

Valproic Acid Exposure During Gestation Alters Synaptic Plasticity and Klotho Expression in the Frontal Cortex

Bianca Andrade Rodrigues¹, Josiane do Nascimento Silva¹, Diana Zukas Andreotti^{1,2},
Thiago Amorim de Souza Lima¹, Cristoforo Scavone², Elisa Mitiko Kawamoto¹

¹Laboratory of Molecular and Functional Neurobiology, Department of Pharmacology,
Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil.

²Laboratory of Molecular Neuropharmacology, Department of Pharmacology, Institute of
Biomedical Sciences, University of São Paulo, São Paulo, Brazil

Corresponding author:

Name: Elisa Mitiko Kawamoto

University of São Paulo

Av. Prof. Lineu Prestes, 1524.

05508-000, São Paulo, SP, Brazil

Phone: +55 (11) 2648-1379

Email: elisamk@usp.br

ORCID

Bianca Andrade Rodrigues: 0000-0002-6542-1629

Josiane do Nascimento Silva: 0000-0001-8102-1177

Diana Zukas Andreotti: 0000-0002-7905-1032

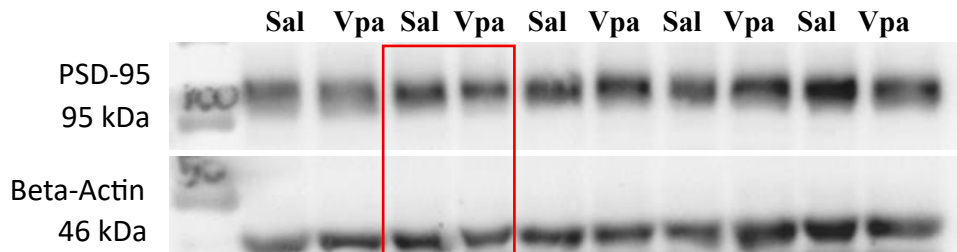
Thiago Amorim de Souza Lima: 0000-0002-78140025

Cristoforo Scavone: 0000-0002-1206-0882

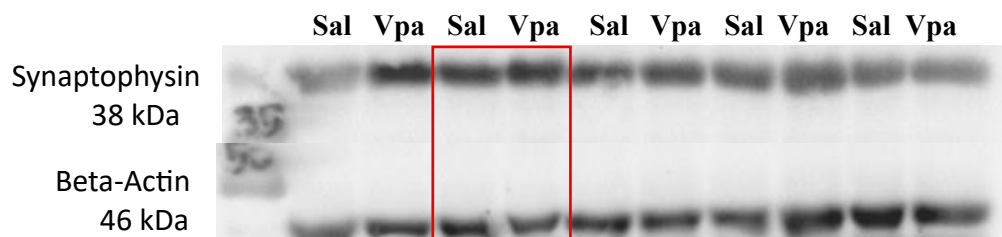
Elisa Mitiko Kawamoto: 0000-0001-8637-414X

Supplementary material

1) Western Blotting – Hippocampus

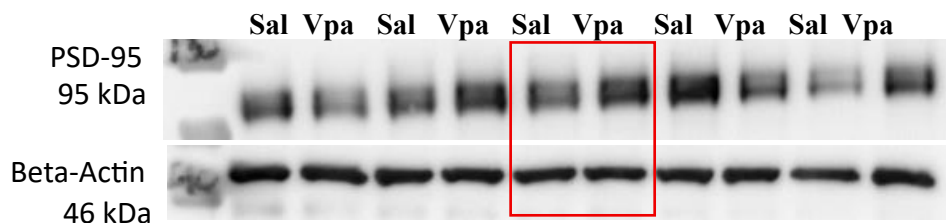


Supplementary Figure S1. Western Blotting membrane images of expression of PSD-95 protein in the hippocampus n = 5 (male). The records of the complete membranes are provided in Supplementary Information Figure 1. **The red boxes refer to the representative images used in the manuscript.** Abbreviations: Sal: Saline; VPA: valproate.

