EVALUATION OF MOTOR DEFICITS AND PERIHAEMATOMAL NEURONAL DEGENERATION IN A MOUSE MODEL OF CEREBELLAR HAEMORRHAGE

BY

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Thesis submitted in partial fulfilment of the requirement for the degree of Master of Neurosciences

April 2016

PENILAIAN DEFISIT MOTOR DAN DEGENERASI/ KEMEROSOTAN NEURON PERIHEMATOMAPADA MODEL TIKUS YANG MENGALAMI PENDARAHAN SEREBELUM

OLEH

ABUBAKAR TIJJANI SALIHU

Tesis diserahkan untuk memenuhi sebahagian keperluan bagi

Ijazah Sarjana Neurosains

April 2016

ACKNOWLEDGEMENT

First of all, I wish to express my thanks to ALLAH Almighty for His endless blessings and help to complete this work. I am greatly indebted to all those who contributed towards successful completion of this work. I would like to express special thanks and sincere appreciation to my supervisor Professor Dr. Jafri Malin Abdullah for his invaluable efforts, mentorship, guidance and encouragement to be an independent researcher and follow through on my ideas. I would also like to express my gratitude to my co-supervisor Dr. Sangu Muthu Raju who gave me wonderful practical suggestion and insight along the way. I am very grateful to Associate Professor Dr. Muzaimi Mustapha for his guidance related to successful completion of dissertation in timely manner. My special thanks go to all staff members at department of Neuroscience and Animal Research and service centre (ARASC), University Sains Malaysia, Health Campus, for their technical supports.

One cannot forget one's friends especially while living away from home. Special thanks to my classmates; Auwal Bello Hassan and Norlyana Samsuri and friends Adamu Ahmad Adamu and Abdullahi Sulaiman Gwani who were in regular contact with me and helped me achieved my aim of being here. Finally, I would like to thanks my entire family for their unwavering love and support. I could not have done this without you.

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ABSTRAK

Pengimejan klinikal yang bertambah baik dan pembedahan awal, apabila ia dikenalpasti, telah menunjukkan penurunan kadar kematian akibat daripada pendarahan serebelum. Namun begitu, hasil fungsi jangka panjang dalam kebanyakan yang terselamat adalah masih sangat membimbangkan; tidak kira sama ada mereka menerima pengurusan secara pembedahan atau konservatif. Jelas sekali, pelaksanaan rawatan perubatan yang digunakan secara khusus atau dalam kombinasi dengan pembedahan bagi meningkatkan hasil terhalang oleh kurangnya pemahaman yang betul bagi patofisiologi penyakit ini. Akibat daripada pendarahan serebelum, tisu otak dalam hematoma boleh disamakan dengan keadaan teras iskemik strok iskemia, dan berkemungkinan tidak akan dapat diselamatkan.Walau bagaimanapun, kehilangan neuron yang lambat disebabkan pelbagai mekanisme kecederaan yang mungkin terus berada di kawasan sekitar pendarahan dan ini boleh menyumbang kepada kecacatan yang berlaku dalam pesakit. Tujuan kajian ini adalah untuk mengetahui keadaan pendarahan sekitar tisu selepas eksperimen pendarahan serebelum pada tikus. Kami membuat hipotesis bahawa degenerasi neuron apoptosis yang lambat berlaku di kawasan perihematoma sejurus selepas pendarahan intraserebrum. Tikus jantan dewasa jenis albino Swiss telah dimasukkan dengan kolagenase jenis VII (0.4U dalam 1 ml saline) secara stereotactic pada sebelah bahagian serebelum, diikuti dengan anestesia. Haiwan dalam kumpulan rekaan telah disuntik dengan saline biasa / air masin, manakala haiwan kawalan tidak disuntik dengan apa-apa. Defisit motor dinilai menggunakan medan terbuka dan skor komposit untuk menilai model tikus bagi serebelum ataxia pada 1, 3, 7, 14 dan 21 hari selepas infusi kolagenase dan haiwan dikorbankan pada selang masa yang sama untuk penilaian degenerasi neuron perihematoma menggunakan haematoxylin dan pewarnaan eosin dan Annexin V-FITC /

Propidium iodida assay. Pada akhir kajian ini, didapati bahawa penyerapan 0.4U kolagenase menghasilkan lokomotor penting dan defisit/kekurangan ataksik pada tikus terutama dalam minggu pertama selepas pembedahan dan ini secara beransur-ansur bertambah baik dalam tempoh tiga minggu.Degenerasi neuron terbukti dengan pengecutan sitoplasmik dan piknosis nuklear diperhatikan di kawasan perihematomaselepas satu hari, terutama pada hari 3 dan 7 selepas pendarahan. Menjelang hari yang ke- 21, kedua-dua hematoma dan kemerosotan neuron di kawasan perihematomal itu telah fagosit dan baki sel-sel neuron di sekitar parut tisu kelihatan normal. Selain itu, sel-sel positif Annexin-V / propidium iodide diperhatikan di kawasan perihematomal pada hari 3 dan 7 menunjukkan bahawa neuron mungkin akan mati melalui apoptosis. Ini dapat disimpulkan bahawa sejumlah neuron yang berpotensi untuk diselamatkan wujud di kawasan perihematomal itu selepas pendarahan serebelum sepanjang masa yang luas yang boleh tahan uji dengan rawatan.

ABSTRACT:

Improved clinical imaging and early surgery, when indicated, have been shown to decrease mortality rate after cerebellar haemorrhage. Nonetheless, the long term functional outcome in the majority of the survivors is still very alarming; irrespective of whether they receive surgical or conservative management. Evidently, the development of definitive medical treatment to use alone or in combination with surgery in order to improve outcome in this condition is hampered by lack of proper understanding of the disease pathophysiology. Following cerebellar haemorrhage, brain tissue inside the hematoma can be considered equivalent to the ischemic core in ischaemic stroke, and is unlikely to be salvageable. However, delayed neuronal loss due to various secondary injury mechanisms might continue in the vicinity of the haemorrhage and this could contribute to the persistent disability in the patients. The aim of this study is to evaluate the histomorphological changes of the perihaemorrhagic tissue after experimental cerebellar haemorrhagen in mice. We hypothesized that delayed neuronal degeneration occurs in the perihaematomal area after intracerebellar haemorrhage. Adult male Swiss albino mice were stereotactically infused with collagenase type VII (0.4U in 1ml saline) unilaterally in to the cerebellum, following anaesthesia. The animals in the sham group were injected with normal saline, while the control animals were not injected with anything. Motor deficits were assessed using open field and composite score for evaluating mouse model of cerebellar ataxia at 1, 3, 7, 14 and 21 days after collagenase infusion and the animals were sacrificed at the same time interval for evaluation of perihaematomal neuronal degeneration using haematoxylin and eosin staining and Annexin V-FITC/ Propidium iodide assay. At the end of the study, it was found that infusion of 0.4U collagenase produces significant locomotor and ataxic deficit in the mice especially within the first week post surgery and this gradually improved within

