Feedback control of cerebellar learning

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2014

FEEDBACK CONTROL OF CEREBELLAR LEARNING

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LUND
UNIVERSITY

DOCTORAL DISSERTATION
by due permission of the Faculty of Medicine, Lund University, Sweden.

To be defended at Segerfalksalen on the 7th of June 2014, at 9.00 am.

Faculty opponent

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Bristol University, United Kingdom

Organization LUND UNIVERSITY	Document name: Doctoral dissertation
	Date of issue 7th of June 2014
Author(s): Anders Rasmussen	Sponsoring organizations: Swedish Research Council and the Krapperup, Söderberg and Åhlen foundations.

Title and subtitle: Feedback Control of Cerebellar Learning

Abstract

The ability to anticipate future events and to modify erroneous anticipatory actions is crucial for the survival of any organism. Both theoretical and empirical lines of evidence implicate the cerebellum in this ability. It is often suggested that the cerebellum acquires "expectations" or "internal models". However, except in a metaphorical sense, the cerebellum, which consists of a set of interconnected nerve cells, cannot contain "internal models" or "have expectations". The aim of this thesis is to untangle these metaphors by translating them back into neurophysiological cause and effect relationships. This task is approached from within the paradigm of classical conditioning, in which a subject, through repeated presentations of a conditional stimulus, followed by an unconditional stimulus, acquires a conditioned response. Importantly, the conditioned response is timed so that it anticipates the unconditioned response. Available neurophysiological evidence suggests that Purkinje cells, in the cerebellar cortex, generate the conditioned response. In addition, Purkinje cells provide negative feedback to the IO, which is a relay for the unconditional stimulus, via the nucleo-olivary pathway. Purkinje cells can therefore regulate the intensity of the signal derived from the unconditional stimulus, which, in turn, decides subsequent plasticity. Hence, as learning progresses, the IO signal will become weaker and weaker due to increasing negative feedback from Purkinje cells. Thus, in an important sense, learning induced changes in Purkinje cell activity constitute an "expectation" or "anticipation" of a future event (the unconditional stimulus), and, consistent with theoretical models, future learning depends on the accuracy of this expectation. Paper 1 in this thesis show that learned changes in Purkinje cells influences subsequent IO activity. The second paper show that, depending on the number of pulses it contains, the signal from the IO to the Purkinje cells can either cause acquisition or extinction. In the third paper we present evidence that can potentially help explain overexpectation, a behavioral phenomenon, which have for long been elusive. Collectively these papers advance our understanding of the feedback mechanisms that govern cerebellar learning and it proposes a potential solution to some long standing behavioral conundrums.

Key words: Feedback, Eyeblink Conditioning, Classical Conditioning, Inferior Olive, Nucleo-Olivary inhibition, Purkinje cells, In vivo Electrophysiology, Anticipation, Rescorla-Wagner, Motor learning,

Classification system and/or index terms (if any)

Supplementary bibliographical information	Language: English
ISSN and key title: 1652-8220	ISBN: 978-91-87651-96-0
Recipient's notes	Number of pages / Price
	Security classification

Signature _____Date _____

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Doctoral Dissertation series 2014:69
ISBN 978-91-87651-96-0
ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University
Lund 2013

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List of original papers

I. Rasmussen, A., Jirenhed, D., Wetmore, D. Z. and Hesslow, G. Changes in complex spike activity during classical conditioning. *Submitted.*

II. Rasmussen, A., Jirenhed, D., Zucca, R., Johansson, F., Svensson, P. and Hesslow, G. (2013). Number of spikes in climbing fibers determines the direction of cerebellar learning, *Journal of Neuroscience.* 33, 13436-13440.

III. Rasmussen, A., Zucca, R., Johansson, F., Jirenhed, D. and Hesslow, G. Cerebellar Purkinje cell activity during eyeblink conditioning with two conditional stimuli. *Manuscript.*

Introduction

Feedback is essential for learning

In science we do experiments to get feedback on our theories and models, which allow us to assess whether these are accurate or need to be modified or discarded. Similarly when we learn to ride a bike we require feedback from various sensory systems to determine whether our behavior achieves its purpose or not. If we were to make up theories without testing their derived predictions, or if we tried riding a bike without attending to sensory feedback, then our theories would be inaccurate and our behavior would not be adaptive. Without feedback, adaptive behaviors cannot be learned.

Learning entails the acquisition of new behavior or the modification of existing behavior. This requires a changed pattern of muscular contractions, which in turn requires a changed pattern of neuronal signaling. Typically, learning is not of a binary nature. Rather, behaviors change gradually until a desired response in a given situation is acquired, after which learning stops. Such modification necessarily involves feedback to the brain, signaling whether or not our behavior achieves its purpose.

This also holds true for cerebellum dependent eyeblink conditioning, which will be the focus of this thesis. Specifically, this thesis will examine how plastic changes in the cerebellum are subject to feedback control by the nucleo-olivary pathway, in the context of eyeblink conditioning. Theoretical work on the cerebellum has improved our understanding considerably, but too often authors stop at a rather abstract level where it is considered sufficient to say that the cerebellum ”generates a model” or ”expects” sensory outcomes. Of course, the cerebellum, which is made up of cells cannot, except metaphorically speaking, contain models, hold expectations or make predictions.

Metaphors can be a great tool for facilitating comprehension. Nevertheless, in science, it is important to be able to translate a metaphor back into the language of cause and effect. While I will not entirely refrain from the use of metaphors, it is my ambition to explain the metaphors that do come up in terms of causal chains of physiological events. How do neurons change their firing during learning? What is the nature of the feedback that prevents further changes when adaptive behavior has been attained? I will argue that some of the mentalistic concepts that are often used to explain learning, such as “predictions”, “internal models” or “expectations”, can be interpreted in terms of the physiology of the cerebellum.

Anticipating consequences

A traditional but ultimately erroneous way of conceptualizing our behavior is to say that first we perceive, then we think and then we act. This is wrong in part because it does not acknowledge the existence of automatic, unconscious brain processes (Eagleman, 2011), but also because it assumes that we rely entirely upon external sensory and motor feedback, which is necessarily noisy and delayed (Koziol et al., 2011). It would be impractical if, to assess the consequences of a certain behavior, one had to wait for feedback on every action. Indeed almost every behavior involves a complicated series of timed muscle contractions and if we were to wait for sensory feedback following every single contraction it would take a very long time to perform even the simplest of actions. For this reason, we must be able to anticipate the consequences of a certain action, prior to its execution.

This ability to anticipate feedback can and has been described within a number of different frameworks. In an early attempt to describe this feature, Kamin suggested that learning depends on the extent to which a certain outcome is surprising (Kamin, 1969). As long as outcomes match our conscious or unconscious expectations, no learning occurs. This makes intuitive sense because if all our behaviors result in the desired consequences then there would be no reason to change our behavior. Rescorla and Wagner subsequently tried to formalize these ideas, stating that changes in associative strength between two paired stimuli, depend on the existing associative strength. If, for example, stimulus A is strongly associated with stimulus B, then presenting these two stimuli together will not induce further learning. If, on the other hand, the association between these two stimuli is weak, paired presentation will induce learning. Another way of putting it is that we learn when events violate our expectations (Rescorla and Wagner, 1972). The mathematical framework they developed gave rise to further predictions, including the subsequently demonstrated overexpectation phenomenon (see below).

