NOVEL ELECTROPHYSIOLOGICAL PHENOTYPE AND ION CHANNEL COMPOSITION IN IPS NEURONS FROM LRRK2 PARKINSON'S PATIENTS

Rodriguez, Leonardo1; Miu, Peter2; Mak, Sally1, Jiang, Xin2, Sun, Faneng1, Marzano, Scott1, Huang, Y. Anne1, Li, Yanping1, Langston, J. William1, Schüle, Birgitt1

1The Parkinson's Institute, Sunnyvale, CA, USA, 2Molecular Devices, Sunnyvale, CA, USA

Introduction

Parkinson's disease (PD) is one of the most common neurodegenerative diseases of aging, affecting specific mid-brain dopaminergic (DA) neurons. There is no cure or effective way to slow disease progression, and the causes of the disease remain obscure. Our goal is to develop a functional experimental tool to study PD using induced pluripotent stem cell (iPSC)-derived dopaminergic (DA) neurons from a patient with LRRK2 parkinsonism which presents the most common form of genetic disease.

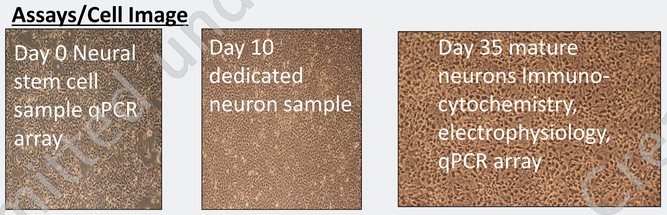
We have shown differences in neurite outgrowth and electrical activity by automated patch clamping. We have tested our LRRK2 G2019S mutant cell line and one control clone to determine if the mutant line shows differences in electrical activity due to variation in ion channel composition. The immunocytochemistry data show the cells express mid-brain markers and a large population of mature neurons. We designed a qPCR array with seventeen genes including Ca^{++} channels, Na^{+} channels and Glutamate receptors. Surprisingly, we see ion channel upregulation in the control line. This is corroborated by our electrophysiology data that suggests ion channels differences and a new phenotype.

Methods

Media

NSC: Neurobasal media, 8ng/ml FGF2(R&D) → (1 passage) → DA1: Neurobasal media, 0.4ng/ml SAG 100ng/ml FGF8 → (10 days) → DA2: Neurobasal media, 1mM dcAMP, 20ng/ml GDNF&BDNF → (25 days)

Assays/Cell Image



•**Cell Culture**: NPCs were plated on Geltrex coated dishes and DA1 media was added for 10 days with passaging as necessary. DA2 was added on day 11. The final passage onto Poly-L-ornithine and Laminin (20ug/ml) coated plates (Sigma) was done on day 14 and the cells were kept in DA2 without passaging for a minimum of two weeks.

•**Biorad Prime PCR Array**: A Prime PCR array was custom designed to include ion channels and genes of interest. Cells were pelleted at day 0 and day 35. The frozen pellet was processed using a QIAshredder and an RNeasy Mini Kit (Quiagen). BioRad DNA Engine Thermal Cycler was used to create cDNA using the iScript Advanced cDNA Synthesis Kit (BioRad). The Prime PCR Array was run on a C1000 Thermal Cycler with a CFX96 Real-time System. CFX Manager was used to analyze data.

•**Automated Electrophysiology**: Cells were dissociated with 0.05% Trypsin and resuspended in external buffer solution. The suspension was transferred to the PatchXpress 7000A Plus, Automated Parallel Patch Clamp System. Tetrodotoxin (TTX) was added to the external solution at 1μM and 4-aminopyridine (4AP) was injected at 5mM and 50 μM.

