Chapter 6

Appendix

Summary

The current thesis sought to discuss the involvement of cerebellum in motor reflexes, ranging from simple vestibulo-ocular reflex to complicated limb movements. We attempted to investigate this question on several levels: how the spiking output of Purkinje could be regulated by synaptic GluR3 containing AMPARs; the spiking properties of Purkinje cell during motor behavior; how different cerebellar cortex neurons contribute to cerebellar dependent motor learning. In addition, we also evaluated the cerebellar dependent motor learning in two autism animal models.

In chapter 2, we evaluated the role of AMPAR subunit GluR3 at both PF-PC and CF-PC synapses. In chapter 2.1, we found that adaptation of compensatory eye movements is impaired in GluR3 but not in GluR1 deficient mice. Thus we went on investigate the role of GluR3 in PF-PC synaptic plasticity, and proposed a novel mechanism for PF-PC LTP, which relies on an increase in GluR3 channel conductance. This form of plasticity is mediated by activation of Epac2 through an increase of cAMP. In chapter 2.2, we studied the role of GluR3 in complex spike related spiking coding. We found that CS pause is reduced and CS waveform is altered in GluR3 deficient mice. And these changes in CS responses might not be the direct consequence of the mild reduced EPSC in GluR3 KO PCs, suggesting that this increased excitability following CS is caused by a compensatory mechanism.

In chapter 3, we shifted our focus form simple reflex to more complex, multi-joint motor behaviours. In chapter 3.1, we attempted to differentiate the distinct role of neurons in cerebellar cortex in limb movements and inter-limb coordination. We investigated these functions using the fully automated ErasmusLadder in cell-specific mouse mutant lines that suffer from impaired Purkinje cell output (Pcd), Purkinje cell potentiation (L7-Pp2b), molecular layer interneuron output (L7-Δ 2), and granule cell output (6-Cacna1a). We found all the four mouse lines exhibited impaired locomotion adaptation and altered front–hind and left–right inter-limb coordination. In chapter 3.2, we sought to establish the contribution of Foxp2 in cortex, striatum and cerebellum, to motor functions by using mice with region- or cell-type specific deletions. A pronounced phenotype was observed in animals lacking Foxp2 in cerebellar PCs (L7-Foxp2). L7-Foxp2 mice showed deficits in executing sequences of all speeds during a lever-pressing task and for skilled walking on the ErasmusLadder. Moreover, we found increased intrinsic excitability of PCs and impaired spiking modulation during locomotion in L7-Foxp2 mice. Whereas striatal-specific and cortical-specific knockouts were predominantly impaired in executing very fast press-sequences and showed deficits on the ErasmusLadder when the course was perturbed. These findings indicate that Foxp2 disruptions may affect motor sequence speed and stereotypy through actions in different brain circuits.

Studies in chapter 4 involved two mouse models of autistic spectrum disorders (ASD), the Neuroligin-3 (NL-3) and Shank2 mutants. ASD patients have been reported to present cerebellum related motor symptoms, such as impairments in eye-blink conditioning and eye movements, as well as balance and postural difficulties. In the NL-3 (chapter 4.1), we found dysfunctional PF-PC LTD and impaired CF elimination. Furthermore, NL-3 animals had difficulties in ErasmusLadder task. In the Shank2 mouse models (chapter 4.2), synaptic AMPARs were reduced and PF-PC LTP was impaired. Consequently, L7-shank2 mice displayed deficits in both VOR adaptation and locomotion.

Samenvatting

De huidige thesis heeft als doel de betrokkenheid van het cerebellum bij motorische reflexen te beschrijven, variërend van de eenvoudige vestibulo-oculaire reflex tot gecompliceerde bewegingen van ledematen. We hebben getracht om deze vraag op verschillende niveaus te onderzoeken: hoe de spiking output van Purkinje cellen gereguleerd kan worden door AMPARs die synaptisch GluR3 bevatten; de vuur-eigenschappen van Purkinje cellen tijdens motorisch gedrag; hoe verschillende neuronen in de cerebellaire cortex bijdragen aan cerebellum afhankelijk motorisch leren. Daarnaast hebben we cerebellum afhankelijk motorisch leren bestudeerd in twee diermodellen voor autisme.

In hoofdstuk 2 hebben we de rol van de AMPAR subunit GluR3 in zowel PF-PC en CF-PC synapsen geëvalueerd. In hoofdstuk 2.1 vonden we dat de adaptatie van compensatoire oog bewegingen gestoord is in GluR3 maar niet in GluR1 deficiënte muizen. Vervolgens hebben we de rol van GluR3 in PF-PC synaptische plasticiteit onderzocht en hebben we een nieuw mechanisme voor PF-PC LTP voorgesteld, wat gebaseerd is op een toename in GluR3 kanaal geleiding. Deze vorm van plasticiteit wordt gemedieerd door de activatie van Epac2 door een toename van cAMP.

In hoofdstuk 2.2 hebben we de rol van GluR3 in complex spike gerelateerde spike codering bestudeerd. We hebben gevonden dat in GluR3 deficiënte muizen de CS pause is verminderd en dat de golfvorm van de CS is veranderd. Deze veranderingen in CS respons zijn mogelijk niet een direct gevolg van de licht verminderde EPSC in GluR3 KO PCs, wat suggereert dat de toename in exciteerbaarheid wordt veroorzaakt door een compensatoir mechanisme.