More recently the brain's capacity to anticipate outcomes has been described within the framework of internal models. All types of actions are preceded by neural activity. If you lift your arm, the muscle contractions are initiated by a signal from the motor cortex, which in turn is initiated by activity in premotor areas (which in turn is caused by other factors). There is convincing evidence suggesting that copies of activity in motor areas, known as efference copies, are used to generate feed-forward models that predict the sensory outcomes of a particular action (Ebner and Pasalar, 2008; Miall et al., 1998; Shadmehr et al., 2010; Wolpert et al., 1998). Using the efference copy, the brain can compute the sensations that the pending motor program will generate. The accuracy of the internal model will be assessed when the external feedback is received. The existence of such internal models would explain why we cannot tickle ourselves, because the tickling sensation only occurs when touch is unpredictable (Blakemore et al., 2000). When trying to tickle oneself we know, ahead of time, which sensations to expect (Wolpert et al., 1998). In the internal models framework, neural plasticity and in

extension behavior modification, is induced when forward models turn out to be wrong (Shadmehr et al., 2010).

One question that is often overlooked in the frameworks described so far, is the nature of a model or an expectation. What brain structures and processes constitute a model? In an attempt to make these concept more concrete, the simulation theory of cognition (Hesslow, 2012; 2002) postulates that anticipation is a result of associative activation of cortical areas. The basic idea is that because certain actions tend to give rise to a certain pattern of sensory input, the action will eventually elicit activity in the sensory cortex, through association. Such associative activity could also be elicited by activity in premotor areas, which occurs irrespective of whether the motor program is translated into actual behavior or not. This activity in the sensory cortex could, in turn, elicit activity in the premotor area, which then elicits a new round of activity in the sensory cortex, and so on. Hence the neural foundation of internal models could be associatively elicited brain activity.

All of these frameworks share the assumption that the brain is capable of anticipating outcomes of actions prior to their execution, and that learning occurs when there is a deviance between the anticipated outcome and the actual outcome. However, whichever framework is used, it is important to remember that neurons do not, in any meaningful sense, have expectations or models, or run simulations. Neurons are "merely" richly interconnected cells, firing at certain rates, influencing other cells they are in contact with. The fundamental goal of any scientific endeavor is to identify causal relationships between different variables and understanding how anticipation of future events works is no exception. When we are speaking of models or expectations we are taking one-step away from this ideal. This is sometimes necessary to aid comprehension, however, ultimately we should seek to understand how one physical event causes the next. In order to achieve this, the first step is to ask what constitutes a model or a simulation? What are we really talking about when we are saying that the brain anticipates future events?

Classical conditioning

In classical conditioning, a subject who is repeatedly presented with a neutral conditional stimulus (CS), followed by reflex eliciting unconditional stimulus (US), will eventually acquire a conditioned response (CR) to the CS. This CR, which is timed so as to anticipate the US, can be extinguished again if the CS is repeatedly presented alone, without the US. The eyeblink-conditioning paradigm has been used extensively to study the neural foundation of classical conditioning. Typically eyeblink conditioning involves the presentation of a tone that precedes an airpuff directed at the cornea of the subject. After a number of repetitions or "trials" the subject acquires a conditioned blink in

response to the tone, before the airpuff hits the cornea. Much of this research has used rabbits' nictitating membrane response, which can be readily elicited by an airpuff stimulus, but which, unlike the eyelid, shows little spontaneous activity. Like other CRs, the conditioned blink response can be extinguished again if the tone is presented alone (Kehoe, 2006; Kehoe and Macrae, 2002). Several factors influence the ease with which CS-US associations are formed. For example, CR acquisition requires that consecutive trials are separated by a minimum delay, reported to be around10 s (Kehoe and Macrae, 2002; Nordholm et al., 1991). Another requirement is that there is a delay of at least 100 ms between the CS and US onset (Salafia et al., 1980; Schneiderman and Gormezano, 1964).

It should be no surprise that it is possible to condition an animal to respond to more than one CS. For example, a subject can learn to blink in response to a tone as well as a light stimulus, as long as these are not presented at the same time. Presenting both CSs simultaneously will result in overshadowing, where conditioning occurs predominantly to one of the two CSs (Gormezano et al., 1983; Kehoe, 1982). Depending on the cricumstances, using multiple CSs can have interesting and sometimes counterintuitive consequences. For example, a subject that has acquired CRs in response to one CS cannot acquire CRs to a second CS if it is only presented together with the first CS. This phenomenon, known as Kamin blocking (Kamin, 1968; Rescorla and Wagner, 1972) is illustrated when a subject has learned to blink in response to a tone, and one then adds a light, presenting the tone and the light simultaneously, still followed by the US. Even though the light stimulus is repeatedly paired with the US, the subject will not acquire a conditioned blink response to this stimulus as long as the tone is also present. Put another way, the learned association to the first CS blocks association to the second CS.

A phenomena related to Kamin blocking is overexpectation. While overexpectation was predicted by Rescorla and Wagner (1972), the phenomenon was first demonstrated in a study by Kehoe & White (2004). When rabbits reliably expressed CRs in response to two different CSs (a tone and a light), they were presented with both stimuli concurrently, followed by the US. After a number of such repetitions the response to the two CSs were tested individually and in accordance with the predictions made by Rescorla and Wagner, the response to the two CSs had decreased. In general overexpectation occurs when two CSs, each of which elicits a CR, are presented simultaneously, followed by the US. Initially the simultaneous presentation results in a stronger CR, however the strength of the CR will gradually decrease, even though the US is still presented.

Kamin blocking and overexpectation may seem like counterintuitive phenomena. However, within Rescorla and Wagner's mathematical framework (Rescorla and Wagner, 1972), we can find satisfying theoretical explanations for both of these phenomena. To appreciate why blocking occurs, imagine that a particular CS is already maximally associated with the US. Adding a second CS will not induce further learning because the subject has already learned to "expect" the US based on the presentation of the first CS, and if expectations are not violated, no learning occurs. Similarly, overexpectation occurs

because when a subject is presented with two CSs, each of which is associated with a US, the "expectations" based on each of these CSs are summed, resulting in an overexpectation. The actual US is consequently weaker than the subject "expected" it to be and therefore the associative strength gradually weakens (Kehoe and White, 2004).

Before discussing the physiological basis of classical conditioning it is worth pointing out that a subject undergoing classical conditioning, in a sense, learns to anticipate a future event, such as the airpuff, based on a certain input, such as the tone. Consequently, studying classical conditioning could potentially help us understand the neural basis, not only of classical conditioning, but also of anticipation and anticipatory action. Indeed several researchers have proposed that anticipation is dependent on the same neural system that is responsible for classical conditioning, namely the cerebellum (Ebner and Pasalar, 2008; Herreros and Verschure, 2013; Wolpert et al., 1998).

