Supplemental Material

Experimentally Induced Convulsive Seizures Are Modulated in Part by Zinc Ions through the Pharmacoresistant $Ca_v2.3$ Calcium Channel

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Supplement for:

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Procedure en détail for the consecutive implantation of transmitter, electrodes and cannula

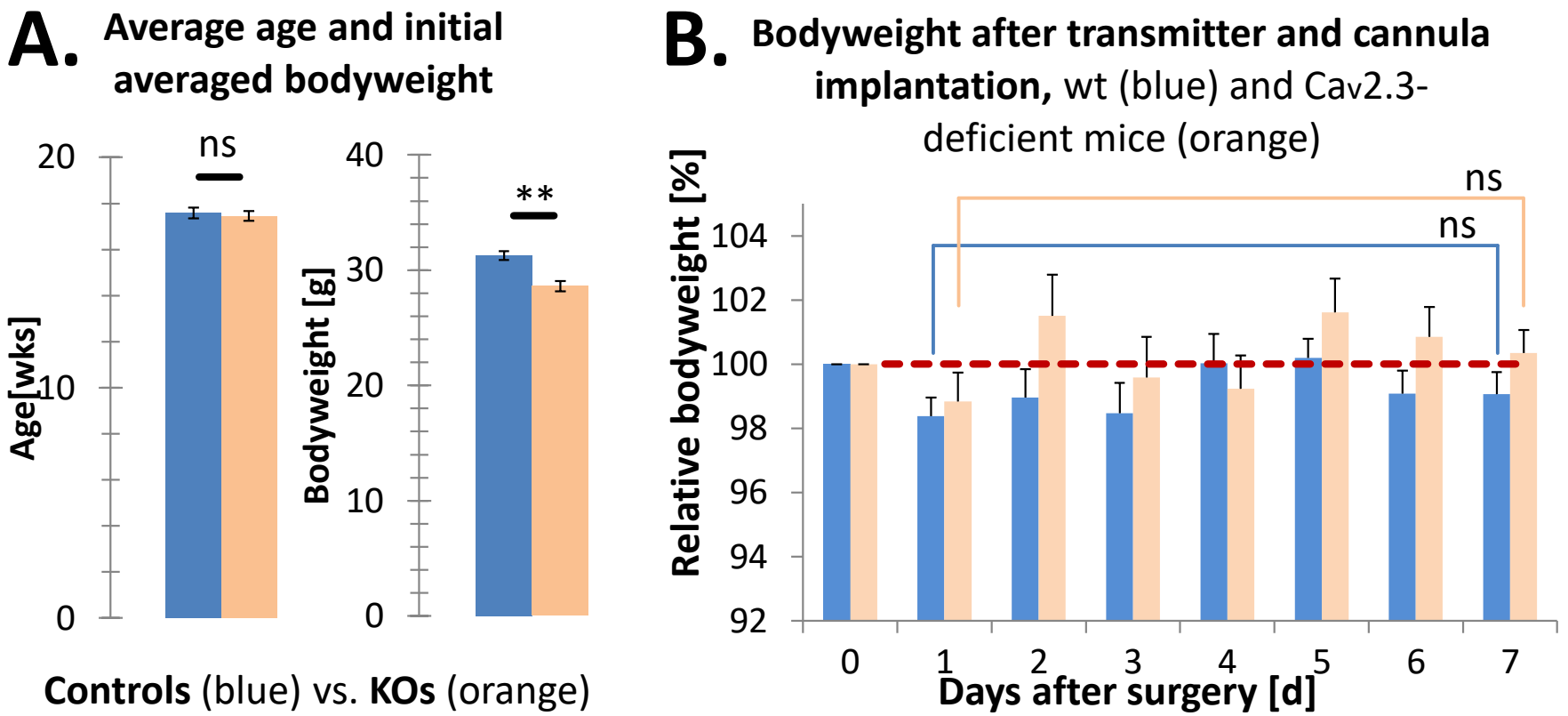
1. Inject ketamine / xylazine – mix intraperitoneally into the mouse isolated in a cage. Make sure that the anesthetized mouse is completely unconscious by checking reflexes. Check it with toe pinches several times throughout the whole surgery. Full surgical tolerance is reached approximately 10 min after i.p. injection of ketamine / xylazine.
2. Before the first incision inject carprofen 5 mg/kg as an analgesic.
3. Place mouse on warming plate on the surgery field and protect eyes with bepanthen ointment. Shave the hair over the skull and disinfect the skin in the surgical area on the head with iodine solution (betaisadona).
4. Make an incision on the skin between the eyes over the skull to the neck with a scalpel. Cutting lenght 1-1.5 cm.
5. Carefully clean up the skull with 3 % hydrogen peroxide (H2O2) to remove tissue from skull.
6. Place mouse in stereotaxic apparatus and fix gently with teeth holder and ear bars.
7. Adjust the drill over the bregma and drill 3 burr holes on selected coordinates. different electrode: from bregma caudal -1 mm, lateral 3 mm (somatosensory cortex), indifferent electrode: from bregma caudal -6.3 mm, lateral 1 mm (close to the sutura saggitalis). For injections: guide canulla with dummy cannula to the lateral ventricle: from bregma -0.34 mm, lateral -1 mm in the os parietale.
8. After drilling all holes place mouse back to warming plate and use the probe head or the curved hemostat to prepare a subcutaneous pouch underneath the skin from the wound over the skull to the same pitch of the right hind limb.
9. Insert the transmitter under the skin to the subcutaneous pouch.
10. Remove a short section of the silicone coat of the electrode wires and bend the wire in a 90° angle.