three weeks. Neuronal degeneration evident by cytoplasmic shrinkage and nuclear pyknosis was observed at the perihaematomal area after one day; especially at 3 and 7 days post haemorrhage. By 21 days, both the haematoma and degenerating neurons in the perihaematomal area were phagocytosed and the remaining neuronal cells around the scar tissue appear normal. Moreover, Annexin-V/propidium iodide–positive cells were observed at the perihaematomal area at 3 and 7 days implying that the neurons likely die via apoptosis. It was concluded that a population of potentially salvageable neurons exist in the perihaematomal area after cerebellar haemorrhage throughout a wide time window that could be amenable to treatment.

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LIST OF ABBREVIATIONS:

CH: Cerebellar haemorrhage

ICH: Intracerebral haemorrhage

IVH: Intraventricular haemorrhage

IVD: Intraventricular drainage

CE: Clot evacuation

NMDA: N-Metthyl-D-Aspartic acid

IL-1β: Interleukin 1 beta

TNF-α: Tumour necrosis factor alpha

CSF: Cerebrospinal fluid

GCS: Glasgow coma scale

FITC: Fluorescein isothiocyanate

MRI/MRA: Magnetic resonance imaging/ Magnetic resonance angiography

iNOS: Inducible Nitric oxide synthase

COX-2: Cycloxygenase-2

MMP: Matrix metalloprotein

FasL: Fas ligand

DSA: Digital subtraction angiography

Src Kinase: Sarcoma Kinase

PLA-2: Phospholipase A2

ICAM-I: Intercellular adhesion molecule 1

DNA: Deoxyribonucleic acid

MPT: Methylpropyltryptamine

Bax: B-cell lymphoma 2 associated x protein

Bad: B-cell lymphoma 2 associated death promoter

Bcl-2: B-cell lymphoma 2

Bcl-xL: B-cell lymphoma extra large

Wnt3a: Wingless-Type MMTV Integration Site Family, Member 3A

s-Fas: soluble Fas

zVADfmk: carbobenzoxy-valyl-alanyl-aspartyl-[O-methyl]- fluoromethylketone)

AT1: Angiotensin 1

ATR: Angiotensin receptor

BBB: Blood brain barrier

COX-2: Cycloxygenase 2

Bak: B-cell lymphoma homologous antagonist killer

BTEB2: basic transcription element binding protein 2

TUNEL: Terminal deoxynucleotidyl transferase (TdT) dUTP Nick-End Labeling

GB: Gingkolide B

TUDCA: Tauroursodeoxycholic Acid

SAH: Subarachnoid haemorrhage

PBS: Phosphate buffered saline

AIF: Apoptosis inducing factor

PKCα: Protein kinase C alpha

PKCδ: Protein kinace C delta

KPNA2: Karyopherin alpha 2

MAO: Medial accessory olive

DAO: Dorsal accessory olive

MAOr: Rostral medial accessory olive

DAOr: Rostral dorsal accessory olive

MedM: Rostromedial nucleus

MedCm: Caudomedial nucleus

MedDL: Dorsolateral nucleus

BAHAGIAN C

Biodata Abstrak penyelidikan

LAMPIRAN A: Contoh Abstrak

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Introduction: Improved clinical imaging and early surgery, when indicated, have been shown to decrease mortality rate after cerebellar haemorrhage. Nonetheless, the long term functional outcome in the majority of the survivors is still very alarming; irrespective of whether they receive surgical or conservative management. Evidently, the development of definitive medical treatment to use alone or in combination with surgery in order to improve outcome in this condition is hampered by lack of proper understanding of the disease pathophysiology. Following cerebellar haemorrhage, brain tissue inside the hematoma can be considered equivalent to the ischemic core in ischaemic stroke, and is unlikely to be salvageable. However, delayed neuronal loss due to various secondary injury mechanisms might continue in the vicinity of the haemorrhage and this could contribute to the persistent disability in the patients.

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Methods: Adult male Swiss albino mice were stereotactically infused with collagenase type VII (0.4U in 1ml saline) unilaterally in to the cerebellum, following anaesthesia. The animals in the sham group were injected with normal saline, while the control animals were not injected with anything. Motor deficits were assessed using open field and composite score for evaluating mouse model of cerebellar ataxia at 1, 3, 7, 14 and 21 days after collagenase infusion and the animals were sacrificed at the same time interval for evaluation of perihaematomal neuronal degeneration using haematoxylin and eosin staining and Annexin V-FITC/ Propidium iodide assay.

Results: At the end of the study, it was found that infusion of 0.4U collagenase produces significant locomotor and ataxic deficit in the mice especially within the first week post surgery and this gradually improved within three weeks. Neuronal degeneration evident by cytoplasmic shrinkage and nuclear pyknosis was observed at the perihaematomal area after one day; especially at 3 and 7 days post haemorrhage. By 21 days, both the haematoma and degenerating neurons in the perihaematomal area were phagocytosed and the remaining neuronal cells around the scar tissue appear normal. Moreover, Annexin-V/propidium iodide–positive cells were observed at the perihaematomal area at 3 and 7 days implying that the neurons likely die via apoptosis.

Conclusion: It was concluded that a population of potentially salvageable neurons exist in the perihaematomal area after cerebellar haemorrhage throughout a wide time window that could be amenable to treatment.

Professor Dato’ Dr Jafri Malin Abdullah: Supervisor

Dr Sangu Muthuraju: Co-Supervisor

CHAPTER ONE

INTRODUCTION

1.1 Introduction

Cerebellar haemorrhage (CH) occur as a results of rupture of blood vessels within the cerebellum (Lekic, Rolland et al. 2013). This type of haemorrhage mostly occur in either of the cerebellar hemispheres (Pong, Chan et al. 2012, Wu, Li et al. 2012), although a vermian location is not uncommon (Zhang, Wang et al. 2014, Ng, Yang et al. 2015). Extensive bleeding involving both the vermis and hemisphere also happen in some cases (Salvati, Cervoni et al. 2001, Zhang, Wang et al. 2014). Superior cerebellar artery is implicated as the commonest source of bleeding in cerebellar haemorrhage (Matsukawa, Shinoda et al. 2012). The predominant cause of cerebellar haemorrhage is hypertension, which is associated with more than 70 percent of cases (Dahdaleh, Dlouhy et al. 2012, Matsukawa, Shinoda et al. 2012, Zhang, Wang et al. 2014, Chang, Lin et al. 2015). Other causes include vascular malformations (arteriovenous malformations, venous angioma, cavernous angioma and aneurysm), hemorrhagic diathesis, cerebellar metastasis, anticoagulation and haemorrhage into a cerebellar infarct (Dinsdale 1964, J. van Loon 1993). The most consistent features of the clinical presentation in this condition are Vomiting, headaches, dizziness, dysarthria, and inability to stand or walk (J. van Loon 1993, Pollak, Rabey et al. 1998).

