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Supplementary Material

The Use Of Stem Cell-derived Neurons For Understanding Development And Disease Of The Cerebellum

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Reference	(Su et al., 2006)	(Salero & Hatten, 2007)	(Muguruma et al., 2010)	(Erceg et al., 2010)	(Srivastava et al., 2013)	(Wang et al., 2015)	(Higuera et al., 2017)
Cells transplanted	D9 Math1-GFP+ (mESC)	D24 *Pde1c-Egfp*-BAC (mESC)	D13+2 GAD-GFP Neph3+ (mESC)	D34+ MATH1-GFP+ (hESC)	D18+ DsRed (Math1-induced) (mESC)	D20 Neph3-GFP+ (hESC)	D20+(p2-6) Actin-GFP+ (mESC)
Age mouse	P2	P4-P6	E15.5	P7	P60	P0	P1 & Adult
Injection site	Cerebellum	EGL	Subventricular space of CP	EGL	Cerebellum	Cerebellum	Cerebellum (vermis & hemisphere)
Cell number injected	5,000-10,000	1,000	10,000	100,000	400,000	20,000	50,000 (P1) 75,000 (Ad)
Volume injected	2-4µl	1µl	2µl	100-200nl	2µl	1µl	1µl (P1) 1.5µl (Ad)
Analysis	5-14 days	1-15 days	1 & 4 wks	4 wks	1 wk	4 wks	25-30 days
Survival rate post-injection	0.1-0.3%		3%	14-18%			0.17% (P1) 2.8% (Ad)
Result	Migratory Math1+ cells, exhibiting leading and trailing processes associating with PF-like bundles. Expression of DCX, PAX6, GIRK2 and later GABAα6R.	*Pde1c-Egfp*-BAC cells migrated across ML into IGL. Interaction with MFs. Expression of ZIC2 and GABAα6R.	Grafted cells integrated into PCL and expressed L7 and Calbindin. Characteristic polarity with dendrites growing towards ML. VGluT2 expression along dendritic shafts.	Cells migrated across ML, past PCL and settled in IGL.	DsRed+ cells were detected in cerebellar lobules (mainly in ML) close to injection site. Cells displayed neuronal shape and expressed TUJ1. Started to colonize GL. Some neurons expressed GABAα6R.	2/12 mice showed GFP+/L7+ cells. Cells were detected in PCL with complex dendrite formation into ML, in ML and extra-cerebellar regions.	P1: 6/8 mice showed GFP labelling in cerebellar cortex. 9% of cells showed morphology of mature PCs and expressed Calbindin and Parvalbumin and received VGluT2-positive inputs.. Adult mice: 11/16 mice showed GFP labelling in cerebellar cortex (most in ML, some in PCL and GL or WM). 3.66% of GFP+ cells were Calbindin+ and showed mature PC morphology.

Supplementary Table 1. Summary of published experiments utilizing transplanted stem cell-derived cerebellar neurons to test integration and cell-fate commitment. Ad (Adult), CP (cerebellar plate), D (day of differentiation) ESC (embryonic stem cells), EGL (external granule layer), h (human), IGL (internal granule layer), GL (granule layer), m (mouse), ML (Molecular layer), PCL (Purkinje cell layer), P (postnatal day), p (passage number), PF (Parallel fiber), MF (Mossy fiber), wk (week), WM (white matter).

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