11. Insert the wire gently into the burr holes as named before.
12. Fix electrodes with dental cement and wait until hardened.
13. Insert the guide cannula with dummy cannula into the ventricle and fix with dental cement and wait until hardened.
14. Suture the wound over the skull and apply betaisadona on the suture.
15. Apply sterile saline for water supply intraperitoneally and place the mouse back into the cage with wet food pellets.
16. Leave the mouse for at least 2 hours on a regulated heating plate and monitor mouse frequently. A video camera for easy and continuous observation is recommended.
17. Check recovery of mouse and treat as needed with 5 mg/kg Carprofen.
18. Check body weight daily.
19. Recovery time at least 7 days.

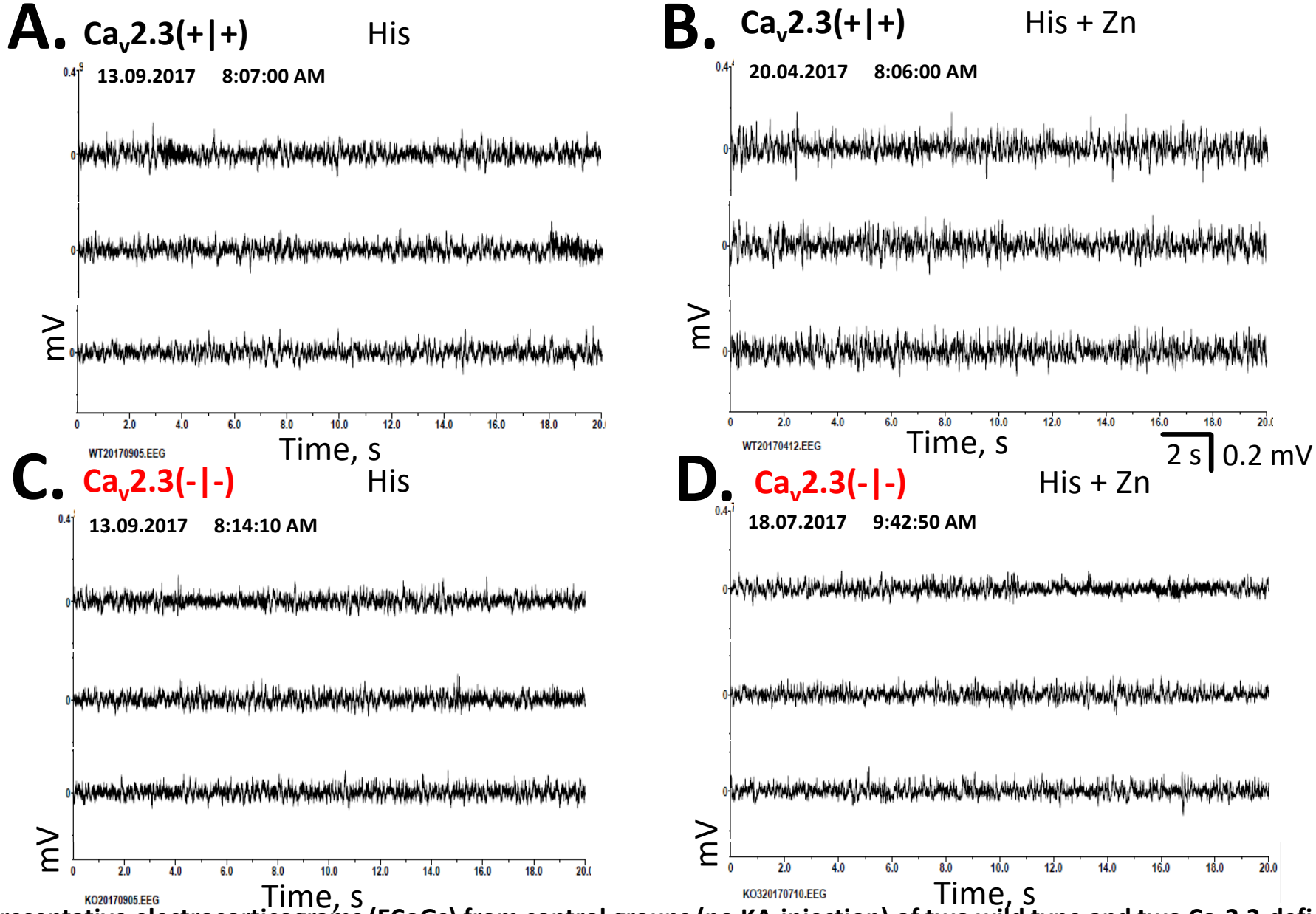
Injection procedure in detail for trace metal cations

