## **Supplementary Material**

## Chronic Lithium Treatment Alters NMDA and AMPA Receptor Synaptic Availability and Dendritic Spine Organization in the Rat Hippocampus

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**Supplementary figure 1**. Cropped immunoblot related to the expression levels of GluN2A, GluN2B, GluN1, Neuroligin-1, GluA1, GluA2, PSD95, pCaMKII<sub>Thr286</sub>, CaMKII,  $\beta$ -actin measured in the **post-synaptic density** of the Hippocampus of rats sacrificed 24 h after the last injection of NaCl or LiCl, presented in figures 1, 3, 4.

250-	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
150-	-	-	-	-	-				-	-	-	-		-	-	-		
250-	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
150-		***	-	-	-	-	-	-	-	-	**		-					**
150- 150-	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
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5 <b>0</b> -	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
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	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
0-		-	-			-	-	-	-		-	-	-	-	-		-	
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	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
)-	1	2	1	2	1	2	1	2	-		-	2	-	2	1	2	1	2
	÷	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
0-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	••
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
)-	-	-	-	-	-	-	-	-	-	-		-	-		-		-	-
	1	2	. 1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
7-	-					-	-		-	-	-	-	-	-	-	-	-	-

**Supplementary figure 2**. Cropped immunoblot related to the expression levels of GluN2A, GluN2B, GluN1, Neuroligin-1, GluA1, GluA2, PSD95, pCaMKII<sub>Thr286</sub>, CaMKII,  $\beta$ -actin measured in **the post-synaptic density** of the Hippocampus of rats sacrificed 24 hours and 7 days after the last injection of NaCl or LiCl, presented in figures 1, 3, 4.

								,						,							, 1				0	1= NaCl 24 h 2= NaCl 7d
250-	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	2	3	2	3	2	3		3= LiCl 7d
150- 250-	1	2	3	1	2	3	-	2	3		-		-		3			- •		2	2	2	2	3	GluN2A (180 kDa)	
	-	2							5	-	2	5	1	-	5	-		5	2	•••					GluN2B (180 kDa)	
150-	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	2	3	2	3	2	3		
150- 100-	18.8	-										-				-	-	-	-		-	-	-		GluN1 <mark>(</mark> 120 kDa)	
150-	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	2	3	2	3	2	3		
				-						**	**	**	**	**	-	**	**	**	14	14	- 4	11	**	**	Neuroligin-1 (120 kDa)	
100-	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	2	3	2	3	2	3		
100-			-	-	-	-	-	-	-		-	-	-	-		-	-	-	-	-	-	-	-		GluA1 (108 kDa)	
100-	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	2	3	2	3	2	3		
					-										2					-	ς.	-	-		GluA2 (108 kDa)	
100-	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	2	3	2	3	2	3		
	-	-	-	-	-	-	-	-	e	-	-	-	-	-	-	-	•	-	-	-	-	-	•	-	PSD95 (95 kDa)	
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	2	3	2	3	2	3		
50-	••		•	••	•											••	•	-					••	**	pCaMKII <sub>Thr286</sub> (50 kDa)	
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	2	3	2	3	2	3		
50-		-		-			-	-	-	-									-	-			-	**	CaMKII (50 kDa)	
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	2	3	2	3	2	3	$\theta_{actin}(42 k D_{a})$	
37-	-		-												-					-	-		-		$\beta$ -actin (43 kDa)	

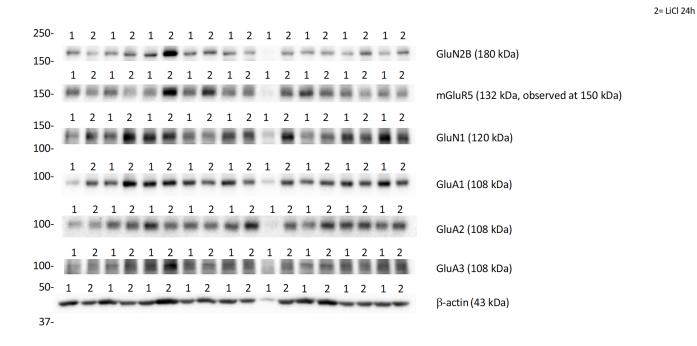
Supplementary figure 3. Cropped immunoblot related to the expression levels of GluA3 and  $\beta$ -actin measured in the **post-synaptic density** of the Hippocampus of rats sacrificed 24 hours (a) and 7 days (b) after the last injection of NaCl or LiCl, presented in figure 3.

a)	100-	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	*	Glu	A3	(102	2 kD	a)		1= NaCl 24 h 2= LiCl 24 h
	37-	1		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	-	β-a	ctin	(43	kDa	a)		
b)	100-	1	2	-	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	2	3	**	**	••	•	GluA3 (102 kDa)	1= NaCl 24 h 2= NaCl 7d
	37-	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	2	3	2	3	2	•••	$\beta$ -actin (43 kDa)	3= LiCl 7d

1= NaCl 24h

1= NaCl 24 h

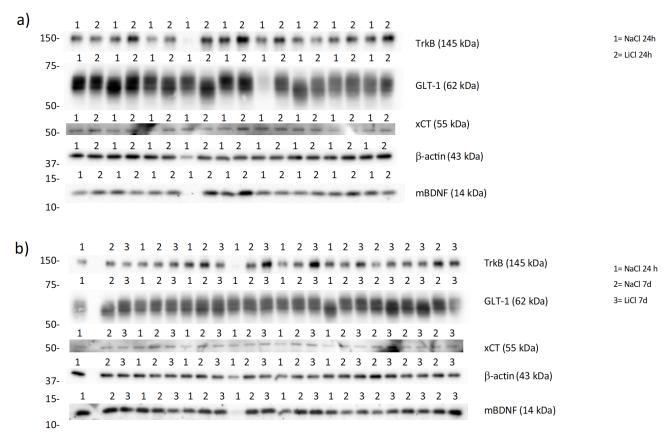
**Supplementary figure 4**. Cropped immunoblot related to the expression levels of GluN2B, mGluR5, GluN1, GluA1, GluA2, GluA3,  $\beta$ -actin measured in the **extra-synaptic fraction** of Hippocampus of rats sacrificed 24 h after the last injection of NaCl or LiCl, presented in figures 1, 3, 4.