Cerebellar haemorrhages accounts for approximately 10% of all intracerebral haemorrhages (ICH) in most populations, and nearly 50% of cerebellar strokes (Lloyd A. Dayes 1986, Flaherty, Woo et al. 2010, Ng, Yang et al. 2015). Most common in the elderly population, the average age of onset reported in most series is greater than sixty years (Matsukawa, Shinoda et al. 2012, Wu, Li et al. 2012, Chang, Lin et al. 2015, Ng,

Yang et al. 2015). Few cases which are often due to angiomatous malformations however exist among teenagers (Firsching, Huber et al. 1991, J. van Loon 1993, Kirollos, Tyagi et al. 2001).

Since the first report of a successful operation for acute cerebellar haemorrhage by Fisher in 1965, a plethora of subsequent studies have recommended surgical therapy, when indicated, for patients with cerebellar haemorrhage to prevent and/or treat life-threatening complications such as hydrocephalus, brainstem compression and CSF blockage caused by obstruction of the fourth ventricle (Fisher, Picard et al. 1965, Pollak, Rabey et al. 1998, Kirollos, Tyagi et al. 2001, Zhang, Wang et al. 2014, Ng, Yang et al. 2015). The indication, timing and type of surgical procedure have, however, remained a subject of controversy (J. van Loon 1993, Salvati, Cervoni et al. 2001, Dahdaleh, Dlouhy et al. 2012, Zhang, Wang et al. 2014). Meanwhile, many patients are still treated conservatively (Lloyd A. Dayes 1986, J. van Loon 1993, Salvati, Cervoni et al. 2001, Ng, Yang et al. 2015). These conservative treatment options (Witsch, Neugebauer et al. 2013) are, nonetheless, neither disease-specific nor standardised and also suffer from lack of evidence (Witsch, Neugebauer et al. 2013). There is similarly no consensus yet regarding their indications.

Improved clinical imaging and early surgical intervention have contributed largely to the decreased mortality rate from cerebellar haemorrhage in recent years (Lui, Fairholm et al. 1985, Dahdaleh, Dlouhy et al. 2012, Ng, Yang et al. 2015), compared to the decades ago (McKissock, Richardson et al. 1960). However, the long term functional outcome in the majority of the surviving patients remain unfavourable, regardless of whether they are treated surgically or conservatively (Matsukawa, Shinoda et al. 2012, Pong, Chan et al. 2012, Wu, Li et al. 2012, Chang, Lin et al. 2015). In a review of clinical outcome among primary cerebellar haemorrhage survivors, Witsch

and colleagues have found that 63.2% of the survivors suffer from moderate to severe disability after data from six case series was pooled together (Witsch, Neugebauer et al. 2013). Therefore, the need for additional medical treatments to use alone or in conjunction with surgery in order to improve outcome in these patients become indispensable. The impact of haemorrhage on the cerebellar tissue might not only be limited to the acute local irritation, tearing and destruction of the tissue due to the direct compressive effect of the clot (Kitaoka, Hua et al. 2002, Babu, Bagley et al. 2012). Other factors such as the body/tissue response to the hematoma through inflammation and the release of clotting factors such as thrombin, as well as the toxic effects of blood components (hemoglobin and iron) could impose strong, delayed cytotoxic effects on the viable neurons surrounding the hematoma leading to their death and thus extending the lesion size. Indeed, evidence from experimental models of supratentorial intracerebral hemorrhage (especially basal ganglia hemorrhage) strongly support the prevailing role of these factors in inducing secondary neuronal loss in the perihaematomal area after hemorrhage into the brain tissue. There is substantial evidence that thrombin participate in intracerebral haemorrhage-induced secondary injury (Cui, Gao et al. 2011, Babu, Bagley et al. 2012, Caliaperumal, Brodie et al. 2014, Liu, Shi et al. 2014, Krenzlin, Lorenz et al. 2016). Gong and his co-workers have found elevated brain thrombin activity in ipsilateral basal ganglia one hour after ICH in rats, and the activated thrombin in turn activate protease-activated receptors which phosphorylate NMDA receptors through Src leading to excitotoxic neuronal injury (Gong, Xi et al. 2008, Sharp, Liu et al. 2008). Additionally, thrombin can stimulate microglia to secrete IL-1β and TNF-α which are potentially toxic to the brain (Wu, Yang et al. 2008). In addition to the important role they play in brain recovery after ICH, multiple animal studies have shown that both resident and infiltrating

inflammatory cells including microglia and neutrophils also contribute to secondary neuronal damage (Zhang, Li et al. 2006, Jung, Chu et al. 2007, Gao, Wang et al. 2008, Lee, Cheng et al. 2015). Evidence indicates that these cells produced reactive oxygen species and proinflammatory cytokines that are damaging to neurons in the perihaematomal region (Wu, Yang et al. 2008, Wang 2010). Other reported contributors of secondary neuronal injury in the perihaematomal area after supratentorial bleeding are haemoglobin degradation products (Babu, Bagley et al. 2012). Iron and bilirubin oxidation products have been shown to induce neuronal damage after experimental haemorrhage in to rodent hippocampus and porcine frontal lobe respectively (Clark, Loftspring et al. 2008, Song, Hua et al. 2008). The mechanism by which they might cause tissue damage is through free radical generation and oxidative stress and the use of free radical scavengers and antioxidants such as metallotheionin and Deforaxamine (an iron chelator) were all successful in reducing neuronal death and improves functional outcome in rodents (Hua, Keep et al. 2008, Rojas, Lekic et al. 2008, Yamashita, Okauchi et al. 2008, Hu, Tao et al. 2016). Moreover, studies in both animals and humans have revealed the prevailing role of apoptotic neuronal death, particularly in the perihaematomal region after haemorrhage in to the striatum (Matsushita, Meng et al. 2000, Qureshi, Suri et al. 2003, Delgado, Cuadrado et al. 2008, Suarez 2008, Chu H 2014, Ling, Li et al. 2014), and this is presumably caused by the secondary injury mechanisms.