1. After recording spontaneous EEGs as a baseline control, the mouse is anesthetized with isoflurane via inhalation mask while applying $ZnCl_2$ (10 µM ZnCl2 + 1 mM histidine in saline) or histidine only in saline via the guide cannula. Injection speed has been 0.15 µl per min and in total 0.3µl.
2. Injection of kainic acid (15 mg/kg body weight in saline) or saline intraperitoneally 0.1 ml / 10 g body weight.
3. Mouse gets placed back into its cage and the receiver plate and recordings and behavioral observations are started.

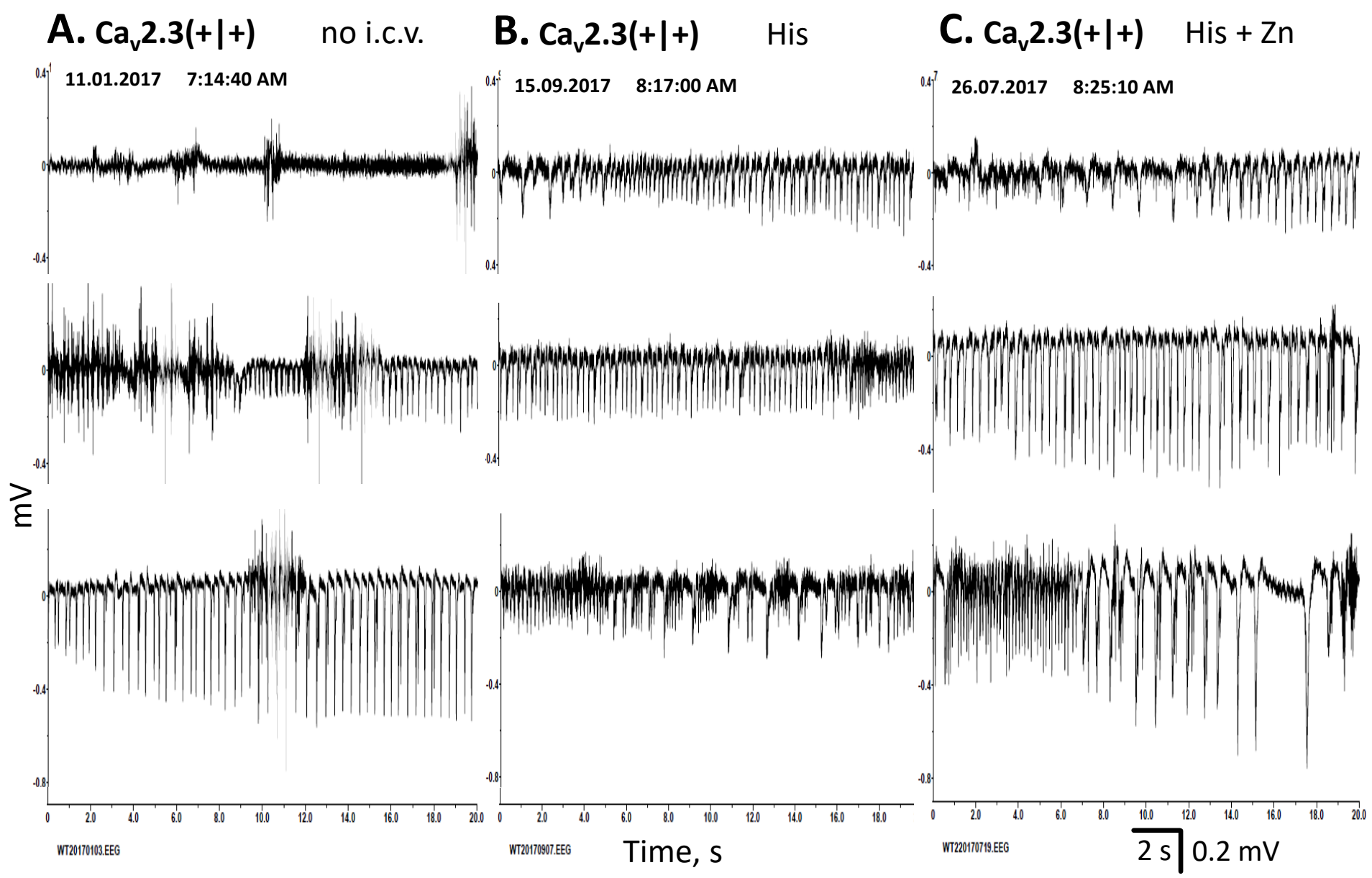
Alpdogan et al.,**Supplement Fig. S1** :