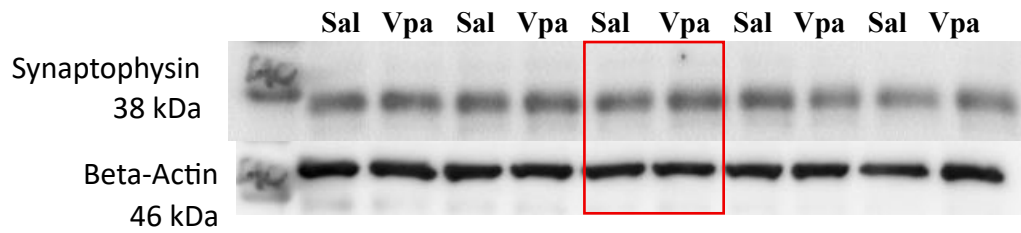


Supplementary Figure S2. Western Blotting membrane images of expression of Synaptophysin protein in the hippocampus n = 5 (male). The records of the complete membranes are provided in Supplementary Information Figure 2. **The red boxes refer to the representative images used in the manuscript.** Abbreviations: Sal: Saline; VPA: valproate.

2) Western Blotting – Cortex

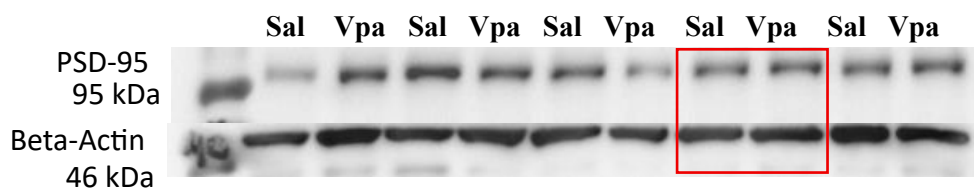


Supplementary Figure S3. Western Blotting membrane images of expression of PSD-95 protein in the cortex n = 5 (male). The records of the complete membranes are provided in Supplementary Information Figure 3. **The red boxes refer to the representative images used in the manuscript.** Abbreviations: Sal: Saline; VPA: valproate.

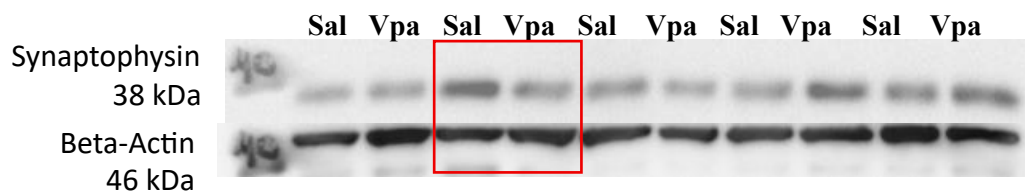


Supplementary Figure S4. Western Blotting membrane images of expression of Synaptophysin protein in the cortex n = 5 (male). The records of the complete membranes are provided in Supplementary Information Figure 4. **The red boxes refer to the representative images used in the manuscript.** Abbreviations: Sal: Saline; VPA: valproate.

3) Western Blotting – Cerebellum



Supplementary Figure S5. Western Blotting membrane images of expression of PSD-95 protein in the cerebellum n = 5 (male). The records of the complete membranes are provided in Supplementary Information Figure 5. **The red boxes refer to the representative images used in the manuscript.** Abbreviations: Sal: Saline; VPA: valproate.



Supplementary Figure S6. Western Blotting membrane images of expression of Synaptophysin protein in the cerebellum n = 5 (male). The records of the complete membranes are provided in Supplementary Information Figure 6. **The red boxes refer to the representative images used in the manuscript.** Abbreviations: Sal: Saline; VPA: valproate.

