A Robust Role for Motor Cortex

Gonçalo C. Lopes

Dissertation presented to obtain the
Ph.D degree in Biology | Neuroscience
Instituto de Tecnologia Química e Biológica António Xavier | Universidade Nova de Lisboa

Oeiras,
December, 2016

A Robust Role
for Motor Cortex

Gonçalo C. Lopes

Dissertation presented to obtain the
Ph.D degree in Biology | Neuroscience
Instituto de Tecnologia Química e Biológica António Xavier | Universidade Nova de Lisboa

Research work coordinated by: Champalimaud Foundation

Oeiras, December, 2016

UNIVERSIDADE
NOVA
DE LISBOA

A Robust Role for Motor Cortex

Gonçalo C. Lopes

A Dissertation
Presented to the Faculty
of Universidade Nova de Lisboa
in Candidacy for the Degree
of Doctor of Philosophy

Supervisors:
Joseph J. Paton
Adam R. Kampff

2016

In loving memory of Kiba

Acknowledgments

An impressively large number of people have accompanied me throughout this journey, and to all of them I am deeply and profoundly thankful. All interactions have a meaning we can never recognize and their combined impact ripples into the future unabated. This is true even if I forget to publicly acknowledge some of them, as I'm sure will be the case.

The Champalimaud Neuroscience Programme has been to me a place of deep personal and intellectual transformation. I can barely recognize the person that six years ago set out on the journey from computer science to neuroscience, intrigued by the mysteries of the brain. The efforts of the institute as a whole to bring together people from widely different cultural and academic backgrounds has created a nexus in which interesting personalities cannot help but be forged and tempered. I will never forget the unique opportunity I had to interact with such a large community of scientists from all over the world, and to discuss nearly every possible topic to my utmost satisfaction. These interactions were always done freely and passionately, in the best spirit of scientific companionship, with no regard for hierarchy or rank.

My first dedication goes to my companions of the International Neuroscience Doctoral Programme, Carolina Doran, Simone Lackner, Tiago Marques, Ivo Marcelo, Bruno Miranda, Raimundo Leong, and Gustavo Moreno, who in the year of 2010 embarked with me on this adventure. Together we have shared much more than just our initial training in neuroscience. Even though we all parted to pursue our own individual projects, there were many crucial points during the journey where, even serendipitously, we were still able to support each other as pillars of sanity in the middle of uncontrollable turbulence. It was an honour and a pleasure to have met each one of you and I hope to have contributed back a small inkling of all the inspiration and admiration you have provided me.

Pavel Itskov, Elena Dreosti, Roberto Medina, Scott Rennie, and Samuel Meyler provided continued drive to collaborate on interesting projects, in addition to their friendship. The academic world would be gentler if there were even more opportunities for this kind of short, sweet and honest collaborations. Bassam Atallah and Cindy Poo have diligently introduced me to many of the subtle complications of animal research. If my transition from desk to bench was any success was mainly due to their patience and support in those early days.

I have to give thanks to Alex Gomez-Marin for sharing with me the passion to ask all the embarrassingly simple questions and to keep searching pure hearted for the truth, no matter how deep or how far back in time the answers must be sought. Also to Niccolò Bonacchi and João Frazão for all the time spent in diligent discussions about all kinds of abstract technicalities that almost no one knows or cares about, but that in the end are critical to keep everyone running; especially Frazão for once again offering me the privilege of his unrivalled sharp and constructive criticism. To João Paulo Gomes for the enduring friendship, creative input and influential discussions.

It is hard to endure long without the kind of safe haven and relaxing times which I found in the short stays with my parents, sister and extended family. Even though I was almost invariably lost to my own thoughts, I found irreplaceable solace in these moments of peace which drove some of the most important insights presented in this thesis. I hope you forgive me for all my absent mindedness and know that I love you all very much.

In the members of the Learning Lab, Gustavo Moreno, Rui Azevedo, Sofia Soares, Thiago Gouvêa and Tiago Monteiro, I always found encouraging support at the bench, as well as very critical debate of ideas in our many lab meetings. I know I was always a bit of an outsider intellectually but rest assured that I took into serious consideration each of your many comments and I thank you for the friendship and sharing spirit.

The Intelligent Systems Lab was quite literally a second home throughout most of the years of this project. To all its members, Joana Neto, João Frazão, Danbee Kim, George Dimitriadis, Lorenza Calcaterra, Pedro Lacerda, and Atabak Dehban I also want to say thank you for the unique companionship and team spirit with which we battled through challenges of any kind. No matter what crazy project was at hand, we were always able to tackle it together, combining our expertise to overcome our individual difficulties. It is not easy to find such equally sharing and devoted spirits that will give so much while expecting so little in return. Thank you for the extraordinary opportunity. Throughout the years there were also many students interning for short projects which helped to shape research in many new weird directions. I want to thank all of them, but in particular to Tim Schröder for a particularly inspiring and fun internship collaboration, where no small measure of work assembling setups, tweaking experiments and trying to make sense of complicated results was achieved.

To the members of my thesis committee, Megan Carey and Leopoldo Petreanu, I want to thank for much needed external perspective and criticism that helped to ground the development of this project. Also to John Krakauer for unparalleled upfront criticism and passionate debate about the hardest conceptual questions surrounding the project. His seemingly unlimited energy and pointers to missing literature did much to invigorate me at a time of disappointment and disillusionment.

To Joseph J. Paton for accepting me as a student and taking up a conceptually complicated project. Even though we did not always see eye-to-eye on questions of methodology and interpretation, I have always taken Joe as the highest standard of rigour against which to sharpen otherwise vague and unsupported ideas. Most, if not all, of the deeper search for answers detailed in this thesis were undertaken directly to try and address the many critically keen concerns that were raised in our meetings and discussions. I hope I have succeeded partly in providing some of the much needed justifications.

To Adam R. Kampff for being more than a mentor, a friend and companion; a fresh and inquisitive mind that never detracts from asking the critical questions. In Adam I always found the incredible ability to point out new interesting directions both for experiments and interpretation of results, even when they initially seemed disappointing or confusing. Also for the tremendous capacity to step back and let students develop their own critical thinking and ability to independently do research. It was thanks to this unique style that I was provided with many opportunities to directly defend difficult ideas in front of a large number of other scientists, and this I now see was absolutely fundamental for intellectual growth. Finally, for the unrelenting support and belief in this project, sometimes even when I had myself lost hope.

And finally, to my wife, Joana Nogueira. It is not uncommon for authors to thank their family and loved ones for shouldering the personal burden and emotional stress of the work, but in this case, the debt runs much deeper. Indeed, early on Joana took a decision that would change both our lives forever, as she joined our experimental group to directly assist in bringing this project to fruition. This work and thesis is every bit hers as it is mine, and I mean it quite literally in every respect, as we built experimental preparations together, performed surgical procedures and took care of animals together, and wrote manuscripts together, often at an exceedingly high burden to our personal life. Producing this manuscript is mostly a victory and testament to her determination and support. I don't know of any greater dedication or demonstration of love than what she has shown me throughout this endeavour. I could not have finished the journey without you by my side and I can only hope that throughout the rest of our lives together I can approximate at least a small percentage of what you have given me these last six years. I am forever yours.

Título

Uma Função Robusta para o Córtex Motor

Resumo

A determinação da função exacta do córtex motor existente no cérebro dos mamíferos tem sido um mistério que persistiu ao longo do tempo. Existe uma longa história de estudos que ligam a actividade desta área do cérebro ao controlo de movimentos "voluntários" mas, curiosamente, existe uma história igualmente longa de estudos em animais descrevendo uma grande variedade de movimentos complexos que *não são* afectados com a remoção total do córtex motor. Qual a razão por detrás desta discrepância? Que tipo de movimentos serão realmente controlados pelo córtex motor? Esta tese procura reconciliar as muitas perspectivas existentes sobre o controlo do córtex sobre os movimentos e sugerir uma estratégia para investigar a teleologia desta região do cérebro.

Começamos por introduzir um conjunto de ferramentas de *hardware* e *software* para facilitar o estudo detalhado de comportamentos motores em situações naturalistas em roedores. Estas ferramentas permitem ao cientista reconfigurar rapidamente o contexto físico e lógico de uma tarefa comportamental em simultâneo com a medição precisa e em tempo-real de vários parâmetros de performance motora.

De seguida investigamos o comportamento de ratos, com e sem o córtex motor, durante a travessia de um percurso de obstáculos em que eram apresentados novos desafios motores inesperados. Surpreendentemente, observámos que os ratos em que o córtex motor havia sido removido demonstraram dificuldade em lidar pela primeira vez com um desafio motor inesperado, apesar de preservarem a sua capacidade de se adaptar com eficácia ao novo ambiente após sucessivas tentativas.

Esta observação levou-nos a propor e discutir uma possível função primordial para o córtex motor: estender a robustez dos sistemas sub-corticais

responsáveis pelo controlo dos movimentos. Especificamente, sugerimos que o córtex motor é a estrutura que permite aos mamíferos ultrapassar situações que requerem uma sucessão de respostas comportamentais rápidas e adaptadas a um novo contexto; uma das capacidades que reconhecemos como característica do reino mamífero.

Abstract

The function of mammalian motor cortex has remained a persistent mystery. There is a long history of research linking activity in this part of the brain with the control of “voluntary” movements but surprisingly there is an equally large body of evidence in non-human animals describing all kinds of complex behaviours that are *not* impaired when motor cortex is fully removed. What is the reason behind this discrepancy? What kind of movements are actually controlled by motor cortex? This thesis attempts to reconcile the many conflicting views on the cortical control of movement and outline a strategy for investigating the teleology of this brain region.

We start out by introducing a new set of hardware and software tools for neuroscience that aim to make it easier to study in detail more naturalistic motor behaviours in rodents. These tools allow the experimenter to quickly reconfigure the physical and virtual environment of a behaviour task while simultaneously tracking in real-time fine-scale measurements of motor performance.

We then set out to investigate the behaviour of rats facing unexpected or unpredictable motor challenges while navigating dynamic obstacle courses with or without motor cortex. Surprisingly, we found that rats without motor cortex show visible impairments when dealing for the first time with an unexpected motor challenge, despite retaining the ability to skilfully adapt to the new environment with repeated trials.

This observation has led us to propose and discuss a primordial role for motor cortex in extending the robustness of sub-cortical movement systems. Specifically, we suggest that motor cortex is the structure that has helped mammals to conquer those situations that require a succession of rapid and adapted behavioural responses to unexpected environmental change; the kind of resourcefulness that is one of the defining characteristics of mammalian phylogeny.

Financial Support

The research leading to these results has received funding from the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement no. 600925 and the Bial Foundation (Grant 190/12). GL was supported by the PhD Studentship SFRH/BD/51714/2011 from the Foundation for Science and Technology, and a Visiting Research Fellow stipend from the Sainsbury Wellcome Centre for Neural Circuits and Behaviour at University College London. The Champalimaud Neuroscience Programme is supported by the Champalimaud Foundation.

Contents

Acknowledgements ix

Título e Resumo xiii

Abstract xv

Financial Support xvi

1 Towards a Teleology of Cortical Motor Control 1

1.1 Chapter Summary 2

1.2 A Dilemma for Cortical Motor Control 3

1.2.1 Discovery of the Motor Cortex 3

1.2.2 The Goltz-Ferrier Debates 4

1.3 The Role of the Corticospinal Tract 8

1.3.1 A Functional Theory for the Motor Cortex 9

1.3.2 The Effects of Lesions in the Corticospinal Tract . . 13

1.4 An Integrative View of the Motor System 20

1.4.1 The Coordinative Role of Inhibition 22

1.4.2 Hierarchical Organization of Motor Behaviour 25

1.5 A Strategy for Probing Cortical Control 30

1.5.1 Outline of the Thesis 32

2 Rapid Prototyping Tools for the Study of Behaviour 47

2.1 Chapter Summary 48

2.2 Introduction 49

2.3 Methods . . . 51
  2.3.1 The Modular Behaviour Box . . . 51
  2.3.2 The Bonsai Framework . . . 55
2.4 Results . . . 71
  2.4.1 Getting Started with Bonsai . . . 71
  2.4.2 Under the Hood . . . 72
  2.4.3 Applications . . . 74
2.5 Discussion . . . 79
2.6 Acknowledgements . . . 82
2.7 Author contributions . . . 83

3 Moving with and without Motor Cortex **87**
3.1 Chapter Summary . . . 88
3.2 Introduction . . . 89
3.3 Methods . . . 91
3.4 Results . . . 103
3.5 Discussion . . . 121
3.6 Acknowledgements . . . 122
3.7 Author contributions . . . 123

4 Extended Discussion **129**
4.1 Chapter Summary . . . 130
4.2 A challenge from robotics . . . 131
4.3 A primordial role for motor cortex . . . 133
4.4 Implications for non-primate mammals . . . 133
4.5 Implications for primate studies . . . 134
4.6 Some speculation on the role of direct cortical control . . . 135
4.7 Some preliminary conclusions . . . 136

Chapter 1

Towards a Teleology of Cortical Motor Control

> *The infinite fertility of the organism as a field for adapted reactions has become more apparent. The purpose of a reflex seems as legitimate and urgent an object for natural inquiry as the purpose of the colouring of an insect or a blossom. And the importance to physiology is, that the reflex reaction cannot be really intelligible to the physiologist until he knows its aim.*
>
> SIR CHARLES S. SHERRINGTON, *The Integrative Action of the Nervous System* (1906)

1.1 Chapter Summary

Motor cortex has 150 years of conflicting history. It was originally defined as the part of cortex where movements can be evoked by low-current stimulation. Stimulated points across the cortical surface were found to be organized into a rough map of the body. A monosynaptic projection system, the corticospinal tract, was found to directly link motor cortex to neurons in contralateral spinal cord. There is a marked decrease in granular layer IV, the main source of inputs from sensory thalamus, across this excitable zone. Lesions of the motor cortex in humans can cause paralysis in any limb and permanently disrupt the execution of basic actions such as standing or walking. Recordings of neural activity in motor cortex correlate with various movement parameters. These lines of evidence support the idea that this part of the brain directly controls movement.

However, lesions of the motor cortex in non-human animals preserve most of the animal's behaviour repertoire. Sectioning of the corticospinal tract in primates is sufficient to reinstate the primary effects of motor cortical lesions, but there are also vast projections from motor cortex to other cortical and sub-cortical areas, including multiple disynaptic parallel descending pathways to spinal centers via brainstem. In most mammals, the corticospinal tract does not target motor neurons in ventral spinal cord, as it does in most primates, but rather spinal interneurons.

It is clear that this part of the brain is somehow involved in movement, but a large number of questions remain strangely unanswered. If motor cortex is a controller, what kind of movements does it control? How does it interact with other existing brain structures to generate behaviour? Why do motor cortical lesions produce such an apparently incomplete effect on movement? This chapter is an attempt to piece together all the fragmentary and contradictory evidence on motor cortical structure and physiology in order to derive a unified functional picture of cortical motor control.

1.2 A Dilemma for Cortical Motor Control

The involvement of the brain and spinal cord in motor control has been recognized since the earliest known clinical records on head and spinal injury, dating back to ancient Egypt (Louis, 1994; van Middendorp, Sanchez & Burridge, 2010). However, the role of the nervous system in generating behaviour was not fully appreciated until Galvani first reported his famous experiments on *animal electricity* (Galvani, 1791). By isolating the sciatic nerve and gastrocnemius muscle in the frog, Galvani clearly demonstrated in a series of stimulation experiments that an electrical process, contained entirely within the biology of the frog's leg, was responsible for the spontaneous generation of muscle contractions. This would lead over the following century to the discovery and physiological characterization of the nerve impulse, the action potential, that travels across the nerve to initiate muscle movement (du Bois-Reymond, 1843; Bernstein, 1868; Schuetze, 1983). The success of these seminal experiments immediately raised a fundamental question regarding nerve conduction: if spontaneous muscle contraction is generated by nerve impulses transmitted throughout the nervous system, how is this transmission coordinated in order to generate the complex patterns of muscle activity observed in natural behaviour?

1.2.1 Discovery of the Motor Cortex

In search of answers to this question, many researchers looked at the brain, the seat of anatomical convergence of the nervous system, for such an integrative role. Following Galvani's footsteps, several attempts were made to stimulate the cerebral cortex electrically, but with little success (Gross, 2007). It wasn't until the 1870s that the first indications of a direct involvement of the cortex in the production of movement came to light, around the time when Hughlings Jackson underwent his studies on epileptic convulsions (Jackson, 1870). He observed that in some patients the fits would start by a deliberate spasm on one side of the body, and that different body parts would become systematically affected one after the other. He connected the orderly march of these spasms to the existence of localized lesions in the *post-mortem* brain of his patients and hypothesized that the origin of these fits was uncontrolled excitation caused by local changes in cortical *grey matter* (Jackson, 1870). In that same year, Fritsch and Hitzig published their famous study demonstrating that it is possible to elicit movements by direct stimulation of the cortex in dogs (Fritsch & Hitzig, 1870). Furthermore, stimulation of different parts of the cortex produced movement in different parts of the body (Fritsch & Hitzig, 1870). It appeared that the causal mechanism for epileptic convulsions predicted by Hughlings Jackson had been found, and with it a possible explanation for how the normal brain might control movement. The cerebral cortex was already considered at the time to be the seat of reasoning and sensation, so if activity over this so-called *motor cortex* was able to exert direct control over the whole musculature of the body, then it might represent in the normal brain the area that connects volition to muscles (Fritsch & Hitzig, 1870).

1.2.2 The Goltz-Ferrier Debates

David Ferrier, a Scottish neurologist deeply impressed by the ideas of Hughlings Jackson and by the positive results of Fritsch and Hitzig's experiments, proceeded to reproduce and expand on their observations with comprehensive stimulation studies showing how activity in the motor cortex was sufficient to produce a large variety of movements across a wide range of mammalian species (Ferrier, 1873). Meanwhile, other researchers across Europe such as Goltz and Christiani were facing a dilemma: in many of the so-called "lower mammals" massive lesions of the cerebral cortex failed to demonstrate any visible long-term impairments in the motor behaviour of animals (James, 1885; Goltz, 1888). These two lines of inquiry first clashed at the seventh International Medical Congress held in London in August 1881, where Goltz of Strassburg and Ferrier of London presented their results in a series of debates on the localization of function in the cerebral cortex (Phillips, Zeki & Barlow, 1984; Tyler & Malessa, 2000).

Goltz assumed a clear anti-localizationist position. He advanced that it was impossible to produce a complete paresis of any muscle, or complete dysfunction of any perception, by destruction of any part of the cerebral cortex, and that he found mostly deficits of general intelligence in his dogs (Tyler & Malessa, 2000). Following Goltz's presentation, Ferrier emphasized the danger of generalizing from the dog to animals of other orders (e.g. man and monkey). He then proceeded to exhibit his own lesion results by means of antiseptic surgery in the monkey, describing how a circumscribed unilateral lesion of the motor cortex produced complete contralateral paralysis of the leg. He also produced a striking series of microscopic sections of Wallerian degeneration (Waller, 1850) of the "motor path" from the cortex to the contralateral spinal cord, the crossed descending projections forming the pyramidal corticospinal tract (Tyler & Malessa, 2000).

The debates concluded with the public demonstration of live specimens: a dog with large lesions to the parietal and posterior lobes from Goltz; and from Ferrier, a hemiplegic monkey with a unilateral lesion to the motor cortex of the contralateral side. As predicted, Goltz's dog showed a clear ability to locomote and avoid obstacles and to make use of its other basic senses, while displaying peculiar deficits of intelligence such as failing to respond with fear to the cracking of a whip or ignoring tobacco smoke blown to its face. On the other hand, Ferrier's monkey showed up severely hemiplegic, in a condition similar to human stroke patients. After the demonstrations, the animals were killed and their brains removed. Preliminary observations revealed that the lesions in Goltz's dog were less extensive than expected, particularly on the left hemisphere. Ferrier's lesions on the other hand were precisely circumscribed to the contralateral motor cortex. These demonstrations secured the triumph of Ferrier, who went on to firmly establish the localizationist approach to neurology and the idea of a somatotopic arrangement over the motor cortex.

The Goltz-Ferrier debates had far-reaching implications throughout the entire research community of the time, and the basic dilemma that was presented has sparked controversy and confusion for over a hundred years since (Phillips et al., 1984; Lashley, 1924; de Barenne, 1933; Tyler & Malessa, 2000; Gross, 2007). In the meantime, views of motor cortex have evolved to suggest it plays a role in "understanding" the movements of others (Rizzolatti & Craighero, 2004), imagining one's own movements (Porro et al., 1996), or in learning new movements (Kawai et al., 2015), but where are we today regarding its suggested primary role in directly controlling movement?

Stimulating motor cortex causes movement; motor cortex is active during movement

Motor cortex is still broadly defined as the region of the cerebral hemispheres from which movements can be evoked by low-current stimulation, following Fritsch and Hitzig's original experiments in 1870 (Fritsch & Hitzig, 1870). Stimulating different parts of the motor cortex elicits movement in different parts of the body, and systematic stimulation surveys have revealed a topographical representation of the entire skeletal musculature across the cortical surface (Leyton & Sherrington, 1917; Penfield & Boldrey, 1937; Neafsey et al., 1986). Electrophysiological recordings in motor cortex have routinely found correlations between neural activity and many different movement parameters, such as muscle force (Evarts, 1968), movement direction (Georgopoulos, Schwartz & Kettner, 1986), speed (Schwartz, 1993), or even anisotropic limb mechanics (Scott, Gribble, Graham & Cabel, 2001) at the level of both single neurons (Evarts, 1968;

Churchland & Shenoy, 2007) and populations (Georgopoulos et al., 1986; Churchland et al., 2012). Determining what exactly this activity in motor cortex controls (Todorov, 2000) has been further complicated by studies using long stimulation durations in which continuous stimulation at a single location in motor cortex evokes complex, multi-muscle movements (Graziano, Taylor & Moore, 2002; Aflalo & Graziano, 2006). However, as a whole, these observations all support the long standing view that activity in motor cortex is involved in the direct control of movement.

Motor cortex lesions produce different deficits in different species

What types of movement require motor cortex? In humans, a motor cortical lesion is devastating. Permanent injury to the frontal lobes of the brain by stroke or mechanical means is often followed by weakness or paralysis of the limbs in the side of the body opposite to the lesion (Louis, 1994). Although the paretic symptoms have a tendency to recover partially by themselves, especially with training and rehabilitation, permanent movement deficits and loss of muscle control in the affected limbs is the common prognosis; movement is permanently and obviously impaired (Laplane, Talairach, Meininger, Bancaud & Bouchareine, 1977; Kwakkel, Kollen, van der Grond & Prevo, 2003). In non-human primates, similar gross movement deficits are observed after lesions, albeit transiently (Leyton & Sherrington, 1917; Travis, 1955). The longest lasting effect of a motor cortical lesion is the decreased motility of distal forelimbs, especially in the control of individual finger movements required for precision skills (Leyton & Sherrington, 1917; Darling, Pizzimenti & Morecraft, 2011). But equally impressive is the extent to which other movements fully recover, including the ability to sit, stand, walk, climb and even reach to grasp, as long as precise finger movements are not required (Leyton & Sherrington, 1917; Darling et al., 2011; Zaaimi, Edgley, Soteropoulos & Baker, 2012). In non-primate mammals, the absence of lasting deficits following motor cortical lesion is even more striking. Careful studies of skilled reaching in rats have revealed an impairment in paw grasping behaviours (Whishaw, Pellis, Gorny & Pellis, 1991; Alaverdashvili & Whishaw, 2008), comparable to the long lasting deficits seen in primates, but this is a limited impairment when compared to the range of movements that *are* preserved (Whishaw et al., 1991; Kawai et al., 2015). In fact, even after complete decortication, rats, cats and dogs retain a shocking amount of their movement repertoire (Goltz, 1888; Bjursten, Norrsell & Norrsell, 1976; Terry, Herbert & Oakley, 1989). If we are to accept the simple hypothesis that motor cortex is the structure responsible for "voluntary movement production", then why is there such a blatant difference in the severity of deficits caused by motor cortical lesions in humans versus other mammals? With over a century of stimulation and electrophysiology studies clearly suggesting that motor cortex is involved in many types of movement, in all mammalian species, how can these divergent results be reconciled?

1.3 The Role of the Corticospinal Tract

It must have felt uncanny to those early researchers to find that surface stimulation of the cortex produces discrete muscle responses, in a way so similar to what Galvani did with the frog's leg. Indeed, Sherrington himself conveys the feeling clearly in the opening of his seminal lecture on the motor cortex (Sherrington, 1906, p.271), confessing "that although it is not surprising that such territorial subdivision of function should exist in the cerebral cortex, it is surprising that by our relatively imperfect artifices for stimulation we should be able to obtain clear evidence thereof."

Of course, it did not go unnoticed that this fact might be due to the massive projection from cortex to the spinal cord, which had been fully traced by Ludwig Türck only twenty years before Fritsch and Hitzig's experiment (Nathan & Smith, 1955). This so-called "pyramidal" tract[1] was found to originate in the anterior regions of the cerebral cortex and terminate directly in the lateral columns of the spinal cord after decussating (i.e. crossing over) at the level of the brainstem's *medulla oblongata*. The presence of this corticospinal tract presented compelling visual evidence of the means by which the motor cortex might be able to exert such a direct influence on movement by electrical conduction of nerve impulses, but the underlying biological mechanism remained elusive.

1.3.1 A Functional Theory for the Motor Cortex

Only four years after the discovery of the motor cortex, the Ukrainian anatomist and histologist Vladimir Betz connected for the first time the macroscopic cerebral organization and function proposed by Hitzig and Ferrier with unique, detailed histological evidence of cells found in the motor region, in his remarkably insightful 1874 publication:

> Such consistency in the region where these cells can be found, manifested as a very definitive cortical layer, as well as in a specific cerebral convolution, prompted me to devote my attention to that particular part of the animal brain, mainly the dog's, in which Fritsch and Hitzig achieved such brilliant physiological results, i.e. the lobe which borders the cruciate sulcus. I now found such cells of the same shape and in exactly the same position in nests in the dog, precisely in the lobe just mentioned. So in the dog, as well as in man, they are imbedded in the fourth cortical layer and occur only in this lobe and in the anterior half of the posterior (postcentral) convolution bordering it. In the dog, they are somewhat smaller, but

---

[1] The name "pyramidal" is derived from the fact that the tract passes through the medullary pyramids, a pair of white matter structures in the brainstem with a roughly pyramidal shape.

> nevertheless are the largest in its entire nervous system. They also possess two large and many small processes, and the inner process runs into a genuine nerve filament. In the area where they are found there are also many axis cylinders visible in the white substance, which run in the same direction as in the human. Undoubtedly these cells have all the attributes of the so-called 'motor cells' and very definitely continue as cerebral nerve fibres. (Betz, 1874; Kushchayev et al., 2012)

Furthermore, in the same article he distinguishes between sensory and motor poles in the brain, placing the division in the central sulcus: "The sulcus of Rolando divides the cerebral surface into two parts; an *anterior* in which the large pyramidal nerve cells predominate, and a *posterior*—including the temporal lobes—in which the cell layers are the same" (Betz, 1874; Clarke & O'Malley, 1996).

In this way, Betz founded the hypothesis that these cells, which he called 'giant pyramids' were the cells of origin of the corticospinal tract, and that it were their impulses propagating down to the spinal cord that initiated the muscle responses evoked by electrical stimulation of the cortical surface. His early assignment of these giant cells to cortical layer four was essentially correct, although his layer four would today be considered layer five due to refinement of the total number of layers in cortical tissue (Kushchayev et al., 2012).

When the landmark works of Campbell and Brodmann elucidated in detail the cytoarchitectural features of the mammalian cortex, they both included extensive treatments of the pre-central 'motor' region and its unique anatomical arrangement (Campbell, 1905; Brodmann, 1909). In regard to the cells of Betz, Campbell in particular provided great clarification on their pattern of distribution and possible physiological function. After extensive histological examination of cortical tissue in the brains of the anthropoid ape and normal human subject, as well as examination of pathological material from cases of Amyotrophic Lateral Sclerosis and from patients that underwent amputation of a body part, he proposed in his monograph a histological basis for assigning the influence of different areas of the pre-central region to different muscle groups, suggesting that "the largest (motor) cells are exactly those whose impulses have to travel down to the muscles of the lower extremity, those for the arm being quite a third smaller" (Campbell, 1905, p.33). He argued on the basis of similarity in anatomical configuration and retrograde degeneration studies that most likely even smaller pyramidal cells can form part of the corticospinal tract, becoming one of the first to suggest that a classification of Betz cells based purely on size was probably erroneous.

In addition to the characterization of the 'motor' cortex by its prominent layer V containing giant pyramidal cells, both Campbell and Brodmann noted clearly that the pre-central region showed a dramatic reduction in granular layer IV[2]. In the canonical layered arrangement of cortical tissue, layer IV is a dense layer of cell bodies known as the granular layer that separates superficial from deep layers. Functionally, layer IV is described as the target of feed-forward projections from sensory thalamus, making it the first layer of the cortex to receive direct sensory input. The observation that this layer was dramatically reduced in the pre-central motor region provided further suggestive evidence that the frontal cortices were more concerned with 'output' than with 'input'.

The last remaining question was to explain how surface stimulation of the cortex was so effective if the subcortically projecting cells were located in the deep layers. This last piece of the puzzle was finally resolved with the development of the Golgi stain and the characterization of the full structure of the cortical pyramidal cell presented in Ramon y Cajal's anatomical masterpiece (Ramón y Cajal, 1894, 1909). In *Histologie du*

---

[2] For this reason, the frontal 'motor' cortices are also sometimes referred to as *agranular* cortex.