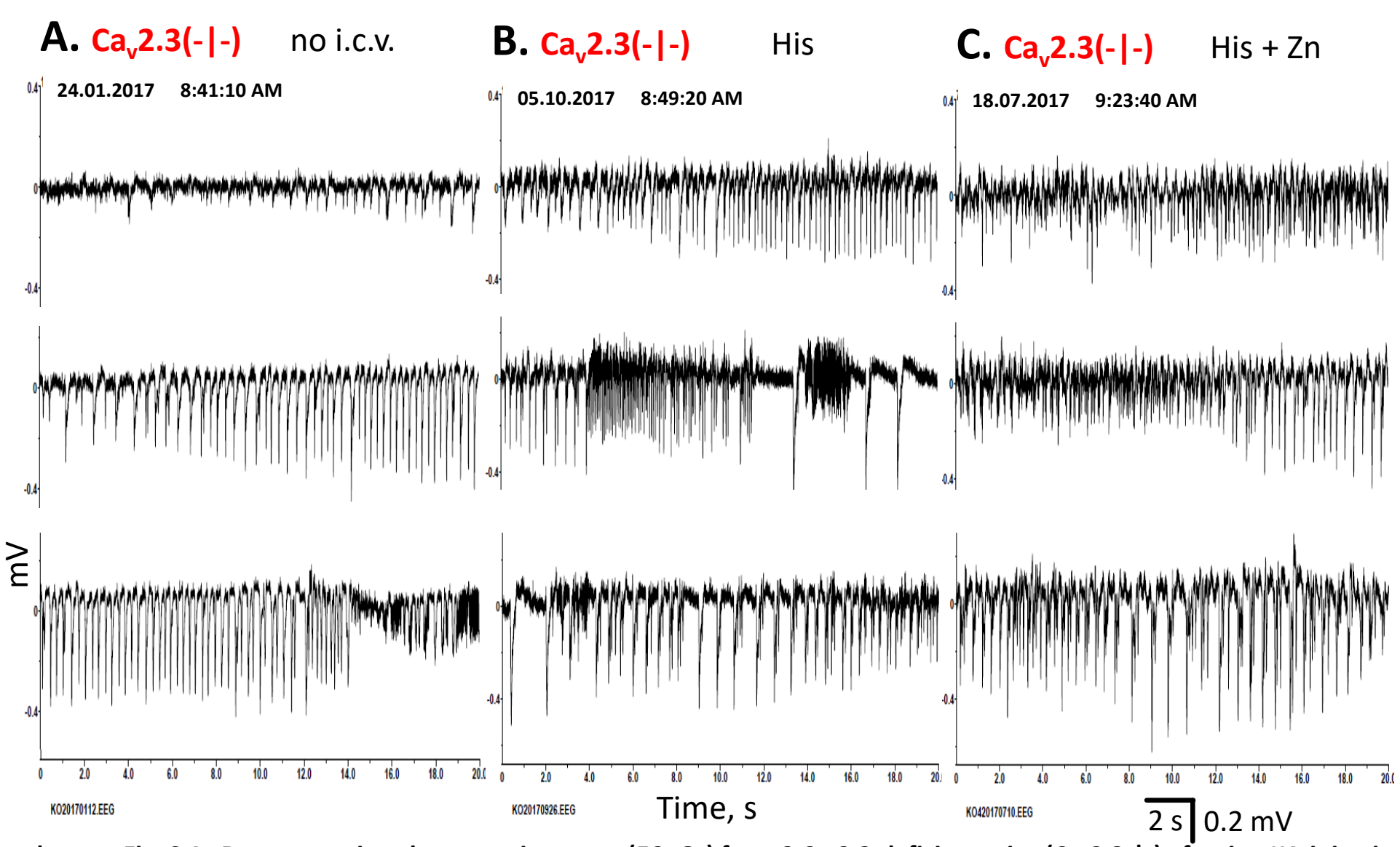
Supplement Fig. S 1: Overview of age, bodyweight and development of bodyweight after transmitter and cannula implantation . A. Mean initial age and bodyweight of the experimental animals for wild type (blue bars , left) and for Cav2.3-deficient mice (orange bars, right) . B. Bodyweight development of wild type (blue) and Cav2.3-deficient mice (orange) after transmitter and cannula implantation . Bodyweight is given as relative value compared to the initial bodyweight (100%) . Significant differences are only labeled between days 1 and 7 for better overview .