Supplementary information

Membranes of Western Blotting in the hippocampus

Figure 1: PSD-95

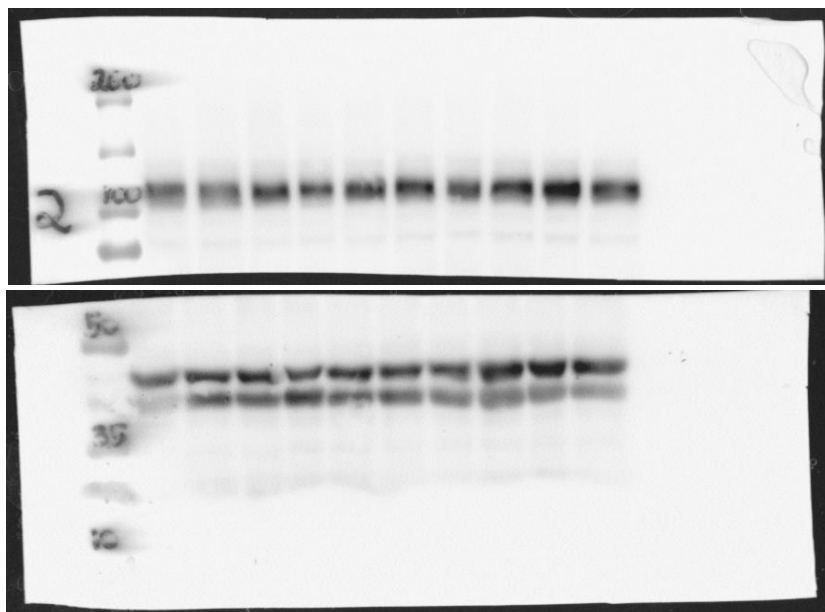
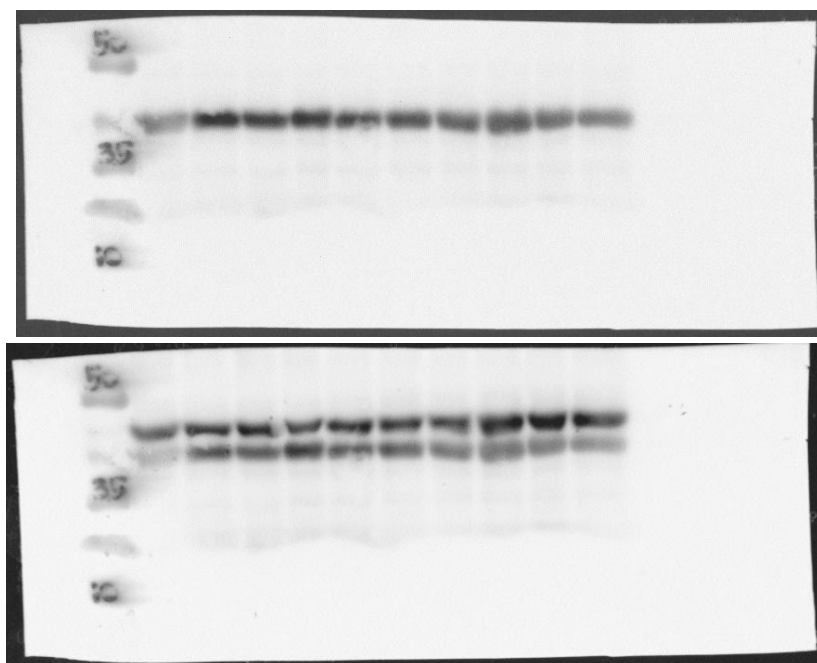