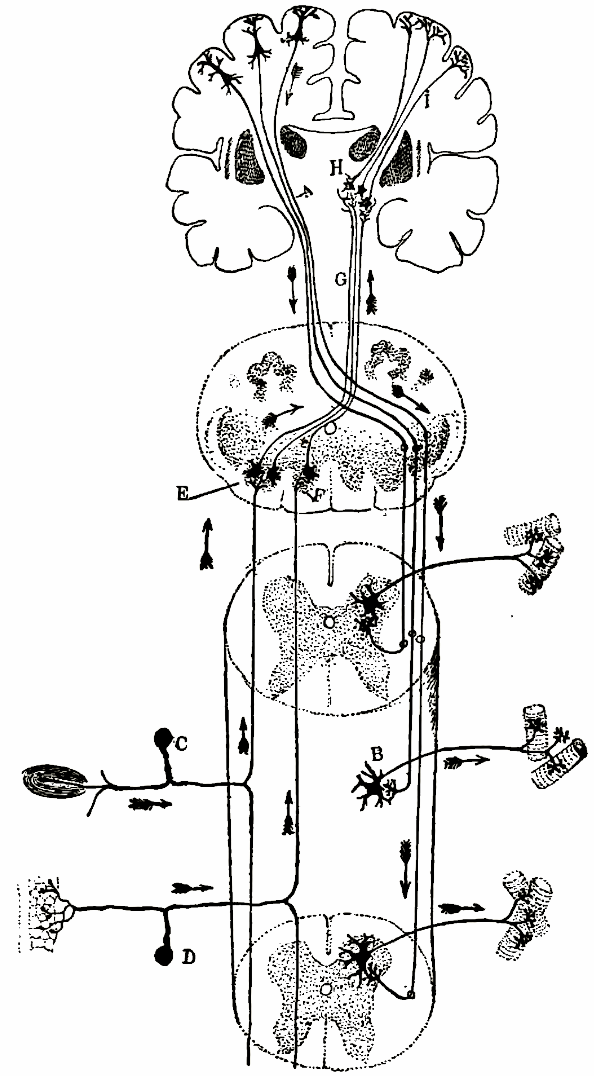


Figure 1.1. Schematic of the direction of impulses in the tactile sensory pathway and the voluntary movements pathway. A, pyramidal pathway; B, motor cells; C, D, sensory cells; E, cuneate nucleus of Burdach; F, gracile nucleus of Goll; G, central sensory pathway. The arrows indicate the direction of impulses (Ramón y Cajal, 1909, p.540).

Système Nerveux de l'Homme et des Vertébrés, Cajal describes in detail the descending circuit for voluntary movements (Figure 1.1): 'centrifugal' excitation originates in the tufts of the long apical dendrites which extend vertically from the soma of deep pyramidal cells all the way into the superficial layers, and then descends to the spinal cord through the axons of the corticospinal pathway, crossing at the medullary pyramids (Ramón y Cajal, 1909). In the same figure he describes the symmetric tactile sensory pathway, flowing in the opposite direction from spinal cord to the somatosensory cortices (Ramón y Cajal, 1909), completing the sketch of the circuit which transported neural impulses from sensory stimulation to the perception and reasoning centres, and from there to muscle activation.

1.3.2 The Effects of Lesions in the Corticospinal Tract

In the wake of the Goltz-Ferrier debates, investigations of the role of the direct corticospinal descending pathway were conducted in multiple animal species. Sherrington himself started out his work by tracing spinal cord degeneration over large periods of time (up to 11 months) following cortical lesions in Goltz's dogs (Langley & Sherrington, 1884; Sherrington, 1885). He confirmed that many of the properties of the corticospinal tract in the primate held for the dog, and furthermore became one of the first to observe the presence of a degenerated "re-crossed" pyramidal tract that travels down the cord ipsilateral to the side of the lesion (Sherrington, 1885). These fibers would later come to be called the ipsilateral, ventral corticospinal tract, and have since been found and described in most mammalian species as forming roughly 10% of the entire corticospinal projections (Kuypers, 1981; Brösamle & Schwab, 2000; Lacroix et al., 2004). However, he also had the chance during this time to observe first hand the negative effects of corticospinal degeneration following lesion, which had been previously reported by Goltz and others in a variety of non-primate specimens. In his own words:

> That the pyramidal tracts are in the dog requisite for volitional impulses to reach limbs and body seems negatived by the fact that the animal can run, leap, turn to either side, use neck and jaws, &c. with ease and success after nearly, if not wholly, complete degeneration of these tracts on both sides. Further, after complete degeneration of one pyramid, there is in the dog no obvious difference between the movements of the right and left sides. (Sherrington, 1885, p.189)

Interestingly, he does note that ‘defect of motion is observable only as a clumsiness in execution of fine movements’ (Sherrington, 1885). These observations once again stood out in stark contrast with lesion experiments reported by Ferrier in the monkey, where cauterization of specific motor cortical areas produced complete and persistent paralysis of the corresponding body parts (Ferrier & Yeo, 1884).

Years later, Sherrington would come back to the motor cortex with a new set of landmark studies on stimulation and ablation of the precentral region (Grünbaum & Sherrington, 1903; Graham Brown & Sherrington, 1913; Leyton & Sherrington, 1917). In these studies together with Grünbaum, Sherrington targeted motor cortical lesions to the excitable area of the arm or the leg and tracked the recovery of the animals over time. Following the initial paresis and loss of muscle control they observed dramatic recovery of most skilled motor acts, such as peeling open a banana or climbing cages (Leyton & Sherrington, 1917). In order to test whether the recovery process was due to cortical reorganization, they systematically stimulated the areas adjacent to the lesion as well as the motor cortex of the opposite hemisphere, but failed to evoke movements in the affected limb (Leyton & Sherrington, 1917), as would be expected if commands were traveling down the corticospinal tract in spared regions. Furthermore, subsequent ablation of those areas failed to produce any new impairments in the recovered limb, leaving Sherrington and his colleagues at a loss to find the locus of recovery (Leyton & Sherrington, 1917).

Confused by these results, which they thought "caused concern to, students of cerebral physiology", Glees and Cole introduced a set of more quantitative behavioural assays in the hope of tracking in detail the recovery of motor control (Glees & Cole, 1950; Cole, 1952). They studied the behaviour of monkeys solving various puzzle boxes following successive circumscribed lesions to the thumb, index and arm areas of the motor cortex. As Sherrington reported, there was a quick recovery after an initial period of paralysis and loss of motor control. However, even though the monkeys fully recovered their ability to skillfully open the puzzle box, some subtle movement deficits and paresis in the control of fine movements of the digits was reported to persist (Glees & Cole, 1950). When stimulating motor cortical areas surrounding the circumscribed lesions, they were able to evoke movements in the impacted digits and reinstate the paretic symptoms after further ablation (Glees & Cole, 1950). This suggested the hypothesis that surrounding areas of the motor cortex could undergo reorganization following the lesion. However, an important difference to emphasize between these experiments and those of Sherrington is the fact that only relatively circumscribed motor cortical regions were removed in each surgery, whereas in the original Sherrington study the entire elbow, wrist, index, thumb and remaining digit motor areas were excised at once (Leyton & Sherrington, 1917), most likely causing degeneration of the entire corticospinal pathway for the affected limb. The presence of an intact corticospinal tract, excitability of movements to low-current stimulation and transient paretic symptoms following ablation thus seem to go hand in hand.

In the hopes of clarifying the confusion of which exact movements were controlled by cortex, other studies focused on lesions restricted to the corticospinal tract, using both unilateral and bilateral section at the level of the medullary pyramids (Tower, 1940; Lawrence & Kuypers, 1968a; Lawrence & Kuypers, 1968b). The goal was to isolate the effects of all the individual descending pathways to the spinal cord and resolve once and for all the question of whether the corticospinal tract of the motor cortex was the source of all "voluntary" movements. Sarah Tower was the first to describe in detail the results of unilateral and bilateral pyramidotomy in primates, with and without lesion of the motor cortex (Tower, 1940). She summarized the condition as "hypotonic paresis", characterized by a loss of skeletal muscle tone and depression of the vasomotor system, along with general weakening of the reflexes involving the affected limb segments. Although all discrete usage of the hand and digits was eliminated, she did emphasize the clear presence of voluntary movements in the various purposeful compensations produced by the animals to deal with the affliction. Tower attributed these compensations to the preserved capacities of brainstem circuits.

A more definitive study to dissociate the effects of direct corticospinal and indirect brainstem descending pathways was conducted by Lawrence and Kuypers, and presented in their now classical publications (Lawrence & Kuypers, 1968a; Lawrence & Kuypers, 1968b). Using the Klüver board, a task where monkeys have to pick morsels of food from differently sized round holes, they observed that while normal monkeys routinely pick up the food by pinching individual bits with their fingers, monkeys with bilateral corticospinal lesions were mostly unable to perform this precise pincer movement, and instead employed coarser compensatory clasping strategies to retrieve the food (Lawrence & Kuypers, 1968a). In addition, lesioned monkeys were consistently reported to be somewhat slower and less agile than normal animals. However, most of their overall movement repertoire was surprisingly preserved. Their final conclusions fit remarkably well with the initial observations of Sherrington in the dog, suggesting that the corticospinal pathways superimpose speed and agility on subcortical mechanisms, and provide the capacity for fractionation of movements such as independent finger movements (Lawrence & Kuypers, 1968a). These observations recapitulate the effects of motor cortical lesions reported by Sherrington, but remain at odds with the primary stated role involving motor cortex, and the direct corticospinal tract, with the control of all voluntary movements.

There are anatomical differences in corticospinal projections between primates and other mammals

In primates, the conspicuous effects of motor cortical lesion can also be induced by sectioning the corticospinal tract, the direct monosynaptic projection that connects motor cortex, and other cortical regions, to the spinal cord (Tower, 1940; Lawrence & Kuypers, 1968a). In monkeys, and similarly in humans, this pathway has been found to directly terminate on spinal motor neurons responsible for the control of distal muscles (Leyton & Sherrington, 1917; Bernhard & Bohm, 1954) and is also thought to support the low-current movement responses evoked by electrical stimulation of the cortex, as evidenced by the increased difficulty in obtaining a stimulation response following section at the level of the medulla (Woolsey et al., 1972).

However, the corticospinal tract is by no means the only pathway from cortex to movement (Figure 1.2). Motor cortex targets many other brain regions that can themselves generate movement. In fact, this specialized connection from telencephalon to spinal cord appeared only recently in vertebrate evolution (ten Donkelaar, 2009), and was further elaborated to include a direct connection from cortex to motor neurons only in some primate species and other highly manipulative mammals such as raccoons (Heffner & Masterton, 1983). In all other mammals, including cats and rats, the termination pattern of the corticospinal tract largely avoids the motor neuron pools in ventral spinal cord and concentrates instead on intermediate zone interneurons and dorsal sensory neurons (Kuypers, 1981; Yang & Lemon, 2003). Why then is there such a large dependency on this tract for human motor control? One possibility is that the rubrospinal tract—a descending pathway originating in the brainstem and terminating in the intermediate zone—is degenerated in humans compared to other primates and mammals (Nathan & Smith, 1955, 1982), and is thought to play a role in compensating for the loss of the corticospinal tract in non-human species (Lawrence & Kuypers, 1968b; Zaaimi et al., 2012).

It thus seems likely that most mammals rely on "indirect" pathways to convey cortical motor commands to muscles. These differences in anatomy might explain the lack of conspicuous, lasting movement deficits following motor cortical lesion in non-primates, but leaves behind a significant question: what is the motor cortex actually controlling in all these other mammals?

What is the role of motor cortex in non-primate mammals?

In the rat, a large portion of cortex is considered "motor" based on anatomical (Donoghue & Wise, 1982), stimulation (Donoghue & Wise, 1982; Neafsey et al., 1986) and electrophysiological evidence (Hyland, 1998). However, the most consistently observed long-term motor control deficit following motor cortical lesion has been an impairment in supination of the wrist and individuation of digits during grasping, which in turn impairs reaching for food pellets through a narrow vertical slit (Whishaw et al., 1991; Alaverdashvili & Whishaw, 2008). Despite the fact that activity in rodent motor cortex has been correlated with movements in every part of the body (not just distal limbs) (Hill, Curtis, Moore & Kleinfeld, 2011; Erlich, Bialek & Brody, 2011), it would appear we are led to conclude that this large high-level motor structure, with dense efferent projections to motor areas in the spinal cord (Kuypers, 1981), basal ganglia (Turner & DeLong, 2000; Wu, Corwin & Reep, 2009), thalamus (Lee, Carvell &

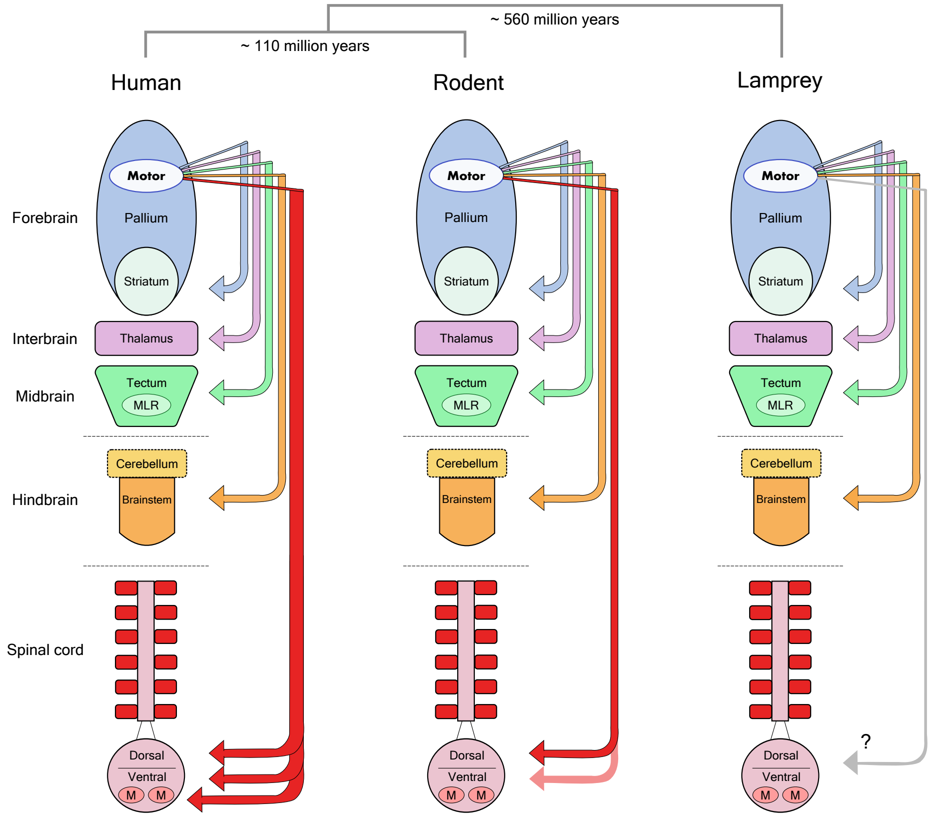


Figure 1.2. Forebrain motor control pathways across different vertebrate taxa. The molecular divergence times between human (primate), rodent and lamprey groups (Kumar & Hedges, 1998) are noted above a schematic view of the major divisions in the vertebrate brain. Arrows indicate the descending monosynaptic projections identified in each group from motor regions of the forebrain pallium to lower motor centres. Note the specialized monosynaptic projection directly targeting spinal motor neurons in human. MLR, Mesencephalic Locomotor Region; M, Motor Neurons.

Simons, 2008), cerebellum (Baker, Javid & Edgley, 2001) and brainstem (Jarratt & Hyland, 1999), as well as to most primary sensory areas (Petreanu et al., 2012; Schneider, Nelson & Mooney, 2014), evolved simply to facilitate more precise wrist rotations and grasping gestures. Maybe we are missing something. Might there be other problems in movement control that motor cortex is solving, but that we may be overlooking with our current assays?

1.4 An Integrative View of the Motor System

A different approach to the problems of motor control developed initially from studies on the integration of spinal reflexes conducted by the Sherrington school. While many researchers continued to look for the integration of complex movements in higher brain structures like the motor cortex, Sherrington turned instead to systematically characterizing anatomically and physiologically the distribution of efferent (Sherrington, 1892) and afferent (Sherrington, 1893a) nerve roots in the spinal cord of multiple species. His goal was to shed light on the so-called *reflex arc*, the nerve pathways involved in muscular reactions like the knee-jerk whereby simple sensory stimuli elicit an immediate, automatic response from the animal, even in the absence of higher brain input (Sherrington, 1893b).

Sherrington and his contemporaries studied in detail a number of long and short spinal reflexes[3] in a variety of model organisms under different levels of anesthesia, pharmacological manipulations and spinal transsection (Sherrington & Laslett, 1903). This systematic approach made abundantly clear a number of facts about how the nervous system organizes motor behaviour.

---

[3] A reflex action in which a stimulus applied to one region elicits a response in another region is termed a *long spinal* reflex, whereas a reflex reaction where the muscular response happens in the same region as the stimulus is termed a *short spinal* reflex.

The first one, and perhaps the most striking, is that complex motor responses can be integrated and coordinated even in the complete absence of the brain (Sherrington, 1906). While the existence of automatisms and fixed action patterns had been recognized since antiquity, systematic stimulation studies in decerebrate animals quickly revealed that the reflex was far from being a rigid and fixed entity, but was rather adaptive and dynamic. In particular, reflex circuits revealed a much wider range of response characteristics than nerve fibers, which were well known since the time of Galvani to exhibit complete stereotypy in their response to a stimulus under various conditions[4].

Indeed, the motor output produced by the massively simplified spinal circuits was remarkably organized and displayed clear ethological meaning: adaptive behaviours such as scratching (Sherrington & Laslett, 1903), shaking (Goltz & Ewald, 1896; Sherrington & Laslett, 1903) or reflex stepping and standing (Sherrington, 1910; Sherrington, 1915) were all available to be elicited from stimulation of the isolated spinal system. Strikingly, these reflexes were also shown to be deployed and modulated appropriately to specific stimuli. The scratch reflex, for example, carries the foot roughly to the place of stimulation (Sherrington, 1904), and in reflex stepping the animal can maintain a rhythmic march through all phases of locomotion over unobstructed surfaces (Sherrington, 1910). Integration of these reflexes with input from the telereceptors is obviously entirely absent, but these observations clarified, beyond any reasonable doubt, that spinal cord circuits alone are sufficient to produce and sustain entire behaviour sequences under the right conditions. Furthermore, deafferentation experiments showed that aspects of these rhythmic network motifs persist

---

[4] Some unique response characteristics of reflex arc conduction include irreversibility of the direction of conduction; fatigability and refractory period; greater variability of threshold; temporal facilitation with successive stimuli; a weaker correspondence of end-effect with intensity and frequency of the stimulus; and a greater susceptibility to metabolic and pharmacological manipulations (Sherrington, 1906, p.14)

even in the absence of sensory input (Graham Brown, 1911). Many of these reflex circuits were later termed *central pattern generators*, or CPGs (Grillner & Zangger, 1975; Grillner, 1981), and found to be present across both vertebrate and invertebrate species (Orlovsky, Deliagina & Grillner, 1999; Selverston, 2010).

1.4.1 The Coordinative Role of Inhibition

One of the aspects of spinal reflexes that most deeply impressed Sherrington was the general capacity of reflex circuits to initiate and switch between concurrent responses despite the existence of a *final common path* from the nervous system to muscles (Sherrington, 1904). Motor neurons in the spinal cord send their axons through the ventral roots of spinal segments to synapse directly on muscle fibres. From his experiments, Sherrington showed that it was common to find multiple motor neurons participating synergistically or antagonistically in a single coordinated reflex response. More importantly, he revealed that the same motor neurons were actually shared among multiple, potentially conflicting, reflex arcs. Sherrington was fascinated by the fact that these antagonistic reflexes, initiated simultaneously from distinct sensory receptors, were still found to be able to coordinate their influence despite sharing this final common path to muscles. That such coordination existed was made clear by stimulation experiments where two or more reflexes were elicited at the same time, generating muscle responses to the combined stimulation that were not a simple summation or linear combination of the responses obtained by stimulation delivered in isolation. Sherrington describes the conception clearly:

> Take the primary retinal reflex, which moves the eye so as to bring the fovea to the situation of the stimulating image. From all the receptors in each lateral retinal half rise reflex

> arcs with a final common path in the nerve of the opposite *rectus lateralis.* Suppose simultaneous stimulation of two of these retinal points, one nearer to, one farther from, the fovea. If the arcs of both points pour their impulses into the final common path together, the effect must be a resultant of the two discharges. If these sum, the shortening of the muscle will be too great and the fovea swing too far for either point. If the resultant be a compromise between the two individual points, the fovea will come to lie between the two points of stimulation. In both cases the result obtained would be useless for the purposes of either. . . .
>
> When two stimuli are applied simultaneously which would evoke reflex actions that employ the same final common path in different ways, in my experience one reflex appears without the other. The result is this reflex or that reflex, but not the two together. (Sherrington, 1904, p.461)

In Sherrington's time the existence of such common paths was a problem for the classic view of reflex control, where the function of the nervous system was conceived in terms of nerve conduction of excitatory impulses. The existence of the final common path mediating multiple reflexes made it necessary to speak openly of the problem of how to coordinate different circuit elements and to describe mechanisms that would allow the same neurons to take on context-dependent roles in generating motor responses. It was during the hunt for such a mechanism of reflex arc coordination that Sherrington hit upon the fundamental role of inhibition in the organization of neural function. Inhibition had always been a complicated topic for physiologists, but following the demonstration of cardiac muscle inhibition by the vagus nerve (Weber, 1846), and Sechenov's grand proposal of a central origin of reflex inhibition (Sechenov, 1863), Sherrington was able to articulate and experimentally validate simple mechanisms for spinal reflex coordination, not only at the level of reciprocal inhibition of antagonistic muscles (Sherrington, 1893b), but also at the level of coordination and maintenance of the so-called central state.

Specifically, Sherrington described the existence of a central inhibitory state that was at least as, if not more, important than central excitatory state. He emphasized the need for discrete regulatory mechanisms in the nervous system which were capable of deciding how much converging neural impulses would influence a target cell. From physiological experiments measuring the latency and amplitude of neural responses in very short reflex arcs, he surmised that the effect could not be a simple *absence* of excitation or shutting down of function, but rather the presence of a fundamentally active force in the nervous system (Sherrington, 1965). He emphasized that the major difficulty in studies of inhibition was the fact that it can only be actively measured in comparison to a baseline of excitation. If this baseline is not set up accurately, inhibition and its effects can be easy to miss. Sherrington was one of the first to suggest that the effects of central inhibitory state acted primarily on the theoretical gap between two neural cells where the nature of conduction changed fundamentally. He named this junction the *synapse* (Foster & Sherrington, 1897, p.929).

Today we know from several detailed descriptions of invertebrate CPGs that different spinal circuits can be delicately super-imposed on the same neuronal elements by using a number of strategies to regulate and coordinate function (Orlovsky et al., 1999; Selverston, 2010). These strategies span all levels of neural organization from networks to molecules, ranging from reciprocal inhibition network motifs gated by specific neuromodulators and cell membrane receptors to overlapping distributions of voltage and ligand gated ion channels with different kinetics. The existence of such finely tuned spinal networks brings significant constraints when thinking about cortical motor control. Ultimately, any descending signals from the brain to muscles are also sharing this final common path and thus should be expected to require some form of coordination with spinal circuits if behavioural output is to remain integrated.

One final implication of the idea of central inhibition was an explanation for the physiological and behavioural changes occurring during phenomena of "shock" and "release", states of generalized depression of activity or over-action in a given area following injury or destruction to distant but related parts of the nervous system. For example, it was well known at the time that circuits in the lower segments of the cat spinal cord underwent "shock" following transsection of fiber tracts projecting from higher segments, or extended lesions to the brain, such as decortication, or decerebration up to the level of the brainstem. The generalized inhibitory state that ensued masked the expression of reflexes organized by spinal centres. Following some period of time, however, the spinal preparation would overcome shock and reflex responses could be reliably and repeatedly elicited. Sherrington and his co-workers described systematically how transient changes in excitatory and inhibitory state ultimately manifest themselves depending on the particular anatomical situation of the influencing and influenced centres. From these results, they emphasized how the presence of such transient changes can be enough to establish that two areas are connected, but complicate inference of the functions located in either area.

1.4.2 Hierarchical Organization of Motor Behaviour

The study of the biological mechanisms underlying adaptive reflexes inspired multiple theories for the organization of behaviour, including Hebbian plasticity (Hebb, 1949) and cybernetics (Wiener, 1948). In cybernetics, the term "feedback" replaced the concept of reflex in technical publications, but the essence of the abstract formulation—the coupling of sensory inputs to motor effectors as the basic unit of adaptive behaviour—remained largely the same. The school of thought initiated by Norbert Wiener, Arturo Rosenblueth, Ross Ashby and others, would come to formulate general properties of adaptive control using negative feedback (Rosenblueth, Wiener & Bigelow, 1943; Wiener, 1948; Ashby, 1960). Specifically, they clarified formally how to design systems that can maintain stable relationships with the environment by comparing incoming sensory input to an internal reference and using the result of the comparison to drive motor responses (i.e. the intensity and direction of motor response should be such as to minimize the difference between the internal reference and the sensory input). Such systems were found to automatically generate adaptive motor outputs that stabilized sensory inputs even in the face of unspecified perturbations (Wiener, 1948; Ashby, 1960) and have since become a staple of the automation industry and robotic control.

Inspired by the success of cybernetics, several writers proposed mechanisms for the adaptive hierarchical integration of multiple such feedback (or reflex) controllers (Powers, 1973). The basic principle of organization was to make the output of a higher level system effectively adjust the internal references of the feedback controllers at the lower level. In this way, the higher level system can act as a regulator without the need to control the details of the behaviour of the lower system. Two basic implications can be derived from this conception. First, the removal of the higher level system does not necessarily compromise the regulatory abilities of the lower systems, which can continue to operate independently. Second, fixing the output of the higher level system will not necessarily fix the output of the lower system, but only its internal reference. The final motor output will also depend on the state of the sensory input to the lower system, and will be regulated appropriately.

The first implication agrees well with the results of motor cortical lesions in non-human mammals, where the vast majority of the behavioural repertoire is preserved (Goltz, 1888; Grünbaum & Sherrington, 1903; Graham Brown & Sherrington, 1913; Leyton & Sherrington, 1917; Bjursten et al., 1976; Terry et al., 1989; Whishaw et al., 1991; Darling et al., 2011; Zaaimi et al., 2012; Kawai et al., 2015). In this view, if subcortical systems are seen as independent controller mechanisms with cortex being their highest level regulator, then it would not be surprising if all of the subcortical capacities would be preserved following extirpation of cortical tissue. In addition, successive decerebration techniques would be in principle capable of revealing the regulatory limits of each stage in the hierarchy, assuming one starts at the lowest level.

As for the second implication, Sherrington himself provided very suggestive evidence in the form of the phenomenon of instability of stimulated cortical points (Graham Brown & Sherrington, 1912; Leyton & Sherrington, 1917). When systematically mapping the somatotopic organization of the motor cortex by low-current electrical stimulation, it was noted that many of the cortical stimulation points were "unstable". Specifically, if the stimulating electrode was kept in the same place and the stimulation protocol repeated, there was a large variability in evoked movements, much larger than what was normally encountered in the study of purely spinal or decerebrate reflexes (Graham Brown & Sherrington, 1912). For example, a cortical point stimulated in rapid succession could reliably produce a flexion response in one isolated muscle in the start of the series, only to completely reverse its response to extension of the same muscle towards the end of the series (Graham Brown & Sherrington, 1912). In addition, even the boundaries of the excitable area itself, as mapped by stimulation, were subject to dramatic change in the same animal, depending on whether the stimulation protocol progressed in the anterior-posterior or posterior-anterior direction (Leyton & Sherrington, 1917). Studies by later authors would suggest that the instability of the point was related to changes in the configuration of the limbs or head, concluding that the stimulation enforced a postural target rather than generating a specific motor response (Ward, 1938).

This implication is also in agreement with studies using prolonged stimulation of individual cortical sites, which were found to reliably evoke entire sequences of behaviourally relevant, purposeful actions (Ferrier, 1873; Clark & Ward, 1937; Graziano et al., 2002). These actions were also found to depend on the current postural configuration of the animal, representing behaviour sequences such as hand-to-mouth or reaching a point in space (Graziano et al., 2002). In this case, if the stimulating electrode is kept in the same place and the arm of the animal moved to a new starting location, the stimulation causes a different pattern of activation in the animal musculature, but with the end result that the hand will be brought to the same specific point in space (Graziano et al., 2002).

Although there are many limitations and even hugely missing gaps in this hierarchical view of motor system organization, the main insight that can be derived from such lines of thinking is that motor cortex does not need to be seen exclusively as a direct driver of motor behaviour, but rather its influences on the lower motor system may be much more subtle and modulatory than was previously appreciated. If this is true, then the question of what exactly is the teleology for cortical motor control becomes all the more difficult. Specifically, it may not be enough to ask which movements does motor cortex control. We may need to ask more generally what is motor cortex doing and what does it allow the organism to achieve over and above the regulatory behaviour patterns managed and adapted by subcortical systems such as cerebellum and brainstem. The capacity of the subcortical systems should not be underestimated, as they have undergone millions of years of evolution and are still used almost exclusively for the control of behaviour in many vertebrate species.

A role in modulating the movements generated by lower motor centres

The idea that the descending cortical pathways superimpose speed and precision on an existing baseline of behaviour has been suggested even in some lines of lesion work in the primate (Lawrence & Kuypers, 1968b), but has been investigated much more thoroughly in the context of studies on the neural control of locomotion in cats. These studies have suggested that the corticospinal tract can play a role in the *adjustment* of ongoing movements, modulating the activity and sensory feedback in spinal circuits in order to adapt a lower movement controller to challenging conditions.

It has been known for more than a century that completely decerebrate cats are capable of sustaining the locomotor rhythms necessary for walking on a flat treadmill utilizing only spinal circuits (Graham Brown, 1911). In addition, there is a general capacity for spinal circuits to modulate network activity with incoming sensory input in order to coordinate and switch between different responses, even during specific phases of movement (Forssberg, Grillner & Rossignol, 1975). Brainstem and midbrain circuits are sufficient to initiate the activity of these spinal central pattern generators (Grillner & Shik, 1973), so what exactly is the contribution of motor cortex to the control of locomotion? Single-unit recordings of pyramidal tract neurons (PTNs) from cats walking on a treadmill have shown that a large proportion of these neurons are locked to the step cycle (Armstrong & Drew, 1984). However, we know from the decerebrate studies that this activity is not necessary for the basic locomotor pattern. What then is its role?