Results

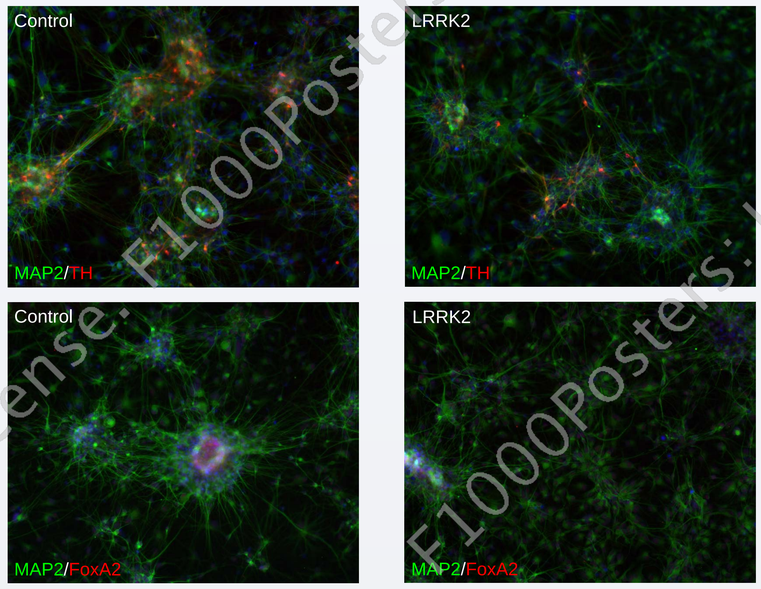


Figure 1. Immunocytochemistry illustrating dopaminergic markers: MAP2/TH, MAP2/FoxA2. Control line on the left, LRRK2 G2019S cell line on the right.

	LRRK2 D35	Cont D35		LRRK2 D35	Cont D35
Target	Regulation	Regulation	Target	Regulation	Regulation
CACNA1B	72	222	SCN11A	3	13
CACNA1C	184	275	SCN2A	35	59
CACNB1	30	115	SCN3A	48	122
CACNB3	5	12	SCN5A	9	-1
CACNG2	221	650	SCN7A	11	20
GRIA1	8	70	SCN9A	74	121
GRIA2	1942	575	SLC18A2	15	5
GRIK1	219	1101	SLC6A3	15	15
GRIK2	37	47			

Table 1. qPCR table showing ion channel expression level differences between the day 0 NSC stage (baseline) and the day 35 mature neuron stage in the LRRK2 and control cell lines. Our control line shows ion channel upregulation compared to the LRRK2 line.

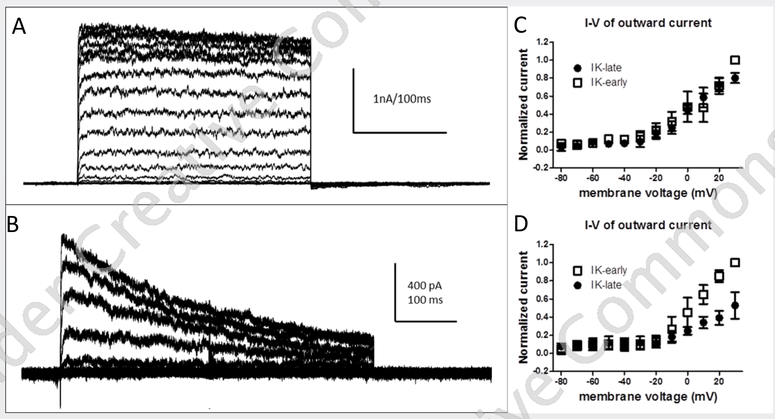


Figure 2. Endogenous currents recorded from the iPSC-derived neurons. **A**. Typical voltage dependent outward currents recorded from a control cell showing no significant inactivation of outward current. **B**. Typical voltage dependent outward currents from a LRRK2 G2019S cell showing significant current inactivation. The difference in current inactivation kinetics was consistent between these two cell lines, thereby suggesting differences in potassium ion channel subunit composition. **C**. I-V relationship of outward currents recorded from the control cells. Current amplitudes were measured at the beginning (IK-early) and end (IK-late) of depolarizing voltage steps. **D**. I-V relationship of outward currents recorded from LRRK2 G2019S cells. Current amplitudes were measured at the beginning (IK-early) and end (IK-late) of depolarizing voltage steps.