Classical conditioning requires the cerebellum

Compelling evidence from numerous lesion and physiological studies leaves little doubt that the cerebellum plays a critical part in eyeblink conditioning (Hesslow and Yeo, 2002; McCormick and Thompson, 1984; Thompson and Steinmetz, 2009). Although few dispute this, key questions regarding the relative roles of the cerebellar cortex and the cerebellar nuclei, and regarding the molecular mechanisms responsible for the acquisition and expression of CRs, are still in need of answers (Gao et al., 2012; Kellett et al., 2010; Ohyama and Mauk, 2001). While some researchers claim that the essential memory trace is stored in the cerebellar nuclei (Thompson and Steinmetz, 2009), almost everyone agrees that the cerebellar cortex is critical for the acquisition and expression of adaptively timed CRs. Indeed there are compelling empirical as well as theoretical reasons to think that Purkinje cells, which provide the sole output from the cerebellar cortex, play an integral role in the acquisition and expression of adaptively timed CRs. On a morphological level, the unequalled size of their dendritic tree permits synaptic input from several hundred thousand parallel fibers (Eccles et al., 1967; Harvey and Napper, 1991). Moreover the climbing fibers, originating in the inferior olive (IO), form the most powerful synapse in the entire central nervous system on the Purkinje cells. If one were to design a neural circuit capable of acquiring associations between a wide range of inputs and a motor response, it would look very much like the circuit in the cerebellar cortex.

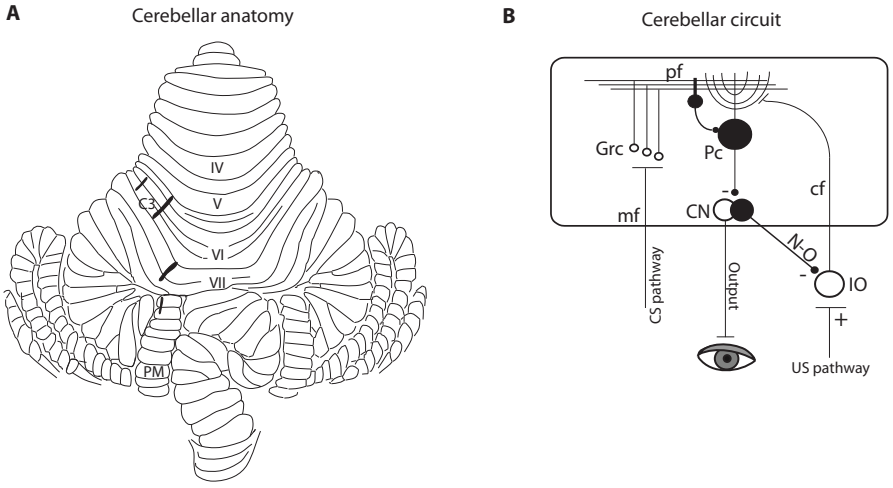

Figure 1. Localization of eyeblink areas on the cerebellar cortex and cerebellar connectivity

A. Cerebellar microzones that show eyeblink related activity. **B.** Cells and pathways in the cerebellar circuit involved in eyeblink conditioning. The CS is delivered via mossy fibers (mf), synapsing on granule cells (Grc), which contact Purkinje cells (PC) via parallel fibers (pf). The US is delivered via climbing fibers (cf), originating in the inferior olive (IO). Purkinje cells project to the cerebellar nuclei (CN), which project to motor nuclei that control eye muscles. In addition the cerebellar nuclei inhibit the inferior olive via the nucleo-olivary pathway (N-O).

There is also abundant empirical evidence showing that lesions of the cerebellar cortex either hinders acquisition of adaptively timed conditioned responses (Bracha et al., 2009; Perrett et al., 1993; Thompson and Steinmetz, 2009), or abolishes them completely (Kellett et al., 2010; Yeo et al., 1984). Although cortical lesions also affect interneurons and glial cells, Purkinje cells are the sole output neurons in the cerebellar cortex. Thus, even if conditioning relies on plastic changes in interneurons, it can only be expressed through Purkinje cells. It also seems wasteful to have the biggest dendritic tree in the entire nervous system attached to cells that only pass on a signal from neighboring interneurons.

Further evidence linking Purkinje cells to eyeblink conditioning comes from extracellular recordings during learning. Prior to training, the CS does not elicit any change in the Purkinje cell firing rate of about 40 spikes per second. However, following repeated, paired presentations of the CS and the US, Purkinje cells develop a pause response to the CS (Jirenhed et al., 2007). Since Purkinje cells are inhibitory, a pause response will disinhibit downstream brain structures, allowing them to activate motor pathways and in the end activate the relevant musculature. This link between Purkinje cell activity and muscle activity has been demonstrated using electrical stimulation (Hesslow, 1994a;

1994b), and more recently using optogenetic stimulation (Heiney et al., 2014; Witter et al., 2013), of the Purkinje cells.

Conditioned blink responses are timed so as to anticipate the US. This means that there is a delay between the CS onset and the eyeblink, so that the maximum eyelid closure occurs near the time of the US arrival (Kehoe and Macrae, 2002). Consistent with their role in eyeblink conditioning, the pause responses in Purkinje cells are also delayed, with the exact timing determined by the interstimulus interval (ISI) between CS onset and US onset (Jirenhed and Hesslow, 2011). For example, in animals trained with a 300 ms ISI, the onset of the Purkinje cell pause response is 59 ± 24 ms and the maximum suppression occurs at 180±40 ms (Jirenhed:2011gn; Jirenhed et al., 2007). Using a different ISI will result in a different time profile so that the pause response always occurs slightly before US onset (Jirenhed and Hesslow, 2011). Similarly, following training with an ISI that does not support CR acquisition, Purkinje cells do not acquire a pause response (Wetmore et al., 2014). Overall, these results demonstrate that there are striking parallels between the criteria that allow for conditioning on a behavioral level, and the conditions that allow Purkinje cells to acquire pause responses. For this reason we often refer to these conditioned pause responses in Purkinje cells, simply as "Purkinje cell CRs"

The cerebellar microcomplex

The basic unit of cerebellar function is the microcomplex. Anatomical and physiological work in the sixties by Voogd and Oscarsson and their collaborators, on the projections from the inferior olive revealed a pattern of sagittal zonation in the cerebellar cortex. Groups of olivary cells project to sagittal bands, typically 1 – 2 mm wide, of Purkinje cells, which in turn project to distinct cell groups, in the cerebellar nuclei. These zones, named A, B, C1, Cx, C2, C3, D, have specific targets in the cerebellar nuclei and are related to different functions (Ito, 1984; Oscarsson, 1979; Voogd and Glickstein, 1998). More detailed analysis of the climbing fiber projections to the C3 and B zones showed that these zones could be further subdivided into what was then termed microzones (Oscarsson, 1979). A microzone is a sagitally oriented strip of the cerebellar cortex, in which the Purkinje cells have the same climbing fiber input, that is, input driven by coupled olivary cells receiving identical peripheral inputs.

A cortical microzone projects to a distinct group of cells in a cerebellar nucleus which controls a single muscle, or a small group of muscles controlling a simple movement. Because of its intimate connections with nuclear and olivary cells, the microzone concept has been replaced by that of a microcomplex or microcircuit (Apps and Garwicz, 2005; Dean et al., 2010; Ito, 1984), which includes the nuclear and olivary cells and their connections.