Lesions of the lateral descending pathways (containing corticospinal and rubrospinal projections) produce a long term impairment in the ability of cats to step over obstacles (Drew, Jiang & Widajewicz, 2002). Recordings of PTN neurons during locomotion show increased activity during these visually guided modifications to the basic step cycle (Drew, Jiang, Kably & Lavoie, 1996). These observations suggest that motor cortex neurons are necessary for precise stepping and adjustment of ongoing locomotion to changing conditions. However, long-term effects seem to require complete lesion of *both* the corticospinal and rubrospinal tracts (Drew et al., 2002). Even in these animals, the voluntary act of stepping over an obstacle does not disappear entirely, and moreover, they can adapt to changes in the height of the obstacles (Drew et al., 2002). Specifically, even though these animals never regain the ability to gracefully clear an obstacle, when faced with a higher obstacle, they are able to adjust their stepping height in such a way that would have allowed them to comfortably clear the lower obstacle (Drew et al., 2002). Furthermore, deficits caused by lesions restricted to the pyramidal tract seem to disappear over time (Liddell & Phillips, 1944), and are most clearly visible only the first time an animal encounters a new obstacle (Liddell & Phillips, 1944).

The view that motor cortex in non-primate mammals is principally responsible for adjusting ongoing movement patterns generated by lower brain structures is appealing. What is this modulation good for? What does it allow an animal to achieve? How can we assay its necessity?

1.5 A Strategy for Probing Cortical Control

It should now be clear that the involvement of motor cortex in the direct control of all “voluntary movement” is human-specific. There is a role for motor cortex across mammals in the control of precise movements of the extremities, especially those requiring individual movements of the fingers, but these effects are subtle in non-primate mammals. Furthermore, what would be a devastating impairment for humans may not be so severe for mammals that do not depend on precision finger movements for survival. Therefore, generalizing this specific role of motor cortex from humans to all other mammals would be misleading. We could be missing another, more primordial role for this structure that predominates in other mammals, and by doing so, we may also be missing an important role in humans.

The proposal that motor cortex induces modifications of ongoing movement synergies, prompted by the electrophysiological studies of cat locomotion, definitely points to a role consistent with the results of various lesion studies. However, in assays used, the ability to modify ongoing movement generally recovers after a motor cortical lesion. What are the environmental situations in which motor cortical modulation is most useful?

Cortex has long been proposed to be the structure responsible for integrating a representation of the world and improving the predictive power of this representation with experience (Barlow, 1985; Doya, 1999). If motor cortex is the means by which these representations can gain influence over the body, however subtle and “modulatory”, can we find situations (i.e. tasks) in which this cortical control is required?

The necessity of cortex for various behavioural tasks has been actively investigated in experimental psychology for over a century, including the foundational work of Karl Lashley and his students (Lashley, 1921, 1950). In the rat, large cortical lesions were found to produce little to no impairment in movement control, and even deficits in learning and decision making abilities were difficult to demonstrate consistently over repeated trials. However, Lashley did notice some evidence that cortical control may be involved in postural adaptations to unexpected perturbations (Lashley, 1921). These studies once again seem to recapitulate the two most consistent observations found across the entire motor cortical lesion literature in non-primate mammals since Hitzig (Fritsch & Hitzig, 1870), Goltz (Goltz, 1888), Sherrington (Sherrington, 1885) and others (Oakley, 1979; Terry et al., 1989). One, direct voluntary control over movement is most definitely not abolished through lesion; and two, certain aspects of some movements are definitely impaired, but only under certain challenging situations. The latter are often reported only anecdotally. It was this collection of intriguing observations in animals with motor cortical lesions that prompted us to expand the scope of standard laboratory tasks to include a broader range of motor control challenges that brains encounter in their natural environments.

1.5.1 Outline of the Thesis

In this work, an attempt to delineate a new role for motor cortex is reported. As many previous efforts, it starts with behaviour, and the realization that controlled exposure of animals to a wider range of environments is of absolute necessity to gain insight into the teleology of the system. To this end, we have developed new tools to make it easier to survey a large range of environments while recording as many fine scale measures of behaviour and physiology as possible. These technical and methodological developments are described in Chapter 2.

In Chapter 3, a set of behaviour and lesion studies is reported in the rat. These studies had the goal of probing the limits of recovery following extensive cortical lesions by exposing animals to more challenging and dynamic environments. Detailed analysis of the moment by moment behaviour of lesioned animals revealed a number of intriguing observations, the implications of which we discuss in Chapter 4.

References

Aflalo, T. N. & Graziano, M. S. A. (2006). Possible origins of the complex topographic organization of motor cortex: reduction of a multidimensional space onto a two-dimensional array. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *26*(23), 6288–97. doi:10.1523/JNEUROSCI.0768-06.2006

Alaverdashvili, M. & Whishaw, I. Q. (2008). Motor cortex stroke impairs individual digit movement in skilled reaching by the rat. *The European journal of neuroscience*, *28*(2), 311–22. doi:10.1111/j.1460-9568.2008.06315.x

Armstrong, D. M. & Drew, T. (1984). Discharges of pyramidal tract and other motor cortical neurones during locomotion in the cat. *The Journal of Physiology*, *346*, 471–495.

Ashby, W. R. (1960). *Design for a brain: The origin of adaptive behavior.* New York: Wiley.

Baker, M. R., Javid, M. & Edgley, S. A. (2001). Activation of cerebellar climbing fibres to rat cerebellar posterior lobe from motor cortical output pathways. *Journal of Physiology*, *536*(3), 825–839. doi:10.1111/j.1469-7793.2001.00825.x

Barlow, H. (1985). Cerebral cortex as model builder. *Models of the visual cortex*, (1985), 37–46.

Bernhard, C. G. & Bohm, E. (1954). Cortical representation and functional significance of the corticomotoneuronal system. *Archives of Neurology And Psychiatry*, *72*(4), 473–502. doi:10.1001/archneurpsyc.1954.02330040075006

Bernstein, J. (1868). Ueber den zeitlichen Verlauf der negativen Schwankung des Nervenstroms. *Pflügers Archiv*, *1*, 173–207. doi:10.1007/BF01640316

Betz, W. (1874). Anatomischer Nachweis zweier Gehirncentra. *Centralblatt für die medizinischen Wissenschaften*, *12*, 578–80, 595–99.

Bjursten, L. M., Norrsell, K. & Norrsell, U. (1976). Behavioural repertory of cats without cerebral cortex from infancy. *Experimental brain research*, *130*(2857), 115–130.

Brodmann, K. (1909). *Localisation in the Cerebral Cortex.* Springer. doi:10.1097/00005053-191012000-00013

Brösamle, C. & Schwab, M. E. (2000). Ipsilateral, ventral corticospinal tract of the adult rat: Ultrastructure, myelination and synaptic connections. *Journal of Neurocytology*, *29*, 499–507. doi:10.1023/A:1007297712821

Campbell, A. W. (1905). *Histological studies on the localisation of cerebral function.*

Churchland, M. M. & Shenoy, K. V. (2007). Temporal complexity and heterogeneity of single-neuron activity in premotor and motor cortex. *J Neurophysiol*, *97*(6), 4235–4257. doi:10.1152/jn.00095.2007

Churchland, M. M., Cunningham, J. P., Kaufman, M. T., Foster, J. D., Nuyujukian, P., Ryu, S. I. & Shenoy, K. V. (2012). Neural population dynamics during reaching. *Nature*, *487*(7405), 51–6. doi:10.1038/nature11129

Clark, S. L. & Ward, J. W. (1937). Electrical stimulation of the cortex cerebri of cats. *Arch NeurPsych*, *38*(5), 927–943.

Clarke, E. & O'Malley, C. (1996). *The Human Brain and Spinal Cord: A Historical Study Illustrated by Writings from Antiquity to the Twentieth Century.* Norman Publishing.

Cole, J. (1952). Three tests for the study of motor and sensory abilities in monkeys. *Journal of Comparative and Physiological Psychology*, *45*(3), 226–230.

Darling, W. G., Pizzimenti, M. A. & Morecraft, R. J. (2011). Functional recovery following motor cortex lesions in non-human primates: experimental implications for human stroke patients. *Journal of Integrative Neuroscience*, *10*(3), 353–384. doi:10.1142/S0219635211002737

de Barenne, J. G. D. (1933). "Corticalization" of function and functional localization in the cerebral cortex. *Archives of Neurology And Psychiatry*, *30*(4), 884–901. doi:10.1001/archneurpsyc.1933.02240160196012

Donoghue, J. & Wise, S. P. (1982). The motor cortex of the rat: cytoarchitecture and microstimulation mapping. *The Journal of comparative neurology*, *212*(1), 76–88. doi:10.1002/cne.902120106

Doya, K. (1999). What are the computations of the cerebellum, the basal ganglia and the cerebral cortex? *Neural networks : the official journal of the International Neural Network Society*, *12*(7-8), 961–974.

Drew, T., Jiang, W., Kably, B. & Lavoie, S. (1996). Role of the motor cortex in the control of visually triggered gait modifications. *Canadian journal of physiology and pharmacology*, *74*(4), 426–42.

Drew, T., Jiang, W. & Widajewicz, W. (2002). Contributions of the motor cortex to the control of the hindlimbs during locomotion in the cat. *Brain research. Brain research reviews*, *40*(1-3), 178–91.

du Bois-Reymond, E. (1843). Vorläufiger Abriss einer Untersuchung über den sogenannten Froschstrom und über die elektromotorischen Fische. *Annalen der Physik und Chemie*, *58*, 1–30.

Erlich, J. C., Bialek, M. & Brody, C. D. (2011). A cortical substrate for memory-guided orienting in the rat. *Neuron*, *72*(2), 330–43. doi:10.1016/j.neuron.2011.07.010

Evarts, E. V. (1968). Relation of pyramidal tract activity to force exerted during voluntary movement. *Journal of neurophysiology*, *31*(1), 14–27.

Ferrier, D. (1873). Experimental Researches in Cerebral Physiology and Pathology. *Journal of anatomy and physiology*, *8*(Pt 1), 152–5. doi:10.1136/bmj.1.643.457

Ferrier, D. & Yeo, G. (1884). A Record of Experiments on the Effects of Lesion of Different Regions of the Cerebral Hemispheres. *Philosophical Transactions of the Royal Society of London (1776-1886)*, *175*(January), 479–564.

Forssberg, H., Grillner, S. & Rossignol, S. (1975). Phase dependent reflex reversal during walking in chronic spinal cats. *Brain Research*, *85*(1), 103–107. doi:10.1016/0006-8993(75)91013-6

Foster, M. & Sherrington, C. S. (1897). *A textbook of physiology* (7th). London: Macmillan.

Fritsch, G. & Hitzig, E. (1870). Über die elektrische erregbarkeit des grosshirns. *Archiv fur Anatomie, Physiologie und Wissenschaftliche*, *37*, 300–332.

Galvani, L. (1791). De viribus electricitatis in motu muscularis commentarius. *De Bononiensi Scientiarum et Artium Instituto atque Academia Commentarii*, *7*, 363–418. doi:10 . 1097 / 00000441 - 195402000-00023

Georgopoulos, A., Schwartz, A. & Kettner, R. (1986). Neuronal population coding of movement direction. *Science*, *233*(4771), 1416–1419. doi:10.1126/science.3749885

Glees, P. & Cole, J. (1950). Recovery of skilled motor functions after small repeated lesions in motor cortex in macaque. *J Neurophysiol.* *13*(2), 137–148.

Goltz, F. (1888). Über die verrichtungen des grosshirns. *Pflügers Archiv gesamte Physiologie des Menschen und der Tiere*, *42*, 419–467.

Goltz, F. & Ewald, J. R. (1896). Der Hund mit verkürztem Rückenmark. *Pflüger's Archiv für die gesamte Physiologie des Menschen und der Tiere*, *63*(7), 362–400.

Graham Brown, T. & Sherrington, C. S. (1912). On the Instability of a Cortical Point. *Proceedings of the Royal Society B: Biological Sciences*, *85*(July), 250–277. doi:10.1098/rspb.1912.0050

Graham Brown, T. (1911). The Intrinsic Factors in the Act of Progression in the Mammal. *Proceedings of the Royal Society B: Biological Sciences*, *84*(572), 308–319. doi:10.1098/rspb.1911.0077. arXiv: NIHMS150003

Graham Brown, T. & Sherrington, C. S. (1913). Note on the functions of the cortex cerebri. *Journal of Physiology*, *46*(suppl), xxii.

Graziano, M. S. A., Taylor, C. S. R. & Moore, T. (2002). Complex Movements Evoked by Microstimulation of Precentral Cortex. *Neuron*, *34*, 841–851.

Grillner, S. & Shik, M. L. (1973). On the descending control of the lumbosacral spinal cord from the "mesencephalic locomotor region". *Acta Physiol Scand.* *87*(0001-6772), 320–333.

Grillner, S. & Zangger, P. (1975). How detailed is the central pattern generation for locomotion? *Brain Research*, *88*(2), 367–371. doi:10.1016/0006-8993(75)90401-1

Grillner, S. (1981). Control of locomotion in bipeds, tetrapods, and fish. *Handbook of Physiology, The Nervous System II*, 1179–1236. doi:10.1002/cphy.cp010226

Gross, C. G. (2007). The discovery of motor cortex and its background. *Journal of the history of the neurosciences*, *16*(3), 320–31. doi:10.1080/09647040600630160

Grünbaum, A. S. F. & Sherrington, C. S. (1903). Observations on the Physiology of the Cerebral Cortex of the Anthropoid Apes. *Proceedings of the Royal Society of London*, *72*(477-486), 152–155. doi:10.1098/rspl.1903.0033

Hebb, D. O. (1949). *The Organization of Behavior: A Neuropsychological Theory*. New York: Wiley.

Heffner, R. S. & Masterton, R. B. (1983). The role of the corticospinal tract in the evolution of human digital dexterity. *Brain, behavior and evolution*, *23*, 165–183.

Hill, D. N., Curtis, J. C., Moore, J. D. & Kleinfeld, D. (2011). Primary motor cortex reports efferent control of vibrissa motion on multiple timescales. *Neuron*, *72*(2), 344–356. doi:10.1016/j.neuron.2011.09.020. arXiv: NIHMS150003

Hyland, B. (1998). Neural activity related to reaching and grasping in rostral and caudal regions of rat motor cortex. *Behavioural Brain Research*, *94*(2), 255–269. doi:10.1016/S0166-4328(97)00157-5

Jackson, J. H. (1870). A study of convulsions. *Transactions of the Saint Andrews Medical Graduates Association*, *3*, 162–204. doi:10.1001/archneur.1970.00480200090012

James, W. (1885). Localization of functions in the brain. *Science*, *6*(143), 379–380. doi:10.1126/science.ns-6.143.379

Jarratt, H. & Hyland, B. (1999). Neuronal activity in rat red nucleus during forelimb reach-to-grasp movements. *Neuroscience*, *88*(2), 629–642. doi:10.1016/S0306-4522(98)00227-9

Kawai, R., Markman, T., Poddar, R., Ko, R., Fantana, A. L., Dhawale, A. K., Kampff, A. R. & Ölveczky, B. P. (2015). Motor Cortex Is Required for Learning but Not for Executing a Motor Skill. *Neuron*, 1–13. doi:10.1016/j.neuron.2015.03.024

Kumar, S. & Hedges, S. (1998). A molecular timescale for vertebrate evolution. *Nature*, *392*, 917–920.

Kushchayev, S. V., Moskalenko, V. F., Wiener, P. C., Tsymbaliuk, V. I., Cherkasov, V. G., Dzyavulska, I. V., Kovalchuk, O. I., Sonntag, V. K. H., Spetzler, R. F. & Preul, M. C. (2012). The discovery of the pyramidal neurons: Vladimir Betz and a new era of neuroscience. *Brain*, *135*(1), 285–300. doi:10.1093/brain/awr276

Kuypers, H. G. J. M. (1981). Anatomy of the descending pathways. *Comprehensive Physiology*, 597–666.

Kwakkel, G., Kollen, B. J., van der Grond, J. & Prevo, A. J. (2003). Probability of Regaining Dexterity in the Flaccid Upper Limb: Impact of

Severity of Paresis and Time Since Onset in Acute Stroke. *Stroke*, *34*(9), 2181–2186. doi:10.1161/01.STR.0000087172.16305.CD

Lacroix, S., Havton, L. a., McKay, H., Yang, H., Brant, A., Roberts, J. & Tuszynski, M. H. (2004). Bilateral corticospinal projections arise from each motor cortex in the macaque monkey: a quantitative study. *The Journal of comparative neurology*, *473*(2), 147–161. doi:10.1002/cne.20051

Langley, J. N. & Sherrington, C. S. (1884). Secondary Degeneration of Nerve Tracts following removal of the Cortex of the Cerebrum in the Dog. *Journal of Physiology*, *5*(2), 49–65. doi:10.1113/jphysiol.1884.sp000151

Laplane, D., Talairach, J., Meininger, V., Bancaud, J. & Bouchareine, A. (1977). Motor consequences of motor area ablations in man. *Journal of the Neurological Sciences*, *31*(1), 29–49. doi:10.1016/0022-510X(77)90004-1

Lashley, K. S. (1921). Studies of cerebral function in learning. III. The motor areas. *Brain*, *44*, 255–285. doi:10.1037/h0070668

Lashley, K. S. (1924). Studies of cerebral function in learning: V. The retention of motor habits after destruction of the so-called motor areas in primates. *Archives of Neurology and Psychiatry*, *12*(3), 249–276. doi:10.1001/archneurpsyc.1924.02200030002001

Lashley, K. S. (1950). In search of the engram. *Symposia of the Society for Experimental Biology*.

Lawrence, D. G. & Kuypers, H. G. J. M. (1968a). The functional organization of the motor system in the monkey. I. The effects of bilateral pyramidal lesions. *Brain*, *91*(1), 1–14.

Lawrence, D. G. & Kuypers, H. G. J. M. (1968b). The functional organization of the motor system in the monkey. II. The effects of lesions of the descending brain-stem pathways. *Brain*, *91*(1), 15–36.

Lee, S., Carvell, G. E. & Simons, D. J. (2008). Motor modulation of afferent somatosensory circuits. *Nature neuroscience*, *11*(12), 1430–8. doi:10.1038/nn.2227

Leyton, A. S. F. & Sherrington, C. S. (1917). Observations on the excitable cortex of the chimpanzee, orang-utan, and gorilla. *Experimental Physiology*, *11*(2), 135–222. doi:10.1113/expphysiol.1917.sp000240

Liddell, E. & Phillips, C. (1944). Pyramidal section in the cat. *Brain*, *67*(1), 1–9.

Louis, E. D. (1994). Contralateral control: Evolving concepts of the brain-body relationship from Hippocrates to Morgagni. *Neurology*, *44*(12), 2398–2400.

Nathan, P. W. & Smith, M. C. (1955). Long descending tracts in man. I. Review of present knowledge. *Brain*, *78*(2), 248–303. doi:10.1093/brain/78.2.248

Nathan, P. W. & Smith, M. C. (1982). The rubrospinal and central tegmental tracts in man. *Brain*, *105*(2), 223–269. doi:10.1093/brain/105.2.223

Neafsey, E., Bold, E., Haas, G., Hurley-Gius, K., Quirk, G., Sievert, C. & Terreberry, R. (1986). The organization of the rat motor cortex: a microstimulation mapping study. *Brain Research Reviews*, *11*(1), 77–96.

Oakley, D. a. (1979). Learning with food reward and shock avoidance in neodecorticate rats. *Experimental neurology*, *63*(3), 627–42.

Orlovsky, G. N., Deliagina, T. G. & Grillner, S. (1999). *Neuronal Control of Locomotion: From Mollusc to Man*. Oxford University Press.

Penfield, W. & Boldrey, E. (1937). Somatic motor and sensory representation in the cerebral cortex of man as studied by electrical stimulation. *Brain*, *60*(4), 389–443.

Petreanu, L., Gutnisky, D. a., Huber, D., Xu, N.-l., O'Connor, D. H., Tian, L., Looger, L. & Svoboda, K. (2012). Activity in motor–sensory projections reveals distributed coding in somatosensation. *Nature*, *489*(7415), 299–303. doi:10.1038/nature11321

Phillips, C. G., Zeki, S. & Barlow, H. B. (1984). Localization of function in the cerebral cortex: past, present and future. *Brain*, *107*(1), 328–361. doi:10.1093/brain/107.1.328

Porro, C. A., Francescato, M. P., Cettolo, V., Diamond, M. E., Baraldi, P., Zuiani, C., Bazzocchi, M. & di Prampero, P. E. (1996). Primary motor and sensory cortex activation during motor performance and motor imagery: a functional magnetic resonance imaging study. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *16*(23), 7688–7698.

Powers, W. T. (1973). *Behavior: The Control of Perception.* Aldine Publishing Company.

Ramón y Cajal, S. (1894). The Croonian Lecture: La Fine Structure des Centres Nerveux. *Proceedings of the Royal Society of London*, *55*(1894), 444–468. doi:10.2307/115494

Ramón y Cajal, S. (1909). *Histologie du système nerveux de l'homme & des vertébrés.*

Rizzolatti, G. & Craighero, L. (2004). The Mirror-Neuron System. *Annual Review of Neuroscience*, *27*(1), 169–192. doi:10.1146/annurev.neuro.27.070203.144230

Rosenblueth, A., Wiener, N. & Bigelow, J. (1943). Behavior, Purpose and Teleology. *Philosophy of Science*, *10*, 18. doi:10.1086/286788

Schneider, D. M., Nelson, A. & Mooney, R. (2014). A synaptic and circuit basis for corollary discharge in the auditory cortex. *Nature*, *513*(7517), 189–194. doi:10.1038/nature13724

Schuetze, S. M. (1983). The discovery of the action potential. *Trends in Neurosciences*, *6*, 164–168. doi:10.1016/0166-2236(83)90078-4

Schwartz, A. B. (1993). Motor cortical activity during drawing movements: population representation during sinusoid tracing. *Journal of neurophysiology*, *70*(1), 28–36.

Scott, S. H., Gribble, P. L., Graham, K. M. & Cabel, W. (2001). Dissociation between hand motion and population vectors from neural activity in motor cortex. *Nature*, *413*(6852), 161–165.

Sechenov, I. M. (1863). *Reflexes of the Brain.*

Selverston, A. I. (2010). Invertebrate central pattern generator circuits. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1551), 2329–2345. doi:10.1098/rstb.2009.0270

Sherrington, C. S. (1885). On Secondary and Tertiary Degenerations in the Spinal Cord of the Dog. *Journal of Physiology*, *6*(4), 177–191. doi:10.1113/jphysiol.1885.sp000195

Sherrington, C. S. (1893a). Experiments in Examination of the Peripheral Distribution of the Fibres of the Posterior Roots of Some Spinal Nerves. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *184*, 641–763. doi:10.1098/rstb.1898.0002

Sherrington, C. S. (1893b). Note on the Knee-jerk and the Correlation of Action of Antagonistic Muscles. *Proceedings of the Royal Society of London*, *52*(315-320), 556–564.

Sherrington, C. S. (1904). The correlation of reflexes and the principle of the common final path. *Nature*, *70*, 460–466.

Sherrington, C. S. (1906). *The Integrative Action of the Nervous System.* Yale University Press. doi:10.1097/00005053-190734120-00021

Sherrington, C. S. (1910). Flexion-reflex of the limb, crossed extension-reflex, and reflex stepping and standing. *The Journal of physiology*, *40*(1-2), 28–121. doi:10.1113/jphysiol.1910.sp001362

Sherrington, C. S. (1915). Postural activity of muscle and nerve. *Brain*, *38*(3), 191–234.

Sherrington, C. S. & Laslett, E. E. (1903). Observations on some spinal reflexes and the interconnection of spinal segments. *Journal of Physiology*, *29*(1), 58–96. doi:10.1113/jphysiol.1903.sp000946

Sherrington, C. S. (1892). Notes on the Arrangement of some Motor Fibres in the Lumbo-Sacral Plexus. *The Journal of physiology*, *13*(6), 621–772.17. doi:10.1097/00005053-189305000-00004

Sherrington, C. S. (1965). Inhibition as a Coordinative Factor. In *Nobel lectures, physiology or medicine 1922-1941*. Amsterdam: Elsevier Publishing Company.

ten Donkelaar, H. J. (2009). Evolution of Motor Systems: Corticospinal, Reticulospinal, Rubrospinal and Vestibulospinal Systems. In *Encyclopedia of neuroscience* (pp. 1248–1254). Berlin, Heidelberg: Springer Berlin Heidelberg. doi:10.1007/978-3-540-29678-2_3131

Terry, P., Herbert, B. A. & Oakley, D. A. (1989). Anomalous patterns of response learning and transfer in decorticate rats. *Behavioural brain research*, *33*(1), 105–9.

Todorov, E. (2000). Direct cortical control of muscle activation in voluntary arm movements: a model. *Nature neuroscience*, *3*(4), 391–8. doi:10.1038/73964

Tower, S. (1940). Pyramidal lesion in the monkey. *Brain*, *63*(1), 36–90.

Travis, A. M. (1955). Neurological deficiencies after ablation of the precentral motor area in Macaca mulatta. *Brain*, *78*(2), 155–173.

Turner, R. S. & DeLong, M. R. (2000). Corticostriatal activity in primary motor cortex of the macaque. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *20*(18), 7096–7108.

Tyler, K. L. & Malessa, R. (2000). The Goltz-Ferrier debates and the triumph of cerebral localizationalist theory. *Neurology*, *55*, 1015–1024. doi:10.1212/WNL.56.10.1424

van Middendorp, J. J., Sanchez, G. M. & Burridge, A. L. (2010). The Edwin Smith papyrus: a clinical reappraisal of the oldest known document on spinal injuries. *European spine journal : official publication of the European Spine Society, the European Spinal Deformity Society, and the European Section of the Cervical Spine Research Society*, *19*(11), 1815–23. doi:10.1007/s00586-010-1523-6

Waller, A. (1850). Experiments on the Section of the glossopharyngeal and hypoglossal nerves of the frog, and observations of the alterations produced thereby in the structure of their primitive fibres. *Philosophical transactions of the Royal Society of London*, *140*(1850), 423–429. doi:10.1016/j.expneurol.2012.01.011

Ward, J. W. (1938). The influence of posture on responses elicitable from the cortex cerebri of cats. *Journal of Neurophysiology*, *1*(5), 463–475.

Weber, E. (1846). Muskelbewegung. *Handwörterbuch der Physiologie mit Rücksicht auf physiologische Pathologie*, *3*(2), 1–122.

Whishaw, I. Q., Pellis, S. M., Gorny, B. P. & Pellis, V. C. (1991). The impairments in reaching and the movements of compensation in rats with motor cortex lesions: an endpoint, videorecording, and movement notation analysis. *Behavioural brain research*, *42*, 77–91.

Wiener, N. (1948). *Cybernetics: or Control and Communication in the Animal and the Machine*. MIT Press.

Woolsey, C. N., Gorska, T., Wetzel, A., Erickson, T. C., Earls, F. J. & Allman, J. M. (1972). Complete unilateral section of the pyramidal tract at the medullar level in Macaca mulatta. *Brain Research*, *40*(1), 119–123.

Wu, J. H., Corwin, J. V. & Reep, R. L. (2009). Organization of the corticostriatal projection from rat medial agranular cortex to far dorsolateral striatum. *Brain Research*, *1280*, 69–76. doi:10.1016/j.brainres.2009.05.044

Yang, H.-W. & Lemon, R. N. (2003). An electron microscopic examination of the corticospinal projection to the cervical spinal cord in the rat: lack of evidence for cortico-motoneuronal synapses. *Experimental brain research*, *149*(4), 458–69. doi:10.1007/s00221-003-1393-9

Zaaimi, B., Edgley, S. A., Soteropoulos, D. S. & Baker, S. N. (2012). Changes in descending motor pathway connectivity after corticospinal tract lesion in macaque monkey. *Brain*, *135*(7), 2277–2289. doi:10.1093/brain/aws115

Chapter 2

Rapid Prototyping Tools for the Study of Behaviour

> *It is not true that "the laboratory can never be like life." The laboratory* must *be like life!*
>
> ---
>
> JAMES J. GIBSON, *The Ecological Approach to Visual Perception* (1979)

2.1 Chapter Summary

The study of animal behaviour has provided tremendous insight into the functions of the brain. However, in the laboratory, behaviour is often studied in impoverished and repetitive regimes in order to control its complexity. In this chapter, we introduce a set of hardware and software tools that make it easier to rapidly survey a larger range of environments without losing control over observable behavioural data.

In the first part of the chapter, we introduce an architecture for a multi-purpose modular behaviour box. This architecture makes it possible to use simple fabrication and rapid prototyping tools to quickly reconfigure a physical environment for different assays requiring complex combinations of sensors and actuators.

In the second part, we present Bonsai, a high-performance visual programming language for controlling and monitoring real-time data streams on a digital computer. We describe Bonsai's core principles and architecture and demonstrate how it allows for the rapid and flexible prototyping of integrated experimental designs in neuroscience. We specifically highlight some applications that require the combination of many different hardware and software components, including video tracking of behavior, electrophysiology and closed-loop control of stimulation.

All the results concerning the Bonsai framework have been published as: Lopes, G., Bonacchi, N., Frazão, J., Neto, J., Atallah, B., Soares, S., Moreira, L., Matias, S., Itskov, P., Correia, P., Medina, R., Calcaterra, L., Dreosti, E., Paton, J. & Kampff, A. (2015). Bonsai: An event-based framework for processing and controlling data streams. *Frontiers in Neuroinformatics*, *9*(7). doi:10.3389/fninf.2015.00007

2.2 Introduction

The formal study of animal behaviour has a long history spanning hundreds of years across the fields of ethology, experimental psychology and neuroscience. While the ethologists mainly endeavoured to study behaviour in its natural environment, the psychologists and neurophysiologists have classically resorted, of necessity, to more controlled laboratory settings. The reason is mainly one of complexity. Behaviour is a highly multi-dimensional, multi-scale phenomenon that often allows no clear separation between relevant and irrelevant variables (Gomez-Marin, Paton, Kampff, Costa & Mainen, 2014). It is in general impossible to predict what an animal is going to do simply because some of the crucial information is not even accessible to measurement. In order to mitigate this problem, neuroscientists resort to making impoverished preparations where the number of variables that are changing at any given moment is low and very carefully controlled. The hope is that in this way the interpretation of brain signals recorded simultaneously with animal behaviour will be facilitated.

