519.14

Characterizing the maturation of electrophysiological properties in stem cell-derived human neurons lacking methyl CpG binding protein 2 (MECP2)

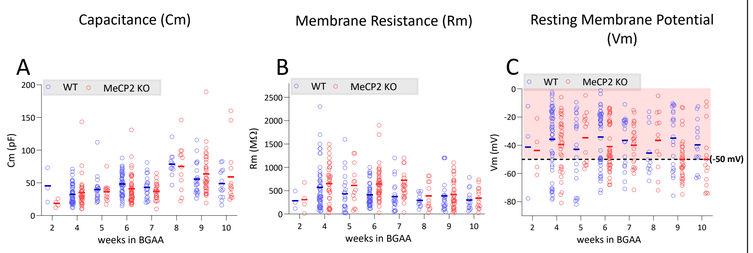
Pauravi J. Gandhi[1], Dong Liu[1], Angela Snyder[1], Chen Tian[1], Christian Petroski[1], Nadia Anastasi[2], Anja Gundlfinger[2], Eva Harde[2], Ravi Jagasia[2], Robert Petroski[1], Eoin C. O'Connor[2]

[1]Neuroservices-Alliance, San Diego CA 92121, [2] Neuroscience and Rare Diseases. Roche Innovation Center Basel, F. Hoffmann-La Roche, Basel, Switzerland

Overview

- **Background:** Rett syndrome (RTT) is a rare, genetic neurodevelopmental disorder that remains without cure. RTT is caused by loss-of-function mutations in the gene encoding methyl CpG binding protein 2 (MECP2), which plays an important role in neuronal maintenance and plasticity.
- **The development of novel therapies for RTT critically depends upon the availability of model systems with robust and disease-relevant phenotypes**.
- Here we generated MECP2 KO human pluripotent stem cell-derived neural stem cells (NSCs) and differentiated them into neurons, as a promising tool for understanding MECP2 loss-of-function on electrophysiological phenotypes in a human neuronal context.
- **Aim:** The objective was to characterize the developmental trajectory of electrophysiological phenotypes human stem cell-derived MECP2 KO neurons, with comparison to isogenic wild-type (WT) Controls.
- **Methods overview:** Whole-cell patch-clamp recordings were performed on over 300 human stem cell-derived neurons (150 WT and 152 MECP2 KO) from 1–2-month-old neuronal cultures. Additionally, gramicidin perforated patch-clamp recordings were conducted to characterize maturation of the GABA reversal potential (E_{GABA}).

1. In vitro development of passive membrane properties



Passive membrane properties						
Weeks	Cm (pF)		Rm (MΩ)		RMP (mV)	
	WT	MeCP2 KO	WT	MeCP2 KO	WT	MeCP2 KO
2-4	33 ± 2 (45)	65 ± 31 (38)	560 ± 62 (45)	612 ± 66 (38)	-34 ± 3.4 (45)	-40 ± 3 (38)
5-8	46 ± 2 (93)	54 ± 11 (92)	399 ± 31 (93)	624 ± 49 (92)	-37 ± 2 (93)	-39 ± 2 (92)
9-10	52 ± 5 (25)	62 ± 5 (48)	355 ± 92 (25)	399 ± 41 (48)	-34 ± 4 (25)	-50 ± 2 (47)

Figure 1: WT and MeCP2 KO neurons mature with longer time in differentiation. (A) Cell capacitance increased with no change in (B) membrane resistance and (C) resting membrane potential (RMP). Most cells did NOT develop a hyperpolarized RMP. No significant difference was observed in recorded properties between WT and MeCP2 KO neurons. Values were binned into 2-4 weeks, 5-8 weeks and 9-10 weeks; and presented as average ± SEM (no. of neurons).