Obviously, the cerebellum which has four times more neurons (Andersen, Korbo et al. 1992) compared to the cerebral cortex is likely to experience greater neuronal loss in the perihaematomal area due to various secondary injury mechanisms. Additionally, cerebellar neurons are discovered to be more susceptible to injury by oxygen glucose deprivation and reoxgenation compared to cerebral cortical neurons (Scorziello,

Pellegrini et al. 2004). However, to date, the impact of cerebellar haemorrhage on the surrounding tissue has not been extensively studied (Rosenberg 2011) and fundamentally, the pathophysiological mechanisms underlying secondary neuronal damage following cerebellar haemorrhage is not yet understood. A proper understanding of pathophysiological mechanism is needed, in order to identify explicit therapeutic targets to minimize secondary neuronal injury following cerebellar haemorrhage. Evidence of ischemic damage within the infratentorial compartment after the induction of experimental cerebellar haemorrhage has been reported in at least one study (Cossu, Pau et al. 1994). But afterward, the progress in the understanding of the effect of bleeding on the cerebellar tissue, and the mechanism of healing has been much slower certainly due to lack of animal models (Rosenberg 2011). A collagenase-induced intracerebellar haemorrhage model has recently being characterized in rats and replications of this model could be used for investigations of injury and subsequent therapeutic mechanisms after cerebellar haemorrhage (Lekic, Rolland et al. 2011).

Mice are increasingly adopted as animals of choice in intracerebral haemorrhage research (Chang, Chen et al. 2011, Chu H 2014, Weng, Tan et al. 2015). It is believed that genetically engineered mice are valuable tools for determining the mechanism of injury following intracerebral haemorrhage (MacLellan, Paquette et al. 2012). Through the use of knock-out or transgenic mice, numerous studies have recently identified the detrimental effect of various factors such as of superoxide, heme oxygenase 1, Toll-like receptor 4, signal transduction molecules such as Caveolin-1, and heme in supratentorial intracerebral haemorrhage (Wang and Doré 2007, Wakisaka, Chu et al. 2010, Chang, Chen et al. 2011, Chen, Zhang et al. 2011, Sansing, Harris et al. 2011). Moreover, using similar approach, the neuroprotective potential of other agents including heme-oxygenase 2, aquaporin-4 expression, haptoglobin and elements of the complement

system in intracerebral haemorrhage have been identified (Nakamura, Xi et al. 2004, Wang and Dore 2008, Zhao, Song et al. 2011, Chu H 2014). Therefore, characterizing a model of intracerebellar haemorrhage in mice could be a gateway to the understanding of injury mechanisms and potential therapeutic targets after cerebellar haemorrhage.

CHAPTER TWO

LITERATURE REVIEW

2.1 Anatomy and functional organisation of mouse cerebellum

2.1.1 Gross features of mouse cerebellum

The anatomy of the mouse cerebellum is recently described in detail (Watson 2012). Constituting averagely more than eleven percent of total brain weight, the external gross features of mouse cerebellum is typical of most mammals. It consists of a series of median vermal lobules and laterally placed extensions called hemispheres. Separating the vermis from the hemispheres on each side is the shallow paramedian sulcus. The relationship between the mouse cerebellum and other parts of the brain is such that its rostral surface is pressed against the inferior colliculus and the caudal pole of the cerebral cortex (Figure 2.1). Cerebellum also forms the roof of the fourth ventricle rostrally by its superior medullary velum. Caudally, the remaining half of the cerebellum overhangs the ependymal roof and choroid plexus of the caudal half of the fourth ventricle. Additionally, three pairs of cerebellar peduncles attached the cerebellum to the brain stem. The cerebellar afferent and efferent fibres pass through the peduncles.