Depending on the kind of question a neuroscientist is after, an appropriate behaviour paradigm is set up. Anaesthetized and head-fixed preparations, as well as classical or operant conditioning boxes are regularly employed to drive the behaviour of the animal to oscillate between a set of repeatedly reproducible states more amenable to statistical analysis. Building such behaviour assays often requires very specialized engineering skills and long development cycles of trial and error in order to ensure all the relevant variables are controlled accordingly. Because of this, the tendency of the field has been to concentrate on a small set of “standardized” assays which have been shown to work for one area of research or other. Small variations to the standard tasks are gradually introduced in order to probe different aspects of the system. The complexity of behaviour studies in neuroscience has thus traditionally progressed by attrition and painstaking accumulation of small perturbations to overall design patterns.

Interestingly, however, many of the most significant conceptual advances in our understanding of brain function have in fact developed *pari passu* with forays into entirely new behaviour spaces. Moving from anaesthetized to awake physiology completely changed the way we understand the neural processing of sensory stimuli (Sellers, Bennett, Hutt, Williams & Fröhlich, 2015). Similarly, moving from head-fixed to freely moving behaviour led to the discovery of place fields in hippocampus (O'Keefe & Dostrovsky, 1971). Single trial analysis of simultaneously recorded responses have revealed patterns of neural activity such as hippocampal ripples that are simply impossible to recover from statistical averages of repetitive behaviour episodes (Foster & Wilson, 2006; Davidson, Kloosterman & Wilson, 2009). Each of these developments has required significant advances in tools used to record and control behavioural data at a fine scale. Unfortunately, the technical cost and scientific risk of trying something novel means that such advances are still much fewer and far between than would be desirable.

From the beginning of this work it was understood that revealing the teleology of cortical control over behaviour would require just this kind of foray into diverse and potentially unknown behaviour spaces. We agreed that it might be worth to try and develop a toolkit for the behavioural neuroscientist that would accelerate the exploration of this vast space. One of the first obvious targets for improvement was the behaviour box. Traditionally, when a given behaviour assay is found to produce interesting results, its design is progressively tweaked so as to exacerbate the features of the original effect. In this work, we started by breaking apart this concept of the polished behaviour box, and wondered what would happen if instead of a standard box, we could have a box of standards.

2.3 Methods

2.3.1 The Modular Behaviour Box

At the outset it was decided that the scale of the modular architecture would probably have to match a given animal model, given the vastly different size scales between rodents, cats and primates. Our animal model of choice is the rodent *rattus norvegicus*, and all of our proposed design choices target its size scale. Small adjustments could, however, be reasonably made up to a point for other mammals of similar stature, such as mice.

The main component and interface of the modular box is the individual 1×1 module (Figure 2.1A). This module defines a standardized footprint ($12\,\text{cm} \times 12\,\text{cm}$), against which all other modules are measured. Every newly fabricated module is built to specification to match a multiple of this standardized footprint (e.g. it is possible to have 2×1, 2×2, 4×1 or any other multiple combination of the standard size). Inside the module footprint the module designer places a single logical component of a behaviour box and ensures that it can operate in isolation. Figure 2.1 shows some examples of reusable modules developed throughout the project.

One of the principal requirements for assembling a box is fastening all its components together. By having a standard footprint, it is possible to design a set of regularly spaced mounting points that allows the experimentalist to quickly generate an entirely new configuration by simply swapping modular components inside the box (Figure 2.2, 2.3). For this work, we took advantage of an existing aluminium structural framing system (Bosch Rexroth, DE) to build the common mounting points (Figure 2.1A). Modules are fastened against post-insertion nuts which are able to slide across the whole length of the aluminium rail. Each of the modules is fastened by four screws, one in each corner. In order to ensure modules

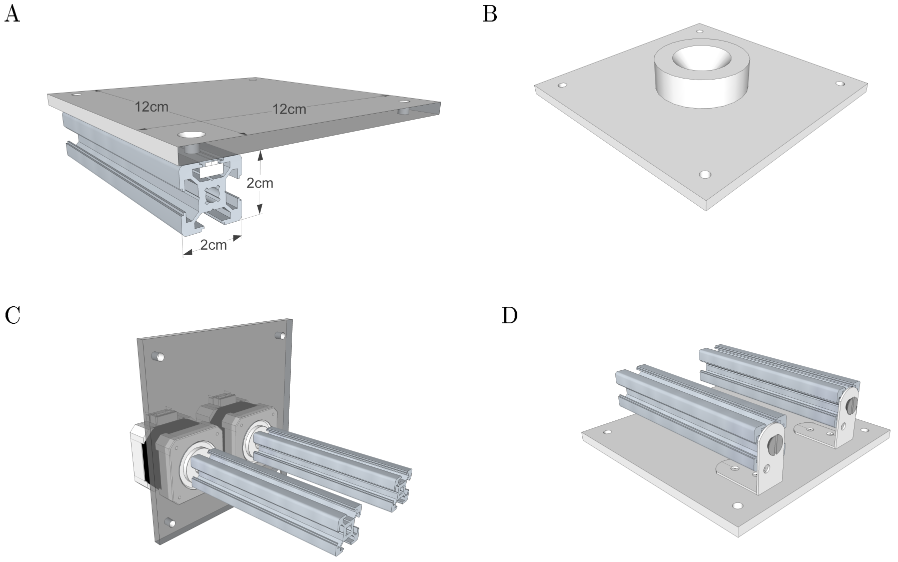


Figure 2.1. Some examples of standardized behaviour modules. (**A**) Detail of a 1 × 1 module mounted in support frame. Fixation is achieved by driving a screw through post-insertion nuts placed in the structural framing (see text). (**B**) Example reward port module which can be floor- or wall-mounted. All relevant electronics and water distribution circuits are assembled on the back of the module (not shown). (**C**) Wall-mounted reconfigurable obstacle course stepper module. Stepper motors mounted on the back of the module allow for dynamic reconfiguration of the orientation of each step. (**D**) Floor-mounted obstacle course step pair. Multiple of these modules can be tiled together to assemble obstacle courses of arbitrary length.

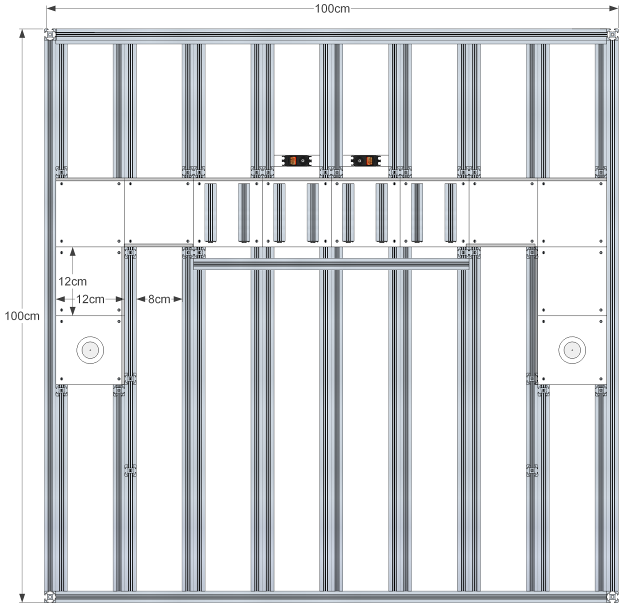


Figure 2.2. Example of a linear shuttling box assembled from a 1 m × 1 m modular structure using reward port and obstacle course step modules.

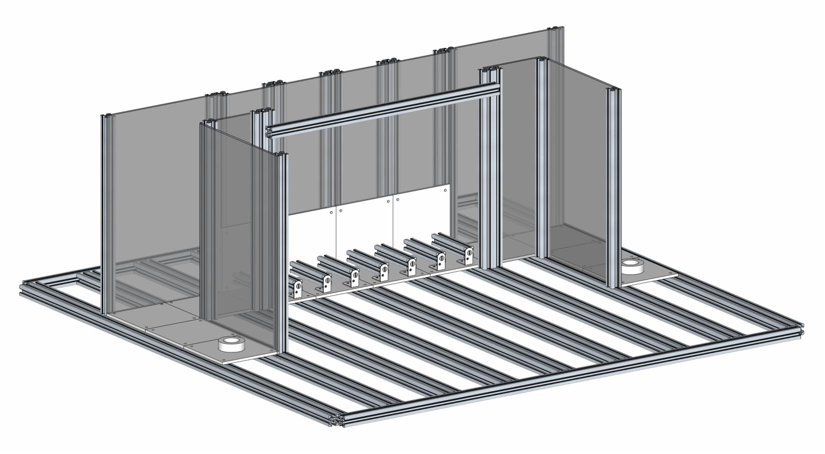


Figure 2.3. Side view of the linear shuttling box.

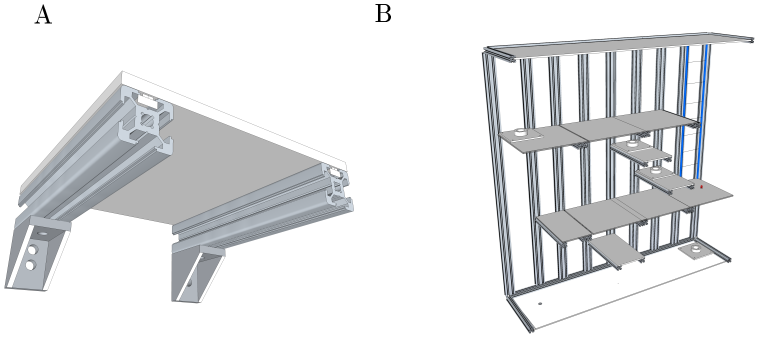


Figure 2.4. Example of vertical assembly. (**A**) Detail of a 1 × 1 wall-mounted platform module. (**B**) Example of a vertical maze configuration.

can be tightly and securely fixed one next to the other, we used a system of regularly spaced double rails (Figure 2.2). This gives the frame the flexibility to easily reposition and rearrange individual modules tiling the entire footprint of any arbitrarily large box.

If the support frame is laid out vertically, it is possible to create modular walls of arbitrary dimensions. Some of the modules can be mounted equally well on a vertical or horizontal configuration, such as reward ports (Figure 2.1B). The three-dimensionality of the design has even been exploited to create vertical mazes (Figure 2.4) to great success.

Throughout the project we made the base of every module from 5 mm acrylic pieces. While not an absolute requirement for the design, this choice of plastic material has the advantage that a laser cutter can be used to very quickly produce a large collection of custom-built modules. In addition, patterns can be engraved or cut on the base to provide additional mounting points for hardware embedded in the module. The use of such rapid prototyping fabrication tools alongside with off the shelf available electronic sensors and actuators meant we were able to completely redesign the entire behaviour box, sometimes in a matter of days.

2.3.2 The Bonsai Framework

Modern scientific experiments crucially depend on the control and monitoring of many parallel streams of data. Multiple measurement devices, from video cameras, microphones, and pressure sensors to neural electrodes, must simultaneously send their data in real-time to a recording system. General purpose digital computers have gradually replaced many of the specialized analog and digital technologies used for this kind of data acquisition and experiment control, largely due to the flexibility of programming and the exponential growth in computing power. However, the serial nature of programming instructions and shared memory makes it a challenge, even for experienced programmers, to develop software that can elegantly deal with the asynchronous, parallel nature of scientific data.

Another challenge arises from the need for software integration. Each hardware vendor provides their own set of drivers and programming interfaces for configuring and acquiring data from their devices. In addition, the growth of the open-source movement has greatly increased the number of freely available technologies for different data processing domains. Integration of these diverse software and hardware components remains a major challenge for researchers.

These difficulties lead to increased development times when setting up an experiment. Moreover, it requires experimenters to pursue specialized training outside their domain of research. This limits the ability to rapidly prototype and try out new designs and can quickly become the factor limiting the kinds of questions that are amenable to scientific investigation.

Here we describe Bonsai, an open-source visual programming framework for processing data streams. The main goal of Bonsai is to simplify and accelerate the development of software for acquiring and processing the many heterogeneous data sources commonly used in (neuro) scientific research. We aim to facilitate the fast implementation of state-of-the-art experimental designs and to encourage the exploration of new paradigms.

The framework has already been successfully used for many applications. In the following we will specifically highlight Bonsai's utility in neuroscience for monitoring and controlling a diverse range of behaviour and physiology experiments.

Architecture

Scientific data, like the world we live in, is inherently parallel. To monitor this complexity, modern experimenters are often forced to use multiple electronic instruments simultaneously, each with their own independent sampling rates. As data arrives at the acquisition computer, there are two main approaches to log and process these asynchronous data streams. The first approach is to use a polling strategy: a single sequential process in the computer runs a processing loop that goes through each device in sequence and gathers the available data. In this case, data from only one device is being collected and manipulated at any point in time. The second approach is to use an event-driven (reactive) architecture: processes are setup in parallel to collect data from all the devices simultaneously. Whenever new data is available, notifications are sent to the appropriate software routines that collect and process the data as soon as possible. When only a single processor is available, the difference between these two strategies is negligible: only one instruction at a time can be executed by the computer. However, with modern multi-processor cores and dedicated data transfer circuits, the performance difference between the two approaches will significantly influence the throughput of a data acquisition and processing system. Unfortunately, software tools to support and facilitate the "reactive" approach to data stream processing are only just now starting to be adopted and most software systems are still built from the sequential composition of simple program routines. Many of the assumptions of the sequential processing scenario do not scale to handle parallel execution, especially when shared memory and resources are involved.

In recent years, a number of advances in programming languages and software frameworks have tried to make it easier to create complex software applications by composition of asynchronous computing elements (Bainomugisha, Carreton, Cutsem, Mostinckx & Meuter, 2013). Bonsai builds upon these new efforts and aims to extend these developments to the rapid-prototyping domain by introducing a visual programming language for composing and processing asynchronous data streams. Bonsai was developed on top of the Reactive Extensions for the.NET framework (Rx) (Microsoft Open Technologies, 2014). Rx provides many built-in operators that transparently deal with the concurrency challenges that inevitably surface when multiple data streams need to be processed and integrated together in a single program. It has become an increasingly popular framework to develop reactive interfaces for next generation mobile and desktop computing platforms, where it is used to handle the growing number of sensors and network communications required by business logic and consumer applications.

Bonsai (via Rx) represents asynchronous data streams using the notion of an observable sequence. An observable sequence represents a data stream where elements follow one after the other. An example would be a sequence of frames being captured by a camera, or a sequence of key presses logged by the keyboard. The name observable simply specifies that the way we access elements in the data stream is by listening to (i.e., observing) the data as it arrives, in contrast with the static database model, in which the desired data is enumerated.

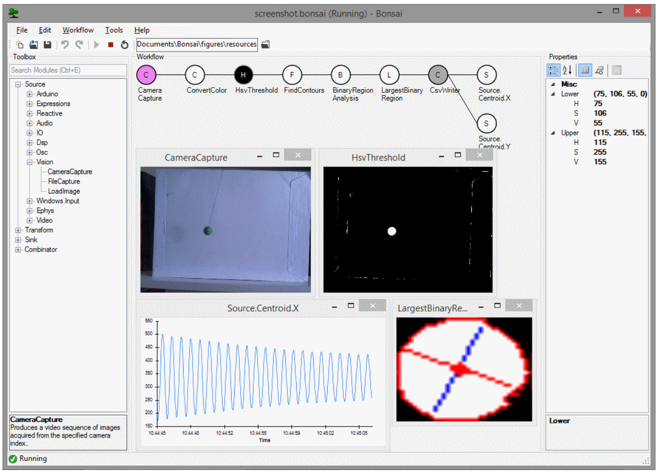
In Bonsai, observable sequences are created and manipulated graphically using a dataflow (Mosconi & Porta, 2000; Johnston, Hanna & Millar, 2004) representation (Figures 2.5, 2.6A, Video 2.1). Each node in the dataflow represents an observable sequence. Nodes can be classified as either observable sources of data or combinators (Table 2.1). Sources deliver access to raw data streams, such as images from a video camera or signal waveforms from a microphone or electrophysiology amplifier. Combinators represent any observable operator that handles one or more of these sequences. This category can be further specialized into transforms, sinks and other operator types depending on how they manipulate their inputs (Table 2.1). Transforms modify the incoming data elements of a single input sequence. An example would be taking a sequence of numbers and generating another sequence of numbers containing the original elements multiplied by two. Sinks, on the other hand, simply introduce processing side-effects without modifying the original sequence at all. One example would be printing each number in the sequence to a text file. The act of printing in itself changes nothing about the sequence, which continues to output every number, but the side-effect will generate some useful action. Combinators that change, filter or merge the flow of data streams are neither transforms nor sinks, and they are simply referred to by the more general term combinator. The Sample combinator illustrated in Figure 2.6A takes two data sequences and produces a new sequence where elements are sampled from the first sequence whenever the second sequence produces a new value. In this example, we use Sample to extract and save single images from a video stream whenever a key is pressed.

A common requirement when designing and manipulating dataflows is the ability to visualize the state of the data at different stages of processing. We have therefore included a set of visualizers to assist debugging and inspection of data elements, including images and signal waveforms (Figure 2.5). These visualizers are automatically associated with the output data type of each node and can be launched at any time in parallel with the execution of the dataflow. Furthermore, it is often desirable to be able to manipulate processing parameters online for calibration purposes. Each node has a set of properties which parameterize the operation of that particular source or combinator (Figure 2.5). This allows, for example, changing the cutoff frequency of a signal processing filter, or setting

Bonsai Node Categories

Color	Category	Description
🟣	Source	**# Inputs: 0** Generates observable sequences of data
🔵	Combinator	**# Inputs: 0..N** Can change both the elements and the sequence; elements may be dropped, shifted, duplicated or merged with other sequences
⚪	Transform	**# Inputs: 1** Transforms the elements in the sequence but does not change the sequence order or timing
🟢	Condition	**# Inputs: 1** Does not change the elements in the sequence but can drop (filter out) elements from the sequence
⚫	Sink	**# Inputs: 1** Does not change neither the elements nor the sequence, but can introduce side-effects
🟠	Property	**# Inputs: 1** Represents a property of another node. Writing to it at runtime will change the value of the property
🟡	Nested	**# Inputs: 0..N** A nested combinator uses an internal (nested) dataflow to implement specific operations

Table 2.1. List of Bonsai node categories. The color of each Bonsai node serves as a visual aid to identify their role in dataflow processing pipelines. Most of these categories are actually specializations of the very general combinator and are meant to visually depict their specific data processing semantics.



Misc
Lower (75, 106, 55, 0)
H 75
S 106
V 55
Upper (115, 255, 155,
H 115
S 255
V 155

Lower

CameraCapture
Produces a video sequence of images acquired from the specified camera index.

Figure 2.5. Screenshot of the Bonsai user interface running a video processing pipeline. An example dataflow for color segmentation and tracking of a moving pendulum is shown. Data sources are colored in violet; transform operators in white; sinks in dark gray. The currently selected node (HsvThreshold) is colored in black and its configuration parameters are displayed in the properties panel on the right. Overlaid windows and graphs represent Bonsai data visualizers for the output of individual nodes.

the name of the output file in the case of data recording sinks. We have also included the possibility of externalizing node properties into the dataflow (Figure 2.6B). Externalizing a property means extracting one of the parameters into its own node in the dataflow, making it possible to connect the output of another node to the exposed property. This allows for the dynamic control of node parameters.

Finally, we have built into Bonsai the ability to group nodes hierarchically. In its simplest form, this feature can be used to encapsulate a set of operations into a single node which can be reused elsewhere (Figure 2.6C). This is similar to defining a function in a programming language and is one of the ways to create new reactive operators in Bonsai. Any named externalized properties placed inside an encapsulated dataflow will also

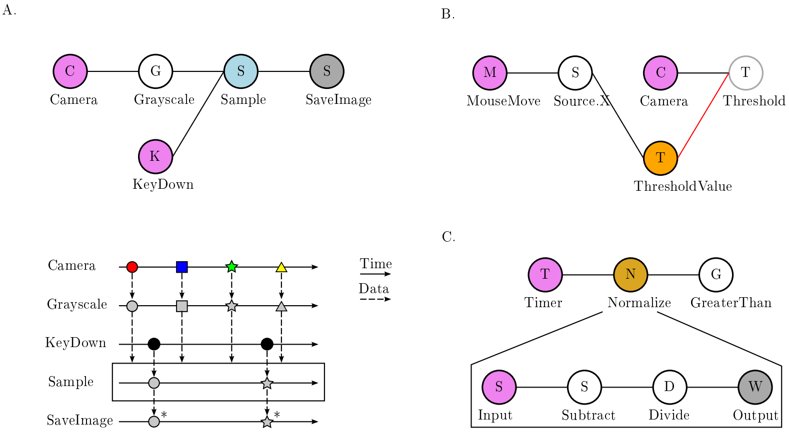


Figure 2.6. Examples of dataflow processing pipelines using Bonsai. **(A)** Taking grayscale snapshots from a camera whenever a key is pressed. Top: graphical representation of the Bonsai dataflow for camera and keyboard processing. Data sources are colored in violet; transform operators in white; combinators in light blue; sinks in dark gray. Bottom: marble diagram showing an example execution of the dataflow. Colored tokens represent frames arriving from the camera. Black circles represent key press events from the keyboard. Asterisks indicate saving of images to permanent storage. **(B)** Dynamic modulation of an image processing threshold using the mouse. The x-coordinate of mouse movements is used to directly set the externalized ThresholdValue property (orange). The updated threshold value will be used to process any new incoming images. **(C)** Grouping a set of complex transformations into a single node. In the nested dataflow, the source represents incoming connections to the group and the sink represents the group output.

show up as properties of the group node itself. This allows for the parameterization of nested dataflows and increases their reuse possibilities. In addition, encapsulated dataflows are used to specify more complicated, yet powerful, operators such as iteration constructs that allow for the compact description of complex data processing scenarios that can be cumbersome to specify in pure dataflow visual languages (Mosconi & Porta, 2000) (see below).

Bonsai was designed to be a modular framework, which means it is possible to extend its functionality by installing additional packages containing sources and combinators developed for specific purposes. New packages can be written using C# or any of the.NET programming languages. Python scripts [via IronPython (IronPython Community, 2014)] can be embedded in the dataflow as transforms and sinks, allowing for rapid integration of custom code. All functionality included in Bonsai was designed using these modular principles, and we hope to encourage other researchers to contribute their own packages and thereby extend the framework to other application domains. At present, the available packages include computer vision and signal processing modules based on the OpenCV library (Itseez, 2014). Drivers for several cameras and interfaces to other imaging and signal acquisition hardware were integrated as Bonsai sources and sinks, including support for Arduino microcontrollers (Banzi, Cuartielles, Igoe, Martino & Mellis, 2014), serial port devices and basic networking using the OSC protocol (Wright, Freed & Momeni, 2003). Given the specific applications in the domain of neuroscience, we also integrated a number of neuroscience technology packages. The Ephys package, for example, builds on the Open Ephys initiative for the sharing of electrophysiology acquisition hardware (Voigts, Siegle, Kemere, Moore & Wilson, 2013) by providing support for the Rhythm open-source USB/FPGA interface (Intan Technologies, US). Therefore, the next generation tools for electrophysiology can already be used inside Bonsai, the acquired physiology data implicitly integrated with other available data streams and thus easily assembled into a powerful and flexible experimental neuroscience platform.

Advanced Operators

The most common application of Bonsai is the acquisition and processing of simple, independent data streams. However, for many modern experiments, basic acquisition and storage of data is often not sufficient. For example, it can be convenient to only record the data aligned on events of interest, such as the onset of specific stimuli. Furthermore, neuroscience experiments often progress through several stages, especially for behavioral assays, where controlled conditions vary systematically across different sessions or trials. In order to enforce these conditions, experiments need to keep track of which stage is active and use that information to update the state of control variables and sensory processing. These requirements often cannot be described by a simple linear pipeline of data, and require custom code to handle the complicated logic and bookkeeping of experimental states. Below we describe a set of advanced Bonsai operators that can be used to flexibly reconfigure data processing logic to cover a larger number of scenarios. These operators and their applications are all built on the single idea of slicing a data stream into sub-sequences, called windows, which are then processed independently and, potentially, in parallel (Figure 2.7).

Bonsai provides different combinators that allow the creation of these sub-sequences from any observable data stream, using element count information, timing, or external triggers (Figures 2.7A–C). The specific set of operations to apply on each window is described by encapsulating a dataflow inside a SelectMany group, as detailed in the signal processing example of Figure 2.7D. The input source in this group represents each of the window sub-sequences, i.e., it is as if each of the windows is a new data source, containing only the elements that are a part of that window.

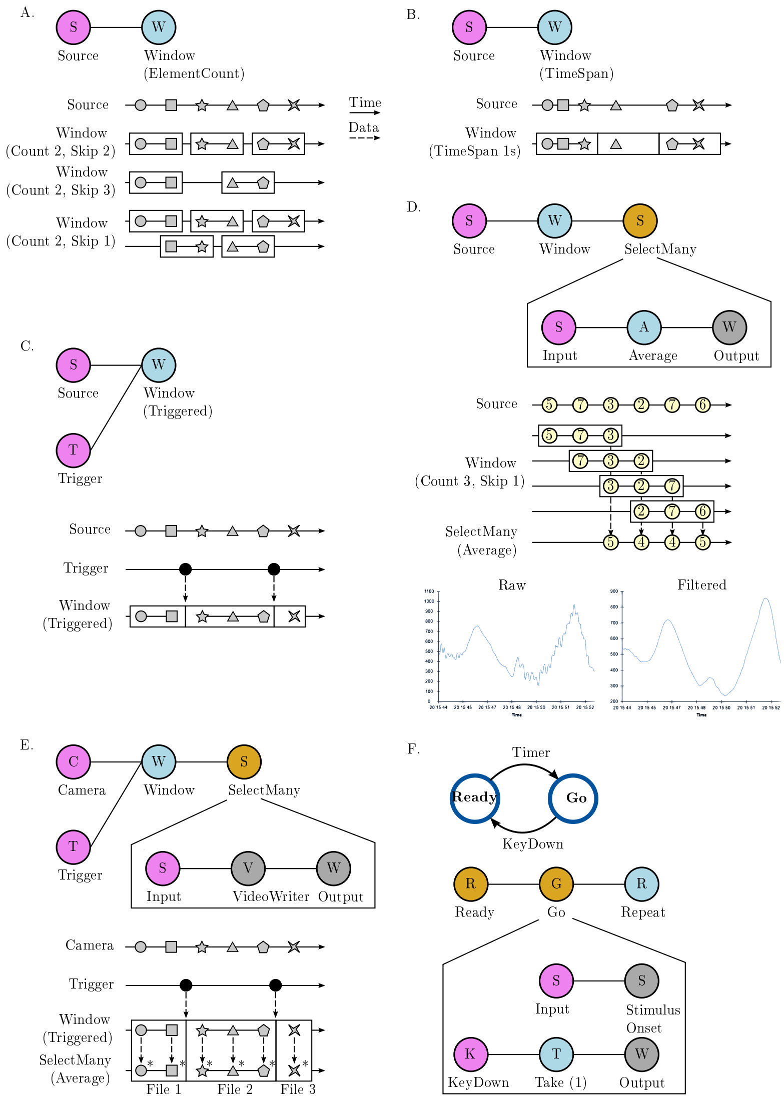


Figure 2.7. Using slicing and window processing combinators in Bonsai.

These elements will be processed as soon as they are available by the encapsulated dataflow. Windows can have overlapping common elements, in which case their processing will happen concurrently. The processing outputs from each window are merged together to produce the final result. In the case of Figure 2.7D, past and future samples are grouped in windows to compute a running average of the signal through time, necessarily time-shifted by the number of future samples that are considered in the average.

The processing of the elements of each window happens independently, as if there was a new isolated dataflow running for each of the sequences. We can exploit this independence in order to dynamically turn dataflows on and off during an experiment. In the video splitting example of Figure 2.7E, we use an external trigger source to chop a continuous video stream into many small video sequences, aligned when the trigger fired. We then nest a VideoWriter sink into the SelectMany group. The VideoWriter sink is used to encode video frames into a continuous movie file. It starts by creating the video file upon arrival of the first frame, and then encoding every frame in the sequence as they arrive. When the data stream is completed, the file is closed. By nesting the VideoWriter inside the SelectMany group, what we have effectively done is to create a new video file for each of the created windows. Whenever a new trigger arrives, a new clip is created and saving proceeds, implicitly parallelized, for that video file.

More generally, we can use this idea to implement discrete transitions between different processing modes, and chain these states together to design complex control structures such as finite state machines (FSMs). FSMs are widely used to model environments and behavioral assays in systems and cognitive neuroscience. One example is illustrated in Figure 2.7F, where we depict the control scheme of a stimulus-response apparatus for a simple reaction time task. In this task, there are only two states: Ready and Go. In the Ready state, no stimulus is presented and a timer is armed. Whenever the timer fires, the task transitions into the Go state, and a stimulus is presented. The subject is instructed to press a key as fast as possible upon presentation of the stimulus. As soon as the key is pressed, the system goes back to the Ready state to start another trial. In a FSM, nodes represent states, e.g., stimulus availability or reward delivery, and edges represent transitions between states that are caused by events in the assay, e.g., a key press. In each state, a number of output variables and control parameters are set (e.g., turning on a light) which represent the behaviour of the machine in that state.

In the Bonsai dataflow model, dataflows encapsulated in a SelectMany group can be used to represent states in a FSM (Figure 2.7F, bottom). Specifically, a state is activated whenever it receives an input event, i.e., the dataflow nested inside the state will be turned on. The dynamics of the nested dataflow determine the dynamics of the state. In the Go state presented in Figure 2.7F, the activation event is used to trigger stimulus onset. In parallel, we start listening for the key press which will terminate the state. Conversely, for the Ready state we would trigger stimulus offset and arm the timer for presenting the next stimulus. An important difference between Bonsai dataflows and pure state machine models is that a dataflow is specified as a directed acyclic graph, i.e., the data stream cannot loop back on itself. However, by taking advantage of the Repeat combinator, we can restart a dataflow once it is completed, allowing us to reset the state machine for the next trial.