Supplement Fig. S2 : **Representative electrocorticograms (ECoGs) from control groups (no KA-injection) of two wild type and two $Ca_v2.3$-deficient mice .** For each animal 3 consecutive 20 sec sections represent a 1 minute lasting ECoG-sequence recorded at the indicated times after i.c.v. injection of either L-histidine (1 mM) only (panel A and C) or in combination with $ZnCl_2$ (10 µM) (panel B and D) . **A.** A 1 minute recording 31 min after injection of histidine plus saline (animal WT20170905) . **B.** A 1 minute recording 30 min after injection of histidine plus saline and $ZnCl_2$ (animal WT20170412) . **C.** A 1 minute recording 30 min after injection of histidine plus saline (KO20170905) . **D.** A 1 minute recording 60 min after injection of histidine plus saline and $ZnCl_2$ (KO320170710) .



Supplement Fig. S 3 : Representative electrocorticograms (ECoGs) from 3 $Ca_v2.3$-competent mice ($Ca_v2.3$+|+) after i.p. KA-injection (15 mg/kg) . For each animal 3 consecutive 20 sec sections represent a 1 minute lasting ECoG-sequence recorded at the indicated times after KA injection . **A.** A 1 minute recording 9 min after KA-injection and no i.c.v. injection (no i.c.v.) (animal WT20170103) . **B.** A 1 minute recording 24 min after KA-injection and i.c.v. injection of L-histidine (1 mM) plus saline (His) (animal WT20170907) . **C.** A 1 minute recording 20 min after KA-injection and i.c.v. injection of histidine plus $ZnCl_2$ (10 µM) (animal WT22017019) .



Supplement Fig. S 4 : Representative electrocorticograms (ECoGs) from 3 $Ca_v2.3$-deficient mice ($Ca_v2.3$-|-) after i.p. KA-injection (15 mg/kg) . For each animal 3 consecutive 20 sec sections represent a 1 minute lasting ECoG-sequence recorded at the indicated times after KA injection . **A.** A 1 minute recording 23 min after KA-injection and no i.c.v. injection (no i.c.v.) (animal KO20170112) . **B.** A 1 minute recording 27 min after KA-injection and i.c.v. injection of L-histidine (1 mM) plus saline (His) (animal KO20170926) . **C.** A 1 minute recording 55 min after KA-injection and i.c.v. injection of histidine plus $ZnCl_2$ (10 µM) (animal KO420170710) .

Supplement Tab. S1: Effect of kainate (15 mg/kg, i.p.) on the EEG spike patterns (no cannulas)

