NCBI Biosample](#)?

Where (section, paragraph #)?

No.

- b. If yes, include in the Methods section a scientific justification of their use--indicate here in which section and paragraph the justification can be found.

- c. For each cell line, include in the Methods section a statement that specifies:

- the source of the cell lines
- have the cell lines been authenticated? If so, by which method?
- have the cell lines been tested for mycoplasma contamination?

Where (section, paragraph #)?

NIH 3T3 cells

Source: American Type Culture Collection (ATCC Manassas, VA, USA)

Authentication: DNA Sanger Sequencing Multiplex PCR Assay

Test: The cells have not been tested for mycoplasma contamination.

Methods section paragraph 5.

▶ Data deposition

Data deposition in a public repository is mandatory for:

- a. Protein, DNA and RNA sequences
- b. Macromolecular structures
- c. Crystallographic data for small molecules
- d. Microarray data

Deposition is strongly recommended for many other datasets for which structured public repositories exist; more details on our data policy are available [here](#). We encourage the provision of other source data in supplementary information or in unstructured repositories such as [Figshare](#) and [Dryad](#).

We encourage publication of Data Descriptors (see [Scientific Data](#)) to maximize data reuse.

1. Are accession codes for deposit dates provided?

Where (section, paragraph #)?

N/A

▶ Computer code/software

Any custom algorithm/software that is central to the methods must be supplied by the authors in a usable and readable form for readers at the time of publication. However, referees may ask for this information at any time during the review process.

1. Identify all custom software or scripts that were required to conduct the study and where in the procedures each was used.

2. If computer code was used to generate results that are central to the paper's conclusions, include a statement in the Methods section under "**Code availability**" to indicate whether and how the code can be accessed. Include version information as necessary and any restrictions on availability.

▶ Human subjects

1. Which IRB approved the protocol?

Where is this stated (section, paragraph #)?

2. Is demographic information on all subjects provided?

Where (section, paragraph #)?

3. Is the number of human subjects, their age and sex clearly defined?

Where (section, paragraph #)?

4. Are the inclusion and exclusion criteria (if any) clearly specified?

Where (section, paragraph #)?

5. How well were the groups matched?

Where is this information described (section, paragraph #)?

6. Is a statement included confirming that informed consent was obtained from all subjects?

Where (section, paragraph #)?

7. For publication of patient photos, is a statement included confirming that consent to publish was obtained?

Where (section, paragraph #)?

► fMRI studies

For papers reporting functional imaging (fMRI) results please ensure that these minimal reporting guidelines are met and that all this information is clearly provided in the methods:

1. Were any subjects scanned but then rejected for the analysis after the data was collected?

a. If yes, is the number rejected and reasons for rejection described?

Where (section, paragraph #)?

2. Is the number of blocks, trials or experimental units per session and/or subjects specified?

Where (section, paragraph #)?

3. Is the length of each trial and interval between trials specified?

4. Is a blocked, event-related, or mixed design being used? If applicable, please specify the block length or how the event-related or mixed design was optimized.

5. Is the task design clearly described?

Where (section, paragraph #)?

6. How was behavioral performance measured?

7. Is an ANOVA or factorial design being used?

8. For data acquisition, is a whole brain scan used?

If not, state area of acquisition.

a. How was this region determined?

9. Is the field strength (in Tesla) of the MRI system stated?
- a. Is the pulse sequence type (gradient/spin echo, EPI/spiral) stated?
- b. Are the field-of-view, matrix size, slice thickness, and TE/TR/flip angle clearly stated?
10. Are the software and specific parameters (model/functions, smoothing kernel size if applicable, etc.) used for data processing and pre-processing clearly stated?
11. Is the coordinate space for the anatomical/functional imaging data clearly defined as subject/native space or standardized stereotaxic space, e.g., original Talairach, MNI305, ICBM152, etc? Where (section, paragraph #)?
12. If there was data normalization/standardization to a specific space template, are the type of transformation (linear vs. nonlinear) used and image types being transformed clearly described? Where (section, paragraph #)?
13. How were anatomical locations determined, e.g., via an automated labeling algorithm (AAL), standardized coordinate database (Talairach daemon), probabilistic atlases, etc.?
14. Were any additional regressors (behavioral covariates, motion etc) used?
15. Is the contrast construction clearly defined?
16. Is a mixed/random effects or fixed inference used?
- a. If fixed effects inference used, is this justified?
17. Were repeated measures used (multiple measurements per subject)?
- a. If so, are the method to account for within subject correlation and the assumptions made about variance clearly stated?
18. If the threshold used for inference and visualization in figures varies, is this clearly stated?
19. Are statistical inferences corrected for multiple comparisons?
- a. If not, is this labeled as uncorrected?

20. Are the results based on an ROI (region of interest) analysis?

a. If so, is the rationale clearly described?

b. How were the ROI's defined (functional vs anatomical localization)?

21. Is there correction for multiple comparisons within each voxel?

22. For cluster-wise significance, is the cluster-defining threshold and the corrected significance level defined?

► Additional comments

Additional Comments