Many of the control tasks in experiments have this sequential trial-based structure, which has allowed us to rapidly prototype complex behaviour assays, such as closed-loop rodent decision making tasks, simply by leveraging the flexibility of the data stream slicing operators.

Alternatives to Bonsai

Although graphical user interfaces have played a crucial role in the widespread proliferation of computing technology throughout various scientific fields, the majority of these interfaces tend to be applied to relatively narrow domains, such as the operation of a specific instrument. Their goal is often to provide access to all the various configuration parameters of the hardware and to provide basic data acquisition functionality. There is often no opportunity to parameterize or condition the behaviour of the instrument beyond the possibilities presented by the interface, and interconnections with other devices are often limited to simple hardware triggers. The alternative, when available, is to access low-level application programming interfaces (APIs), and program the desired behaviour from scratch.

In the more flexible domains of data analysis, behaviour control and software simulations, the use of more versatile graphical interfaces has become increasingly prevalent. In these scenarios, it is not uncommon to encounter the development of domain-specific languages (DSLs), where graphical building blocks related to the domain of application can be combined together by the user to generate new behaviors, such as the sequence of steps in a psychophysics experiment or a state-machine diagram used to control stimuli and rewards in operant conditioning. While providing more flexibility to the end user, such DSLs are usually not conceived, at their core, to be applied to wildly different domains (e.g., an operant conditioning state machine is not expected to be able to filter continuous electrophysiology signals). In fact, most DSLs will not even allow the user to extend the set of built-in operations. In those that do, the developer may find a customization pit (Cook, Jones, Kent & Wills, 2007), where concepts and operations that are within the range of what the DSL can express are easy to develop, whereas tasks that are a little bit outside of the boundaries of the language quickly become impossible or too cumbersome to implement.

As the level of flexibility of a graphical user interface increases, we start to approach the space occupied by general purpose visual programming languages (GPVPL). These are languages that are designed from the outset to be capable of solving problems across a wide variety of domains using a general set of operations. Ideally, the core building blocks of the language will themselves be domain-independent, so that the user can easily apply the same set of operations to the widest possible class of inputs. In order to better illustrate the feel and expressive power of GPVPLs, and to clarify where Bonsai itself is positioned, we will give two examples of popular languages that have succeeded in this niche: LabVIEW (National Instruments, 2014) and Simulink (MathWorks, 2014).

LabVIEW is one of the best examples of a GPVPL applied to the design and control of experiments (Elliott, Vijayakumar, Zink & Hansen, 2007). In LabVIEW, users create virtual instruments (VIs) which are composed of a graphical front-panel containing an assortment of buttons, dials, charts and other objects; as well as a back-panel where a flowchart-like block diagram can be used to specify the behaviour of the VI. In this back-panel, nodes and terminal elements can represent hardware components, numerical operations or front-panel objects, which are connected together using virtual wires that specify the flow of data between them. The popularity of LabVIEW grew initially from its support for state-of-the-art data acquisition cards and hardware as well as its data visualization capabilities. The modularity of its architecture also allowed users to quickly develop and implement new nodes within the language itself by using VIs themselves as nodes.

Although the LabVIEW back-panel is a dataflow visual programming language, its execution model tends to follow a polling, rather than event-driven, strategy for dealing with multiple data streams. In order to properly scale this model to the increasing number of available processor cores, LabVIEW has implemented sophisticated code analysis tools that attempt to identify parallelizable portions of block diagrams automatically (Elliott et al., 2007). Once these sections are identified, LabVIEW will automatically generate parallel processes depending on the number of available cores and will manage the bottlenecks in the code accordingly. Although this mitigates the limitations of the sequential polling programming model, it is important to realize that the goal of such automatic parallelization is still to provide the user with a logically synchronized programming model.

Simulink is a popular dataflow visual programming language for modeling, simulating and analyzing multi-domain dynamic systems. It has become extremely popular for modeling response characteristics of control systems, allowing not only for the rapid prototyping of algorithms, but also the automatic generation of microcontroller code for embedded systems. Again, the success of the language stemmed primarily from the flexibility and ease of use of the block diagrams, as well as the number of prebuilt operations and data visualization tools which quickly took care of many crucial but tedious aspects of control systems modeling.

Like LabVIEW, the execution model for Simulink generated code is still based on polling strategies, where ready to execute dataflow nodes are updated in turn as inputs become available. Again, strategies to scale the output of Simulink to multiple cores have been proposed based on analyzing and segmenting the model into parallelizable sections which can be converted into equivalent parallel execution code for microcontrollers (Kumura, Nakamura, Ishiura, Takeuchi & Imai, 2012).

Similar to LabVIEW and Simulink, Bonsai was designed as a general purpose modular language. The core architecture of Bonsai is domain-independent and provides a general framework to compose asynchronous data streams. A general set of composition operators, or combinators, provides support for iteration, segmentation and merging of parallel data streams, as well as other common manipulations on observable sequences. Both the sources of data and available processing operations can be extended within the language itself using nesting of dataflows. Data visualizers and a growing library of data stream acquisition, processing and logging modules are provided to allow rapid prototyping of a large number of different applications.

However, in contrast to LabVIEW or Simulink, Bonsai adopts a very different strategy to implement dataflow execution. Rather than trying to derive a global sequential execution order of dataflow nodes based on the number of active inputs, Bonsai nodes simply react to incoming inputs immediately, without the need to wait for all of them to be active. When multiple observable sequences are present, this allows for a choice of different concurrency composition strategies. Nevertheless, as the result of the composition is an observable sequence itself, such concurrency management can remain functionally isolated from the combinator that is handling the composition. From the point of view of downstream operators, they are simply receiving an observable sequence. There is a tradeoff, of course, that more responsibility for managing the flow of data is passed to the end user, but it also allows for a finer grained control of concurrency that is critical to the specification of parallel applications.

One important caveat of developing asynchronous systems is that debugging can be more difficult in situations where the precise timing and ordering of events is required to reproduce an offending behaviour. In synchronized and sequential execution environments, one can easily go step by step through the precise cascade of transformations that resulted in a problem. In contrast, when multiple processes are executing concurrently, it can be harder to analyze the program flow in a similarly reproducible, deterministic manner. However, it should be noted that this issue is not unique to reactive environments with real asynchronous devices. A sequential polling strategy will be equally deficient in reproducing a particular execution sequence when data from parallel input devices is being accessed.

Another important caveat is that Bonsai currently runs exclusively in Windows operating systems. However, Microsoft has recently open-sourced the execution engine of the.NET framework and will pursue implementations for all the major operating systems (Linux/Mac). This raises the interesting possibility of eventually extending the Bonsai user base into these important platforms.

2.4 Results

2.4.1 Getting Started with Bonsai

Community

The Bonsai framework can be downloaded at https://bitbucket.org/horizongir/bonsai and installed on Windows operating systems starting with Windows 7 and above. The website is organized into different sections: Downloads (where the latest installer is located), Wiki (with a "Getting Started" guide, tutorials and (FAQ) frequently asked questions), and Issues (where bugs can be reported). We have also created a user forum (address is listed in the FAQ section) where the community of Bonsai users have been sharing their feedback, questions and experiences.

A video tutorial introduction to Bonsai is included with this publication (Video 2.1).

Extending Bonsai

Bonsai was designed from the outset to support many different layers of extensibility:

(a) Dataflows: The first layer is through the creation of Bonsai dataflow files themselves. Existing dataflows can be directly reused inside other dataflows as nested nodes. This allows for the sharing of reusable dataflow design patterns between applications.

(b) Python Scripting: Bonsai supports embedded scripting using IronPython 2.7. Specifically, Bonsai includes three types of Python nodes: PythonTransform, PythonCondition, and PythonSink, which all operate by calling a user-defined Python function described by a script. Below we include a simple example of a PythonTransform for rescaling data:

```
# Declare transform output type
@returns(float)
def process(value):
    return value / 255.0
```

(c) NuGet: Bonsai modules are natively written in C# or other.NET languages. The NuGet package manager has emerged as the defacto standard for the sharing of code between.NET developers. Bonsai includes a full NuGet client which manages local package versions, provides access to the curated feed of standard Bonsai packages, and allows for the quick sharing of modules between Bonsai users through either NuGet or other remote and local package sources. Tutorials and examples on how to create new Bonsai modules are included in the Wiki.

2.4.2 Under the Hood

Computational Overhead

Bonsai takes full advantage of the flexibility of C# and its Just-In-Time (JIT) compiler to bring the computational overhead of running the framework to zero. This is possible due to the fact that the graphical dataflows in Bonsai are actually specifying syntactically correct C# code by means of an expression tree. When the dataflow is executed, C# code is generated for assembling and running the pipeline. This code is ultimately compiled into native machine language before execution, which has the consequence that running a Bonsai dataflow is as fast as if one wrote the equivalent Rx code manually. In fact, this also means every Bonsai module is just a standard C# class exposing methods working on Rx's observable interface, which makes it possible to reference every single Bonsai package from a standard.NET application and just use the module functionality directly.

Concurrency

The level of concurrency and parallelism in Bonsai entirely depends on the structure of each individual dataflow and the specific computer hardware involved. Typically, each hardware device source (e.g., a camera) runs independently in its own logical thread. Some sources can occasionally share threads when the underlying device architecture allows for it. For example microcontroller sources coming from the same USB port effectively require sharing a single communications channel, but this is logically abstracted from the developer so there is no need to worry about handling multiplexed messages.

The specialized handling of concurrency introduced by merging different processing streams is done using dedicated Rx concurrency operators that are exposed graphically through the language. Operators located downstream from the merge point can treat the merged sequence as if it was a single sequential data source. This means most Bonsai operators are actually concurrency-agnostic, meaning they don't have to worry about concurrency at all: they simply assume their inputs are processed sequentially. This functional approach allows Bonsai operators to be simple to program, reliable and extremely performant.

Finally, some Bonsai operators introduce local concurrency implicitly to maximize performance. For example, many of the data logging sinks actually write to disk in parallel with the arrival of data. This prevents processor-heavy routines, such as video compression, to stall the pipeline and allow for online execution to proceed as fast as possible. From the point of view of the developer, however, such optimizations happen transparently.

Time

Being a fully asynchronous framework, Bonsai has to deal with code executing logically in many different processors. There is no particular assumption about time in the framework other than the sequential flow of data through the pipeline, but facilities are in place to help the synchronization and timing of data. For example, the Timestamp operator provides access to hardware performance timers included in modern processors to timestamp event notifications, across the pipeline, using a shared high resolution clock. However, it should be noted that this only applies to processes occurring centrally: for precise sub-millisecond synchronization of physical events happening outside the computer (e.g., stimulation pulse train and electrophysiology data) we still recommend the classical sharing of voltage or optical sync pulses logged simultaneously in each device.

2.4.3 Applications

The validation of Bonsai was performed by using the framework to implement a number of applications in the domain of neuroscience (Figure 2.8). The breadth of technologies at use in this field demands that modern experiments be able to handle many heterogeneous sources of data. Experimenters need to routinely record video and sensor data monitoring the behaviour of an animal simultaneously with electrophysiology, optical reporters of neural activity or other physiological measures. Online manipulation and visualization of data is a fundamental part of the experiment protocol for many of the reported techniques. In the following, we highlight some of these practical applications of Bonsai in more detail in order to illustrate both "best practices" and implementation strategies.

One of the first use cases driving the development of Bonsai was the automated online tracking of animal behaviour using video. The most common tracking application involves chaining together operators for image segmentation and binary region analysis to allow the extraction of the spatial location of an animal over time (Figures 2.8A,B). The same technique can easily be extended to track different kinds of objects, such as eyes or experimental manipulanda in human psychophysics experiments (Figure 2.8C), provided adequate illumination contrast and the appropriate choice of a method for segmentation. These image processing tools can also be used to acquire and process physiological data in neural imaging setups, where it is now possible to record bioluminescent or fluorescent reporters of neural activity during behaviour. For example, Figure 2.8B demonstrates simultaneous measurement of animal behaviour and neural activity using bulk fluorescence calcium imaging in the mouse brain recorded with a CCD sensor and a fiberoptic system (Tecuapetla, Matias, Dugue, Mainen & Costa, 2014).

Raw video data from modern high-resolution, high-speed cameras can be expensive and cumbersome to store. Online video compression and storage sinks were implemented taking advantage of parallelism to avoid frame loss. Video compression is processing intensive and can compromise data acquisition if reading the next frame has to wait for the previous frame to be fully encoded. One solution is to buffer incoming frames and compress them in parallel with the rest of the processing stream. By encapsulating this behaviour into a Bonsai sink, it became easy to incorporate video recording and compression functionality into any image processing pipeline (Figures 2.8A–E,G,H).

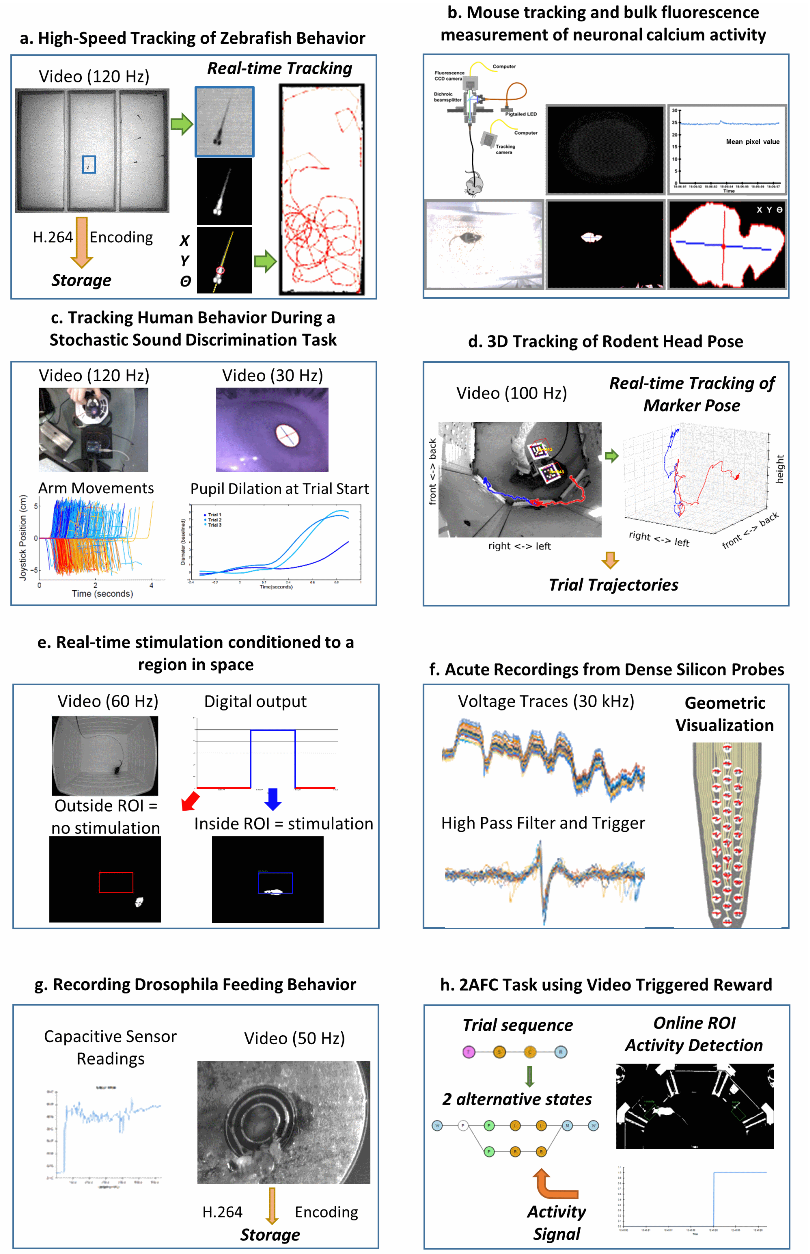


Figure 2.8. Example neuroscience experimental setups using Bonsai.

While simple image processing techniques can easily extract continuous two-dimensional measures of animal location over time, it often becomes the case that the experimenter is concerned with tracking the detailed behaviour of specific features in the animal's body, such as head pose. This is an essential component in neurophysiology or stimulation experiments in freely moving animals, where ongoing behaviour is the central constraint in interpreting neural responses and manipulations. However, identifying such features and reconstructing their position and orientation in 3D space is a challenging computer vision problem. A common solution is to use planar fiducial markers of known geometry (Kato & Billinghurst, 1999; Garrido-Jurado, Munõz-Salinas, Madrid-Cuevas & Marín-Jiménez, 2014) (Figure 2.8D). The computer vision research community has developed some open-source software solutions to this problem (Garrido-Jurado et al., 2014), which have been integrated into Bonsai to allow the possibility of easily and flexibly incorporating online 3D fiducial tracking in video streams. This approach has been used successfully to record 3D head movements of a mouse under optogenetic stimulation in a decision-making task (Figure 2.8D).

One final, but important application of video stream processing is in the development of closed-loop interfaces, where the actions of an animal directly modulate manipulations under the experimenter's control. This kind of experiment requires fast online analysis of behaviour and physiological variables of interest that are subsequently coupled to hardware control interfaces. In Figure 2.8E, real-time stimulation conditioned to a region in space was implemented by analyzing the position of an animal in a square arena. Whenever the animal found itself inside a specified region of interest, a signal was sent to an Arduino controller which was then used to drive optogenetic stimulation of specific neural circuits.

Another key data type that is commonly processed by Bonsai dataflows is buffered time-series data. This type of data usually arises from audio, electrophysiology or other digital acquisition systems where multiple data samples, from one or more channels, are synchronously acquired, buffered and streamed to the computer. These buffers are often represented as data matrices, where rows are channels and columns represent individual data samples through time, or vice-versa. Support for simple band-pass filters, thresholding and triggering allowed us to build flexible spike detection and waveform extraction systems (Figure 2.8F). Using Intan's Rhythm API, we integrated into Bonsai support for a variety of next-generation electrophysiology devices using Intan's digital amplifier technology, such as the Open Ephys acquisition system (Voigts et al., 2013) or Intan's evaluation board (RHD2000, Intan Technologies, US). This system was successfully used to acquire and visualize simultaneous recordings from dense silicon probes where spikes from a loose-patch juxtacellular pipette were used as triggers to align and extract waveform data appearing on the multi-channel extracellular probe. Responses from every silicon probe site could then be superimposed on an accurate rendition of the probe geometry, in real-time.

The ability to rapidly integrate new modules allowed us to support the development and cross-validation of new tools for behavioral neuroscience. A paradigmatic example was the flyPAD, a new method for quantifying feeding behaviour in Drosophila melanogaster by measuring changes in electrode capacitance induced by the proboscis extension of a fly (Itskov et al., 2014). The integration of the flyPAD in Bonsai allowed researchers to quickly get started using this approach to design new experiments. Furthermore, it also allowed the validation of the tool by enabling simultaneous acquisition of high-speed video recordings of fly behaviour which were later used for annotation and classification of the sensor feeding traces (Figure 2.8G).

In a different set of experiments, Bonsai was used to implement a variation on a popular two-alternative forced choice (2AFC) decision-making task for rodents (Figure 2.8H). In this type of task, animals are placed in an environment with three “ports.” They are presented with a stimulus in the center port and afterwards report their perception of the stimulus by going either to the left or right choice ports. In the variation we present in this work, the two choice ports were replaced by regions of interest where the activity of the animal is analyzed using computer vision. This example offered unique challenges as it combined sophisticated sequential control of a task environment with continuous data stream processing of video and sensor data.

The integration of all these diverse components for data acquisition and experiment control does not only allow for the rapid deployment of established protocols. In fact, the modular nature of their integration (i.e., how they can be combined together) opens up new avenues for research, by allowing a rich, rapid exploration of novel methodologies. To demonstrate this, we created a dynamic virtual environment for freely moving rodents where the visual presentation of a stimulus is tightly controlled in closed-loop to the actions of the animal. We used a projection setup similar to the low-cost multi-touch sensing table proposed by (Han, 2005), where a visible light rear-projection system is coupled with infrared illumination and an infrared imaging sensor to detect in real-time where the animal is located with respect to the visual display surface (Video 2.2).

2.5 Discussion

After about a year of using Bonsai in an active neuroscience research institute, dozens of different experimental protocols and data analysis pipelines have been successfully implemented using the provided building blocks (Gouvêa, Monteiro, Soares, Atallah & Paton, 2014; Itskov et al., 2014; Tecuapetla et al., 2014). We were surprised by the diversity of applications and by the pace at which new modules and devices were developed and integrated.

The performance achieved by Bonsai dataflow processing was an important consideration throughout. Video processing can be particularly challenging to handle given the bandwidth required to quickly acquire and process large data matrices. In order to correlate continuous measures of behaviour with neural activity, it is useful for those measurements to have both high spatial and high temporal resolution. Using Bonsai, we were able to simultaneously process and compress grayscale image sequences from high resolution (1280×960) and high frame rate (120 Hz) cameras using standard off-the-shelf desktop computers (Intel Core i7, 8 GB RAM). In fact, many of the reported assays use multiple (>2) such video streams with success and actually process the behaviour video online either to control states of the behaviour protocol or to pre-process video data for offline analysis.

One of the areas where we see the application of Bonsai becoming most significant is in the development of dynamic behaviour assays (environments) using reactive control strategies. Brains evolved to generate and control behaviors that can deal with the complexity of the natural world. However, when neuroscientists try to investigate these behaviors in the lab, it is often difficult to design equivalent environmental complexity in a controlled manner. As an example, consider a simple foraging scenario in which a land animal must collect, in a timely manner, food items that become available at random intervals in many sites. If the item is not collected in time, it rots or gets eaten by competitors. In the case of a single foraging site, a FSM description intuitively represents the workings of the environment (Figure 2.9A). However, let us now consider a situation where the environment has two of these food sites operating independently, thus introducing the possibility of different events occurring simultaneously at each of the sites. If our environment is modeled as a finite-state machine, then we must represent every possible combination of states and transitions, as in Figure 2.9B. In the classical state machine

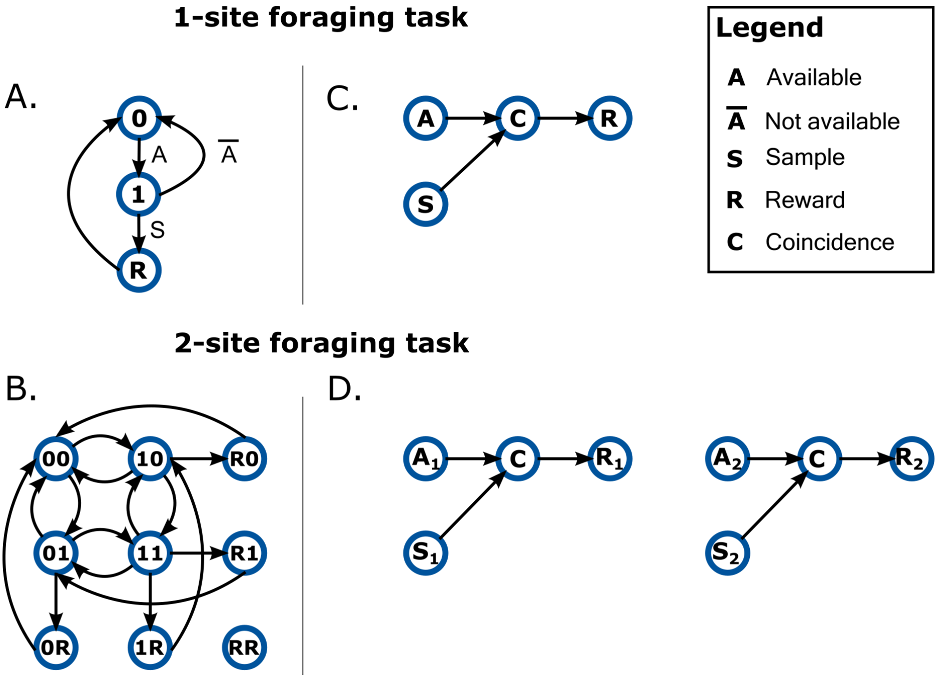


Figure 2.9. Describing the behaviour of dynamic environments using either state-machines or dataflows.

formalism the machine can only be in one state at a time, which means we now need to model each state as the combination of the individual independent states at each reward location. Furthermore, because transitions between these states are asynchronous and independent, we thus have edges between nearly every pair of nodes, as each reward site can change its state at any point in time relative to the other.

How would designing such a scenario feel like in a reactive programming language? Figure 2.9C shows a possible specification of the 1-site foraging task in reactive terms. In this case, we have two sources of events from the environment: one timer signaling the availability of reward (A); and a sampling event (S) which is triggered every time the animal checks the location for food. Both of these events can occur independently of each other, but when a sampling event coincides with reward availability (C), then reward (R) is delivered. Because this description is intrinsically asynchronous and parallel, it makes it extremely easy to scale the task to a larger set of locations: just replicate the dataflow for each of the other locations (Figure 2.9D). In this example, the design space was made more intuitive by introducing the parallel and asynchronous nature of a real-world situation into our modeling formalism.

Another difficulty of the classical state machine formalism is dealing with continuous variables. The natural environment provides constant real-time feedback that tightly correlates with the actions of an animal. Reproducing such closed-loop interaction and manipulating its dynamics is a necessary tool for fully investigating brain function. Such models are virtually impossible to represent in a machine of finite states, given the potential infinitude of feedback responses. However, the dataflow formalism of asynchronous event sources can easily accommodate such models. In fact, this is their natural battleground; nodes represent reactive operators that promptly respond to input values broadcasted by event sources. These models of asynchronous computation are thus ideal for recreating the complex discrete and continuous aspects of natural environments that brains evolved to master. We thus propose Bonsai as a new tool for neuroscientists trying to understand how the brain deals with real world complexity.

2.6 Acknowledgements

We thank João Bártolo Gomes for suggesting the name Bonsai; Danbee Kim for early discussions on creating virtual environments for rodents; Joana Nogueira, George Dimitriadis and all the members of the Intelligent Systems Laboratory for helpful discussions and comments on the manuscript. We also thank all the members of the Champalimaud Neuroscience

Programme who used Bonsai to setup their data analysis and acquisition experiments and in so doing provided valuable feedback to improve the framework. The research leading to these results has received funding from the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement no. 600925 and the Bial Foundation (Grant 190/12). GL is supported by the PhD Studentship SFRH/BD/51714/2011 from the Foundation for Science and Technology. The Champalimaud Neuroscience Programme is supported by the Champalimaud Foundation.

2.7 Author contributions

Conceived and designed the modular box: GL, ARK; Conceived and developed the Bonsai framework: GL; Technical advisory board: NB, JF; Conceived and developed the experimental applications: GL, NB, JF, JPN, BVA, SS, LM, SM, PMI, PAC, REM, LC, ED, JJP, ARK; Performed and analyzed experiments: GL, JF, JPN, BVA, SS, LM, SM, PMI, PAC, REM, LC, ED; Wrote the Bonsai manuscript: GL, ARK.

References

Bainomugisha, E., Carreton, A. L., Cutsem, T. V., Mostinckx, S. & Meuter, W. D. (2013). A survey on reactive programming. *ACM Computing Surveys*, *45*(4), Article No. 52. doi:10.1145/2501654.2501666. arXiv: 1005.3014

Banzi, M., Cuartielles, D., Igoe, T., Martino, G. & Mellis, D. (2014). Arduino.

Cook, S., Jones, G., Kent, S. & Wills, A. C. (2007). *Domain Specific Development with Visual Studio DSL Tools*. Addison-Wesley Professional.

Davidson, T. J., Kloosterman, F. & Wilson, M. A. (2009). Hippocampal Replay of Extended Experience. *Neuron*, *63*(4), 497–507. doi:10.1016/j.neuron.2009.07.027

Elliott, C., Vijayakumar, V., Zink, W. & Hansen, R. (2007). National Instruments LabVIEW: A Programming Environment for Laboratory Automation and Measurement. *Journal of Laboratory Automation*, *12*(1), 17–24. doi:10.1016/j.jala.2006.07.012

Foster, D. J. & Wilson, M. a. (2006). Reverse replay of behavioural sequences in hippocampal place cells during the awake state. *Nature*, *440*(7084), 680–3. doi:10.1038/nature04587

Garrido-Jurado, S., Munõz-Salinas, R., Madrid-Cuevas, F. J. & Marín-Jiménez, M. J. (2014). Automatic generation and detection of highly reliable fiducial markers under occlusion. *Pattern Recognition*, *47*(6), 2280–2292.

Gomez-Marin, A., Paton, J. J., Kampff, A. R., Costa, R. M. & Mainen, Z. F. (2014). Big behavioral data: psychology, ethology and the foundations of neuroscience. *Nature Neuroscience*, *17*(11), 1455–1462. doi:10.1038/nn.3812

Gouvêa, T. S., Monteiro, T., Soares, S., Atallah, B. V. & Paton, J. J. (2014). Ongoing behavior predicts perceptual report of interval duration. *Frontiers in neurorobotics*, *8*(March), 10. doi:10.3389/fnbot.2014.00010

Han, J. (2005). Low-cost multi-touch sensing through frustrated total internal reflection. *Proceedings of the 18th annual ACM symposium on User interface software and technology (UIST '05)*, 115–118.

IronPython Community. (2014). IronPython.

Itseez. (2014). OpenCV (Open Source Computer Vision).

Itskov, P. M., Moreira, J.-M., Vinnik, E., Lopes, G., Safarik, S., Dickinson, M. H. & Ribeiro, C. (2014). Automated monitoring and quantitative analysis of feeding behaviour in Drosophila. *Nature Communications*, *5*, 4560. doi:10.1038/ncomms5560

Johnston, W. M., Hanna, J. R. P. & Millar, R. J. (2004). Advances in dataflow programming languages. *ACM Computing Surveys*, *36*(1), 1–34. doi:10.1145/1013208.1013209

Kato, H. & Billinghurst, M. (1999). Marker tracking and hmd calibration for a video-based augmented reality conferencing system. In *Augmented reality, 1999.(iwar'99) proceedings. 2nd ieee and acm international workshop on* (pp. 85–94). IEEE.

Kumura, T., Nakamura, Y., Ishiura, N., Takeuchi, Y. & Imai, M. (2012). Model Based Parallelization from the Simulink Models and Their Sequential C Code. In *Proc. of the 17th workshop on synthesis and system integration of mixed information technologies (sasimi 2012)* (pp. 186–191).