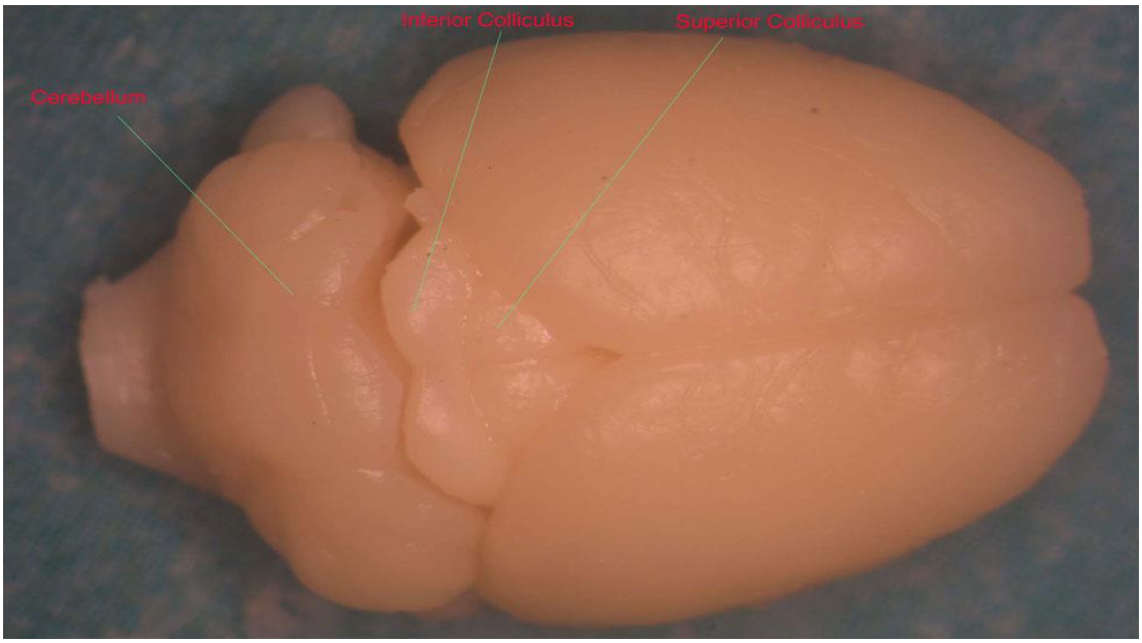

Figure 2.1 Gross features of mouse cerebellum. Adapted from www.mbl.org

2.1.2 Mouse cerebellar lobules

The mouse cerebellum is divided rostrocaudally by many fissures and sulci into a chain of folds called lobules (Watson 2012). The primary fissure divides the cerebellum into anterior and posterior lobes which are further subdivided into lobules (Figure 2.2). The mouse cerebellar vermal lobules include the lingual (I), ventral lobule of the lobules centralis (II), dorsal lobule of the lobules centralis (III), ventral lobule of culmen (IV), dorsal lobule of culmen (V), declive (VI), tuber (VII), pyramis (VIII), uvula (IX) and nodulus (X) (Inouye and Oda 1980). In the hemispheres, the lobules are arranged from rostral to caudal as simple lobule, crus 1 of the ansiform lobule, crus 2 of the ansiform lobule, the paramedian lobule and the copula of the pyramis. The vermal segment of the simple lobule is denoted as VICb. VIICba is extended laterally as crus 1 and crus 2 of the ansiform, while the paramedian lobule is regarded as the lateral extension of VIICbb. VIIICb extends laterally as the copula of the pyramis in the hemisphere. The simple lobule occupied the small frontal part of the cerebellar posterior lobe. On the other hand, the major part of the cerebellar hemisphere is occupied by the ansiform lobule which is divided into a rostral (crus 1) and caudal (crus 2) parts by the intracrural fissure. Rostrally, the paramedian lobule is separated from the caudal border of crus 2 by the ansoparamedian fissure, while on its caudal end, it is demarcated from the rostral side of the copula of the pyramis by the prepyramidal fissure. On the lateral side, the copula of the pyramis continues with the paraflocculus. The paraflocculus is a small round structure located inside a bony fossa bounded by the semicircular canals. At the bottom of the posterior lateral fissure, the cortex of the paraflocculus is continous with that of the flocculus. In the posterior lobe, the vermis is divided by the deep posterior fissure into a rostral division containing VICb to VIIICb and a caudal part

containing IXCb and XCb. The posterior lateral fissure separates lobules IXCb and XCb, and also separate paraflocculus from the flocculus (Watson 2012).

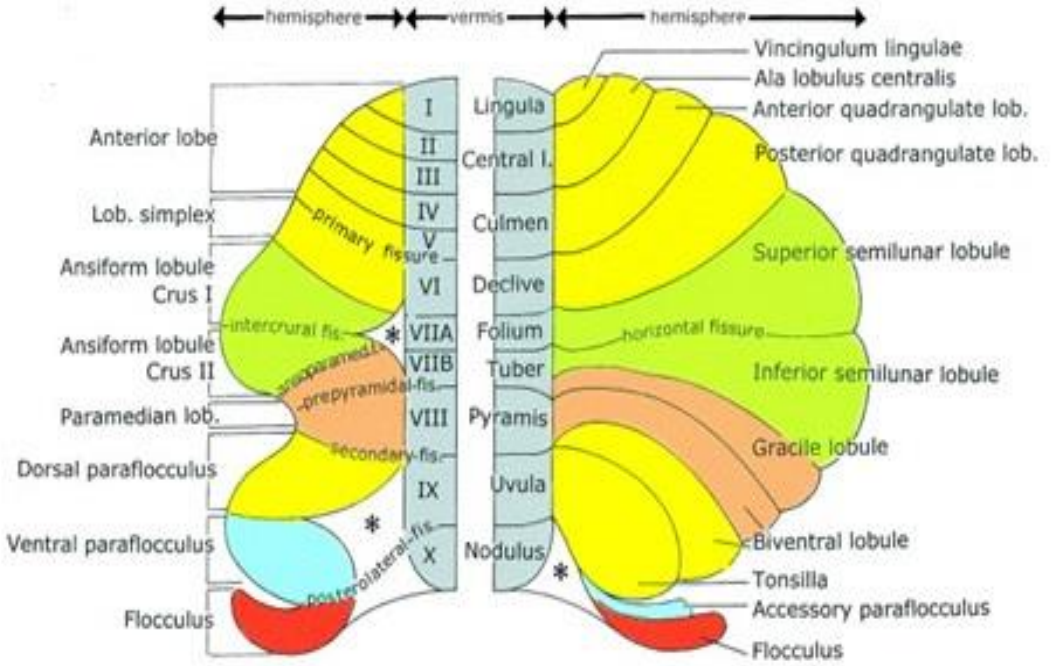

Figure 2.2: Mouse cerebellar lobules (right side) and comparable areas in human cerebellum (left side). Adapted from (Voogd and Glickstein 1998).

2.1.3 Histology of mouse cerebellar cortex

The mouse cerebellar coretex is made up of three different cellular layers each with a unique cellular contents (Figure 2.3) (Watson 2012). The most superficial of these layers is the molecular layer. Primarily, this layer is made up of purkinje cell dendrites and granule cells axons (parallel fibres). It also contains the stellate and basket cell interneurons (Voogd and Glickstein 1998). Next to the molecular layer is the purkinje cell layer. This is a monolayer of purkinje cell somata, but it also contain the cell bodies of candelabrum and Bregman glia. Finally, the deepest layer boardering the medullary centre of the cerebellum is the granular layer. The majority of this layer is occupied by the small granule cells residing together with somata of Golgi cells and Lugaro cells interneurons. Additionally, unipolar brush cells recognised only recently as as a dinstict neuronal type are also located in the granular layer (Diño, Nunzi et al. 2000).