An additional reason to regard the microcomplex or microcircuit as the basic functional cerebellar unit, is the fact that some microzones are functionally connected (Apps and Garwicz, 2005; Oscarsson, 1979). For instance, climbing fibers from the dorsal accessory olive branch to innervate microzones in both the C3 and C1 zones. These microzones in turn project to the same cells in the anterior interpositus nucleus. An example of this principle is the identification of, at least, four distinct areas of the cerebellar cortex that receive climbing fiber input from the periorbital area and that project to the muscles controlling the eyelid (Hesslow, 1994b; 1994a). Overall, the evidence suggests that the microcomplexes form independent units, where each microcomplex has its own olivocerebellar connections but it also seems probable that the nucleo-olivary fibers project to those olivary cells that supply the Purkinje cells controlling the corresponding nuclear cells (Andersson and Hesslow, 1987).

CS and US pathways

For Purkinje cells to play a major role in conditioning, it is necessary that they receive information about the CS and the US. In the context of eyeblink conditioning, there is a large body of evidence showing that mossy fibers from the pontine nuclei that run in the middle cerebellar peduncle, carry information about the CS (Hesslow et al., 1999; Hesslow and Yeo, 2002; Steinmetz et al., 1986; Svensson and Ivarsson, 1999), and that climbing fibers, from the IO that run in the inferior cerebellar peduncle, carry the US signal (Hesslow and Yeo, 2002; Mauk et al., 1986; Steinmetz et al., 1989).

The bulk of the mossy fibers synapse on the granule cells, of which there are about 50 billion in humans (Azevedo et al., 2009). From these granule cells, parallel fibers project up to the molecular layer of the cerebellar cortex, where they run parallel to the foliae (hence the name). The sheer number of granule cells explains how a single Purkinje cell, with its impressive dendritic tree can receive input from several hundred thousand parallel fibers (Harvey and Napper, 1991). Presumably, the parallel fibers provide each Purkinje cell with information about a huge range of events from external and internal sensory systems as well as from other brain regions.

The climbing fibers derive their name from the way they entangle the dendritic tree of the Purkinje cell. A single climbing fiber can form hundreds of synapses with a single Purkinje cell, resulting in a massive excitation of the Purkinje cell following activation of climbing fiber afferents (Eccles et al., 1966). While there is some controversy regarding the type of information that is relayed by the climbing fibers, the predominant view is that climbing fibers provide some form of “error” or “teaching” signal. This signal indicates that something significant has happened or that there was a deviation between the intended and the actual outcome of a particular action (Simpson et al., 1996). This error signal may be of a sensory or motor nature (Ito, 2013), or it may represent a

prediction error (Herreros and Verschure, 2013; Shadmehr et al., 2010). This climbing fiber teaching signal, in combination with contextual information from the mossy/parallel fibers, can induce plasticity in the cerebellar cortex that would modify future behavior in similar contexts. There is ample evidence showing that when climbing fibers and mossy/parallel fibers are activated either concurrently, or shortly after one another, the result is some form of plasticity in the cerebellar cortex (Daniel and Crepel, 2013; Jirenhed et al., 2007; Safo and Regehr, 2008).

Some findings, however, indicate that this is an oversimplification. When cats trained at a certain task performed clearly erroneous actions, it did not lead to activation of the climbing fibers (Gibson et al., 2004). This challenges the notion that climbing fibers relay an error/teaching signal. The authors do not exclude the possibility that the climbing fibers are still involved in the plasticity that causes behavior modification. However, when it comes to eyeblink conditioning, the consensus view is that mossy/parallel fibers relay information about the CS, and that the climbing fibers relay information about the US to the Purkinje cells (Hesslow and Yeo, 2002). When Purkinje cells receive input from these two afferent systems in a certain temporal order, plasticity occurs and the cell changes its firing pattern in response to further input.

Molecular mechanisms for generating pause responses

The molecular mechanisms responsible for the Purkinje cell plasticity induced during classical conditioning is still a topic of discussion. A long standing view has been that concomitant input from the parallel fibers and climbing fibers to the Purkinje cells results in long term depression of parallel-Purkinje cell synapses, and that this depression causes the observed pause responses (Aiba et al., 1994; Albus, 1971; Ito, 2001; Marr, 1969). Superficially, this might seem like a plausible explanation. However, upon closer inspection there are several problems with it. One problem is that long-term depression of an excitatory synapse cannot reduce the firing rate of the receiving cell, unless the firing rate depends on activation of that synapse. Evidence shows that an intrinsic spike generator is responsible for Purkinje cell activity, and that blocking input from parallel fibers with CNQX (an antagonist of ionotropic glutamate receptors), has no effect on Purkinje cell activity (Cerminara and Rawson, 2004). In other words, Purkinje cell activity does not rely on parallel fiber input and therefore long term depression of parallel-Purkinje cell synapses should not have any direct effects on spontaneous Purkinje cell activity.

Results from studies on genetically modified knockout mice also question whether long-term depression is the molecular mechanism responsible for the learning that occurs during eyeblink conditioning. One study from 2003 found that a strain of knockout mice, in which long term depression cannot occur at the parallel fiber-Purkinje cell

synapse, could not be conditioned (Koekkoek et al., 2003). However, mice in a subsequent study using knockouts in which long-term depression was more precisely targeted, did not show any learning deficit (Schonewille et al., 2011). In addition there are several discrepancies between the conditions that result in conditioned eyeblinks and the conditions that give rise to long term depression (Hesslow et al., 2013).

What alternative mechanisms could explain the changes that occur in Purkinje cells during eyeblink conditioning? One possibility is that motor learning is dependent on plastic changes that occur within the Purkinje cell (Steuber and Willshaw, 2004). Another possibility is that adaptively timed conditioned responses relies on plastic changes in many places throughout the cerebellar network, including changes within the Purkinje cell (Gao et al., 2012). Regardless of the molecular mechanisms responsible for their pauses, Purkinje cells appear to have the potential to associate almost any type of information, whether it is unprocessed information from sensors, or information from other brain regions, with various reflexive actions.

Feedback in cerebellar learning

The nucleo-olivary pathway

Because Purkinje cells are GABAergic, a pause in their intrinsic firing will disinhibit the cerebellar nuclei, the primary target of Purkinje cell axons. The cerebellar nuclei project to other nuclei in the brainstem that control motor output (Bengtsson and Hesslow, 2006; Berthier and Moore, 1990; Witter et al., 2013). Importantly, the cerebellar nuclei also project to the IO, which is the origin of the climbing fibers that relay the US signal. The nucleo-olivary pathway is also GABAergic (De Zeeuw et al., 1989; Nelson and Mugnaini, 1989), and if the pathway is stimulated prior to the US, then the signal reaching the cerebellar cortex will be weaker than it otherwise would have been (Bengtsson and Hesslow, 2006; Hesslow, 1986; Svensson et al., 2006). This may explain why CRs are extinguished if the US is consistently preceded by stimulation of the nucleo-olivary pathway (Bengtsson et al., 2007). This essentially blocks the US, thus robbing the cerebellar cortex of input that is necessary for learning.