Figure 2: Synaptophysin



Membranes of Western Blotting in the cortex

Figure 3: PSD-95

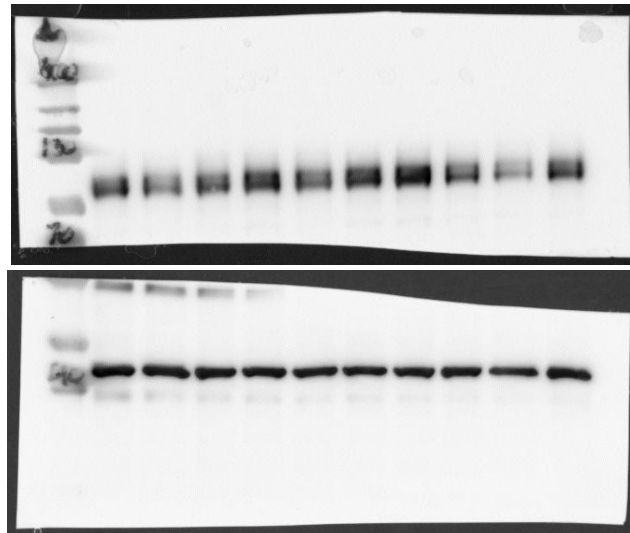
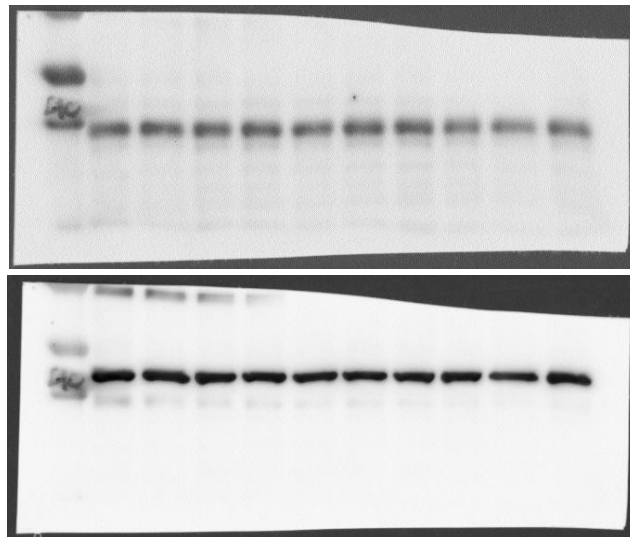


Figure 4: Synaptophysin



Membranes of Western Blotting in the cerebellum

Figure 5: PSD-95

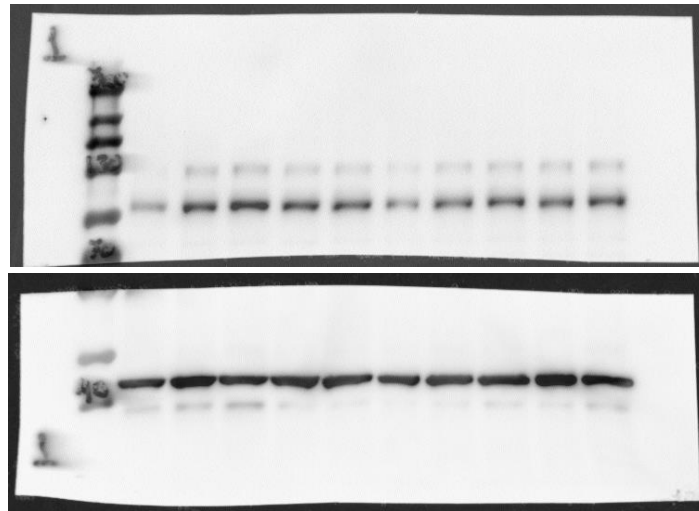
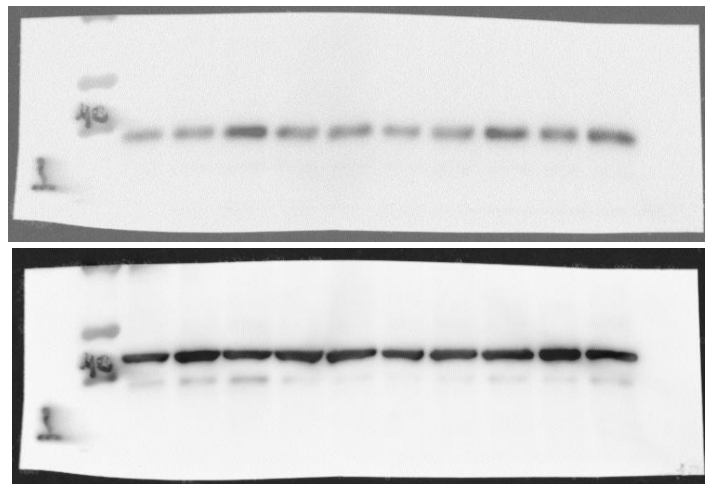


Figure 6: Synaptophysin



Supplementary tables

Table1-Statistical analysis of behavioral tests results.