No cannulas — **up to 2 hours after injection (calculated for 2 hours)**

A.	Injection of kainate	Neuroscore:	1	2	3	4	5	6
Genotype	no or yes		Total Number of Spike Trains:	Total Spike Train Duration, min	Average Spike Train Duration, sec	Longest Spike Train Duration, sec	Shortest Spike Train Duration, sec	Average # of Spikes per Train:
$Ca_v2.3$(+|+)	no	**mean**	**0.8**	**0.02**	**0.4**	**0.5**	**0.3**	**1.4**
		SEM	0.7	0.0	0.3	0.3	0.2	0.9
		n =	6	6	6	6	6	6
	yes	**mean**	**184.1**	**15.0**	**14.7**	**106.2**	**1.4**	**52.7**
		SEM	53.0	4.3	4.8	34.4	0.4	19.0
		n =	7	7	7	7	7	7
	MW-U Test	p =	0.00	no Value	0.00	0.00	0.01	0.00
$Ca_v2.3$(-|-)	no	**mean**	**11.5**	**0.3**	**1.1**	**1.9**	**0.5**	**3.8**
		SEM	5.8	0.1	0.4	0.7	0.2	1.3
		n =	6	6	6	6	6	6
	yes	**mean**	**150.0**	**6.1**	**3.1**	**12.7**	**0.9**	**9.4**
		SEM	75.1	3.0	0.9	3.6	0.3	3.0
		n =	9	9	9	9	9	9
	MW-U Test	p =	0.09	0.07	0.09	0.05	0.53	0.22
+|+	no	p =	0.18	no Value	0.39	0.13	0.70	0.18
vs. -|-	yes	p =	0.35	0.05	0.01	0.00	0.25	0.01

Supplement Tab. S2: Effect of kainate (15 mg/kg, i.p.) on the EEG spike patterns (with 1 mM histidine)

Observation of group B revealed no significant differences between both genotypes after KA injection. But histidine alone seems to increase the total spike train duration in $Ca_v2.3$-deficient mice (0.9 sec ±0.9) compared to WT mice (0.3 sec ±0.1, p = 0.04). However 4 parameters changed dependent on injection of KA in KO mice. TSTD, ASTD, LSTD,ANST)

With 1 mM Histidine:

	Neuroscore:		1	2	3	4	5	6
B.	Injection of kainate		Total Number of	Total Spike Train	Average Spike Train	Longest Spike Train	Shortest Spike Train	Average # of Spikes
Genotype	no or yes		Spike Trains:	Duration, min	Duration, sec	Duration, sec	Duration, sec	per Train:
$Ca_v2.3$(+|+)	no	**mean**	**3.1**	**0.3**	**3.8**	**11.8**	**0.8**	**15.6**
		SEM	1.2	0.1	1.1	4.1	0.2	5.0
		n =	9	9	9	9	9	9
	yes	**mean**	**93.5**	**9.9**	**5.2**	**37.7**	**0.9**	**14.0**
		SEM	40.6	4.2	1.2	15.5	0.1	3.0
		n =	11	11	11	11	11	11
	MW-U Test	p =	**0.05**	**0.11**	**0.79**	**0.32**	**0.85**	**0.94**
$Ca_v2.3$(-|-)	no	**mean**	**25.8**	**0.9**	**0.8**	**1.9**	**0.4**	**3.2**
		SEM	23.9	0.9	0.3	0.9	0.1	1.0
		n =	9	9	9	9	9	9
	yes	**mean**	**102.1**	**6.9**	**3.1**	**20.7**	**0.6**	**8.6**
		SEM	50.5	3.2	0.8	11.6	0.2	2.2
		n =	10	10	10	10	10	10
	MW-U Test	p =	**0.08**	**0.04**	**0.02**	**0.04**	**0.50**	**0.04**
+|+	no		**0.60**	**0.04**	**0.05**	**0.08**	**0.55**	**0.09**
vs. -|-	yes	p =	**0.97**	**0.67**	**0.20**	**0.31**	**0.78**	**0.24**

Supplement Tab. S3: Effect of kainate (15 mg/kg, i.p.) on the EEG spike patterns (10 µM $ZnCl_2$)

With 10 µM $ZnCl_2$ plus 1 mM Histidine:

		Neuroscore:	1	2	3	4	5	6
C.	Injection of kainate		Total Number of	Total Spike Train	Average Spike Train	Longest Spike Train	Shortest Spike Train	Average # of Spikes
Genotype	no or yes		Spike Trains:	Duration, min	Duration, sec	Duration, sec	Duration, sec	per Train:
$Ca_V2.3$(+|+)	no	**mean**	**12.7**	**0.4**	**0.9**	**1.9**	**0.5**	**3.0**
		SEM	8.5	0.3	0.4	0.9	0.3	1.2
		n =	9	9	9	9	9	9
	yes	**mean**	**146.6**	**8.9**	**5.6**	**40.3**	**1.2**	**17.7**
		SEM	41.9	2.7	0.7	12.5	0.2	3.3
		n =	11	11	11	11	11	11
	MW-U Test	p =	0.001	0.001	0.000	0.001	0.01	0.000
$Ca_V2.3$(-|-)	no	**mean**	**3.7**	**0.1**	**0.9**	**2.9**	**0.4**	**3.8**
		SEM	1.8	0.1	0.3	1.8	0.2	1.3
		n =	11	11	11	11	11	11
	yes	**mean**	**36.9**	**3.5**	**4.1**	**25.7**	**1.0**	**10.5**
		SEM	14.6	1.7	0.8	14.5	0.2	2.3
		n =	11	11	11	11	11	11
	MW-U Test	p =	0.01	0.02	0.00	0.01	0.02	0.03
+|+	no	p =	0.94	0.18	0.88	1.00	0.41	0.85
vs. -|-	yes	p =	0.02	0.05	0.15	0.05	0.79	0.07

Supplement Tab. S4: were differences were also tested in between the individual groups.