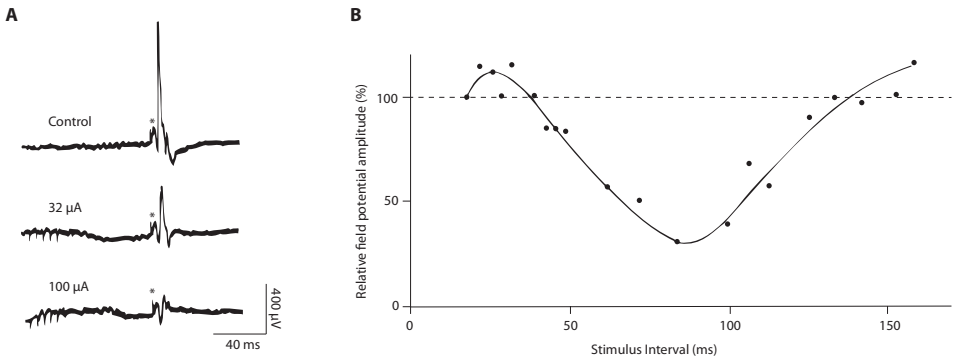

Figure 2. Stimulation of the nucleo-olivary pathway causes a suppression of perioribitally elicited field potentials on the cerebellar cortex.
A. Field potentials elicited by periorbital stimulation. The amplitude of the field potential was significantly reduced when the periorbital stimulation was preceded by stimulation of the nucleo-olivary pathway (adapted from Hesslow, 1986). **B.** The suppression of the periorbital field potential was substantially larger when the stimulation of the nucleo-olivary pathway preceded the periorbital stimulation by at least 40 ms (adapted from Hesslow, 1986).

An unusual but highly interesting feature of the nucleo-olivary pathway is the long delay between activation of the nucleo-olivary pathway and the inhibition of the IO. When the nucleo-olivary pathway is stimulated directly using electrical stimulation, the maximum inhibition of the IO occurs with a 25-75 ms delay (Svensson et al., 2006). This long delay, which appears to be caused by asynchronous GABA release onto the IO (Best and Regehr, 2009), has important implications for cerebellar learning and feedback regulation of learning. For example, because of this delay the olivary inhibition resulting from acquired Purkinje cell CRs should reach its maximum at about the same time that the US arrives at the IO. Had this delay not existed, the inhibition would arrive too early to have any effect on the US (Lepora et al., 2010). In general, the existence of the nucleo-olivary pathway means that activity in the IO will become suppressed when activity in the cerebellar nuclei increase. This is presumably the reason why Purkinje cell activity correlates with subsequent complex spike activity (Miall et al., 1998). Moreover, it can explain why conditioning leads to a suppression of the climbing fiber field potential (Apps and Lee, 2002; Hesslow and Ivarsson, 1996) and why complex spike frequency, which reflects olivary activity, go down when a Purkinje cell CR is acquired (Rasmussen et al., 2008).

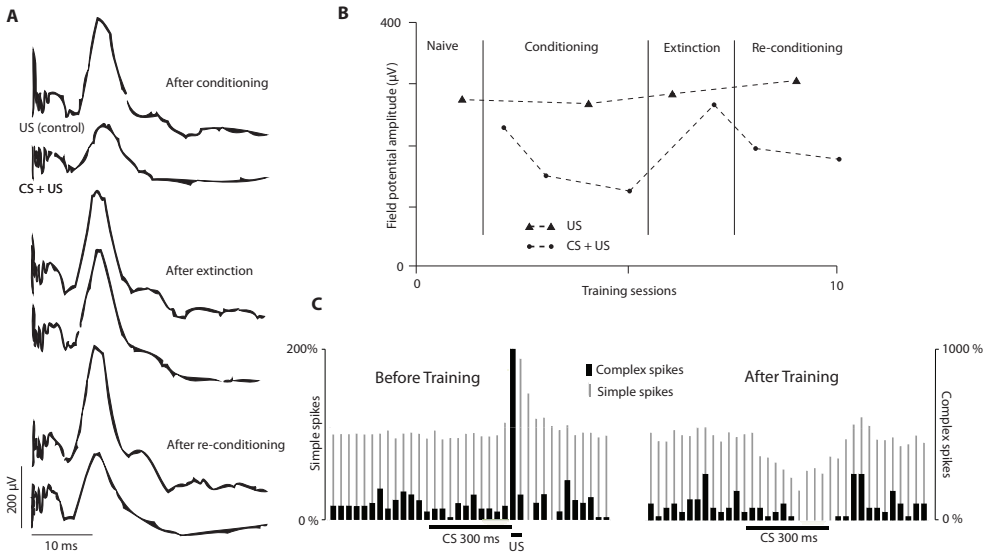

Figure 3. Conditioned responses suppress olivary activity.
A. Sample sweeps demonstrating that in after acquisition of conditioned eyeblink responses, the field potential elicited by periorbital stimulation is suppressed when preceded by the conditional stimulus (adapted from Hesslow & Ivarsson, 1996). **B.** The average amplitude of the periorbitally elicited field potential during different phases of conditioning (adapted from Hesslow & Ivarsson, 1996). **C.** Complex spike activity, which reflects olivary activity, is suppressed when Purkinje cells have acquired a conditioned pause response (adapted from Rasmussen et al. 2008).

When the IO receives input from another part of the brain it typically elicits more than one action potential in the climbing fibers. The fact that the IO fires in high frequency bursts (>250 Hz) was observed several decades ago (Armstrong and Rawson, 1979; Eccles et al., 1966). However, despite this, many researchers have overlooked the potential implications of this observation. Indeed, many researchers have implicitly or explicitly assumed that the IO fires in an "all-or-none" fashion (Ito, 2001). However, recently a handful of papers specifically addressing the burst firing nature of the IO and its functional implications, have been published (Maruta et al., 2007; Mathy et al., 2009; Najafi et al., 2014; Najafi and Medina, 2013; Rasmussen et al., 2013). These papers show that the olive fires in a graded, rather than an all-or-none, fashion, although they disagree as to why this is the case. Morevover, the evidence demonstrate that olivary signals elicited by stimulation result in more EPSPs (Maruta et al., 2007) and generate enhanced calcium signals (Najafi et al., 2014), compared to spontaneous signals.

Reaching equilibrium

So far we have shown that eyeblink conditioning results in a Purkinje cell pause response that disinhibits the cerebellar nuclei, resulting in increased inhibition of the IO via the nucleo-olivary pathway. The cerebellum appears to be organized in micro-complexes where the activity of a particular Purkinje cell provides feedback to the same cells in the IO that project to that particular Purkinje cell (Apps and Garwicz, 2005; Apps and Hawkes, 2009; Chaumont et al., 2013). Combining these two observations suggests that Purkinje cells are able to control their own IO input (Andersson et al., 1988; Bengtsson and Hesslow, 2006; Chaumont et al., 2013). As learning gives rise to stronger and more consistent pause responses, the strength of the teaching signal diminishes because of increased nucleo-olivary inhibition. This implies that an equilibrium level can be reached where the inhibition of the IO that is generated by the pause response matches the strength of the teaching signal. This idea is in many ways similar to Oscarsson's comparator hypothesis (Oscarsson, 1980).

A model with "all-or-none" complex spikes (Ito, 2001) would permit learning, assuming that US elicited complex spikes are suppressed when preceded by a CR. The direction of learning would then depend on the probability that a complex spike is elicited as a result of the US, which in turn would depend on the strength of the nucleo-olivary inhibition. For example, acquisition might occur when there is a greater than 50% probability that a US results in a climbing fiber signal, while extinction might occur when this proportion is smaller than 50%. However, all-or-none complex spikes cannot provide information about the size of an error (Herreros and Verschure, 2013; Najafi and Medina, 2013). The fact that the IO fires in bursts thus potentially enables the negative feedback from the cerebellar cortex to alter the number of pulses in the IO burst. Such a graded US signal not only allows a more fine tuned system, but is actually a criterion for some theoretical models of cerebellar function (Herreros and Verschure, 2013; Lepora et al., 2010; Najafi and Medina, 2013).