Behavioral test	Parameter	Statistical test	Group	Distribution value	p value	Significant
Open field	Distance	Unpaired t-test	Sal vs. Vpa	t=0,4292, df=30	P=0.6708	Ns
Open field	Time in the center	Unpaired t-test	Sal vs. Vpa	t=1,318, df=30	P=0.1976	Ns
Open field	<i>Freezing</i>	Mann-Whitney	Sal vs. Vpa	U: 93,50	P=0.1691	Ns
Open field	Time in the periphery	Unpaired t-test	Sal vs. Vpa	t=1,330, df=30	P=0.1935	Ns
Open Field	Time of immobility	Unpaired t-test	Sal vs. Vpa	t=4,106, df=30	P=0.0003	Yes
Elevated Plus Maze	Closed arms entries	Mann-Whitney	Sal vs. Vpa	U: 93,50	P=0.1939	Ns

Elevated Plus Maze	Open arms entries	Unpaired t-test	Sal vs. Vpa	t=2,591, df=30	P=0.0146	Yes
Elevated Plus Maze	Time in closed arms	Mann-Whitney	Sal vs. Vpa	U: 64.00	P=0.0147	Yes
Elevated Plus Maze	Time in open arms	Mann-Whitney	Sal vs. Vpa	U: 65.50	P=0.0174	Yes
Social Interaction	S.I with unknown animal	Unpaired t-test	Sal vs. Vpa	t=7,240, df=30	P=<0.0001	Yes
Social Interaction	time in the unknown animal compartment	Unpaired t-test	Sal vs. Vpa	t=1,437, df=30	P=0.1611	Ns
Social Interaction	Interaction with object	Unpaired t-test	Sal vs. Vpa	t=3,637, df=30	P=0.0010	Yes
Social Memory	Preference for the known animal	Unpaired t-test	Sal vs. Vpa	t=6,763, df=30	P=<0.0001	Yes
Social Memory	Time in the compartment	Unpaired t-test	Sal vs. Vpa	t=3,474, df=30	P=0.0016	Yes
Inhibitory Avoidance	Time effect Day 1 and 2	Two-Way ANOVA	Sal vs. Vpa	F (1, 30) = 33,38	P=<0,0001	Yes

Table 2 - Statistical analysis of Western Blotting results.

Protein	Region	Statistical test	Group	Distribution value	p value	Significant
PSD-95	Hippocampus	Unpaired t-test	Sal vs. Vpa	t=0,09678, df=8	P=0.9253	Ns
Synapto	Hippocampus	Unpaired t-test	Sal vs. Vpa	t=1,296, df=8	P=0.2310	Ns
PSD-95	Cortex	Unpaired t-test	Sal vs. Vpa	t=2,306, df=8	P=0.0500	Yes
Synapto	Cortex	Unpaired t-test	Sal vs. Vpa	t=1,637, df=8	P=0,1402	Ns
PSD-95	Cerebellum	Unpaired t-test	Sal vs. Vpa	t=0,09504, df=8	P=0,9266	Yes
Synapto	Cerebellum	Mann-Whitney	Sal vs. Vpa	t=0,3270, df=8	P=0,7521	Ns

Table 3 – Statistical analysis of ELISA results.

Protein	Region	Statistical test	Group	Distribution value	p value	Significant
Klotho	Hippocampus	Unpaired t-test	Sal vs. Vpa	t=1,242, df=8	P=0,2495	Ns
Klotho	Cortex	Unpaired t-test	Sal vs. Vpa	t=3,906, df=8	P=0,0045	Yes
Klotho	Cerebellum	Unpaired t-test	Sal vs. Vpa	t=0,7879, df=8	P=0,4535	Ns

Table 5 – Statistical analysis of Immunofluorescence results.

Protein	Region	Statistical test	Group	Distribution value	p value	Significant
Klotho	Cortex	Unpaired t-test	Sal vs. Vpa	$t = 5.233, df = 28$	$P < 0.0001$	Yes