In hoofdstuk 3 verschoof onze focus van simpele reflexen naar meer complex, multi-gewricht motorisch gedrag. In hoofdstuk 3.1 hebben we getracht de verschillende rollen van neuronen in de cerebellaire cortex bij beweging van ledematen en coördinatie tussen ledematen te differentiëren. We hebben deze functies onderzocht met behulp van de vol-automatische ErasmusLadder in cel-specifieke gemuteerde muis lijnen welke leden aan verminderde Purkinje cel output (Pc d), verminderde Purkinje cel potentiëring (L7-Pp2b), verminderde moleculaire laag interneuron output (L7-Δγ2) en verminderde korrel cel output (α6-Cacna1a). We vonden dat alle vier de muis lijnen een gestoorde locomotie adaptatie hadden en een veranderde voor-achter en links-rechts coördinatie tussen ledematen.

In hoofdstuk 3.2 hebben we gepoogd om de bijdrage van Foxp2 in cortex, striatum en cerebellum aan motorfuncties op te helderen door muizen met regio of cel-type specifieke deleties te gebruiken. Een uitgesproken fenotype werd geobserveerd bij dieren zonder Foxp2 in cerebellaire PCs (L7-Foxp2). L7-Foxp2 muizen lieten gebreken zien in de uitvoering van reeksen op alle snelheden gedurende een hefboom-druk test en bij het lopen op de ErasmusLadder. Daarbij vonden we een toename in de intrinsieke exciteerbaarheid van PCs en gestoorde modulatie van vuren gedurende locomotie in L7-Foxp2 muizen. De striatum-specifieke en cortex-specifieke knockout waren voornamelijk gestoord in het uitvoeren van zeer snelle druk-sequenties en vertoonden gebreken op de ErasmusLadder als het verloop verstoord werd. Deze bevindingen wijzen er op dat verstoringen in Foxp2 zowel de snelheid van motorische reeksen kan aantasten als ook stereotypie door acties in verschillende hersencircuits.

Studies in hoofdstuk 4 betroffen twee muis modellen van autistisch spectrum ziekten (ASD), Neuroligin-3 (NL-3) en Shank2 mutanten. Het is beschreven dat ASD patiënten cerebellum gerelateerde motorische symptomen vertonen, zoals beperkingen in oog-knipper conditionering en oogbewegingen, als ook balans en houdingsproblemen. In de NL-3 muizen (hoofdstuk 4.1) vonden we disfunctionele PF-PC LTD en gestoorde CF eliminatie. Tevens vertoonden NL-3 dieren moeilijkheden bij het uitvoeren van de ErasmusLadder taken. In de Shank2 muis modellen (hoofdstuk 4.2), waren de synaptische AMPARs verminderd en was PF-PC LTP gestoord. Dientengevolge vertoonden L7-shank2 muizen gebreken in zowel VOR adaptatie als locomotie.

Curriculum vitae

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Education

2013 –	PhD student, Department of Neuroscience, Erasmus MC, NL
2011-2013	Master of Neuroscience, Erasmus MC, NL
2005-2010	Medicine, Shanghai Jiao Tong University, CN

Articles in preparation

1. Zhou K, Gao Z, Boele HJ, Lin Z, Schonewille M and De Zeeuw CI. AMPA-Receptor subunit GluA3 regulates climbing fiber signaling in Purkinje cells. In preparation.

2. French CA*, Zhou K*, Vinueza Veloz MF *, Fisher SE, Costa RM and De Zeeuw CI. Foxp2 disruptions in specific brain circuits differentially affect motor-skill learning. Submitted.
   * These authors contributed equally to this work.

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1. Gutierrez-Castellanos N, Silva-Matos C, Zhou K, Canto CB, Renner C, Koene L, Ozyildirim O, Sprengel R, Kessels HW and De Zeeuw CI (2016). Motor Learning Requires Purkinje Cell Synaptic Potentiation through Activation of AMPA-Receptor subunit GluA3. Neuron

2. Peter S, Ten Brinke MM, Stedehouder J, Reinelt CM, Wu B, Zhou H, Zhou K, Boele HJ, Kushner SA, Lee MG, Schmeisser MJ, Boeckers TM, Schonewille M, Hoebeek FE, De Zeeuw CI (2016). Dysfunctional cerebellar Purkinje cells contribute to autism-like behaviour in Shank2-deficient mice. Nature Communication.

3. Vinueza Veloz MF*, Zhou K*, Bosman LW, Potters JW, Negrello M, Seepers RM, Strydis C, Koekkoek SK, De Zeeuw CI (2015) Cerebellar control of gait and interlimb coordination. Brain Structure and Function. * These authors contributed equally to this work.

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PhD Portfolio

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General Courses

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2012	Imaging life at the molecular level (ONWAR microscopy course)
2014	MGC epigenetic regulation in health and disease

Conferences

2014	FENS meeting (Milan)
2015	Society of Neuroscience meeting (Chicago)
2016	Barrel Cortex Function (Amsterdam)

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