If the level of activity in the nucleo-olivary pathway can indeed alter the number of spikes in the climbing fiber signal it means that the number of pulses in the climbing fiber signal reflects the degree of learning. The number of pulses in the climbing fiber signal may in turn determine which, if any, plastic changes are triggered in the cerebellar cortex. In support of this idea, we recently demonstrated that whereas a US consisting of two sets of five climbing fiber impulses causes acquisition of Purkinje cell CRs, a US consisting of a single impulse resulted in extinction of previously acquired pause responses (Rasmussen et al., 2013).

The idea that learned pauses in Purkinje cell activity can alter the number of spikes in the climbing fiber signal, which in turn influences plastic processes in Purkinje cells, gives rise to a number of predictions, some of which remain untested. For example, given that complex spike appearance depends on the number of spikes in the climbing fiber signal (Mathy et al., 2009), the appearance of a peripherally elicited complex spikes ought to

depend on whether it is preceded by a Purkinje cell pause response. We further predict that the appearance of complex spikes changes gradually as learning progresses. Another prediction, which may be difficult to test, is that a Purkinje cell CR, when it is present, should be able to reduce the number of EPSPs generated by the US.

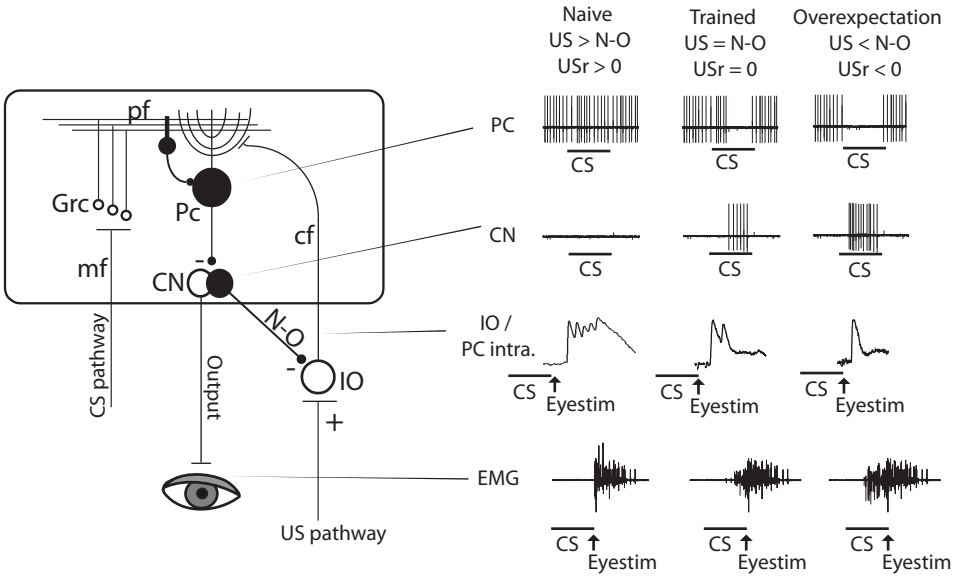

Figure 4. Feedback model
Predicted activity in Purkinje cells (PC), cerebellar nuclei (CN), Inferior olive as reflected by EPSPs in Purkinje cell dendrites (IO/PC intra.), and eyelid muscle (EMG), during different stages of conditioning (Naïve, Trained an Overexpectation). The reinforcing value of the US signal (USr) depends on the balance between the US and the nucleo-olivary inhibition (N-O). In a naïve state, the CS does not cause any change in Purkinje cell activity, and because of this, the cerebellar nuclei remain inhibited. Since there is little nucleo-olivary inhibition, eye stimulation results in a burst of EPSPs in the Purkinje cell dendrites, which drives plastic changes in the cerebellar cortex, resulting in gradually increasing nucleo-olivary inhibition. After training, Purkinje cells disinhibit the cerebellar nuclei, resulting in an EMG response as well as increased nucleo-olivary inhibition, which in turn reduces the number of EPSPs elicited by eye stimulation. The circuit has reached equilibrium when the climbing fiber input does not induce further plasticity. When two CSs (both generating CRs), are presented simultaneously, there will be a stronger pause response in the Purkinje cells. This results in more disinhibition of the cerebellar nuclei as well as a stronger overt CR (EMG activity). In addition, more nucleo-olivary inhibition suppresses the burst from the olive below the equilibrium point, driving plasticity in the opposite direction (extinction).

Classical or instrumental conditioning?

The focus of this thesis is feedback regulation of cerebellar learning, within the context of classical conditioning. This actually raises a type of a paradox because part of the definition of classical conditioning is that the subject is unable to affect the input he or she receives. If, on the other hand, the response of the subject affects the input received then learning is strictly speaking operant or instrumental (Mackintosh, 1974). For example, if a subject learns to blink in response to a tone and thereby prevents an air puff from hitting the cornea then the learning paradigm has changed from being classical to being operant. Using operant terminology one could say that the closing of the eyelid will be negatively reinforced because it helps to avoid an unpleasant stimulus.

On a behavioral level this is not normally an issue in studies on eyeblink conditioning, because animals are typically trained with electrical stimuli instead of air puffs, rendering them incapable of reducing the US intensity through eyelid closure. However, the feedback loops within the brain remain active, and from the perspective of individual Purkinje cells, which play an integral part in conditioning, the learning is still, in a certain sense, operant. As Purkinje cells acquire a Purkinje cell CR (see below), the strength of the US signal that reaches the Purkinje cell will be reduced. Whether the learning should be defined as classical or operant thus depends on which perspective you take. While the animal cannot prevent the US by blinking, the Purkinje cell can respond in a way that inhibits the signal from the eye. It might even be the case that this operant feature is a crucial part of the learning process (Lepora et al., 2010).

Back to behavior

How do these neurophysiological theories and discoveries improve our understanding of behavior? In a conditioned animal, the CS results in inhibition of the IO, the strength of which is proportional to the strength of the association between the CS and the US. Due to the unique timing properties of the nucleo-olivary pathway, GABAergic input from the cerebellar nuclei to the IO coincides with the arrival of the US (if present). Ultimately this means that the stronger the association between the CS and US is, the stronger the inhibition of the teaching signal or US will be. Based on this we can begin to explain several behavioral findings as well as make some additional predictions.

There is already evidence implicating nucleo-olivary feedback in blocking (Kim et al., 1998). As previously mentioned, blocking refers to the observation that CRs are not acquired to a particular CS if that CS has only been presented together with a different CS that already elicits CRs. A straightforward explanation of blocking is that the nucleo-olivary inhibition that the first CS generates, inhibits the IO, depriving the cerebellar cortex of the teaching signal that is necessary to form an association between the second CS and the US. Nucleo-olivary feedback can also explain the fact that reducing US

intensity following CR acquisition results in partial extinction (Kehoe and White, 2002). Reducing the strength of the teaching signal in a situation where the negative feedback matches the strength of the teaching signal would disrupt the equilibrium, triggering further plasticity, in this case extinction.