Cav2.3+|+ Plus His vs. No $ZnCl_2$	no	p =	0.181	no Value	0.050	0.026	0.145	0.026	B to A
Plus His vs. No $ZnCl_2$	yes	p =	0.070	0.160	0.046	0.057	0.365	0.024	B to A
Cav2.3-|- Plus His vs. No $ZnCl_2$	no	p =	0.456	0.607	0.689	0.607	0.864	0.864	B to A
Plus His vs. No $ZnCl_2$	yes	p =	0.661	0.968	1.000	0.720	0.842	0.905	B to A
Cav2.3+|+ Plus His-$ZnCl_2$ vs. His	no	p =	0.730	0.340	0.094	0.094	0.077	0.094	C to B
Plus His-$ZnCl_2$ vs. His	yes	p =	0.168	0.555	0.844	0.555	0.393	0.511	C to B
Cav2.3-|- Plus His-$ZnCl_2$ vs. His	no	p =	0.848	0.935	0.939	0.939	0.410	0.908	C to B
Plus His-$ZnCl_2$ vs. His	yes	p =	0.751	0.671	0.377	0.622	0.333	0.778	C to B
Cav2.3+|+ Plus His-$ZnCl_2$ vs. no $ZnCl_2$	no	p =	0.529	no Value	0.607	0.529	0.776	0.456	C to A
Plus His-$ZnCl_2$ vs. no $ZnCl_2$	yes	p =	0.651	0.239	0.057	0.103	0.587	0.057	C to A
Cav2.3-|- Plus His-$ZnCl_2$ vs. no $ZnCl_2$	no	p =	0.264	0.427	0.647	0.477	0.378	0.960	C to A
Plus His-$ZnCl_2$ vs. no $ZnCl_2$	yes	p =	0.403	0.594	0.323	0.761	0.403	0.648	C to A

Supplement Tab. S5: Effect of kainate (15 mg/kg, i.p.) on ECoG power (no cannulas)

No cannulas

A.	Injection of kaina	Frequencies	(δ)	(θ)	(α)	(σ)	(β)	(γ)	(R)	(FR)
Genotype	no or yes	Hz	0.5 - 4	4 - 8	8 - 12	12 - 16	16 - 24	30 - 80	80 - 200	200 - 250
$Ca_V2.3(+\|+)$	no	**mean**	**26.2**	**24.9**	**11.8**	**5.1**	**4.9**	**8.8**	**7.9**	**8.3**
		SEM	2.6	1.3	0.7	0.3	0.3	0.8	0.9	1.0
		n =	6	6	6	6	6	6	6	6
	yes	**mean**	**29.6**	**16.3**	**7.5**	**4.3**	**5.6**	**11.1**	**12.9**	**9.4**
		SEM	4.1	1.1	1.4	0.8	1.2	0.7	2.4	1.8
		n =	7	7	7	7	7	7	7	7
	t-test:	p =	0.73 *	0.00	0.03	0.40	0.73 *	0.05	0.09	0.64
$Ca_V2.3(-\|-)$	no	**mean**	**23.7**	**22.7**	**9.9**	**4.4**	**4.4**	**9.8**	**11.0**	**11.9**
		SEM	1.5	2.4	1.2	0.6	0.5	0.2	1.5	2.1
		n =	6	6	6	6	6	6	6	6
	yes	**mean**	**36.8**	**19.5**	**6.9**	**3.4**	**3.7**	**9.0**	**10.8**	**8.5**
		SEM	2.5	2.4	0.7	0.3	0.3	0.8	2.0	2.0
		n =	9	9	9	9	9	9	9	9
	t-test:	p =	0.00 *	0.37	0.04	0.09	0.22 *	0.40	0.95	0.28
+|+ vs. -|-	no	p =	0.43	0.46	0.21	0.31	0.46	0.24	0.11	0.16
	yes	p =	0.14	0.29	0.71	0.35	0.10	0.07	0.50	0.76