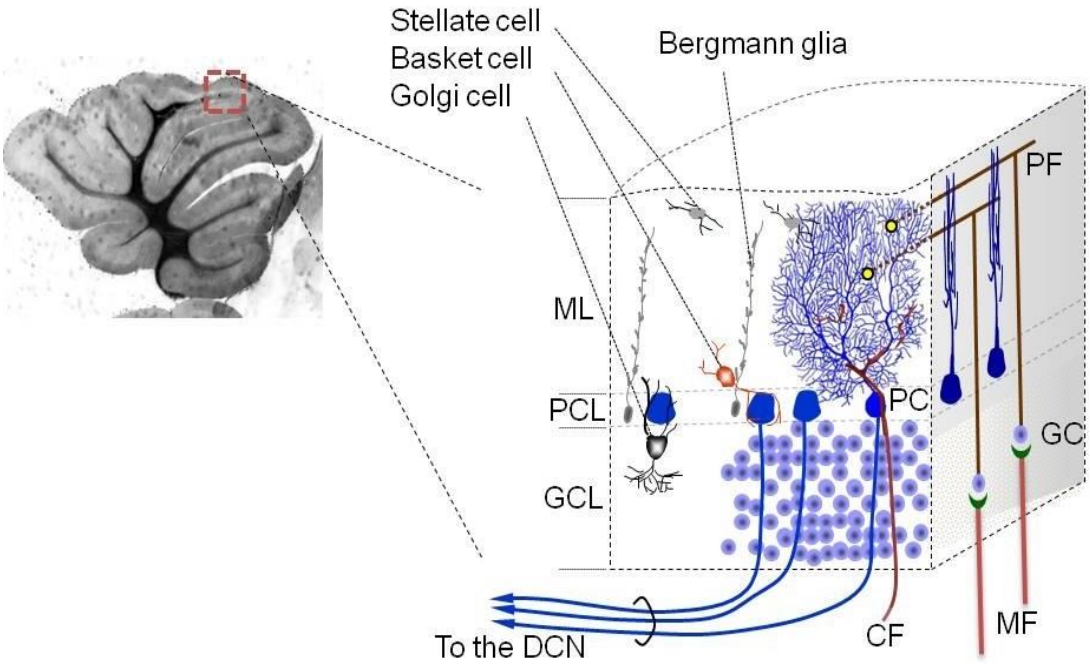

Figure 2.3: Cellular layers of mouse cerebellar cortex (ML: Molecular layer; PCL: Purkinje cell layer; GCL: Granule cell layer; DCN: Deep cerebellar nuclei; CF: Climbing fibre; MF: Mossy fibre; GC: Granule cell; PF: Parallel fibre). Adapted from (Hirai and Iizuka 2011).

2.1.4 Afferent and efferent connections of the mouse cerebellum

Input to the cerebellar cortex is carried by three major afferent systems (Watson 2012). Originating from the contralateral inferior olivary nucleus of the medulla oblongata are the climbing fibres. These fibres synapse within the proximal dendrites of purkinje cells. The second afferent system comprises of the mossy fibres which arises from many sites including the brainstem, the spinal cord and the pons. The connection of mossy fibres to purkinje cells is indirect (Itō 1984). Their axons terminate on granule cells which in turn send axons to the molecular layer where they bifurcate into parallel fibres before making synaptic contact with the spiny branches of purkinje cells dendrites. Consequently, a single mossy fibre may influence many purkinje cells. The third afferent system comprises of the noradrenergic afferents from the locus coereleus (Abbott and Sotelo 2000), cholinergic afferents from the pedunculopontine nucleus (Jaarsma, Ruigrok et al. 1996) and serotonergic afferents from the raphe nucleus (Strahlendorf and Hubbard 1983). The distribution of these fibres within the cerebellum has not been mapped in detail. Nevertheless, the few that have been mapped were shown to be terminating within all the three layers of the cerebellar cortex and in some cases localized to particular lobules (Watson 2012).

Essentially, the three major afferent systems converge upon the purkinje cells. Hence, the output of the cerebellar cortex conveys an integration of all incoming information. The purkinje cells send inhibitory efferents to the cerebellar nuclei and the vestibular nuclei which are located in the brainstem. In turn, the cerebellar nuclei serve as the major cerebellar output sending descending projections back to afferent sources such as the spinal cord and inferior olive. Additionally, cerebellar nuclei send efferent axons via the thalamus to the cerebral cortex. Through the integration and modulation

of afferent information, the cerebellum plays critical role in the regulation of fine motor control, sensory motor learning, memory and perhaps cognition (Watson 2012).

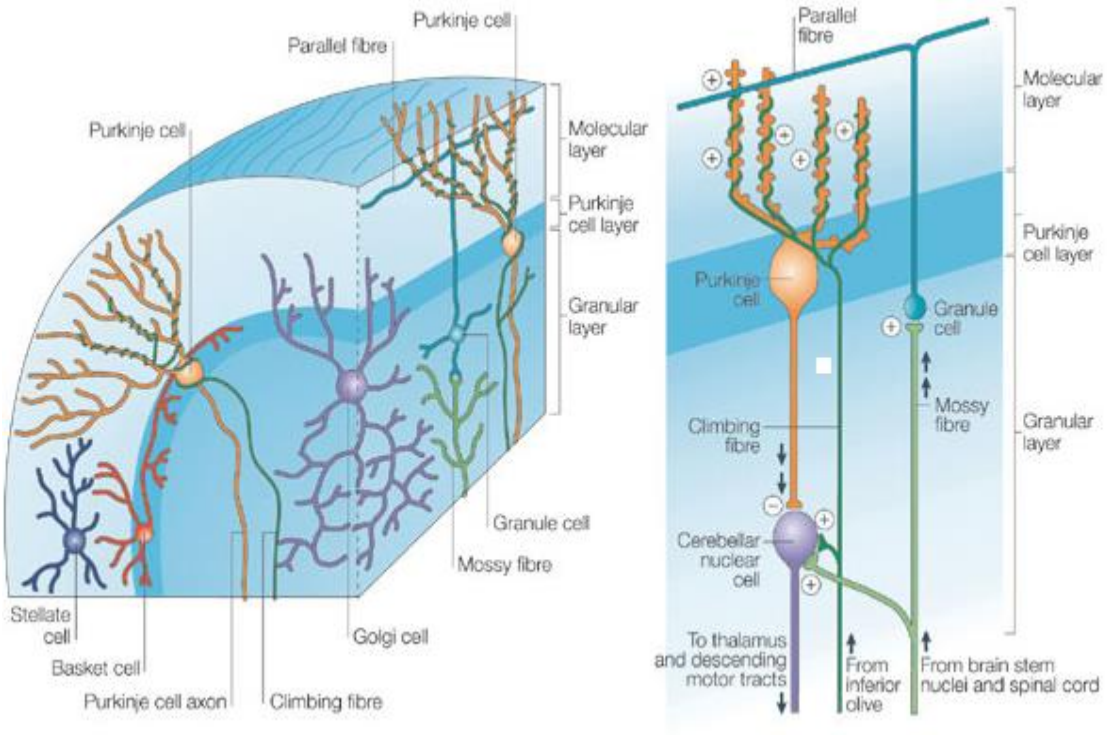

Figure 2.4. Afferent and efferent connections of mouse cerebellum. Adapted from (http://goo.gl/17c0U)