Lopes, G., Bonacchi, N., Frazão, J., Neto, J., Atallah, B., Soares, S., Moreira, L., Matias, S., Itskov, P., Correia, P., Medina, R., Calcaterra, L., Dreosti, E., Paton, J. & Kampff, A. (2015). Bonsai: An event-based framework for processing and controlling data streams. *Frontiers in Neuroinformatics*, *9*(7). doi:10.3389/fninf.2015.00007

MathWorks. (2014). Simulink.

Microsoft Open Technologies. (2014). Reactive Extensions for the .NET framework (Rx).

Mosconi, M. & Porta, M. (2000). Iteration constructs in data-flow visual programming languages. *Computer Languages*, *26*(2000), 67–104.

National Instruments. (2014). LabVIEW.

O'Keefe, J. & Dostrovsky, J. (1971). The hippocampus as a spatial map: Preliminary evidence from unit activity in the freely moving rat. *Brain research*, *34*(1), 171–175.

Sellers, K. K., Bennett, D. V., Hutt, A., Williams, J. H. & Fröhlich, F. (2015). Awake vs. anesthetized: layer-specific sensory processing in visual cortex and functional connectivity between cortical areas. *Journal of Neurophysiology*, *113*(10), 3798–3815. doi:10.1152/jn.00923.2014

Tecuapetla, F., Matias, S., Dugue, G. P., Mainen, Z. F. & Costa, R. M. (2014). Balanced activity in basal ganglia projection pathways is critical for contraversive movements. *Nature communications*, *5*, 4315. doi:10.1038/ncomms5315

Voigts, J., Siegle, J. H., Kemere, C., Moore, C. & Wilson, M. (2013). A low-cost, open-source system for combining high-channel count electrophysiology with closed-loop optogenetic feedback. In *Society for neuroscience (san diego, ca, 13 november)*.

Wright, M., Freed, A. & Momeni, A. (2003). Open Sound Control: State of the Art 2003. In *Proceedings of the 2003 conference on new interfaces for musical expression (nime-03)* (pp. 153–159). Montreal, Canada.

Chapter 3

Moving with and without Motor Cortex

> *I first became sceptical of the supposed path of the conditioned reflex when I found that rats, trained in a differential reaction to light, showed no reduction in accuracy of performance when almost the entire motor cortex, along with the frontal poles of the brain, was removed.*
>
> KARL S. LASHLEY, *In Search of the Engram* (1950)

3.1 Chapter Summary

The role of motor cortex in the direct control of movement remains unclear, particularly in non-primate mammals. More than a century of research using stimulation, anatomical and electrophysiological studies has implicated neural activity in this region with all kinds of movement. However, following the removal of motor cortex, or even the entire cortex, rats retain the ability to execute a surprisingly large range of adaptive behaviours, including previously learned skilled movements. In this chapter we revisit these two conflicting views of motor cortical control by asking what the primordial role of motor cortex is in non-primate mammals, and how it can be effectively assayed. In order to motivate the discussion we present a new assay of behaviour in the rat, challenging animals to produce robust responses to unexpected and unpredictable situations while navigating a dynamic obstacle course. Surprisingly, we found that rats with motor cortical lesions show clear impairments in dealing with an unexpected collapse of the obstacles, while showing virtually no impairment with repeated trials in many other motor and cognitive metrics of performance. Finally, we present the results of a preliminary investigation on the neurobiological basis of robust responses using electrocorticography in freely moving rodents and report the existence of large amplitude evoked potentials in the rat motor cortex following exposure to occasionally unstable obstacles.

The behaviour and lesion experiments discussed in this chapter have been prepared for submission and are currently stored as the following preprint: Lopes, G., Nogueira, J., Paton, J. J. & Kampff, A. R. (2016). A robust role for motor cortex. *bioRxiv*, 058917. doi:10.1101/058917

Electrocorticography experiments were made possible thanks to vital collaboration from G. Dimitriadis, who introduced the technique and performed all implantation surgeries. J. Menendez provided critical assistance in behaviour classification and preliminary analysis of physiology data.

3.2 Introduction

In the natural world, an animal must be able to adapt locomotion to any surface, not only in anticipation of upcoming terrain, but also in response to the unexpected perturbations that often occur during movement. This allows animals to move robustly through the world, even when navigating a changing environment. Testing the ability of the motor system to generate a robust response to an unexpected change can be difficult as it requires introducing a perturbation without cueing the animal about the altered state of the world. Marple-Horvat and colleagues built a circular ladder assay for cats that was specifically designed to record from motor cortex during such conditions (Marple-Horvat, Amos, Armstrong & J.M., 1993). One of the modifications they introduced was to make one of the rungs of the ladder fall unexpectedly under the weight of the animal. When they recorded from motor cortical neurons during the rung drop, they noticed a marked increase in activity, well above the recorded baseline from normal stepping, as the animal recovered from the fall and resumed walking. However, whether this increased activity of motor cortex was necessary for the recovery response has never been assayed.

Some remarks on lesion techniques

The original methods used to induce a permanent lesion to the motor cortex were very crude, often involving gross mechanical aggression to the neural tissue by using surgical knife cuts or ablation by water-jet, aspiration, and thermo- or electrocoagulation. These methods are still widely used in lesion studies for their simplicity and bluntness, but have the disadvantage of making it hard to limit the lesion to a single area because of possible damage to subcortical areas or the destruction of fibers of passage. Fibers of passage are nerve fibers passing through the lesioned area which neither originate nor terminate in the region of interest. These limitations made it more difficult to interpret the effects of cortical lesions, and eventually led to the development of new techniques designed to work around such problems. Chemical injections of neurotoxic compounds such as ibotenic acid or kainic acid aim to increase selectivity of the lesion by limiting damage to neural cell bodies in the target area while leaving the fibers of passage intact (Schwarcz et al., 1979). Photothrombosis (Watson, Dietrich, Busto, Wachtel & Ginsberg, 1985) or devascularization by pial stripping (Meyer & Meyer, 1971) aim to reproduce the effects of clinical stroke while avoiding extension of the lesion to subcortical areas as much as possible.

The early studies of Broca localizing the function of articulate language to a specific region in the cerebral hemispheres (Broca, 1861) established a long tradition of correlating the location of surgical brain injury with detailed analysis of any subsequent behavioural deficits. This method is not without its difficulties. The problems of plasticity and diaschisis will forever complicate conclusions based on injury and manipulation of nervous tissue (Lashley, 1933). Many recent methods for reversible chemical or optogenetic inactivation of the cortex have been proposed to improve statistical power of behavioural assessments (DeFeudis, 1980; Dong, Allen, Farrell & Roth, 2010; Guo et al., 2015). Unfortunately, given that the cortex maintains a tight balance of excitation and inhibition during normal functioning and is also densely interconnected with the rest of the brain, the effects of such transient manipulations are prone to cause multiple downstream effects that can confound inferences about behavioural relevance (Otchy et al., 2015). In this respect, they are similar to stimulation experiments in that they are very useful in determining that two areas are connected in a circuit, but not necessarily what the connection means. Of course, permanent lesions themselves can induce plasticity changes in the function of downstream and upstream circuits. The expectation, however, is that such changes represent a homeostatically stable state of the system, allowing simultaneous investigation of the limits of recovery, as well as the kinds of problems for which a fully intact structure is definitely required.

3.3 Methods

All experiments were approved by the Champalimaud Foundation Bioethics Committee and the Portuguese National Authority for Animal Health, Direcção-Geral de Alimentação e Veterinária (DGAV).

Lesions: Ibotenic acid was injected bilaterally in 11 Long-Evans rats (ages from 83 to 141 days; 9 females, 2 males), at 3 injection sites with 2 depths per site ($-1.5\,\mathrm{mm}$ and $-0.75\,\mathrm{mm}$ from the surface of the brain). At each depth we injected a total amount of 82.8 nL using a microinjector (Drummond Nanoject II, 9.2 nL per injection, 9 injections per depth). The coordinates for each site, in mm with respect to Bregma, were: +1.0 AP / 2.0 ML; +1.0 AP / 4.0 ML; +3.0 AP / 2.0 ML, following the protocol reported by Kawai et al. for targeting forelimb motor cortex (Kawai et al., 2015). Five other animals were used as sham controls (age-matched controls; 3 females, 2 males), subject to the same intervention, but where ibotenic acid was replaced with physiological saline. Six additional animals were used as wildtype, no-surgery, controls (age-matched controls; 6 females).

For the frontal cortex aspiration lesions, the margins of the craniotomy were extended to cover from -2.0 to +5.0 mm AP relative to Bregma and laterally from 0.5 mm up to the temporal ridge of the skull. After removal of the skull, the exposed dura was cut and removed, and the underlying tissue aspirated to a depth of 2 to 3 mm with a fine pipette (Whishaw, 2000). For the frontoparietal cortical lesions, the craniotomy extended from -6.0 to +4.0 mm AP relative to Bregma and laterally from 0.5 mm up to the temporal ridge. Two of these animals underwent aspiration lesions as described above. In the remaining animal, the lesion was induced by pial stripping in order to further restrict the damage to cortical areas. After removal of the dura, the underlying pia, arachnoid and vasculature were wiped with a sterile cotton swab until no vasculature was visible (Farr & Whishaw, 2002).

Recovery period: After the surgeries, animals were given a minimum of one week (up to two weeks) recovery period in isolation. After this period, animals were handled every day for a week, after which they were paired again with their age-matched control to allow for social interaction during the remainder of the recovery period. In total, all animals were allowed at least one full month of recovery before they were first exposed to the behaviour assay.

The three largest frontoparietal lesioned animals were originally prepared for a study of behaviour in a dynamic visual foraging task, which they were exposed to for one month in addition to the recovery period described above. This task did not, however, require any challenging motor behaviours besides locomotion over a completely flat surface. This period was also used to monitor the overall health condition of the animals and to facilitate sensorimotor recovery as much as possible. The animal with the largest lesion (Extended Lesion F) was prevented from completing the behaviour protocol due to deteriorating health conditions following the first two days of testing.

Histology: All animals were perfused intracardially with 4% paraformaldehyde in phosphate buffer saline (PBS) and brains were post-fixed for at least 24 h in the same fixative. Serial coronal sections (100 µm) were Nissl-stained and imaged for identification of lesion boundaries. In two of the largest frontoparietal lesions (Extended Lesions D and E), serial sections were taken sagittally.

In order to reconstruct lesion volumes, the images of coronal sections were aligned and the outlines of both brain and lesions were manually traced in Fiji (Schindelin et al., 2012) and stored as two-dimensional regions of interest. Lesion volumes were calculated by summing the area of each region of interest multiplied by the thickness of each slice. The stored regions were also used to reconstruct a 3D polygon mesh for visualization of lesion boundaries.

Electrocorticography: Recording of electrophysiological signals from the intact rodent cortex was performed using two high-density 64-channel micro-electrocorticography (micro-ECoG) grids using the method reported by Dimitriadis et al. for freely moving animals (Dimitriadis, Fransen & Maris, 2014). The particular grids used in these experiments were fabricated at the International Iberian Nanotechnology Laboratory by depositing microelectrode gold contacts through a custom designed layout mask on a flexible thin-film polyimide substrate (Figure 3.15A). The soft connectors at the end of each grid are inserted during the implantation surgery into a custom made breakout board in the recording chamber, which exposes groups of 32-channels via Omnetics connectors to the recording amplifier (see data acquisition section).

The microelectrode grids were implanted epidurally into the right hemisphere of three male Long-Evans rats at almost two years of age. The margins of the craniotomy for implantation extended from -3.3 to +5.0 mm AP relative to Bregma and laterally from 1.5 to 4.0 mm. The anterior grid was first placed carefully on top of the brain, and then slowly inserted below the anterior and medial margins of the craniotomy until the first rows of electrodes were fully covered. The second grid was placed posterior to the first one and inserted below the medial margin of the craniotomy, taking care that the first rows of electrodes were kept equidistant from the last row of electrodes in the anterior grid. Two zirconium hooks were inserted in the anterior and posterior margins of the craniotomy and fixed to the recording chamber in order to hold it firmly in place relative to the skull. With the aid of a micromanipulator and video feedback system, the coordinates of different electrodes in each quadrant of both grids were measured relative to Bregma, and later used to reconstruct the precise placement of all grid electrodes in the brain. At the end of the surgery, a titanium screw was inserted posteriorly to the craniotomy in contact with the brain in order to be used as reference for the recording system. The stability of the implant depends critically on the absence of movement in the bony plates of the skull during development, which can compromise the mechanical fixation of the recording chamber to the head (Dimitriadis et al., 2014). For this reason, it is recommended that rats undergoing this procedure should be older than 7 months (Dimitriadis et al., 2014).

Behaviour assay: During each session the animal was placed inside a behaviour box for 30 min, where it could collect water rewards by shuttling back and forth between two nose pokes (Island Motion Corporation, USA). To do this, animals had to cross a 48 cm obstacle course composed of eight 2 cm aluminium steps spaced by 4 cm (Figure 3.1). The structure of the assay and each step in the obstacle course was built out of aluminium structural framing (Bosch Rexroth, DE, 20 mm series). The walls of the arena were fabricated with a laser-cutter from 5 mm thick opaque black acrylic and fixed to the structural framing. A transparent acrylic window partition was positioned in front of the obstacle course in order to provide a clear view of the animal. All experiments were run in the dark by having the behavioural apparatus enclosed in a light tight box.

A motorized brake allowed us to lock or release each step in the obstacle course (Figure 3.2). The shaft of each of the obstacles was coupled to an acrylic piece used to control the rotational stability of each step. In order to lock a step in a fixed position, two servo motors are actuated to press against the acrylic piece and hold it in place. Two other acrylic pieces were used as stops to ensure a maximum rotation angle of approximately +/- 100°. Two small nuts were attached to the bottom of each step to work as a counterweight that gives the obstacles a tendency to return to their original flat configuration. In order to ensure that noise from servo

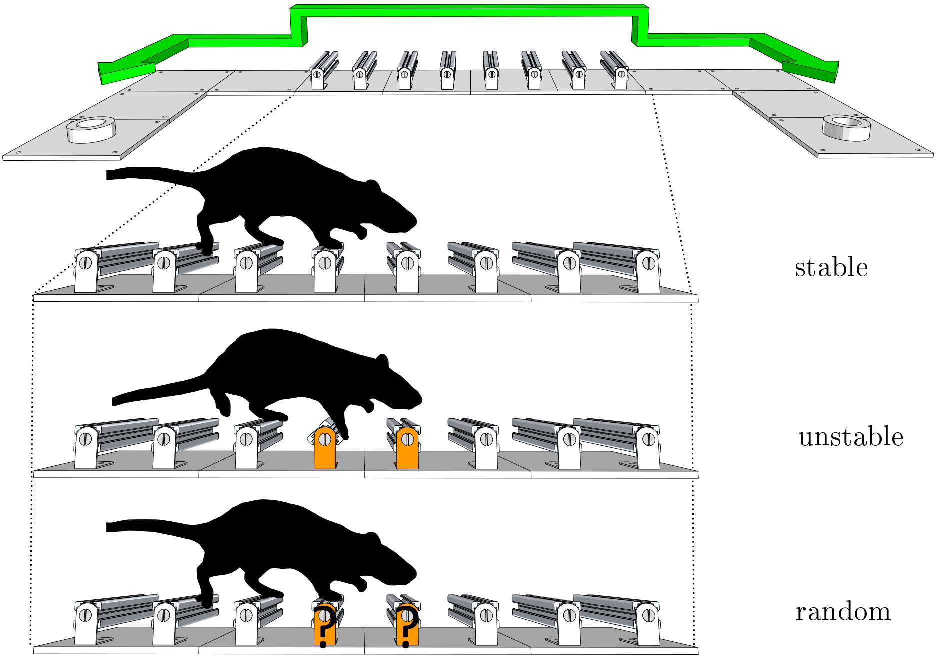


Figure 3.1. Schematic of the apparatus and summary of the different conditions in the shuttling protocol.

motor actuation could not be used as a cue to tell the animal about the state of each step, the motors were always set to press against an acrylic piece, either the piece that keeps the step stabilized, or the acrylic stops. At the beginning of each trial, the motors were run through a randomized sequence of positions in order to mask information about state transitions and also to ensure the steps were reset to their original configuration. Control of the motors was done using a Motoruino board (Artica, PT) along with a custom workflow written in the Bonsai visual programming language (Lopes et al., 2015).

Prior to the micro-ECoG recordings, each step in the obstacle course was outfitted with a micro load cell (CZL616C, Phidgets, CA) secured between the step front holder and the base (Figure 3.2). This allowed us

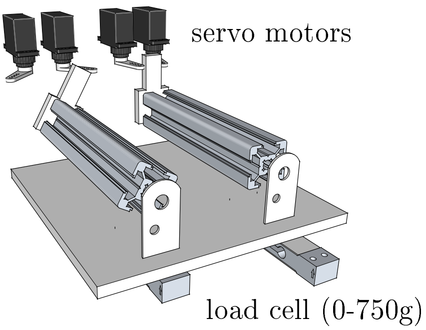


Figure 3.2. Schematic of the step locking mechanism and load cell sensor.

to record a varying voltage signal proportional to the load applied by the animal on each step. This load signal was acquired simultaneously on all eight steps and digitized synchronously with the ECoG data acquisition system.

Data acquisition: The behaviour of the animals was recorded with a high-speed and high-resolution videography system (1280x680 @ 120 Hz) using an infrared camera (Flea3, PointGrey, CA), super-bright infrared LED front lights (SMD5050, 850 nm) and a vari-focal lens (Fujinon, JP) positioned in front of the transparent window partition. A top view of the assay was simultaneously recorded with the same system at a lower frame-rate (30 Hz) for monitoring purposes. All video data was encoded with MPEG-4 compression for subsequent offline analysis. Behaviour data acquisition for the nose poke beam breaks was done using an Arduino board (Uno, Arduino, USA) and streamed to the computer via USB. All video and sensor data acquisition was recorded in parallel using the same Bonsai workflow used to control the behaviour assay.

For the micro-ECoG recordings, all electrophysiological signals were amplified, digitized and multiplexed using two 64-channel amplifier boards (RHD2164, Intan Technologies, US) connected to the electrode interface board (EIB) on the recording chamber. The amplifier boards were then connected through a dual headstage adapter (C3440, Intan Technologies, US) to the main data acquisition USB interface board (RHD2000-Eval, Intan Technologies, US). In order to facilitate the free movement of the animal in the behaviour box, the single cable connecting the head of the animal to the USB interface board was passed through a slip ring (MMC235, Moflon, CN) and hooked into a nylon string crossing the top of the assay. In this way, movement and rotation of the tethered animal were compensated to avoid unwanted strain and twisting on the cables during the entire recording period.

In order to synchronize the videography and ECoG recording systems, we connected the strobe output of the camera to a digital input in the Intan USB interface board using a GPIO cable (ACC-01-3000, PointGrey, CA). The camera strobe output is electronically coupled to individual frame exposures (i.e. shutter opening and closing events), and can be used for sub-millisecond readout of individual frame acquisition times. The strobe signal was acquired and digitized synchronously with ECoG data acquisition, and used for *post-hoc* reconstruction of precise frame timing. Data acquisition from the USB interface board was recorded using a Bonsai workflow and care was taken that it was always started first and terminated last in order to ensure that no external synchronization events were lost.

Behaviour protocol: The animals were kept in a state of water deprivation for 20 h prior to each daily session. For every trial, rats were delivered a 20 µL drop of water. At the end of each day, they were given free access to water for 10 min before initiating the next deprivation period. Sessions lasted for six days of the week from Monday to Saturday, with a day of free access to water on Sunday. Before the start of the water deprivation protocol, animals were run on a single habituation session where they were placed in the box for a period of 15 min.

The following sequence of conditions were presented to the animals over the course of a month (see also Figure 3.1): day 0, habituation to the box; day 1-4, all the steps were fixed in a stable configuration; day 5, 20 trials of the stable configuration, after which the two centre steps were made unstable (i.e. free to rotate); day 6-10, the centre two steps remained unstable; day 11, 20 trials of the unstable configuration, after which the two centre steps were again fixed in a stable state; day 12, all the steps were fixed in a stable configuration; day 13-16, the state of the centre two steps was randomized on a trial-by-trial basis to be either stable or unstable. Following the end of the random protocol, animals continued to be tested in the assay for a variable number of days (up to one week) in different conditions. At the end of the testing period, all animals were exposed to a final session where all steps were made free to rotate in order to assay locomotion performance under challenging conditions.

For the micro-ECoG recordings, the basic behaviour protocol was adjusted to allow for extra recording time during conditions of interest. First, all session times were doubled for the recordings (e.g. 30 min for the habituation session, and 60 min for all other sessions). Second, the number of days on each condition was also extended to allow extracting more trials from each animal for analysis. Finally, the condition where the centre two steps were reliably unstable was replaced with a condition of rare instability. In this condition, after the animal is exposed to an unstable configuration, the steps are reverted back to being stable for another 20 trials, after which they become again unstable for one trial, and so on.

Data analysis: All scripts and custom code used for data analysis are available online[1]. The raw video data was first pre-processed using a custom Bonsai workflow in order to extract features of interest (Figure 3.3). Tracking of the nose was achieved by background subtraction and connected component labelling of segmented image elements. First we

---

[1] https://bitbucket.org/kampff-lab/shuttling-analysis

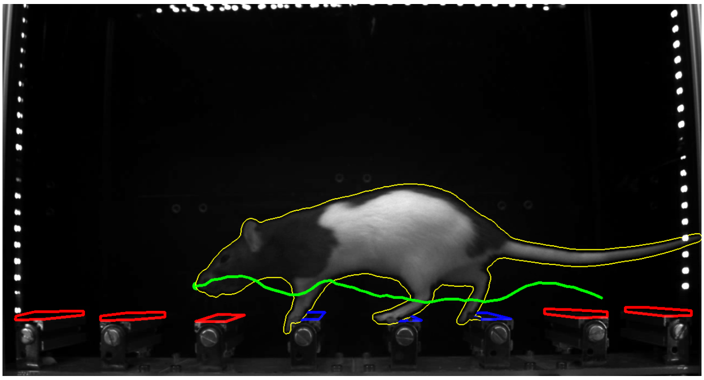


Figure 3.3. Example video frame from the behaviour tracking system. Coloured overlays represent regions of interest and feature traces extracted automatically from the video.

compute the ellipse best-fit to the largest object in the image. We then mark the tip of the nose as the furthermost point, in the segmented shape of the animal, along the major axis of the ellipse. In order to analyse stepping performance, regions of interest were defined around the surface of each step and in the gaps between the steps. Background subtracted activity over these regions was recorded for every frame for subsequent detection and classification of steps and slips.

Analysis routines were run using the NumPy scientific computing package (van der Walt, Colbert & Varoquaux, 2011) and the Pandas data analysis library (McKinney, 2010) for the Python programming language. Crossings were automatically extracted from the nose trajectory data by first detecting consecutive time points where the nose was positively identified in the video. In order for these periods to be successfully marked as crossings, the starting position of the nose must be located on the opposite side of the ending position. Inside each crossing, the moment of stepping with the forelimb on the centre steps was extracted by looking at the first peak above a threshold in the first derivative of the activation signal in the corresponding region of interest. False positive classifications due to hindlimb or tail activations were eliminated by enforcing the constraint that the position of the head must be located before the next step. Visual confirmation of the classified timepoints showed that spurious activations were all but eliminated by this procedure as stepping with the hindlimb or tail requires the head to be further ahead in space unless the animal turned around (in which case the trajectory would not be marked as a crossing anyway). The position of the nose at the moment of each step was extracted and found to be normally distributed, so statistical analysis of the step posture in the random condition used an unpaired t-test to check for independence of different measurement groups.

In order to evaluate the dynamics of crossing in the random condition, we first measured for every trial the speed at which the animals were moving on each spatial segment of the assay. To minimize overall trial-by-trial variation in individual animal performance, we used the average speed at which the animal approached the manipulated step as a baseline and subtracted it from the speed at each individual segment. To summarize differences in performance between stable and unstable trials, we then computed the average speed profile for each condition, and then subtracted the average speed profile for unstable trials from the average speed profile for stable trials. Finally, we computed the sum of all these speed differences at every segment in order to obtain the speedup index for each animal, i.e. an index of whether the animal tends to accelerate or decelerate across the assay on stable versus unstable trials.

For the micro-ECoG experiments, evoked potentials were analysed by splitting the raw physiological voltage traces into 750 ms windows, where time zero was aligned to the moment of stepping with the forelimb on one of the obstacles in the course (see below). Each individual time series was low-pass filtered at 50 Hz (4th order Butterworth filter, two-pass) and baselined by subtracting the average of the first 250 ms before event onset in order to compensate for constant voltage shifts between the two grids. Some of the channels in each grid were entirely excluded from the analysis due to potentially damaged surface contacts, as evidenced by wide amplitude, random oscillatory behaviour, which was often matched by the presence of high impedance measurements extracted from the electrode site in vivo. In one of the sessions, the cable connecting the headstage to the interface board was accidentally removed by the animal, and all the trials falling during this period had to be excluded from analysis. Correspondence between individual ECoG samples and video frames was computed by matching the individual hardware frame counter with the sequence of falling edges detected in the shutter strobe signal acquired from the infrared camera.

Video classification: Classification of paw placement faults (i.e. slips) was performed in semi-automated fashion. First, possible slip timepoints were detected automatically using the peak detection method outlined above. All constraints on head position were relaxed for this analysis in order to exclude the possibility of false negatives. A human classifier then proceeded to manually go through each of the slip candidates and inspect the video around that timepoint in order to assess whether the activation peak was a genuine paw placement fault. Examples of false positives include tail and head activations as well as paw activations that occur while the animal is actively engaged in exploration, rearing, or other activities that are unrelated to crossing the obstacles.

A similar technique was used to detect and classify the event onsets for the analysis of evoked potentials in the micro-ECoG experiments. In this case, a preliminary classification of each video frame into left and right forelimb was achieved by first computing the brightness histogram of each frame, which was used to encode the image as a lower-dimensional vector. The vectors for all step frames were subsequently clustered using K-means and then manually inspected for label correction.

Classification of behaviour responses following first exposure to the unstable condition was done on a frame-by-frame analysis of the high-speed video aligned on first contact with the manipulated step. The frame of first contact was defined as the first frame in which there is noticeable movement of the step caused by animal contact. Three main categories of behaviour were observed to follow the first contact: compensation, investigation and halting. Behaviour sequences were first classified as belonging to one of these categories and their onsets and offsets determined by the following criteria. Compensation behaviour is defined by a rapid and adaptive postural correction to the locomotion pattern in response to the perturbation. Onset of this behaviour is defined by the first frame in which there is visible rapid contraction of the body musculature following first contact. Investigation behaviour consists of periods of targeted interaction with the steps, often involving manipulation of the freely moving obstacle with the forepaws. The onset of this behaviour is defined by the animal orienting its head down to one of the manipulated steps, followed by subsequent interaction. Halting behaviour is characterized by a period in which the animal stops its ongoing motor program, and maintains the same body posture for several seconds, without switching to a new behaviour or orienting specifically to the manipulated steps. This behaviour is distinct from a freezing response, as occasional movements of the head are seen. Onset of this behaviour is defined by the moment where locomotion and other motor activities besides movement of the head come to a stop. A human classifier blind to the lesion condition was given descriptions of each of these three main categories of behaviour and asked to note onsets and offsets of each behaviour throughout the videos. These classifications provide a visual summary of the first response videos; the complete dataset used for this classification is included as supplementary movies.

3.4 Results

To investigate whether the intact motor cortex is required for the robust control of movement in response to unexpected perturbations, we designed a reconfigurable dynamic obstacle course where individual steps can be made stable or unstable on a trial-by-trial basis (Figure 3.1, also see Methods). In this assay, rats shuttle back and forth across the obstacles, in the dark, in order to collect water rewards. We specifically designed the assay such that modifications to the physics of the obstacles could be made covertly. In this way, the animal has no explicit information about the state of the steps until it actually contacts them. Water deprived animals were trained daily for 4 weeks, throughout which they encountered increasingly challenging states of the obstacle course. Our goal was to characterize precisely the conditions under which motor cortex becomes necessary for the control of movement, and this motivated us to introduce an environment with graded levels of uncertainty.

We compared the performance of 22 animals: 11 with bilateral ibotenic acid lesions to the primary and secondary forelimb motor cortex, and 11 age and gender matched controls (5 sham surgery, 6 wild-types). Animals were given ample time to recover, 4 weeks post-surgery, in order to specifically isolate behaviours that are chronically impaired in animals lacking the functions enabled by motor cortical structures. Histological examination of serial coronal sections revealed significant variability in the extent of damaged areas (Figure 3.4), which was likely caused by mechanical blockage of the injection pipette during lesion induction at some sites. Nevertheless, volume reconstruction of the serial sections allowed us to accurately quantify the size of each lesion, identify each animal (from Lesion A to Lesion K; largest to smallest), and use these values to compare observed behavioural effects as a function of lesion size.

