Electrophysiological profiling the functional properties of human NGN2 neurons

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Abstract

Human iPSC-derived neurons promise to be a relevant in vitro model of human neuronal function and are hypothesized to predict the efficacy of novel therapeutics for neuroscience indications. The phenotypes of hiPSC-derived neurons have been primarily characterized by mRNA expression. Very few reports describe the functional properties by electrophysiological methods. We describe the functional properties of an NGN2-derived cell line by both patch clamp and microelectrode array recording (HD-MEA).

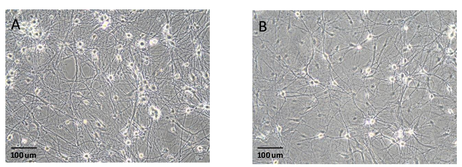
NGN2 neurons were plated at low density on a confluent monolayer of rat astrocytes. The acquisition of a mature electrophysiological phenotype was monitored with time culture. The neurons exhibited a negative resting membrane potential very early. Not surprisingly, the neurons grew larger with time in culture as measured by increased whole cell capacitance and decreased input resistance. The percentage of neurons exhibiting spontaneous action potential firing also increased with time in culture. Spontaneous postsynaptic currents were also detected indicating that the NGN2 neurons formed functional synapses. We detected NMDA receptor currents in ~50% of the NGN2 neurons.

We also profiled the activity of NGN2 neurons using the MaxTwo HD-MEA (MaxWell Biosystems) that simultaneously records up to 1,020 neurons from 6 independent wells. In agreement with the patch clamp results, spontaneous activity increased with time in culture. The mean firing rate was ~1 Hz. NGN2 neurons in culture exhibited network connectivity as seen by coherent bursting with a frequency of ~0.2 Hz and a burst duration of ~0.8 sec. Co-culturing the excitatory NGN2 neurons with human iCellGABA inhibitory neurons (Fujifilm) decreased the spontaneous activity of NGN2 neurons.

Methods

- Human NGN2 neurons and E18 rat cortical neurons were plated at a very low density (50-100 cells/mm2) on monolayers of rat astrocytes.
- Whole cell patch clamp recordings were made using standard methods. The composition of external recording solution was: 140 mM NaCl, 2.5 mM KCl, 2 mM $CaCl_2$, 1.3 mM $MgCl_2$, 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 $MgCl_2$, 5 mM EGTA, 0.5 mM $CaCl_2$, 4 mM Na_2-ATP, 0.3 mM Li-GTP, 10 mM HEPES pH 7.3.
- We measured spontaneous action potentials using the Maxwell MaxTwo high-density multielectrode array (HD-MEA). The development of spontaneous action potential firing was monitored from 1-7 weeks in vitro using the Activity Scan recording protocol (40 sec recording). Bursting activity was recorded from 1,020 electrodes simultaneously per well using the Network recording protocol (5 min recording).

Human iPSC-derived NGN2 neurons resemble the morphology of primary rat CNS neurons



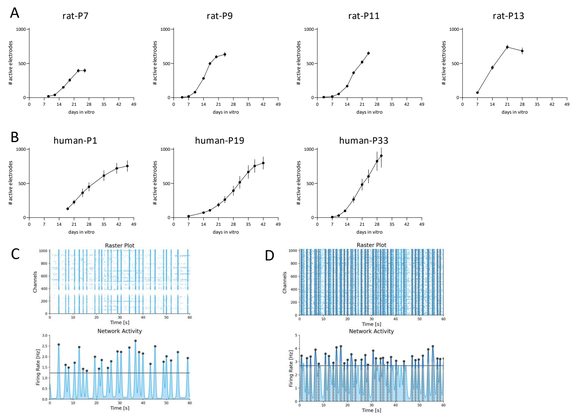
Photomicrographs of human NGN2 neurons at 27 div (A) and rat cortical neurons at 27 div (B).

Functional properties of human iPSC-derived NGN2 neurons and primary rat CNS neurons