2.1.5 Mouse deep cerebellar nuclei

Three major nuclei (medial, interposed and lateral nuclear groups) are usually present in the deep cerebellar white matter of all mammals (Figure 2.5) (Watson 2012). These are equivalent to the fastigial, interposed and dentate nuclei in primates and the names are even used interchangeably (Bauer, Kerr et al. 2011, Uusisaari and Knöpfel 2011). However, the cerebellar nuclei in rodents are not as discrete as those found in humans and other primates (Treuting and Dintzis 2011). Cerebellar nuclei are subdivided into smaller groups forming the major cerebellar output to the thalamus and spinal cord. In the mouse, the subdivisions are similar to those that have been described in detail in the rats (Voogd and Ruigrok 2004, Chung, Marzban et al. 2009). Medial cerebellar nucleus is subdivided into three; the caudomedial nucleus (MedCM), the rostromedial nucleus (MedM) and the dorsolateral protuberance (MedDL). The caudomedial, rostromedial and dorsolateral subdivisions of medial cerebellar nucleus contain small, intermediate and large cells respectively. Moreover, MedDL receives afferents from the posterior lobe of cerebellar cortex, whereas the rest of the medial nucleus receives corticonuclear projections from the vermis (Voogd and Ruigrok 2004). Functionally, it is believed that the medial nucleus is involved in adaptive control of balance, posture and autonomic functions through its rostral part which send projections to vestibular nuclei and reticular formation, while the caudal part is involved in oculomotor control via its connection to the cerebral cortex (Bagnall, Zingg et al. 2009). The interpositus nucleus is divided into posterior and anterior parts. The main part of posterior interpositus nucleus contains large neurons, while its ventral part called the pervicellular part contains small cells (Voogd and Ruigrok 2004). A small triangular region (interstitial cell groups) is located between the rostromedial nucleus, posterior interpositus and pervicellular part. Posterior interpositus receives input from the red

nucleus, the superior colliculus, the suprafasciular nucleus, thalamus, and the zona incerta (Voogd and Ruigrok 2004). On the other hand, the anterior part of the interpositus nucleus is further divided into medial and lateral parts. Anterior interpositus efferent join the ipsilateral superior cerebellar peduncle and is thought to be involved in the learning of conditioned eye blink response (Chen, Bao et al. 1999). The lateral nucleus consists of ventral and dorsal parts. The main portion of lateral nucleus is formed by its dorsal part. The ventral part contains mainly small cells and is named the pervicellular part of lateral nucleus. Efferent from the lateral nucleus leave the cerebellum via the ipsilateral superior cerebellar peduncle (Watson 2012).

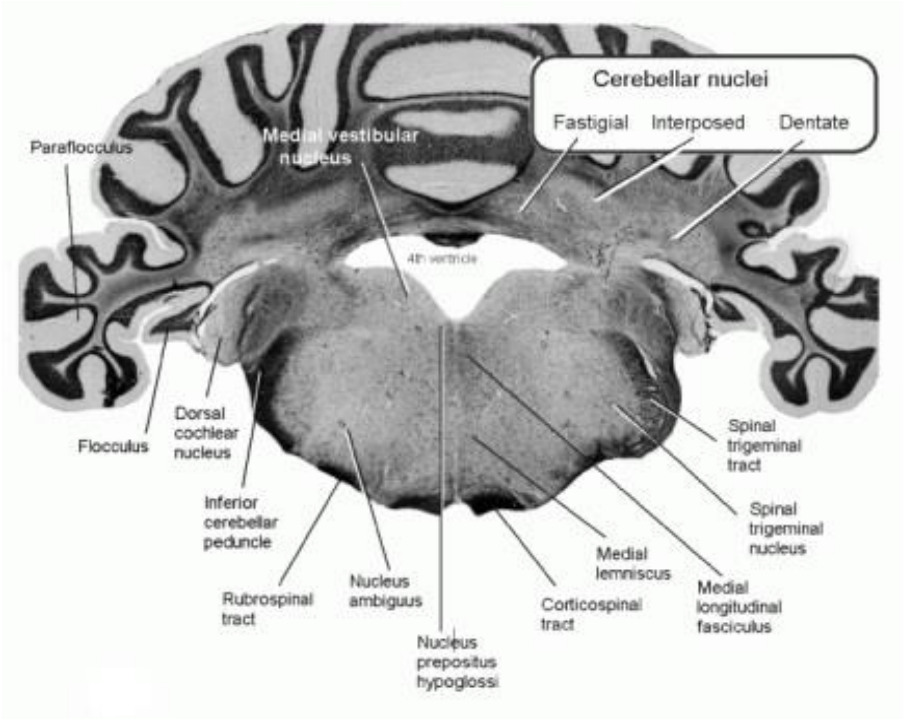

Figure 2.5: Mouse deep cerebellar nuclei.

Adapted from https://instruct.uwo.ca/anatomy

2.1.6 Functional organization of mouse cerebellum

There is a close relationship between structure and function in mammalian cerebellum (Watson 2012). Each anatomically distinct area is supplied by a subset of functionally unique afferent fibres, implying the possibility of functional specificity within different cerebellar regions. In the antero-posterior axis, specific mossy fibre subset projects mainly to lobules in the anterior zone and posterior zone (the spinocerebellum) (Watson 2012). Other mossy fibres projects heavily to the central zone and nodal zone (pontocerebellum and archicerebellum respectively). In the medio-lateral axis, certain mossy fibres project only to the vermis and not to the hemisphere (eg spinocerebellar tracts). Moreover, the input of climbing fibres into the purkinje cells leads to the division of the cerebellar cortex into a series of longitudinally oriented strips or 'sagittal zones'(Ruigrok 2011). Individual zones are typically 1–2 mm in width, running across the cerebellar lobules for many millimetres in the rostrocaudal plane (Voogd and Glickstein 1998, Manni and Petrosini 2004). However, these smaller zones are encompassed in the larger subdivisions of the cerebellum namely the vermis (medial zone), paravermis (intermediate zone) and the lateral zone situated laterally in the hemisphere (Morton and Bastian 2004). In each zone, the Purkinje cells receives climbing fibre input from a circumscribed region of the inferior olive and, in turn, send output to a circumscribed region in the cerebellar nuclei, thereby forming discrete olivo–cortico–nuclear complexes (Itō 1984). Thus inputs and outputs are highly compartmentalized into distinct anatomical divisions leading to functional specialization i.e. purkinje cells from each zone contributes to specific behavioural response in the animal (Watson 2012). Although purkinje cells microzones have mainly being studied in rats and cats, they are likely the functional counterparts of the parasagittal molecular stripes which have been studied extensively in mice. The basic

modular arrangement as observed in the rat is shown in Figure 2.6. In the vermis, the A, X, and B zones are distinguished that, by definition project to fastigial nucleus, interstitial cell groups and lateral vestibular nucleus, respectively. They receive climbing fiber input from respectively the caudal part of medial accessory olive (MAO), the intermediate part of MAO and the dorsal fold of dorsal accessory olive (DAO) (Voogd and Ruigrok, 2004). In the paravermis, the C1, C2 and C3 zones are located. They receive input from rostral medial accessory olive (MAOr) and rostral dorsal accessory olive (DAOr) and send efferent mainly to the interpose nuclei (anterior and posterior). In the hemispheres the D zones (D1, D0 and D2) are found but their precise relation with inferior olive and cerebellar nuclei are debated (Buisseret-Delmas and Angaut 1993, Ruigrok 1996, Ruigrok and Voogd 2000). The highly organized input-output relations of each of these zones determine their functional roles and as a whole they are called modules, which possibly reflect the functional entities of the cerebellum (Oscarsson 1980). Although much is known about the neuronal wiring of individual cerebellar modules, their behavioural significance remains to be fully elucidated (Pijpers 2007, Cerminara and Apps 2011). Recent data from neural recording studies on the functional role of three different cerebellar modules: the vermal A module, the paravermal C2 module and the lateral D2 module suggests that A module is concerned with balance and the postural base for voluntary movements, while the C2 module is concerned more with limb coordination (Cerminara and Apps 2011). This is not to imply that these are the only functions of these modules. Indeed, the available evidence points that they may also have some responsibilities in common—notably a shared or perhaps complementary role in the control of eye and head movements (Cerminara and Apps 2011). In addition, Purkinje-cell recordings in the lateral cerebellar D2 module have provided evidence of the operation of an internal model associated with