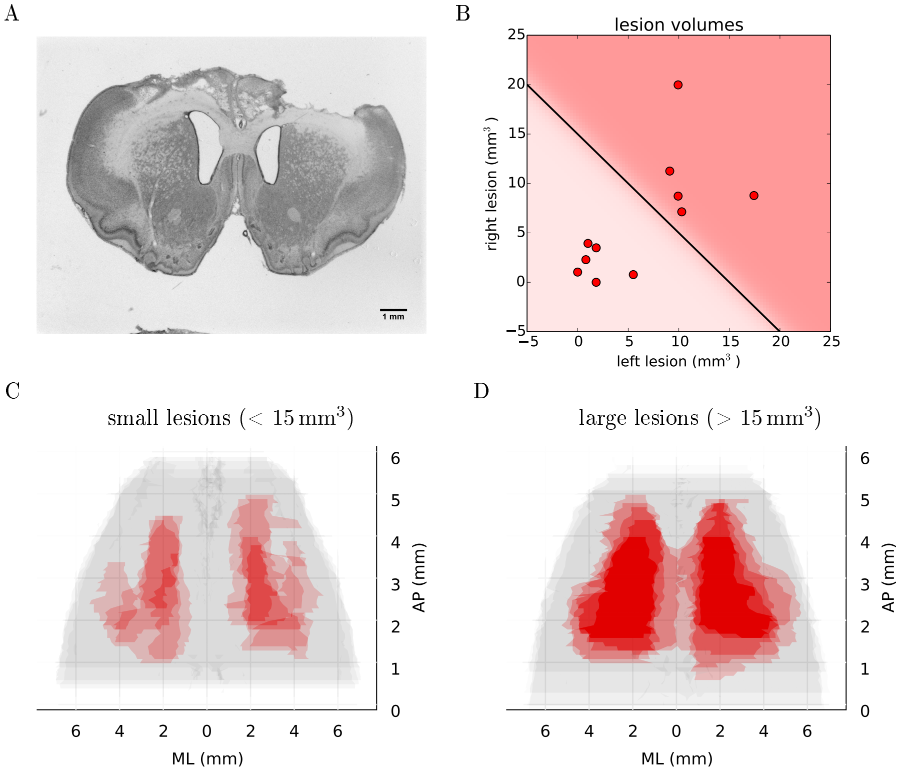


Figure 3.4. Histological analysis of lesion size. (**A**) Representative example of Nissl-stained coronal section showing bilateral ibotenic acid lesion of primary and secondary forelimb motor cortex. (**B**) Distribution of lesion volumes in the left and right hemispheres for individual animals. A lesion was considered "large" if the total lesion volume was above 15 mm^3. (**C**) Super-imposed reconstruction stacks for all the small lesions ($n = 6$). (**D**) Super-imposed reconstruction stacks for all the large lesions ($n = 5$).

During the first sessions in the “stable” environment, all animals, both lesions and controls, quickly learned to shuttle across the obstacles, achieving stable, skilled performance after a few days of training (Figure 3.5). Even though the distance between steps was fixed for all animals, the time taken to adapt the crossing strategy was similar irrespective of body size. When first encountering the obstacles, animals adopted a cautious gait, investigating the location of the subsequent obstacle with their whiskers, stepping with the leading forepaw followed by a step to the same position with the trailing paw (Video 3.1: “First Leftwards Crossing”). However, over the course of only a few trials, all animals exhibited a new strategy of “stepping over” the planted forepaw to the next obstacle, suggesting an increased confidence in their movement strategy in this novel environment (Video 3.1: “Second Leftwards Crossing”). This more confident gait developed into a coordinated locomotion sequence after a few additional training sessions (Video 3.1: “Later Crossing”). The development of the ability to move confidently and quickly over the obstacle course was observed in both lesion and control animals (Video 3.2).

In addition to the excitotoxic lesions, in three animals we performed larger frontal cortex aspiration lesions in order to determine whether the remaining trunk and hindlimb representations were necessary to navigate the elevated obstacle course. Also, in order to exclude the involvement of other corticospinal projecting regions in the parietal and rostral visual areas (Miller, 1987), we included three additional animals which underwent even more extensive cortical lesion procedures (Figure 3.6, see Methods). These *extended* lesion animals were identified following chronological order (from Extended Lesion A to Extended Lesion F; where the first three animals correspond to frontal cortex aspiration lesions and the remaining animals to the more extensive frontoparietal lesions). In these extended cortical lesions, recovery was found to be overall slower than in lesions lim-

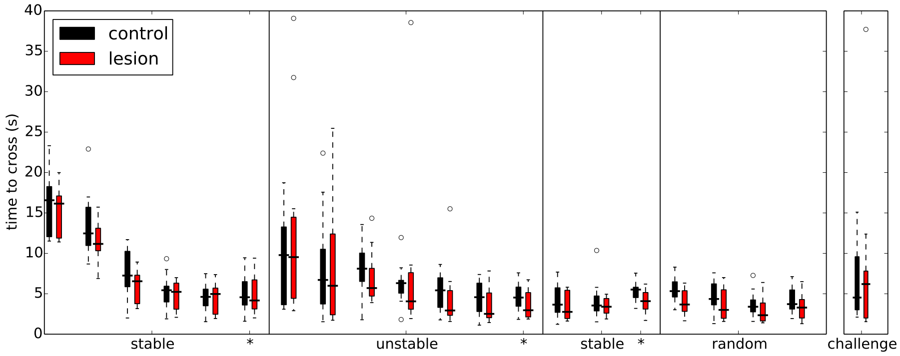


Figure 3.5. Overall performance on the obstacle course is similar for both lesion ($n = 11$) and control animals ($n = 11$) across the different protocol stages. Each set of coloured bars represents the distribution of average time to cross the obstacles on a single session. Asterisks indicate sessions where there was a change in assay conditions during the session (see text). In these transition sessions, the average performance on the 20 trials immediately preceding the change is shown to the left of the solid vertical line whereas the performance on the remainder of that session (after the change) is shown to the right.

ited to the motor cortex, and animals required isolation and more extensive care during the recovery period.

Nevertheless, when tested in the shuttling assay, the basic performance of these extended lesion animals was similar to that of controls and animals with excitotoxic motor cortical lesions (Figure 3.7A). Animals with large frontoparietal lesions did exhibit a very noticeable deficit in paw placement throughout the early sessions (Figure 3.7B). Interestingly, detailed analysis of paw placement behaviour revealed that this deficit was almost entirely explained by impaired control of the hindlimbs. Paw slips were much more frequent when stepping with a hindlimb than with a forelimb (Figure 3.7C,D). In addition, when a slip did occur, these animals failed to adjust the affected paw to compensate for the fall (e.g. keeping their digits closed),

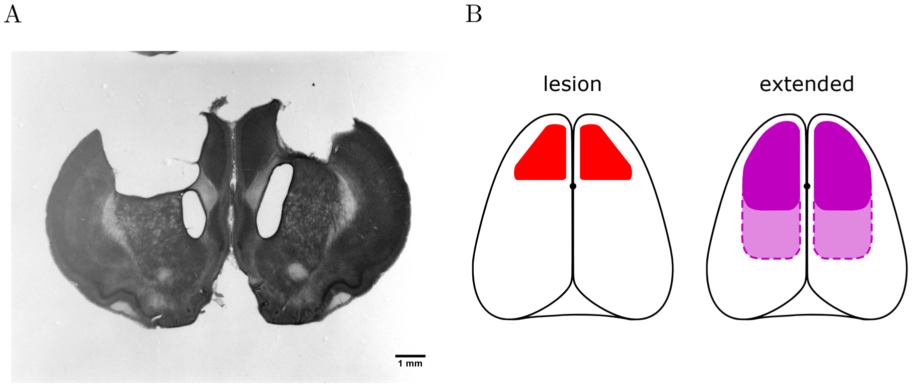


Figure 3.6. Extent of frontoparietal cortex lesions. (**A**) Representative example of Nissl-stained coronal section showing bilateral aspiration lesion of forelimb sensorimotor cortex. (**B**) Schematic depicting targeted lesion areas in the different animal groups. Left: outline of bilateral ibotenic acid lesions to the motor cortex. Right: outline of extended bilateral frontoparietal cortex lesions. Solid outline represents frontal cortex targeted lesions and dotted outline the more extensive frontoparietal lesions.

which significantly impacted their overall posture recovery. These deficits in paw placement are consistent with results from sectioning the entire pyramidal tract in cats (Liddell & Phillips, 1944), and reports in ladder walking following motor cortical lesion in rodents (Metz & Whishaw, 2002), but surprisingly we did not observe deficits in paw placement in animals with ibotenic acid lesions limited to forelimb motor cortex (Figure 3.7B). Furthermore, despite this initial impairment, animals with extended lesions were still able to improve their motor control strategy up to the point where they were moving across the obstacles as efficiently as controls and other lesioned animals (Figure 3.7A, Video 3.2). Indeed, in the largest frontoparietal lesion, which extended all the way to rostral visual cortex, recovery of a stable locomotion pattern was evident over the course of just ten repeated trials (Video 3.3). The ability of this animal to improve its motor control strategy in such a short period of time seems to indicate the presence of motor learning, not simply an increase in confidence with the new environment.

In subsequent training sessions we progressively increased the difficulty of the obstacle course, by making more steps unstable. The goal was to compare the performance of the two groups as a function of difficulty. Surprisingly, both lesion and control animals were able to improve their performance by the end of each training stage even for the most extreme condition where all steps were unstable (Figure 3.5, Video 3.4). This seems to indicate that the ability of these animals to fine-tune their motor performance in a challenging environment remained intact.

One noticeable exception was the animal with the largest ibotenic acid lesion. This animal, following exposure to the first unstable protocol, was unable to bring itself to cross the obstacle course (Video 3.5). Some other control and lesioned animals also experienced a similar form of distress following exposure to the unstable obstacles, but eventually all these animals managed to start crossing over the course of a single session. In order to test whether this was due to some kind of motor disability, we lowered the difficulty of the protocol for this one animal until it was able to cross again. Following a random permutation protocol, where any two single steps were released randomly, this animal was then able to cross a single released obstacle placed in any location of the assay. After this success, it eventually learned to cross the highest difficulty level in the assay in about the same time as all the other animals, suggesting that there was indeed no lasting motor execution or learning deficit, and that the disability must have been due to some other unknown, yet intriguing, (cognitive) factor.

Having established that the overall motor performance of these animals was similar across all conditions, we next asked whether there was any difference in the strategy used by the two groups of animals to cross the unstable obstacles. We noticed that during the first week of training, the

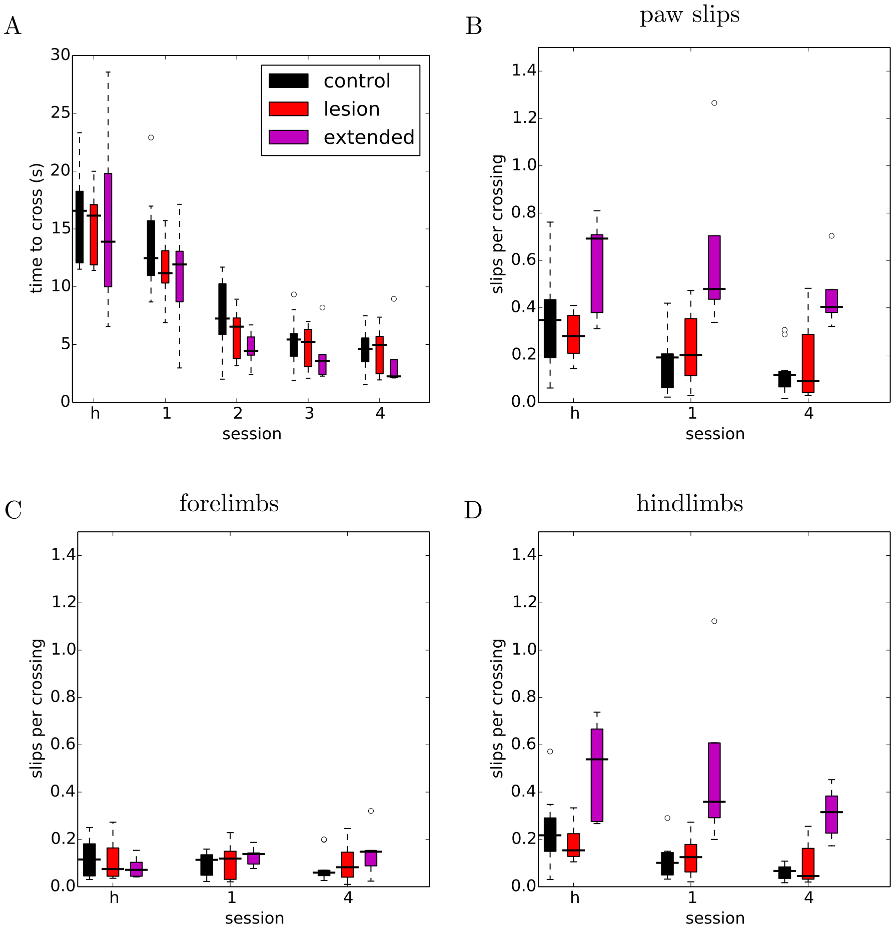


Figure 3.7. Extended frontoparietal cortex lesions perform as well as control animals despite impaired hindlimb control. (**A**) Average time required to cross the obstacles in the stable condition for extended lesions ($n = 5$). Performance of the other groups is shown for comparison. (**B**) Average number of slips per crossing in early versus late sessions of the stable condition. (**C**) Same data showing only forelimb slips. (**D**) Same data showing only hindlimb slips.

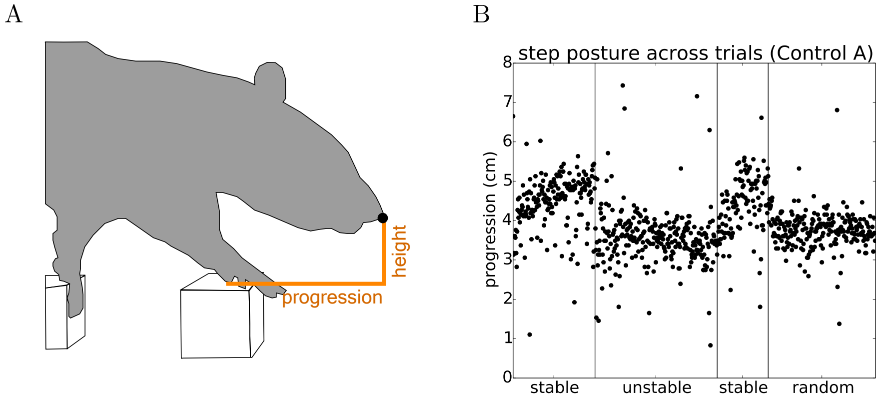


Figure 3.8. Measuring postural approach to the obstacles. (**A**) Schematic of postural analysis image processing. The position of the animal's nose is extracted whenever the paw activates the ROI of the first manipulated step (see methods). (**B**) The horizontal position, i.e. progression, of the nose in single trials for one of the control animals stepping across the different conditions of the shuttling protocol.

posture of the animals when stepping on the obstacles changed significantly over time (Figure 3.8B, 3.9A). Specifically, the centre of gravity of the body was shifted further forward and higher during later sessions, in a manner proportional to performance. However, after the obstacles changed to the unstable state, we observed an immediate and persistent adjustment of this crossing posture, with animals assuming a lower centre of gravity and reducing their speed as they approached the unstable obstacles (Figure 3.9A,B). Interestingly, we also noticed that a group of animals adopted a different strategy. Instead of lowering their centre of gravity, they either kept it unchanged or shifted it even more forward and performed a jump over the unstable obstacles (Figure 3.10A,B). These two strategies were remarkably consistent across the two groups, but there was no correlation between the strategy used and the degree of motor cortical lesion (Figure

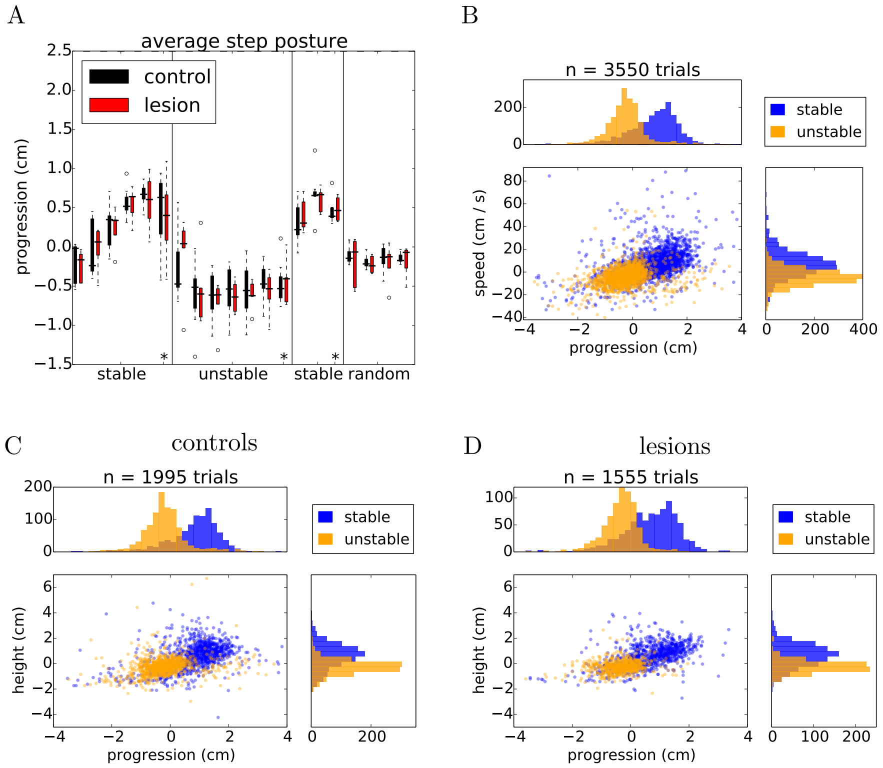


Figure 3.9. Rats adapt their postural approach to the obstacles after a change in physics. (**A**) Average horizontal position of the nose across the different protocol stages for both lesion and control animals. Asterisks indicate the average nose position on the 20 trials immediately preceding a change in protocol conditions (see text). (**B**) Distribution of horizontal position against speed for the last two days of the stable (blue) and unstable (orange) protocol stages. (**C-D**) Distribution of nose positions for control and lesion animals over the same sessions.

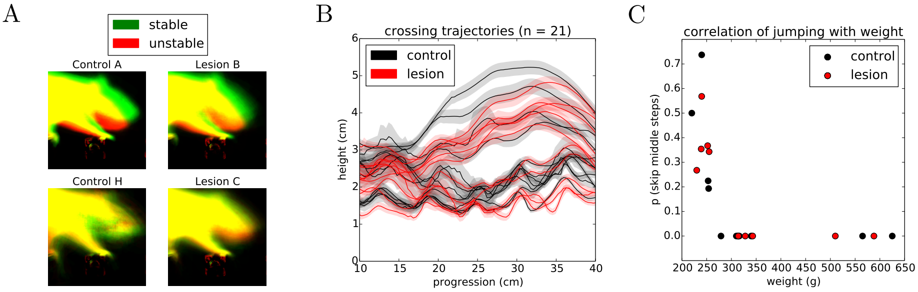


Figure 3.10. Animals use different strategies for dealing with the unstable obstacles. (**A**) Example average projection of all posture images for stable (green) and unstable (red) sessions for two non-jumper (top) and two jumper (bottom) animals. (**B**) Average nose trajectories for individual animals crossing the unstable condition. The shaded area around each line represents the 95% confidence interval. (**C**) Correlation of the probability of skipping the center two steps with the weight of the animal.

3.9C,D, 3.10C). In fact, we found that the use of a jumping strategy was best predicted by the body weight of the animal (Figure 3.10C).

During the two days where the stable state of the environment was reinstated, the posture of the animals was gradually restored to pre-manipulation levels (Figure 3.8B, 3.9A), although in many cases this adjustment happened at a slower rate than the transition from stable to unstable. Again, this postural adaptation was independent of the presence or absence of forepaw motor cortex.

We next looked in detail at the days where the state of the obstacle course was randomized on a trial-by-trial basis. This stage of the protocol is particularly interesting as it reflects a situation where the environment has a persistent degree of uncertainty. For this analysis, we were forced to exclude the animals that employed a jumping strategy, as their experience with the manipulated obstacles was the same irrespective of the state of the world. First, we repeated the same posture analysis comparing all the stable and unstable trials in the random protocol in order to control for whether there was any subtle cue in our motorized setup that the animals might be using to gain information about the current state of the world. There was no significant difference between randomly presented stable and unstable trials on the approach posture of the animal (Figure 3.11A). However, classifying the trials on the basis of past trial history revealed a significant effect on posture (Figure 3.11B). This suggested that the animals were adjusting their body posture when stepping on the affected obstacles on the basis of their current expectation about the state of the world, which is updated by the previously experienced state. Surprisingly, this effect again did not depend on the presence or absence of frontal motor cortical structures (Figure 3.11C,D).

Finally, we decided to test whether general motor performance was affected by the randomized state of the obstacles. If the animals do not know what state the world will be in, then there will be an increased challenge to their stability when they cross over the unstable obstacles, possibly demanding a quick change in strategy when they learn whether the world is stable or unstable. In order to evaluate the dynamics of crossing, we compared the speed profile of each animal across these different conditions (Figure 3.12, see Methods). Interestingly, two of the animals with the largest lesions appeared to be significantly slowed down on unstable trials, while controls and the animals with the smallest lesions instead tended to accelerate after encountering an unstable obstacle. However, the overall effect for lesions versus controls was not statistically significant (Figure 3.12C).

Nevertheless, we were intrigued by this observation and decided to investigate, in detail, the first moment in the assay when a perturbation is encountered. In the random protocol, even though the state of the world is unpredictable, the animals know that the obstacles might become unstable. However, the very first time the environment becomes unstable,

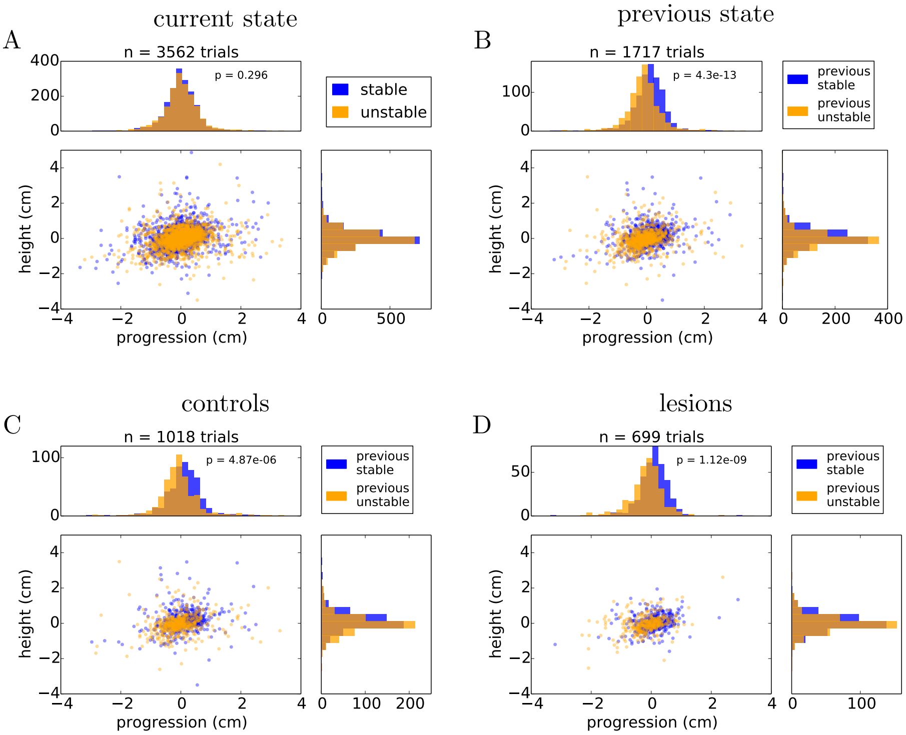


Figure 3.11. Animals adjust their posture on a trial-by-trial basis to the expected state of the world. (**A**) Distribution of nose positions on the randomized protocol when stepping on the first manipulated obstacle, for trials in which the current state was stable (blue) or unstable (orange). (**B**) Distribution of nose positions for trials in which the previous two trials were stable (blue) or unstable (orange). (**C-D**) Same data as in (**B**) split by the control and lesion groups. p values from Student's unpaired t-test are indicated.

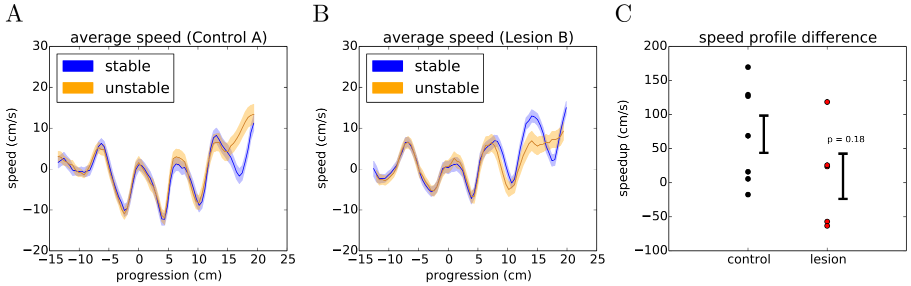


Figure 3.12. Encountering different states of the randomized obstacles causes the animals to quickly adjust their movement trajectory. (**A**) Example average speed profile across the obstacles for stable (blue) and unstable (orange) trials in the randomized sessions of a control animal (see text). The shaded area around each line represents the 95% confidence interval. (**B**) Respectively for one of the largest lesions. (**C**) Summary of the average difference between the speed profiles for stable and unstable trials across the two groups of animals. Error bars show standard error of the mean. p value from Student's unpaired t-test is indicated.

the collapse of the obstacles is completely unexpected and demands an entirely novel motor response.

A detailed analysis of the responses to the first collapse of the steps revealed a striking difference in the strategies deployed by the lesion and control animals. Upon the first encounter with the manipulated steps, we observed three types of behavioural responses from the animals (Video 3.6): investigation, in which the animals immediately stop their progression and orient towards, whisk, and physically manipulate the altered obstacle; compensation, in which the animals rapidly adjust their behaviour to negotiate the unexpected instability; and halting, in which the ongoing motor program ceases and the animals' behaviour simply comes to a stop for several seconds. Remarkably, these responses depended on the presence or absence of motor cortex (Figure 3.13). Animals with the largest motor cortical lesions, upon their first encounter with the novel environmental

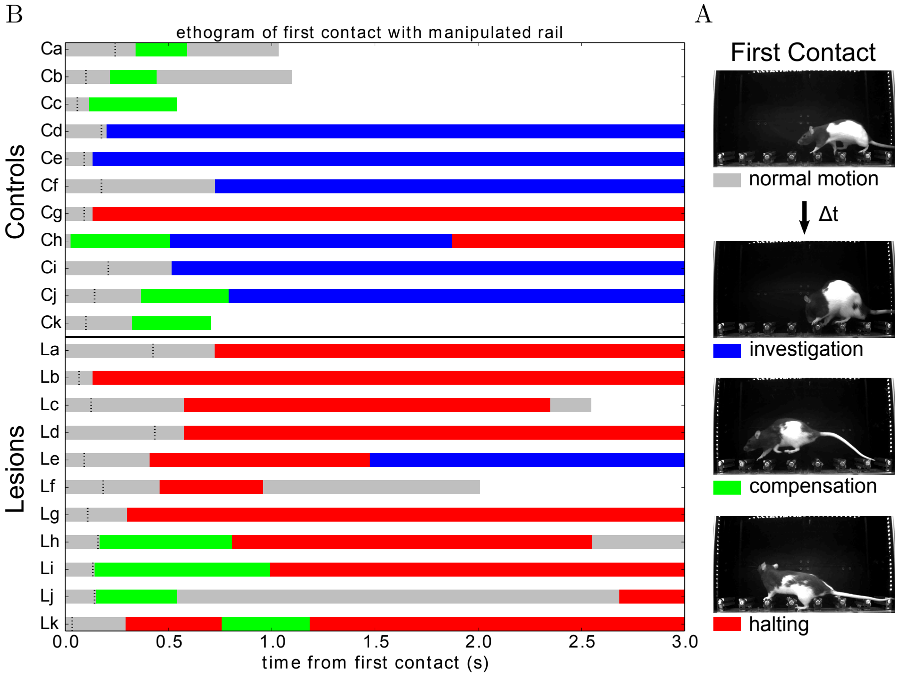


Figure 3.13. Responses to an unexpected change in the environment. (**A**) Response types observed across individuals upon first encountering an unpredicted instability in the state of the centre obstacles. (**B**) Ethogram of behavioural responses classified according to the three criteria described in (**A**) and aligned (0.0) on first contact with the newly manipulated obstacle. Black dashes indicate when the animal exhibits a pronounced ear flick. White indicates that the animal has crossed the obstacle course.

obstacle, halted for several seconds, whereas animals with an intact motor cortex, and those with the smallest lesions, were able to rapidly react with either an investigatory or compensatory response (Video 3.7,3.8).

The response of animals with extended lesions was even more striking. In two of these animals, there was a failure to recognize that a change had occurred at all (Video 3.9). Instead, they kept walking across the now unstable steps for several trials, never stopping to assess the new situation. One of them gradually noticed the manipulation and stopped his progression, while the other one only fully realized the change after inadvertently hitting the steps with its snout (Video 3.9: Extended Lesion A). This was the first time we ever observed this behaviour, as all animals with or without cortical lesions always displayed a clear switch in behavioural state following the first encounter with the manipulation. In the remaining animals with extended lesions, two of them clearly halted their progression following the collapse of the obstacles, in a way similar to the large motor cortex ibotenic lesions (Video 3.10). The third animal (Extended Lesion B) actually collapsed upon contact with the manipulated step, falling over its paw and digits awkwardly and hitting the obstacles with its snout. Shortly after this there was a switch to an exploratory behaviour state, in a way similar to Extended Lesion A.

Towards a neurobiological basis of robust responses

In order to investigate the neurophysiological correlates of these robust responses in the motor cortex, in three animals we implanted flexible surface electrode grids above the dura in one hemisphere of the intact brain (Figure 3.15A, also see Methods). Each step of the obstacle course was outfitted with a load cell sensor to measure the precise timing of contact and the amount of weight placed on each limb during locomotion. The entire electrocorticography (ECoG) system was synchronized on a frame-by-frame basis with the high-speed video acquisition so we could reconstruct the detailed behaviour of the animal at any point of the physiological trace as well as relate the continuous load profile on individual steps with different phases in the locomotion cycle (Figure 3.14).