Overexpectation is another phenomenon that can be explained by a close examining of cerebellar feedback mechanisms. In overexpectation simultaneous presentation of two CSs, each of which elicits CRs, results in partial extinction (Kehoe and White, 2004; Rescorla and Wagner, 1972). Each of the two CSs elicits a Purkinje cell CR, resulting in disinhibition of the cerebellar nuclei, which in turn results in IO inhibition. Therefore simultaneous presentation of two CSs presumably results in more nuclear disinhibition and stronger IO inhibition, than any of the two CSs would cause individually. This increased inhibition disrupts the equilibrium and causes extinction in the Purkinje cells.

Feedback, anticipation and nucleo-olivary inhibition

By understanding the cerebellar feedback mechanisms that are active during learning we can begin to understand what it really means to say that the brain is anticipating future events. In essence, the Purkinje cell CR and the resulting inhibition of the IO, *is* an anticipation of the coming US signal. The suppression of the US signal, assuming it is delivered, will be proportional to the amount of learning that has taken place. If the anticipated US intensity matches the actual US intensity, there will be no further plasticity in the cerebellar cortex, and in extension, there will be no further behavior modification. In other words, the cerebellar network will be at an equilibrium level where subsequent input, given that it does not differ from prior input, will not induce further plasticity. However, if the anticipated US intensity deviates from the actual US intensity then the signal from the IO to the cortex will be above or below the equilibrium level, which will induce plasticity.

For example, suppose that, following a number of paired CS-US presentations, a subject has acquired conditioned responses. This means that Purkinje cells in the subject's cerebellar cortex pause following presentation of the CS. This pause response in turn inhibits the US signal. When this stage has been reached the system is at equilibrium, meaning that additional paired CS-US presentations will induce no further neural plasticity, nor behavior modification. Now suppose that we change the intensity of the US stimulation. In this case the inhibition of the IO will not match the intensity of the US and therefore the signal that reaches the cerebellar cortex will deviate from the equilibrium. In other words, the predicted US intensity will not be accurate.

We have now come full circle and should be able to tie everything together. Anticipation and prediction of future events is crucial for survival. Learning occurs when expectations are violated or when our predictions are erroneous, or in other words, when our anticipations fail. Since nerve cells cannot really "anticipate" or "predict" anything we must try to find the neurophysiological basis of these events. Here I hope to have shown that the Purkinje cell CR is a potential candidate for neural activity that, in an important sense, anticipates future outcomes.

Broadening the perspective

The extent to which the general principles described here apply to other parts of the brain is also an open question. With a few exceptions, the conclusions drawn here have been based on studies of eyeblink conditioning, which is thought to rely on a relatively discrete part of the cerebellum. Other parts of the cerebellum are involved in other types of learning. We know for instance that the flocculus is involved in adaptation of the vestibulo-ocular reflex (VOR) (Ito, 1998), and still other parts of the cerebellum contribute to other brain functions. It is plausible that other parts of the cerebellum also have a feedback system that shares features with the system that has been described here. However, it is possible, and perhaps even likely, that there are also differences between the feedback system that controls the acquisition of conditioned eyeblinks and the feedback systems that control other cerebellar functions.

Broadening the perspective even further we may ask how the feedback system described here relates to feedback systems regulating different types of learning that may or may not rely on the cerebellum. Take for example operant conditioning in which the frequency of a behavior can be changed through rewards or punishments. It has been shown that rewards are associated with activity in dopaminergic neurons. What is of particular interest in this context, is that the change in activity of dopaminergic neurons following a reward, is greater if the reward was unexpected, based on the history of rewards (Fiorillo et al., 2003). Indeed Schultz (2006) suggests that the activity dopamine neurons is consistent with the Rescorla Wagner model (Rescorla and Wagner, 1972), discussed in detail above.

In other words, just like the signal from the IO to the cerebellar cortex decreases as learning progresses, and the teaching signal becomes predictable, so the activity of dopamine neurons decrease as the reward becomes increasingly predictable. If the actual teaching signal and the predicted teaching signal in eyeblink conditioning is "compared" in the IO, where is the predicted reward and the actual reward compared that allow dopamine neurons to fire in the way they do? Is there a separate anatomical system filling this function or are other brain structures recruited? It is not inconceivable that dopamine

neurons, via the pathways connecting the basal ganglia and the cerebellum (Bostan and Strick, 2010), recruit the cerebellar circuitry to perform a comparison between the expected and actual reward signals. Indeed, it would be more economical if different parts of the brain shared a common neural circuitry to perform comparisons between anticipated outcomes and actual outcomes.

In conclusion, there is little doubt that the cerebellum plays an important role in various forms of learning and that there are feedback mechanisms in place to regulate this learning process. Specifically, we suggest that the Purkinje cell CR, apart from generating the overt CR, can push the intensity of the US signal above or below an equilibrium level, which, in turn, determines subsequent plasticity. This means that, in an important sense, learning induced changes in Purkinje cell activity constitute an "expectation" or "anticipation" of a future event (the unconditional stimulus), and, consistent with theoretical models, future learning depends on the accuracy of this expectation.

Methods

The methods used in the individual studies are described in detail in the publications that follow. Here I will present a brief, general, description of the methods shared in all three studies.

Animal model

All experiments were performed on decerebrated ferrets, in which brain regions that are necessary for experience of consciousness and pain are disconnected from the rest of the body. There were two primary reasons why ferrets, rather than some other species, were used for these experiments. One reason is that many other species, including mice and rats, do not cope as well with decerebration, which is preferred to various forms of anesthesia for reasons stated below. The second reason was that, with the exception of cats, there is probably no other species that we have as much knowledge about when it comes to the functional anatomy of the cerebellum. It has been shown that the cerebellum is organised in functionally distinct zones (Apps and Garwicz, 2005) (Garwicz et al., 1996), which receive input from and project to different parts of the body.

Though it should perhaps be obvious, many researchers seem to overlook the fact that when studying the cellular basis of eyeblink conditioning, it is essential to record from cells that are actually involved in eyeblinks. There is no reason to expect plastic changes in Purkinje cells that control for instance the musculature in the forelimb, following eyeblink conditioning. In the ferret physiological criteria verifying that a Purkinje cell is connected to the muscles controlling eye movements have been established (Hesslow, 1994b; 1994a). The fact that the studies presented in this thesis as well as prior studies from our laboratory are based on a functionally homogenous population of cells means that a central confounding variable have been excluded. Presumably, this also means that cells will change in a more homogenous fashion, which in extension means that we need fewer animals to ensure that changes in firing patterns are not random fluctuations. This is probably why virtually all Purkinje cells that fulfill our pre-defined criteria, develop a pause response during classical conditioning (Jirenhed et al., 2007), whereas Purkinje cells with unverified input change their activity in a inconsistent fashion (Kotani et al., 2006).

Experimental procedure

The experiments start when a male ferret (0.75-1.5 kg) is brought to the lab from a licensed breeder. Following a short period, in which the ferrets are allowed to habituate to the lab environment, they are allowed to walk into a gas chamber in which they are anesthetized with a mixture of air, oxygen and isoflurane.