**Supplementary figure 5**. Cropped immunoblot related to the expression levels of GluN2B, mGluR5, GluN1, GluA1, GluA2, GluA3,  $\beta$ -actin measured in the **extra-synaptic fraction** of Hippocampus of rats sacrificed 24 and 7 days after the last injection of NaCl or LiCl, presented in figures 1, 3, 4

250-	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	2	3	2	3	2	3	2= NaCl 7d 3= LiCl 7d
150-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	GluN2B (180 kDa)
150-	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	2	3	2	3	2	3	mGluR5 (132 kDa, observed at 150 kDa)
130-	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	2	3	2	3	2	3	
100-	н.		•	•	**	**	*	-	•	÷.	-	-	H	-	-	7	**	-	•	-	•	•	-	•	GluN1 (120 kDa)
100-	1	2	3	1	2	3	-	2	-	-	-	3	-	-	-	-	-	3	2	-	-	3	2	3	GluA1 (108 kDa)
100-	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	2	3	2	3	2	3	GluA2 (108 kDa)
100	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	2	3	2	3	2	3	
100-		-	•	•	-	*	-	-	-	8	H	ú	8	iii.	2	4	**	65	ņ	4	•	•	*		GluA3 (108 kDa)
37-	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	2	3	2	3	2	3	β-actin (43 kDa)

**Supplementary figure 6**. Cropped immunoblot related to the expression levels of TrkB, GLT-1, xCT,  $\beta$ -actin, and mBDNF measured in the **homogenate** of the Hippocampus of rats sacrificed 24 hours (a) and 7 days (b) after the last injection of NaCl or LiCl, presented in figure 2.



**Supplementary figure 7**. Cropped immunoblot related to the expression levels of pCREB<sub>Ser133</sub>, CREB, and  $\beta$ -actin measured in the **nuclear fraction** of the Hippocampus of rats sacrificed 24 hours (a) and 7 days (b) after the last injection of NaCl or LiCl, presented in figure 2.

a)		2	1	2	1	2	1	2	2	1	2	1	2	1	L	2	1	2	1	2							1= NaCl 24 h
	37-		-	**	P	-	•	1	11	10	14	-		1	1	1	1	•	-	Ŀ			pCl	REB	Ser13	<sub>3</sub> (43 kDa)	2= LiCl 24 h
		2	1	2	1	2	1	2		1	2	1	2	1	. 2	2	1	2	1	2							
	37-		-	••	**	-	•	• •	• •		**	-	***	-	••	•••	•	••	**	**			С	REB	(43	kDa)	
		2	1	2	1	2	1	2		1	2	1	2	1	2	2	1	2	1	2							
	37-	-	-	-	-	-	-	-		-	-	-	-	-	-	•••	-	-	-	-			β	-act	in (4	3 kDa)	
	57-																										
b)		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	2	3	2	3	2	3		1= NaCl 24 h
	37-	łł.		B		**	н	44	Ŀł.	Đ.		66	6	**	ķā i		44	н	łł.	tt	**	1	1	1	-	pCREB <sub>Ser133</sub> (43 kDa)	2= NaCl 7d
	0,	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	2	3	2	3	2	3		3= LiCl 7d
	37-	**	•		**	••	**	**		**	••	-		••	**	-	**	**	**	••	••	-	-		***	CREB (43 kDa)	
	57	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	2	3	2	3	2	3		
	37-	-	-	-	-	-	-	-	-	-	-	-	-	~	-	-	-	-	~	-	-	-	-	-	-	β-actin (43 kDa)	

Supplementary Table 1. Effects of LiCl treatment on dendritic spine morphology in the hippocampus of rats sacrificed 24 hours or 7 days after the last exposure. Table (a) shows the percentage of thin-, stubby-shaped spines and filopodia, expressed as mean percentage  $\pm$  mean standard error. Table (b) shows the length and head width of thin- and stubby-shaped spines, expressed as  $\mu m \pm$  mean standard error.

## a)

	%	Гhin	% Stu	bby	% filopodia			
	NaCl	LiCl	NaCl	LiCl	NaCl	LiCl		
24 hours after the last treatment	13,63 ± 3,71	13,70 ± 4,03	21,03 ± 2,80	21,09 ± 2,22	6,14 ± 2,68	4,82 ± 2,09		
7 days after the last treatment	7,45 ± 1,98	12,22 ± 3,50	22,31 ± 1,94	21,72 ± 1,24	6,22 ± 1,84	4,74 ± 2,06		

## b)

		Thin	(μm)			Stubb	y (μm)	
	Na	Cl	Li	Cl	Na	ıCl	Li	Cl
	Spine length	Head width	Spine length	Head width	Spine length	Head width	Spine length	Head width
24 hours after the last treatment	1,27 ±0,04	0,51 ± 0,05	1,19 ± 0,07	0,43 ±0,02	0,80 ± 0,01	0,41 ±0,02	0,79 ± 0,01	0,37 ±0,02
7 days after the last treatment	1,25 ±0,02	0,57 ±0,09	1,27 ±0,05	0,53 ±0,03	0,76 ± 0,01	0,36 ±0,01	0,78 ± 0,01	0,37 ±0,01