	NGN2 8-14 div	NGN2 15-21 div	NGN2 22-28 div	NGN2 29-35 div	NGN2 36-42 div	NGN2 43-49 div	E18 cort 3-4 weeks (5-2023-033)
Cm	34 ± 2 pF (54)	45 ± 3 pF (21)	62 ± 3 pF (119)	63 ± 3 pF (59)	91 ± 11 pF (17)	107 ± 8 pF (40)	132 ± 5 pF (132)
Rm	437 ± 30 MΩ (54)	283 ± 26 MΩ (21)	223 ± 11 MΩ (119)	198 ± 14 MΩ (59)	142 ± 17 MΩ (17)	112 ± 8 MΩ (40)	109 ± 4 MΩ (132)
Vm	-47.3 ± 1.1 mV (54)	-51.0 ± 1.9 mV (21)	-58.1 ± 0.8 mV (102)	-58.2 ± 0.7 mV (59)	-58.2 ± 1.7 mV (12)	-59.5 ± 1.0 mV (32)	-61.0 ± 0.5 mV (117)
% cells spontaneous APs	51 % (23 of 45)	43% (6 of 14)	43% (38 of 88)	43% (23 of 53)	82% (9 of 11)	64% (16 of 25)	81% (95 of 117)
Firing rate	0.16 ± 0.11 (23)	0.10 ± 0.07 (6)	0.14 ± 0.07 (38)	0.12 ± 0.08 (23)	0.14 ± 0.04 (9)	0.15 ± 0.03 (15)	0.39 ± 0.04 Hz (95)
Rheobase	21.5 ± 2.9 pA (43)	15.8 ± 2.5 pA (12)	56.7 ± 5.5 pA (61)	57.3 ± 5.2 pA (51)	43.5 ± 19.3 pA (10)	220 ± 33.9 pA (23)	85 ± 8 pA (101)
Max APs	6 ± 1 APs (44)	15 ± 2 APs (12)	22 ± 2 APs (62)	22 ± 2 APs (53)	17 ± 4 APs (11)	25 ± 3 APs (25)	18 ± 1 (100)
Na current	1,878 ± 169 pA (36)	3,079 ± 446 pA (9)	5,293 ± 400 pA (52)	4,949 ± 395 pA (47)	9,402 ± 937 pA (10)	8,749 ± 778 pA (24)	5,695 ± 340 pA (134)
K current	1,845 ± 148 pA (36)	3,083 ± 502 pA (9)	2,876 ± 245 pA (52)	2,615 ± 237 pA (47)	7,576 ± 827 pA (10)	5,225 ± 564 pA (24)	4,054 ± 284 pA (134)

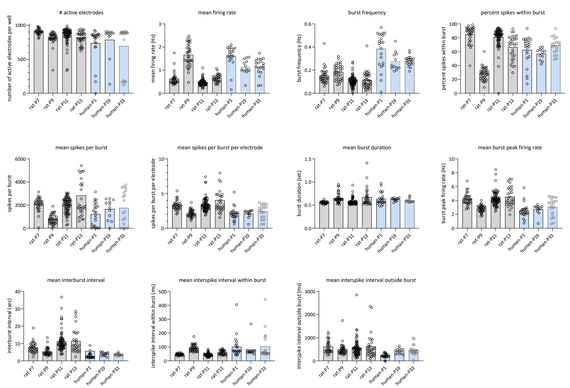
Table summarizing the functional properties of human NGN2 neurons by patch clamp recording at 2, 3, 4, 5, 6, 7 weeks *in vitro*. The properties of primary rat cortical neurons at 4 weeks *in vitro* are shown for comparison. Membrane capacitance and input resistance reflect neuron size. Human NGN2 neurons are smaller than rat CNS neurons. Resting membrane potential is comparable. A lower percentage of human NGN2 neurons exhibited spontaneous action potential firing and the firing rate was lower. Intrinsic excitability was comparable as evidenced by the maximum number of action potentials elicited by a 1 sec depolarizing current injection. Voltage-gated Na and K current amplitudes were similar.

Spontaneous action potentials and network bursting in primary rat CNS neurons and human NGN2 neurons



The number of active electrodes from high-density MEA recording reflects the number or neurons exhibiting spontaneous action potential firing. Four independent batches of primary rat CNS neurons reached a plateau in activity between 3-4 weeks *in vitro* (A).
Three independent batches of human NGN2 neurons developed spontaneous firing and reached a plateau at 5-7 weeks *in vitro* (B). Representative raster plots show the synchronous firing of 1,020 electrodes from rat CNS neurons (C) and human NGN2 neurons (D).

Comparison of the network bursting endpoints in human NGN2 neurons and rat CNS neurons



Scatter plots illustrating the experimental variance of eleven network bursting endpoints recorded by HD-MEA. The results from four independent batches of primary rat CNS neurons (gray bars) and three independent batches of human NGN2 neurons (blue bars) are shown. Each symbol represents the data from one MEA well; bars are the mean.