visuomotor control (Cerminara and Apps 2011). Similarly, lesion studies in various animals including rats and mice provide useful insight into the overall function of cerebellum and the possible role of the various zones (especially the larger subdivisions) in movement control and other functions. Intraperitoneal or oral injection of Neurotoxin 3-acetylpyridine (3-AP) and benzene in rats causes extensive neuronal loss in all the three deep cerebellar nuclei as well as the cellular layers and induces cerebellar ataxia and locomotor reduction (Jiang, Cao et al. 2015, Rafati, Erfanizadeh et al. 2015). Behavioural assessment in various cerebellar mutant mice such as staggerer, hot-foot, and lurcher also revealed significant disturbances in posture and equilibrium and impaired sensorimotor learning (Lalonde, Bensoula et al. 1995, Lalonde, Filali et al. 1996). Additonally, lesion to the ponto-cerebellar and olivo-cerebellar pathways, the major afferent pathways to the cerebellum causes deficits in learning complex motor sequences and spatial tasks in rats (Gasbarri, Pompili et al. 2003). The rats also showed static equilibrium deficiencies.

Influences of the medial zone of the cerebellum on balance and locomotion are well documented in animals. Lesions of the vestibular or fastigial nuclei in cats and monkeys cause abnormal changes in upright postural tone, impairments in maintaining sitting and standing balance and difficulty walking (Chambers and Sprague 1955). Specifically, cats with damage to either the fastigial or vestibular nuclei produce a standing and walking posture of ipsilateral limb flexion and adduction combined with contralateral limb extension and abduction (Sprague and Chambers 1953, Chambers and Sprague 1955). Balance deficits that include impaired righting responses and frequent falls backward and toward the side of the lesion are also common. In rats, unilateral and bilateral electrolytic lesions of fastigial nuclei produces motor deficits characterized by significantly increased grid and narrow beam runway time, increased hindlimb and

forelimb error on grid and narrow runway and decreased rotarod retention time (Choudhary, Gajalakshmi et al. 2014, Wankhar and Rathinasamy 2015). Taken together, these data indicate that the midline regions of the cerebellum play a strong role in the control of balance and locomotion. Generally, the midline cerebellar regions control extensor tone thereby maintaining upright balance and stance. They also modulate the rhythmic flexor and extensor muscle activations of the reticular and vestibular nuclei that generate portions of the locomotor pattern (Morton and Bastian 2004). In addition, the medial cerebellar region may also participate in altering the locomotor pattern based on sensory feedback from the limbs. The role of the intermediate region of the cerebellum in locomotion is not as well understood. Studies have shown that walking deficits are less pronounced after damage to intermediate cerebellar regions compared to more medial regions (Thach, Goodkin et al. 1992). Small intermediate zone cerebellar cortical lesion in mice produce subtle gait abnormalities that were only detectable on treadmill gait analysis (Stroobants, Gantois et al. 2013). However, in the rotarod test, cerebellar-lesioned mice performed at the level of control animals (Stroobants, Gantois et al. 2013). Balance and upright posture show little or no impairment during walking and standing in cats and monkeys with intermediate zone cerebellar damage (Chambers and Sprague 1955). Cats with intermediate anterior lobe or interpositus lesions can often walk rather effectively over ground but have difficulty walking on a treadmill or on rungs of a ladder (Chambers and Sprague 1955, Thach, Goodkin et al. 1992). Some notable abnormalities of walking that have been reported include a loss of placing reflexes, ipsilateral limb hypermetria during swing phase and abnormal timing of forelimb stance and swing durations (Udo, Matsukawa et al. 1980, Thach, Goodkin et al. 1992). With respect to this latter impairment, lesions of the intermediate cerebellar cortex have been shown to result in

an increased swing phase and a decreased stance phase in the ipsilateral limb and a decreased swing phase and an increased stance phase in the contralateral limb (Udo, Matsukawa et al. 1980). Therefore, the intermediate cerebellar region is involved in locomotor control, although in a different way than the medial cerebellar region. The consistent finding that over-ground locomotion is less impaired than treadmill or ladder walking suggests that one main function of the intermediate region might relate to the specific control of limb placement (e.g., regulating the timing, amplitude, and trajectory of limb elevation and descent), particularly when more than the usual amount of precision is required (Morton and Bastian 2004). Regarding the lateral cerebellar zone, animal lesion studies show that over-ground walking deficits are minimal after lateral cerebellar damage. Bilateral lesions targeting lateral deep cerebellar nuclei significantly impaired visuospatial processing, but not visuomotor coordination in rats (Noblett and Swain 2003). It similarly slowed down the acquisition of the hidden platform task of the Morris water maze, without affecting any of several motor performance scores including vertical grid, suspended wire, and rotarod tests (Joyal, Strazielle et al. 2001). Electrolytic lesion of cerebellar dentate nuclei lower breaking points on an operant conditioning progressive ratio schedule and reduce open field exploration in rats in another study (Bauer, Kerr et al. 2011). But the reduction in open field exploration was in the absence of motor impairment, implying that it is as a result of hedonic and purposive motivational reduction rather than difficulty in walking. Further illustrating the minimal effect of lateral lesion in producing motor deficits in animals is the study by Joyal and colleagues. They demonstrated that rats with midline lesions had difficulty in maintaining their equilibrium on a bridge and were slower before turning upward and traversed less squares on an inclined grid while those with lateral lesions had milder deficits on the bridge and were not affected in the other two tests (Joyal, Meyer et al.

1996). In the Morris water maze test, however, rats with lateral lesions were deficient in spatial orientation, whereas rats with midline lesions were deficient in visuomotor coordination. Evidently, the most consistent finding from lateral lesion studies is that animals display more of cognitive deficits than motor deficits (Figure 2.7).