2. Most WT and MeCP2 KO neurons do not fire spontaneous action potentials.

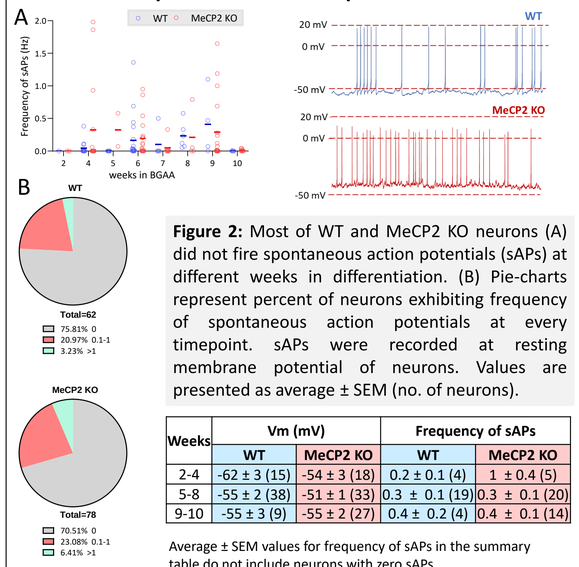
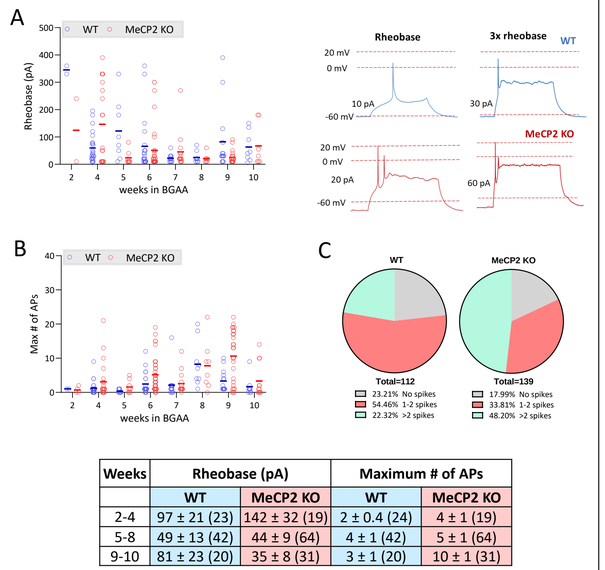


Figure 2: Most of WT and MeCP2 KO neurons (A) did not fire spontaneous action potentials (sAPs) at different weeks in differentiation. (B) Pie-charts represent percent of neurons exhibiting frequency of spontaneous action potentials at every timepoint. sAPs were recorded at resting membrane potential of neurons. Values are presented as average ± SEM (no. of neurons).

Weeks	Vm (mV)		Frequency of sAPs	
	WT	MeCP2 KO	WT	MeCP2 KO
2-4	-62 ± 3 (15)	-54 ± 3 (18)	0.2 ± 0.1 (4)	1 ± 0.4 (5)
5-8	-55 ± 2 (38)	-51 ± 1 (33)	0.3 ± 0.1 (19)	0.3 ± 0.1 (20)
9-10	-55 ± 3 (9)	-55 ± 2 (27)	0.4 ± 0.2 (4)	0.4 ± 0.1 (14)

Average ± SEM values for frequency of sAPs in the summary table do not include neurons with zero sAPs.

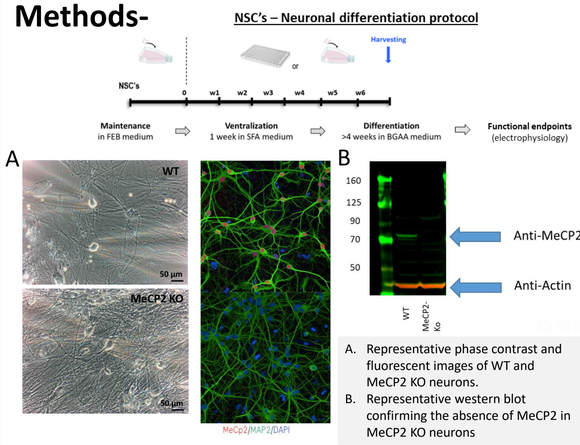
3. Action potentials can be evoked in both WT and MeCP2 KO neurons



Weeks	Rheobase (pA)		Maximum # of APs	
	WT	MeCP2 KO	WT	MeCP2 KO
2-4	97 ± 21 (23)	142 ± 32 (19)	2 ± 0.4 (24)	4 ± 1 (19)
5-8	49 ± 13 (42)	44 ± 9 (64)	4 ± 1 (42)	5 ± 1 (64)
9-10	81 ± 23 (20)	35 ± 8 (31)	3 ± 1 (20)	10 ± 1 (31)

Figure 3: WT and MeCP2 KO neurons could (A) fire action potentials with depolarizing current injection. (C) Pie charts represent number of evoked action potentials in WT and MeCP2 KO neurons. Values were binned into 2-4 weeks, 5-8 weeks and 9-10 weeks; and presented as average ± SEM (no. of neurons). Average ± SEM values for maximum # of APs in the summary table do not include neurons with zero spikes.

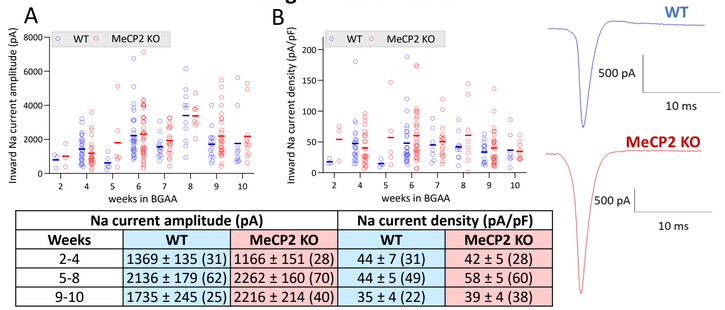
Methods-



A. Representative phase contrast and fluorescent images of WT and MeCP2 KO neurons.
B. Representative western blot confirming the absence of MeCP2 in MeCP2 KO neurons