We first asked whether there were responses in the ECoG signal over forelimb motor cortex that were modulated by stepping behaviour. Aligning the ECoG traces to the event of stepping on a permanently stable step with the contralateral paw revealed the distinct presence of an evoked po-

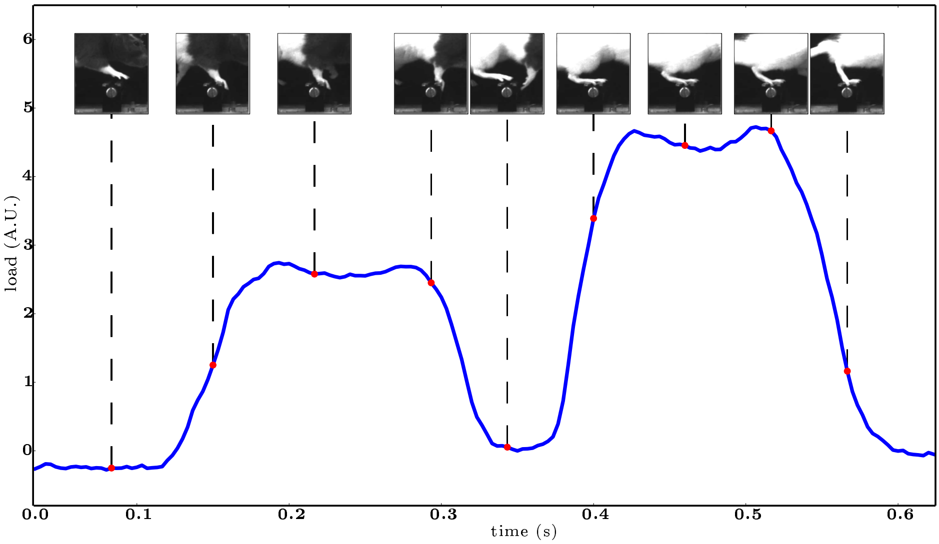


Figure 3.14. Example load cell activation profile during stepping on a rung.

tential on the anterior grid channels that was absent when stepping with the ipsilateral paw (Figure 3.15B, top trace). On close inspection, it could be seen that the beginning of the negative deflection slightly precedes the time of contact with the step, suggesting a non-sensory contribution to the evoked response. Synaptic activity in the long and thick apical dendrites of pyramidal cells are thought to be one of the main contributors to cortically recorded extracellular field potentials (Buzsáki, Anastassiou & Koch, 2012). In the cat, a sizeable proportion of pyramidal tract neurons in the motor cortex have been found to discharge rhythmically during unimpeded locomotion (Armstrong & Drew, 1984; Drew, Jiang, Kably & Lavoie, 1996), a phenomenon that is very likely to be coupled with observable synaptic activity in the potential traces and could account for the step-aligned evoked responses that we observed during locomotion of rats in the stable obstacle course.

Next, we asked whether there was any modulation of the evoked response when navigating the unstable obstacle course. In order to try and maximize the number of trials in which the encounter with the unstable step is unexpected, we adjusted the behavioural protocol at the transition between the stable and unstable test periods. This time, instead of permanently switching the centre steps to the unstable configuration, we decided to immediately revert the steps back to the stable state after the first exposure to the instability. After 20 subsequent trials in the stable state, the steps were again made unstable, and this pattern was repeated for several days.

Surprisingly, when we aligned the ECoG traces to contralateral paw steps on the manipulated obstacle in unstable trials, we observed a second evoked negativity, delayed in time relative to the previously observed stable step evoked response, and with a much larger amplitude across the channels in the anterior grid (Figure 3.15B, middle left trace). Remarkably, even in the presence of such a small number of trials, the consistency of the response in every trial provided a good enough signal-to-noise ratio for the average response to be clearly visible. Interestingly, this negativity was found to be rapidly followed by an equally large positive deflection in the potential which decayed to baseline with a much larger time constant, a response that was entirely absent from the evoked potential to stepping on a stable step. In contrast, the response to unstable steps with the ipsilateral paw did not reveal such large deflections from the baseline, although a consistent negativity could still be seen across the grid around the same time point (Figure 3.15B, middle right trace). The amplitude and timing of evoked responses when stepping with the contralateral paw on the same manipulated step in stable trials was largely identical to the condition where the step was permanently stable, and again was found to be absent when stepping with the ipsilateral paw (Figure 3.15B, bottom trace).

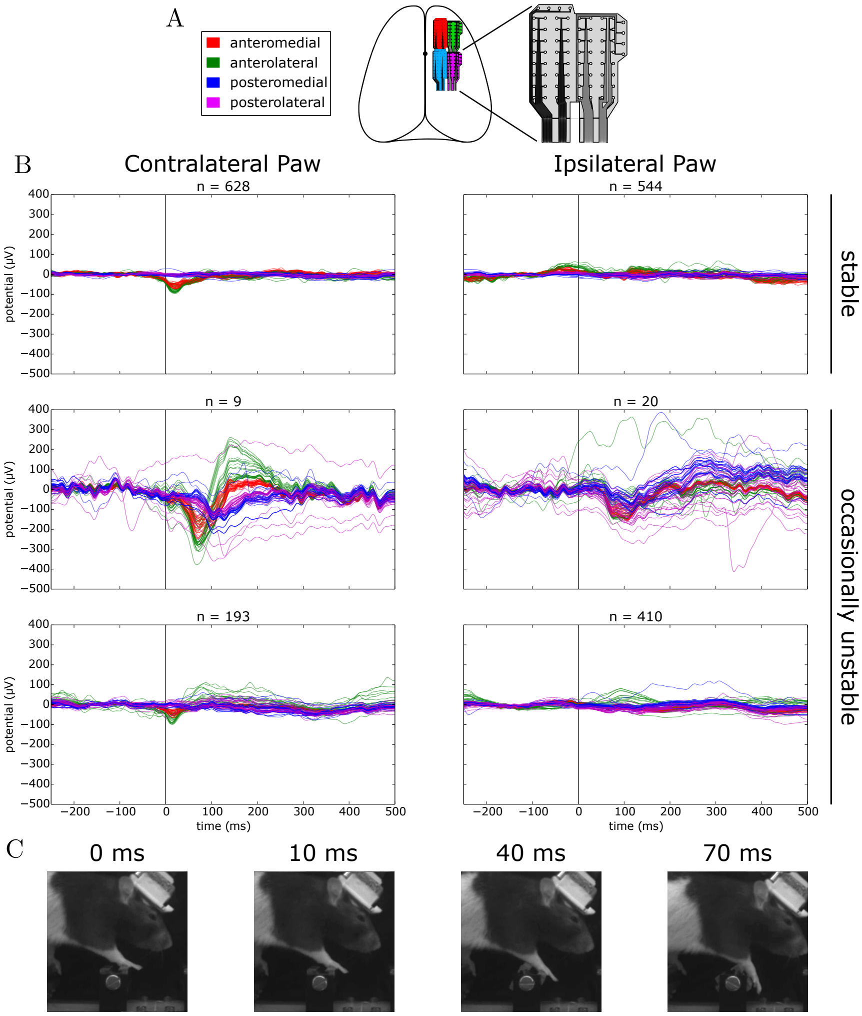


Figure 3.15. Evoked responses to stepping on stable versus occasionally unstable steps. (**A**) Schematic depicting the location of implanted ECoG grids. (**B**) Average voltage traces aligned on stepping with the contra- or ipsilateral paw on a manipulated step. Top: sessions where the step was permanently stable. Bottom: sessions where the step was occasionally made unstable. The middle row shows traces for unstable trials and the lower row the traces for the remaining stable trials. (**C**) Example frames of the behaviour of the animal at different time points of an unstable trial.

To investigate whether such a large evoked response correlated with an equally pronounced change in the overt behaviour of the animal, we extracted successive frames in the high-speed video corresponding to different time points of the trace (Figure 3.15C). Interestingly, there was no obvious motor response from the animal up to the point where the negativity peaks at around 70 ms. In fact, the affected paw was seen to mostly follow the inertia of the rotating step and no further motor response was observed before 100 ms, roughly consistent with the compensation reaction times observed in the responses to an unexpected collapse of the steps in control animals (Figure 3.13). The basic features of these evoked potential profiles were recapitulated across all the remaining animals (data not shown).

3.5 Discussion

In these experiments, we assessed the role of motor cortical structures by making targeted lesions to areas responsible for forelimb control (Kawai et al., 2015; Otchy et al., 2015). Consistent with previous studies, we did not observe any conspicuous deficits in movement execution for rats with bilateral motor cortex lesions when negotiating a stable environment. Even when exposed to a sequence of unstable obstacles, animals were able to learn an efficient strategy for crossing these more challenging environments, with or without motor cortex. These movement strategies also include a preparatory component that might reflect the state of the world an animal expected to encounter. Surprisingly, these preparatory responses also did not require the presence of motor cortex.

It was only when the environment did not conform to expectation, and demanded a rapid adjustment, that a difference between the lesion and control groups was obvious. Animals with extensive damage to the motor cortex did not deploy a change in strategy. Rather, they halted their progression for several seconds, unable to robustly respond to the new motor challenge. In an ecological setting, such hesitation could easily prove fatal. Control animals, on the other hand, were able to rapidly and flexibly reorganize their motor response to an entirely unexpected change in the environment.

Our preliminary investigations of the neurophysiological basis of these robust responses with ECoG have revealed the presence of large amplitude evoked potentials in the motor cortex arising specifically in response to an unexpected collapse of the steps during locomotion. Compared with evoked responses obtained from normal stepping under stable conditions ($-100\,\mu V$ peak at $10\,ms$), these potentials are both much larger ($-300\,\mu V$) and delayed in time (peak at $70\,ms$). Still, they preceded any overt behaviour corrections from the animal following the perturbation, as observed in the high-speed video recordings. The onset of these evoked potentials is in the range of the long-latency stretch reflex, which has been suggested to involve a transcortical loop through the motor cortex (Phillips, 1969; Matthews, Farmer & Ingram, 1990; Capaday, Forget, Fraser & Lamarre, 1991). However, the simultaneous complexity and rapidity of adaptive motor responses we observed in control animals is striking, as they appear to go beyond simple corrective responses to reach a predetermined goal and include a fast switch to entirely different investigatory or compensatory motor strategies adapted to the novel situation. What is the nature of these robust responses that animals without motor cortex seem unable to deploy? What do they allow an animal to achieve? Why are cortical structures necessary for their successful and rapid deployment?

3.6 Acknowledgements

We thank Lorenza Calcaterra for the extended frontoparietal cortical lesion preparations; João Gaspar of the International Iberian Nanotechnology Laboratory for kindly providing the fabrication process for the micro-ECoG grids; João Frazão, Pedro Lacerda and Tiago Monteiro for invaluable help in extending the behaviour assay for the micro-ECOG recordings and all the members of the Intelligent Systems Lab for constant feedback on the ideas, experiments and manuscript as well as help annotating behaviour videos. G.L. is supported by the PhD Studentship SFRH/BD/51714/2011 from the Foundation for Science and Technology. The Champalimaud Neuroscience Programme is supported by the Champalimaud Foundation.

3.7 Author contributions

Conceived and designed the lesion experiments: GL, ARK, JP; Performed the lesion experiments: GL, JN; Analysed the lesion data: GL, ARK; Conceived and designed the ECoG experiments: GL, GD, ARK, JP; Performed the ECoG implantation surgeries: GD, JN; Performed the ECoG behaviour experiments: GL; Analysed the ECoG data: GL, GD, JM, ARK. Wrote the manuscript: GL, ARK.

References

Armstrong, D. M. & Drew, T. (1984). Discharges of pyramidal tract and other motor cortical neurones during locomotion in the cat. *The Journal of Physiology*, *346*, 471–495.

Broca, P. (1861). Remarques sur le siège de la faculté du language articulé; suivies d'une observation d'aphemie. *Bulletin de la société anatomique de Paris*, *6*, 330–357.

Buzsáki, G., Anastassiou, C. A. & Koch, C. (2012). The origin of extracellular fields and currents – EEG, ECoG, LFP and spikes. *Nat Rev Neurosci*, *13*(6), 407–420. doi:10.1038/nrn3241. arXiv: NIHMS150003

Capaday, C., Forget, R., Fraser, R. & Lamarre, Y. (1991). Evidence for a contribution of the motor cortex to the long-latency stretch reflex of the human thumb. *J Physiol*, *440*, 243–255. doi:10.1113/jphysiol.1991.sp018706

DeFeudis, F. V. (1980). Physiological and behavioral studies with muscimol. *Neurochemical research*, *5*(10), 1047–1068.

Dimitriadis, G., Fransen, A. M. M. & Maris, E. (2014). Sensory and cognitive neurophysiology in rats. Part 1: Controlled tactile stimulation and micro-ECoG recordings in freely moving animals. *Journal of Neuroscience Methods*, *232*, 63–73. doi:10.1016/j.jneumeth.2014.05.001

Dong, S., Allen, J. a., Farrell, M. & Roth, B. L. (2010). A chemical-genetic approach for precise spatio-temporal control of cellular signaling. *Molecular BioSystems*, *6*(8), 1376. doi:10.1039/c002568m

Drew, T., Jiang, W., Kably, B. & Lavoie, S. (1996). Role of the motor cortex in the control of visually triggered gait modifications. *Canadian journal of physiology and pharmacology*, *74*(4), 426–42.

Farr, T. D. & Whishaw, I. Q. (2002). Quantitative and qualitative impairments in skilled reaching in the mouse (Mus musculus) after a focal motor cortex stroke. *Stroke*, *33*(7), 1869–1875. doi:10.1161/01.STR.0000020714.48349.4E

Guo, J.-Z., Graves, A. R., Guo, W. W., Zheng, J., Lee, A., Rodríguez-González, J., Li, N., Macklin, J. J., Phillips, J. W., Mensh, B. D., Branson, K. & Hantman, A. W. (2015). Cortex commands the performance of skilled movement. *eLife*, *4*(December), 160. doi:10.7554/eLife.10774. arXiv: arXiv:1011.1669v3

Kawai, R., Markman, T., Poddar, R., Ko, R., Fantana, A. L., Dhawale, A. K., Kampff, A. R. & Ölveczky, B. P. (2015). Motor Cortex Is Required for Learning but Not for Executing a Motor Skill. *Neuron*, 1–13. doi:10.1016/j.neuron.2015.03.024

Lashley, K. S. (1933). Integrative functions of the cerebral cortex. *Physiological Reviews*, *13*, 1–43.

Liddell, E. & Phillips, C. (1944). Pyramidal section in the cat. *Brain*, *67*(1), 1–9.

Lopes, G., Bonacchi, N., Frazão, J., Neto, J., Atallah, B., Soares, S., Moreira, L., Matias, S., Itskov, P., Correia, P., Medina, R., Calcaterra, L., Dreosti, E., Paton, J. & Kampff, A. (2015). Bonsai: An event-based framework for processing and controlling data streams. *Frontiers in Neuroinformatics*, *9*(7). doi:10.3389/fninf.2015.00007

Lopes, G., Nogueira, J., Paton, J. J. & Kampff, A. R. (2016). A robust role for motor cortex. *bioRxiv*, 058917. doi:10.1101/058917

Marple-Horvat, D., Amos, A., Armstrong, D. & J.M., C. (1993). Changes in the discharge patterns of cat motor cortex neurones during unexpected perturbations of on-going locomotion. *Journal of Physiology*, *462*, 87–113.

Matthews, P. B., Farmer, S. F. & Ingram, D. A. (1990). On the localization of the stretch reflex of intrinsic hand muscles in a patient with mirror movements. *The Journal of Physiology*, *428*(1), 561–577. doi:10.1113/jphysiol.1990.sp018228

McKinney, W. (2010). Data Structures for Statistical Computing in Python. *Proceedings of the 9th Python in Science Conference*, 51–56.

Metz, G. A. & Whishaw, I. Q. (2002). Cortical and subcortical lesions impair skilled walking in the ladder rung walking test: a new task to evaluate fore- and hindlimb stepping, placing, and co-ordination. *Journal of neuroscience methods*, *115*(2), 169–79.

Meyer, P. M. & Meyer, D. R. (1971). Neurosurgical procedures with special reference to aspiration lesions. In R. D. Myers (Ed.), *Methods in psychobiology, vol. i* (pp. 91–130). London: Academic Press.

Miller, M. W. (1987). The origin of corticospinal projection neurons in rat. *Exp Brain Res*, *67*(2), 339–351. doi:10.1007/BF00248554

Otchy, T. M., Wolff, S. B. E., Rhee, J. Y., Pehlevan, C., Kawai, R., Kempf, A., Gobes, S. M. H. & Ölveczky, B. P. (2015). Acute off-target effects of neural circuit manipulations. *Nature*. doi:10.1038/nature16442

Phillips, C. G. (1969). The Ferrier Lecture, 1968: Motor Apparatus of the Baboon's Hand. *Proceedings of the Royal Society B: Biological Sciences*, *173*(1031), 141–174. doi:10.1098/rspb.1969.0044

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D. J., Hartenstein, V., Eliceiri, K., Tomancak, P. & Cardona, A. (2012). Fiji: an open-source platform for biological-image analysis. *Nature methods*, *9*(7), 676–82. doi:10.1038/nmeth.2019

Schwarcz, R., Hökfelt, T., Fuxe, K., Jonsson, G., Goldstein, M. & Terenius, L. (1979). Ibotenic acid-induced neuronal degeneration: A morphological and neurochemical study. *Experimental Brain Research*, *37*(2), 199–216. doi:10.1007/BF00237708

van der Walt, S., Colbert, S. C. & Varoquaux, G. (2011). The NumPy Array: A Structure for Efficient Numerical Computation. *Computing in Science & Engineering*, *13*(2), 22–30. doi:10.1109/MCSE.2011.37

Watson, B. D., Dietrich, W. D., Busto, R., Wachtel, M. S. & Ginsberg, M. D. (1985). Induction of reproducible brain infarction by photochemically initiated thrombosis. *Annals of Neurology*, *17*(5), 497–504. doi:10.1002/ana.410170513

Whishaw, I. Q. (2000). Loss of the innate cortical engram for action patterns used in skilled reaching and the development of behavioral compensation following motor cortex lesions in the rat. *Neuropharmacology*, *39*(5), 788–805. doi:10.1016/S0028-3908(99)00259-2

Chapter 4

Extended Discussion

> *Let us take a couple of examples from physical culture and sports. Downhill skiing and slalom make high demands on a skier's dexterity. What is the difference between slalom and plain cross-country skiing, which does not require any particular dexterity? It is in a conglomerate of unexpected, unique complications in the external situation, in a quick succession of motor tasks that are all unlike each other.*
>
> NICHOLAI A. BERNSTEIN, *Dexterity and its Development* (1996)

4.1 Chapter Summary

We propose a new role for motor cortex: extending the robustness of subcortical movement systems, specifically to unexpected situations demanding rapid motor responses adapted to environmental context. The implications of this idea for current and future research are discussed.

4.2 A challenge from robotics

Is "robust control" a problem worthy of high level cortical input? Recovering from a perturbation, to maintain balance or minimize the impact of a fall, is a role normally assigned to our lower level postural control systems. The corrective responses embedded in our spinal cord (Sherrington, 1893, 1910), brainstem (Arshian et al., 2014) and midbrain (Grillner & Shik, 1973) are clearly important components of this stabilizing network, but are they sufficient to maintain robust movement in the dynamic environments that we encounter on a daily basis? Some insight into the requirements for a robust control system can be gained from engineering attempts to build robots that navigate in natural environments.

In the field of robotics, feats of precision and fine movement control (the most commonly prescribed role for motor cortex), are not a major source of difficulty. Industrial robots have long since exceeded human performance in both accuracy and execution speed (Senoo et al., 2009). More recently, using reinforcement learning methods, they are now able to automatically learn efficient movement strategies, given a human-defined goal and many repeated trials for fine-tuning (Coates, Abbeel & Ng, 2008). What then are the hard problems in robotic motor control? Why are most robots still confined to factories, i.e. controlled, predictable environments? The reason is that as soon as a robot encounters natural terrain, a vast number of previously unknown situations arise. The resulting "perturbations" are dealt with poorly by the statistical machine learning models that are currently used to train robots in controlled settings.

Let's consider a familiar example: You are up early on a Sunday morning and head outside to collect the newspaper. It is cold out, so you put on a robe and some slippers, open the front door, and descend the steps leading down to the street in front of your house. Unbeknownst to you, a thin layer of ice has formed overnight and your foot is now quickly sliding out from underneath you. You are about to fall. What do you do? Well, this depends. Is there a railing you can grab to catch yourself? Were you carrying a cup of coffee? Did you notice the frost on the lawn and step cautiously, anticipating a slippery surface? Avoiding a dangerous fall, or recovering gracefully, requires a rich knowledge of the world, knowledge that is not immediately available to spinal or even brainstem circuits. This rich context relevant for robust movement is readily available in cortex, and cortex alone.

Imagine now that you are tasked with building a robot to collect your morning newspaper. This robot, in order to avoid a catastrophic and costly failure, would need to have all of this contextual knowledge as well. It would need to know about the structure of the local environment (e.g. hand railings that can support its weight), hot liquids and their viscosities, and even the correlation of frozen dew with icy surfaces. To be a truly robust movement machine, a robot must *understand* the physical structure of the world. Moreover, it needs to understand it in about 100 ms after its "foot" starts to slip.

Reaching to stop a fall while holding a cup of coffee is not exactly the kind of feat for which we praise our athletes and sports champions, and this might explain why the difficulty of such "feats of robustness" is often overlooked. However, it would not be the first time that we find ourselves humbled by the daunting complexity of a problem that we naively assumed was "trivial". Vision, for example, has remained an impressively hard task for a machine to solve at human-level performance, yet it was originally proposed as an undergraduate summer project (Papert, 1966). Perhaps a similar misestimate has clouded our designation of the hard motor control problems worthy of cortical input.

Inspired by the challenges confronting roboticists, as well as our rodent behavioural results, we are now in a position to posit a new role for motor cortex.

4.3 A primordial role for motor cortex

We are seeking a role for motor cortex in non-primate mammals, animals that do not require this structure for overt movement production. The struggles of roboticists highlight the difficulty of building movement systems that robustly adapt to unexpected perturbations, and the results we report in this study suggest that this is, indeed, the most conspicuous deficit for rats lacking motor cortex. So let us propose that, in rodents, motor cortex is primarily responsible for extending the robustness of the subcortical movement systems. It is not required for control in stable, predictable, non-perturbing environments, but instead specifically exerts its influence when unexpected challenges arise. This, we propose, was the original selective pressure for evolving a motor cortex, and thus, its primordial role. This role persists in all mammals, mediated via a modulation of the subcortical motor system (as is emphasized in studies of cat locomotion), and has evolved in primates to include direct control of the skeletal musculature. Our proposal of a "robust" teleology for motor cortex has a number of interesting implications.

4.4 Implications for non-primate mammals

One of the most impressive traits of mammals is the vast range of environmental niches that they occupy. While most other animals adapt to change over evolutionary time scales, mammals excel in their flexibility, quickly evaluating and responding to unexpected situations, and taking risks even when faced with challenges that have never been previously encountered (Spinka, Newberry & Bekoff, 2001). This success requires more than precision, it requires resourcefulness: the ability to quickly come up with a motor solution for any situation and under any condition (Bernstein, 1996). The Russian neurophysiologist Bernstein referred to this ability with an unconventional definition of "dexterity", which he considered to be distinct from a simple harmony and precision of movements. In his words, dexterity is required only when there is 'a conglomerate of unexpected, unique complications in the external situations, [such as] in a quick succession of motor tasks that are all unlike each other' (Bernstein, 1996).

If Bernstein's "robust dexterity" is the primary role for motor cortex, then it becomes clear why the effects of lesions have thus far been so hard to characterize: assays of motor behaviour typically evaluate situations that are repeated over many trials in a stable environment. Such repeated tasks were useful, as they offer improved statistical power for quantification and comparison. However, we propose that these conditions specifically exclude the scenarios for which motor cortex originally evolved. It is not easy to repeatedly produce conditions that animals have not previously encountered, and the challenges in analysing these unique situations are considerable.

The assay reported here represents our first attempt at such an experiment, and it has already revealed that such conditions may indeed be necessary to isolate the role of motor cortex in rodents. We thus propose that neuroscience should pursue similar assays, emphasizing unexpected perturbations and novel challenges, and we have developed new hardware and software tools to make their design and implementation much easier (Chapter 2).

4.5 Implications for primate studies

In contrast to other mammals, primates require motor cortex for the direct control of movement. However, do they also retain its role in generating robust responses? The general paresis, or even paralysis, that results from motor cortical lesions in these species obscures the involvement of cortex in directing rapid responses to perturbations. Yet there is evidence that a role in robust control is still present in primates, including humans. For example, stroke patients with partial lesions to the distributed motor cortical system will often recover the ability to move the affected musculature. However, even after recovering movement, stroke patients are still prone to severe impairments in robust control: unsupported falls are one of the leading causes of injury and death in patients surviving motor cortical stroke (Jacobs, 2014). We thus suggest that stroke therapy, currently focused on regaining direct movement control, should also consider strategies for improving robust responses.

Even if we acknowledge that a primordial role of motor cortex is still apparent in primate movement control, it remains to be explained why the motor cortex of these species acquired direct control of basic movements in the first place. This is an open question.

4.6 Some speculation on the role of direct cortical control

What happens when cortex acquires direct control of movement? First, it must learn how to use this influence, bypassing or modifying lower movement controllers. While functional corticospinal tract connections may be established prenatally (Eyre, Miller, Clowry, Conway & Watts, 2000), the refinement of corticospinal dependent movements, which must override the lower motor system, takes much longer and coincides with the lengthy maturation period of corticospinal termination patterns (Lawrence & Hopkins, 1976). Humans require years of practice to produce and refine basic locomotion and grasping (Thelen, 1985; von Hofsten, 1989), motor behaviours that are available to other mammals almost immediately after birth. This may be the cost of giving cortex direct control of movement—it takes more time to figure out how to move the body—but what is the benefit?

Giving motor cortex direct control over the detailed dynamics of movement might simply have extended the range and flexibility of robust responses. This increased robustness may have been required for primates to negotiate more difficult unpredictable environments, such as the forest canopy. Direct cortical control of the musculature may have evolved because it allowed primates to avoid their less “dexterous” predators simply by ascending, and robustly negotiating, the precarious branches of tree tops. However, the consequences of this cortical “take-over” might be even more profound.

With motor cortex in more direct control of overt movements, the behaviour of a primate is a more direct reflection of cortical state: when you watch a primate move you are directly observing cortical commands. For species that live in social groups, this would allow a uniquely efficient means of communicating the state of cortex between conspecifics, a rather significant advantage for group coordination and a likely prerequisite for human language. This novel role for motor cortex—communication—might have exerted the evolutionary pressure to give cortex more and more control over basic movements, ultimately obscuring its primordial, and fundamental, role in robust control.

4.7 Some preliminary conclusions

Clearly our results are insufficient to draw any final conclusion, but that is not our main goal. We present these experiments to support and motivate our attempt to distil a long history of research, and ultimately suggest a new approach to investigating the role of motor cortex. This approach most directly applies to studies of non-primate mammals. There is now a host of techniques to monitor and manipulate cortical activity during behaviour in these species, but we propose that we should be monitoring and manipulating activity during behaviours that actually require motor cortex.

This synthesis also has implications for engineers and clinicians. We suggest that acknowledging a primary role for motor cortex in robust control, a problem still daunting to robotics engineers, can guide the development of new approaches for building intelligent machines, as well as new strategies to assess and treat patients with motor cortical damage. We concede that our results are still naïve, but propose that the implications are worthy of further consideration.

References

Arshian, M. S., Hobson, C. E., Catanzaro, M. F., Miller, D. J., Puterbaugh, S. R., Cotter, L. a., Yates, B. J. & McCall, a. a. (2014). Vestibular nucleus neurons respond to hindlimb movement in the decerebrate cat. *J Neurophysiol*, *111*(March 2014), 2423–2432. doi:10.1152/jn.00855.2013

Bernstein, N. A. (1996). *Dexterity and its Development* (M. L. Latash & M. Turvey, Eds.). L. Erlbaum Associates.

Coates, A., Abbeel, P. & Ng, A. Y. (2008). Learning for control from multiple demonstrations. *Proceedings of the 25th international conference on Machine learning - ICML '08*, 144–151. doi:10.1145/1390156.1390175

Eyre, J. A., Miller, S., Clowry, G. J., Conway, E. A. & Watts, C. (2000). Functional corticospinal projections are established prenatally in the human foetus permitting involvement in the development of spinal motor centres. *Brain : a journal of neurology*, *123 (Pt 1*, 51–64. doi:10.1093/brain/123.1.51

Grillner, S. & Shik, M. L. (1973). On the descending control of the lumbosacral spinal cord from the "mesencephalic locomotor region". *Acta Physiol Scand. 87*(0001-6772), 320–333.

Jacobs, J. V. (2014). Why we need to better understand the cortical neurophysiology of impaired postural responses with age, disease, or injury. *Frontiers in integrative neuroscience*, *8*(August), 69. doi:10.3389/fnint.2014.00069

Lawrence, D. G. & Hopkins, D. A. (1976). The development of motor control in the rhesus monkey: evidence concerning the role of corticomotoneuronal connections. *Brain : a journal of neurology*, *99*(2), 235–54. doi:10.1093/brain/99.2.235

Papert, S. (1966). The summer vision project.

Senoo, T., Yamakawa, Y., Mizusawa, S., Namiki, A., Ishikawa, M. & Shimojo, M. (2009). Skillful manipulation based on high-speed sensory-motor fusion. *2009 IEEE International Conference on Robotics and Automation*, 1611–1612. doi:10.1109/ROBOT.2009.5152852

Sherrington, C. S. (1893). Note on the Knee-jerk and the Correlation of Action of Antagonistic Muscles. *Proceedings of the Royal Society of London*, *52*(315-320), 556–564.

Sherrington, C. S. (1910). Flexion-reflex of the limb, crossed extension-reflex, and reflex stepping and standing. *The Journal of physiology*, *40*(1-2), 28–121. doi:10.1113/jphysiol.1910.sp001362

Spinka, M., Newberry, R. C. & Bekoff, M. (2001). Mammalian Play: Training for the Unexpected. *Source: The Quarterly Review of Biology*, *76*(2), 141–168. doi:10.1086/393866

Thelen, E. (1985). Developmental origins of motor coordination: leg movements in human infants. *Developmental psychobiology*, *18*(1), 1–22. doi:10.1002/dev.420180102

von Hofsten, C. (1989). Mastering Reaching and Grasping: The Development of Manual Skills in Infancy. *Advances in Psychology*, *61*(100), 223–258. doi:10.1016/S0166-4115(08)60023-0

ITQB-UNL | Av. da República, 2780-157 Oeiras, Portugal
Tel (+351) 214 469 100 | Fax (+351) 214 411 277

www.itqb.unl.pt