* Calculated with Mann Whitney U test

Supplement Tab. S6: Effect of kainate (15 mg/kg, i.p.) on ECoG power (1 mM histidine)

With 1 mM Histidine:

B.	Injection of kaina	Frequencies	(δ)	(θ)	(α)	(σ)	(β)	(γ)	(R)	(FR)
Genotype	no or yes	Hz	0.5 - 4	4 - 8	8 - 12	12 - 16	16 - 24	30 - 80	80 - 200	200 - 250
$Ca_V2.3(+\|+)$	no	mean	**25.5**	**24.8**	**12.5**	**5.7**	**5.3**	**8.8**	**7.4**	**7.6**
		SEM	2.5	1.1	0.6	0.3	0.3	0.5	0.6	1.3
		n =	9	9	9	9	9	9	9	9
	yes	mean	**24.2**	**17.1**	**6.9**	**3.9**	**5.7**	**16.9**	**12.2**	**9.4**
		SEM	2.3	1.2	0.7	0.4	0.5	1.4	1.6	1.2
		n =	11	11	11	11	11	11	11	11
	t-test:	p =	0.71	0.00 *	0.00	0.00	0.55	0.00	0.01*	0.15 *
$Ca_V2.3(-\|-)$	no	mean	**27.6**	**20.8**	**11.0**	**4.9**	**4.8**	**10.0**	**8.7**	**9.9**
		SEM	3.6	1.5	0.9	0.4	0.4	1.0	1.5	2.2
		n =	9	9	9	9	9	9	9	9
	yes	mean	**33.1**	**19.9**	**8.1**	**3.9**	**4.1**	**10.5**	**9.7**	**8.3**
		SEM	3.4	2.2	1.2	0.5	0.5	1.0	1.6	1.3
		n =	10	10	10	10	10	10	10	10
	t-test:	p =	0.28	0.76	0.04 *	0.11 *	0.27	0.73	0.64	0.90*
+|+	no	p =	0.64	0.05	0.11 *	0.11 *	0.34	0.28	0.45	0.44*
vs. -|-	yes	p =	0.04	0.46*	0.40	0.96	0.05	0.00	0.29*	0.55

* Calculated with Mann Whitney U test

Supplement Tab. S7: Effect of kainate (15 mg/kg, i.p.) on ECoG power (10 µM $ZnCl_2$)

With 10 µM $ZnCl_2$ plus 1 mM Histidine:

C.	Injection of kaina	Frequencies	(δ)	(θ)	(α)	(σ)	(β)	(γ)	(R)	(FR)
		Hz	0.5 - 4	4 - 8	8 - 12	12 - 16	16 - 24	30 - 80	80 - 200	200 - 250
Genotype	no or yes									
$Ca_v2.3$(+|+)	no	**mean**	**24.4**	**22.9**	**11.8**	**5.5**	**5.1**	**10.2**	**10.3**	**7.7**
		SEM	2.8	3.0	1.6	0.7	0.6	0.6	3.0	2.0
		n =	9	9	9	9	9	9	9	9
	yes	**mean**	**32.0**	**18.4**	**6.0**	**3.3**	**4.3**	**12.4**	**12.0**	**8.7**
		SEM	3.3	2.0	0.5	0.3	0.4	1.3	2.8	1.6
		n =	11	11	11	11	11	11	11	11
	t-test:	p =	0.11	0.22	0.00	0.01	0.24	0.16	0.25*	0.59 *
$Ca_v2.3$(-|-)	no	**mean**	**23.0**	**24.7**	**12.0**	**5.2**	**4.7**	**9.6**	**8.5**	**10.0**
		SEM	1.6	1.6	0.9	0.3	0.3	0.4	1.1	2.4
		n =	11	11	11	11	11	11	11	11
	yes	**mean**	**32.6**	**22.2**	**7.9**	**4.0**	**4.6**	**11.7**	**8.4**	**5.9**
		SEM	3.1	1.3	0.6	0.4	0.4	1.2	1.2	0.9
		n =	11	11	11	11	11	11	11	11
	t-test:	p =	0.01	0.05 *	0.00 *	0.05 *	0.45 *	0.28 *	0.84 *	0.15 *
+|+	no	p =	0.66	0.30 *	0.05 *	0.08 *	0.41 *	0.39	0.39 *	0.94 *
vs. -|-	yes	p =	0.88	0.13	0.02	0.12	1.00 *	0.55 *	0.32 *	0.13

* Calculated with Mann Whitney U test