- The composition of external recording solution was: 140 mM NaCl, 2.5 mM KCl, 2 mM CaCl2, 1.3 mM MgCl2, 10 mM glucose, 10 mM HEPES pH 7.3. The composition of internal recording solution was: 120 mM K-gluconate, 20 mM KCl, 3 mM MgCl2, 5 mM EGTA, 0.5 mM CaCl2, 4 mM Na2-ATP, 0.3 mM Li-GTP, 10 mM HEPES pH 7.3.
- Upon establishing the whole cell configuration, we measured the passive membrane properties for every cell as well as spontaneous action potential firing, intrinsic excitability and the expression of voltage-gated sodium currents.
- We performed gramicidin perforated patch clamp recording using 50 µg/mL gramicidin in KCl containing internal solution. GABAA receptors current were elicited with 100 ms puff of 100 µM GABA every 30 secs.

4. WT and MeCP2 KO neurons exhibited larger sodium currents with longer differentiation



Na current amplitude (pA)			Na current density (pA/pF)	
Weeks	WT	MeCP2 KO	WT	MeCP2 KO
2-4	1369 ± 135 (31)	1166 ± 151 (28)	44 ± 7 (31)	42 ± 5 (28)
5-8	2136 ± 179 (62)	2262 ± 160 (70)	44 ± 5 (49)	58 ± 5 (60)
9-10	1735 ± 245 (25)	2216 ± 214 (40)	35 ± 4 (22)	39 ± 4 (38)

Figure 4: Both WT and MeCP2 KO neurons showed increased (A) sodium (Na) current amplitude and (B) no change in current density with longer differentiation. Values were binned into 2-4 weeks, 5-8 weeks and 9-10 weeks; and presented as average ± SEM (no. of neurons).

5. WT and MeCP2 KO neurons did not exhibit sPSCs at week 8 and 9 in differentiation

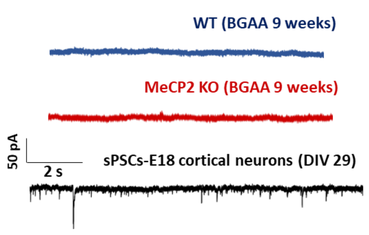


Figure 5: Both WT and MeCP2 KO neurons did not elicit any spontaneous post-synaptic currents (sPSCs) at week 8 and 9, compared to E18 rat cortical neurons (positive control). sPSCs were recorded from 25 WT and 19 MeCP2 neurons at week 8 and week 9 timepoint.

6. Both WT and MeCP2 KO neurons exhibited hyperpolarized E_{GABA}

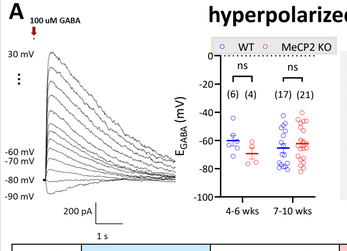


Figure 6A: WT and MeCP2 KO neurons showed hyperpolarizing E_{GABA} at 4-6- and 7-10-weeks developmental time, under perforated patch clamp configuration. Values presented as average ± SEM (no. of neurons).

Weeks	WT	Cl^- concentration	MeCP2 KO	Cl^- concentration
4-6	-60.12 ± 3.8 (6)	14.6 mM	-69.24 ± 3.8 (4)	10.2 mM
7-10	-65.43 ± 3.16 (17)	11.9 mM	-62.18 ± 2.7 (21)	13.5 mM

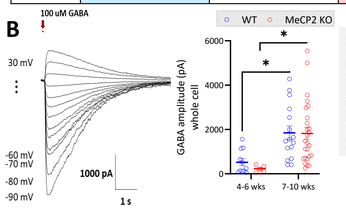


Figure 6B: Both WT and MeCP2 KO neurons showed increased GABA amplitude at 4-6- and 7-10-weeks time, under whole-cell configuration. Values presented as average ± SEM (no. of neurons).

Conclusions-

1. **A very low percentage of all human stem cell-derived neurons develop a mature functional phenotype,** evidenced by 1) great variability in functional properties and 2) absence of synaptic activity even with up to 2 months of in vitro differentiation. Only 23% of WT NSCs and 34% MeCP2 KO NSCs met the minimal criteria of exhibiting a hyperpolarized resting membrane potential (≤-50 mV) and could fire at least one evoked action potential.
2. **Rigorous electrophysiological characterization was unable to identify conspicuous differences between the MeCP2 KO and isogenic control genotypes.** We could not confirm differences in spontaneous post-synaptic currents in WT and MeCP2 KO neurons as reported by Mok et al (2022), nor differences in the chloride reversal the reversal potential (E_{GABA}) between WT and MeCP2 KO neurons, as reported by Tang et al (2015).
3. **These results emphasize the importance of robust validation of functional properties of human iPSC-derived neuronal lines and their culture methods**, before deploying them for target validation and/or drug optimization efforts in drug discovery research.