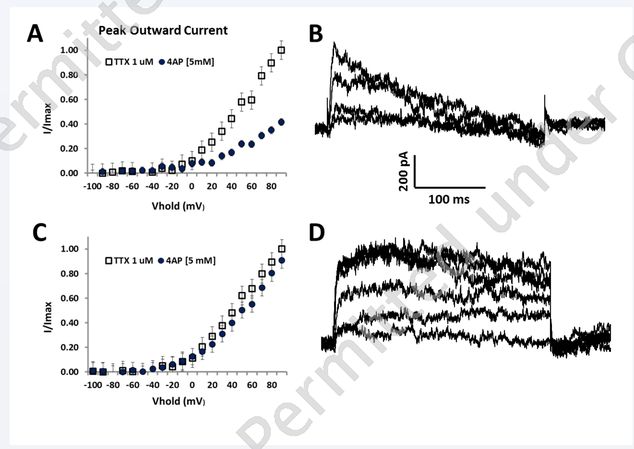


Figure 3. Pharmacological characterization of endogenous potassium currents recorded from the control iPSC-derived neurons. The potassium currents were first isolated by blocking sodium channels with 1uM TTX (open squares in panels **A** and **C**), before the cells were subjected to 5mM of 4-aminopyridine (4-AP) (closed circles in panels **A** and **C**). One group of cell displayed higher sensitivity to 4-AP than the other, suggesting variable expression of potassium channels among neurons. **A** and **C**: I-V relationship of potassium currents for group of neurons with higher (**A**) and lower (**C**) sensitivity to 4-AP; **B** and **D**: representative traces of 4-AP (5mM) sensitive currents from panel **A** (**B**) and panel **C** (**D**).

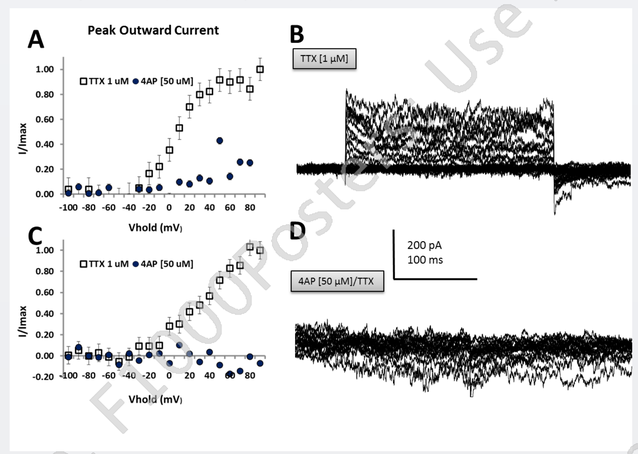


Figure 4. Pharmacological characterization of endogenous potassium currents recorded from the LRRK2 iPSC-derived neurons. The potassium currents were first isolated by blocking sodium channels with 1uM TTX (open squares in panels **A** and **C**), and a subsequent application of 50uM of 4-AP completely blocked the potassium currents. Panels **B** and **D** are representative currents remaining after the additions of TTX and 4-AP.

Discussion

Our immunocytochemistry images depict mature neurons stained with MAP2, FoxA2 and a subset expressed tyrosine hydroxylase. At the molecular level, preliminary data from qPCR experiments identified a number of ion channels that appear to be upregulated in the control line versus the mutant line. At the functional level, evidence from electrophysiology study demonstrates profound difference of kinetic profiles between two cell lines, with in the LRRK2 G2019S cell line displaying significant current inactivation. Further characterization of potassium channel pharmacology confirmed different sensitivity to channel blocker, suggesting the composition of potassium channels are different between two cell lines. Taken together our data suggests that we have a novel phenotype for our iPCS derived LRRK2 G2019S cells.

References

Mak et al, 2012 Stem Cell International 140427; Nguyen et al, 2011 Cell Stem Cell; Haythornthwaite et al, 2012 J. of Biomolecular Screening

Acknowledgments

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