Network bursting endpoints in human NGN2 neurons and rat CNS neurons

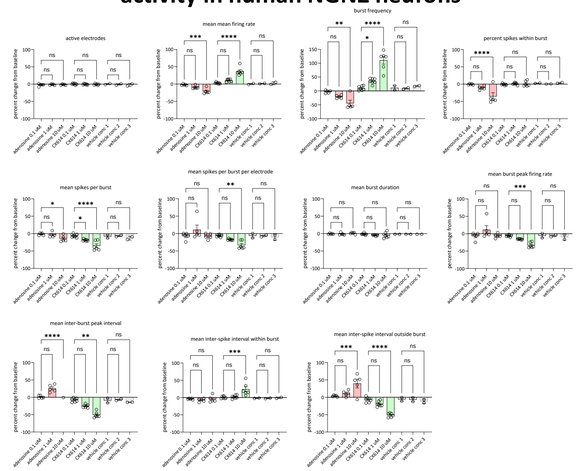
	rat-P7	rat-P9	rat-P11	rat-P13	human-P1	human-P19	human-P33
# active electrodes	905 ± 7 (32)	815 ± 16 (36)	852 ± 12 (72)	814 ± 15 (35)	737 ± 51 (23)	785 ± 67 (12)	694 ± 75 (19)
Mean firing rate	0.62 ± 0.04 Hz (32)	1.66 ± 0.07 Hz (36)	0.57 ± 0.10 Hz (72)	0.65 ± 0.03 Hz (30)	1.71 ± 0.14 Hz (22)	1.13 ± 0.09 Hz (12)	1.14 ± 0.08 Hz (19)
Mean burst frequency	0.15 ± 0.01 Hz (32)	0.18 ± 0.01 Hz (36)	0.10 ± 0.01 Hz (72)	0.11 ± 0.01 Hz (35)	0.38 ± 0.03 Hz (23)	0.27 ± 0.02 Hz (12)	0.28 ± 0.01 Hz (19)
Percent spikes within burst	84 ± 2% (32)	29 ± 2% (36)	81 ± 2% (72)	66 ± 3% (30)	62 ± 4% (23)	57 ± 2% (12)	69 ± 3% (19)
Mean spikes per burst	2,100 ± 101 (32)	780 ± 69 (36)	1,994 ± 76 (71)	2,824 ± 338 (30)	1,209 ± 206 (23)	1,647 ± 251 (12)	1,754 ± 339 (19)
Mean spikes per burst per electrode	3.2 ± 0.1 (32)	2.2 ± 0.1 (36)	3.4 ± 0.1 (71)	4.0 ± 0.4 (30)	2.1 ± 0.2 (23)	2.1 ± 0.2 (12)	2.4 ± 0.2 (19)
Mean burst duration	0.55 ± 0.01 sec (32)	0.65 ± 0.01 sec (36)	0.57 ± 0.01 sec (71)	0.67 ± 0.04 sec (30)	0.63 ± 0.02 sec (23)	0.62 ± 0.01 sec (12)	0.60 ± 0.01 sec (19)
Mean burst peak firing rate	4.2 ± 0.2 Hz (32)	2.8 ± 0.1 Hz (36)	4.3 ± 0.1 Hz (71)	4.5 ± 0.3 Hz (30)	2.5 ± 0.2 Hz (23)	2.7 ± 0.2 Hz (12)	3.1 ± 0.3 Hz (19)
Mean interburst interval	7.7 ± 0.5 sec (32)	5.3 ± 0.4 sec (36)	11.2 ± 1.3 sec (71)	11.3 ± 1.3 sec (30)	5.6 ± 2.6 sec (23)	3.9 ± 0.3 sec (12)	3.7 ± 0.1 sec (19)
Mean interspike interval within burst	46 ± 1 ms (32)	93 ± 4 ms (36)	43 ± 1 ms (71)	60 ± 3 ms (30)	100 ± 17 ms (22)	81 ± 17 ms (12)	105 ± 23 ms (19)
Mean interspike interval outside burst	604 ± 47 ms (32)	493 ± 42 ms (36)	549 ± 45 ms (71)	619 ± 98 ms (30)	218 ± 20 ms (23)	415 ± 35 ms (12)	473 ± 52 ms ((19)

Table of eleven network bursting endpoints from four independent batches of primary rat CNS neurons (gray) and three independent batches of human NGN2 neurons (blue).

Summary

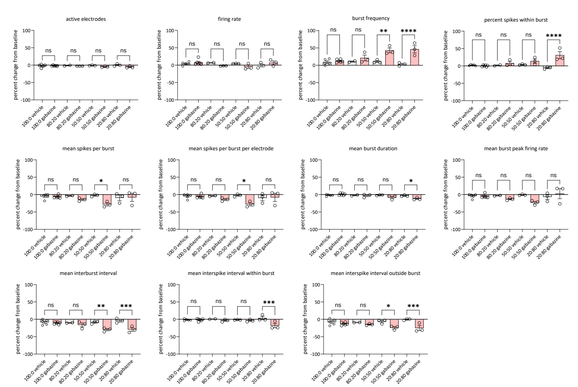
1. Human iPSC-derived NGN2 neurons are suitable for patch clamp recording and MEA recording endpoints.
2. Human NGN2 neurons exhibited functional properties expected of "*bona fide*" neurons isolated from rat CNS.
3. Human NGN2 neurons responded to test compounds (adenosine and CX614) as expected from primary rat neuron cultures.
4. Human NGN2 neurons can be used to support lead optimization (SAR) for drug discovery programs.

Adenosine reduces activity and CX614 increases activity in human NGN2 neurons



Concentration-response of adenosine (pink bars) and CX614 (green bars) and vehicle (gray bars) on eleven (11) MEA network endpoints in human excitatory NGN2 neurons recorded at 30 div. Adenosine reduces excitability through a presynaptic site of action via adenosine A1 receptors. CX614 increases activity through a postsynaptic site of actino by slowing deactivation of AMPA receptors. Bars represent the percent change from baseline.

Effect of gabazine in NGN2-iCell GABA co-cultures



Human NGN2 excitatory neurons were co-cultured with human iCell GABA inhibitory neurons at different ratios (100:0, 80:20, 50:50, 20:80). The effect of 10 uM gabazine on the percent change from baseline on 11 MEA network endpoints at 42 div. Vehicle treated shown in gray bars and gabazine treated shown in pink. Gabazine exhibited a greater effect in co-cultures with more GABAergic neurons.