After this initial anesthesia has put the ferret to sleep, a number of surgical procedures are performed. We insert tubes for administration of drugs and infusion, and we apply sensors that allow us to monitor blood pressure, expiratory CO2 and temperature. To decerebrate the ferret we first aspirate ~2/3 of the ipisilateral hemisphere, as well as ~1/3 of the contralateral hemisphere. When the colliculi have been exposed we use a blunt spatula to cut the brainstem, 1-2 mm rostral to the superior colliculi. Following decerebration, all functions associated with brain areas rostral to the red nucleus will be disabled. A major advantage associated with decerebration is that it renders the animal incapable of experiencing pain or anxiety, which in turn allows anesthesia to be discontinued. This is important because we know that anesthesia interferes with cerebellar function (Bengtsson and Jorntell, 2007), which complicates interpretation of results. Nevertheless, decerebration also disables some brain functions, which must be compensated for, in the remaining part of the experiment. For example, because the hypothalamus is aspirated/disconnected, the ferret will not be able to control its own temperature, which we must therefore maintain within physiological limits using an external thermostat and external heaters.

One of the main strengths of our setup is that it allows for very long recordings from single Purkinje cells (the current record is 21 hours), which have allowed us to observe the entire Purkinje cell CR acquisition process (Jirenhed et al., 2007). Recordings of this length require stability. Minor tissue movements, even in distal parts of the body, can propagate through the tissue and cause the cell to move, relative to the electrode, rendering the recording useless. A number of steps are taken to diminish movements and thus increase stability of the preparation. When the ferret has been decerebrated, the animal is suspended from the spine, and then a bilateral pneumothorax is performed to provide an outlet for the pressure that builds up during lung expansion. A pool made of cotton-reinforced agar is constructed on top of the ferrets head and filled with FC40 with the dual purpose of protecting and maintaining pressure on the brain surface. Following identification of the functionally relevant area of the cerebellum, we cover the cerebellar surface with a thin layer (1-2 mm) of agarose gel that further stabilizes the cerebellar surface.

Stimulation and recording

The position of stimulation electrodes depends on the type of experiment planned, and therefore differs between the three studies included in this thesis. However, as CS we used either peripheral stimulation (forelimb & whiskers), or central stimulation (pontine nuclei, superior colliculus, or mossy fiber stimulation), or both. The US consisted of electrical stimulation of the periocular region, or direct stimulation of the pathway that convey the signal from the eye to the cerebellum. For more details on electrode positioning and stimulation parameters see the included publications.

Most data presented in this thesis come from single unit, extracellular Purkinje cell recordings during various types of training protocols. Extracellular recordings were performed using 30–40 μm metal core diameter, quartz glass-coated platinum–tungsten fiber microelectrodes with an impedance ranging from 5-10 mΩ (Thomas Recording, Giessen, Germany). Upon discovery, it was verified that Purkinje cells (identified by the presence of complex spikes), were located in the blink controlling area of the C3 zone of the ipsilateral hemispheral lobule VI. After verification we proceeded to apply a stimulation protocol, the details of which depended on which type of experiment was done.

The signal from the microelectrode was fed into a pre-amplifier (NL104) + filter module (NL125), via a NL100 headstage, all from Digitimer Ltd. To remove remaining hum noise (50 Hz), the signal was passed through a humbug (Digitimer Ltd), before entering a Power 1401 analog/digital converter interface (Cambridge Electronics Design), which sampled the signal at ~30 kHz and passed it on, via a USB interface, to a PC running Spike2 v7 software. Online analysis and offline spike sorting was performed using Spike2, version 7 (Cambridge Electronics Design). All data analysis as well as statistical calculations were done using custom-made Matlab scripts (MathWorks).

Results

Purkinje cell CRs influences subsequent complex spike activity

In the first paper we examined changes in complex spike firing during classical conditioning. We reasoned that the pause response acquired by Purkinje cells during classical conditioning (Jirenhed et al., 2007), should result in disinhibition of the cerebellar nuclei which in turn should cause increased inhibition of the IO, via the nucleo-olivary pathway. Specifically, we predicted that the simple spike pause response would lead to decreased complex spike activity, which reflects IO activity. The results obtained confirm this prediction. During acquisition, when the Purkinje cell pause response develops, there was a parallel decrease in complex spike activity. We also found that the maximum complex spike suppression occurred approximately 60 ms after the maximum simple spike suppression (Jirenhed et al., 2007), which is consistent with the delay in the nucleo-olivary pathway (Svensson et al., 2006). More evidence suggesting that simple spike activity influences subsequent complex spike activity was found when we analyzed complex spike activity in cells that had been trained with short CS-US intervals (ISI). We have recently shown that, in these cells, simple spike activity increases, rather than decreases (Wetmore et al., 2014). In line with our predictions, and with the logic underlying this entire thesis, this increase in simple spike activity was associated with a corresponding increase in complex spike activity.

Burst of spikes in climbing fibers required for cerebellar learning

In the second paper we examined whether the number of spikes in the IO signal affects the acquisition of the Purkinje cell CR. This study was inspired by previous studies demonstrating that the IO does not fire in an all-or-none fashion, suggesting that perhaps the number of spikes in the signal carries important information. We knew from earlier studies that Purkinje cells readily acquire pause responses when a burst of impulses is

applied to the climbing fibers. In this study we found that replacing the burst stimulus with a single stimulus pulse resulted in extinction of the Purkinje cell pause response. Furthermore, when we subsequently switched back to a burst like stimulus, the pause response was re-acquired. This suggests that the number of spikes in the IO discharge is a critical variable in determining whether a Purkinje cell acquires a conditioned pause response or not.

Stronger pause response when two conditional stimuli are combined

In the third study we conditioned Purkinje cells to two different CSs. Once they had acquired conditioned responses to both stimuli we examined how they reacted when we presented both stimuli simultaneously. Compared to the individual CSs, simultaneous presentation of both CSs resulted in a stronger pause response in the Purkinje cell. This finding provides a plausible explanation for the overexpectation phenomenon (Kehoe and White, 2004), where combined presentation of two CSs, each of which elicits a CR, results in a gradual extinction. That is, after a number of combined presentations, the response to the individual conditional stimuli will decrease. The finding that Purkinje cells respond with a stronger pause response means that there will be more disinhibition of the cerebellar nuclei, which should lead to more inhibition of the IO. This inhibition suppresses and thereby deprives the cortex of the teaching signal, triggering extinction.

Discussion

Collectively, the three papers in this thesis advance our understanding of the feedback mechanisms that govern cerebellar learning. The first paper, which contains a detailed analysis of changes in complex spike activity during classical conditioning, shows that the activity of Purkinje cells influences subsequent complex spike activity in a bidirectional manner. The second paper presents evidence that the number of pulses in the IO discharge, which has often been a neglected variable, plays a crucial role in determining the direction of learning in Purkinje cells. The third paper shows that a single Purkinje cell can acquire pause responses to two separate CSs and that combining them results in a stronger pause response. These findings can be incorporated into a broader theory in which IO inhibition from the Purkinje cell CR adjusts the strength of the teaching signal by altering the number of spikes in the climbing fiber signal. This would allow for a fine tuned negative feedback regulation of cerebellar learning. Further empirical tests are required to test this theory, but if it turns out to be correct, it could provide an answer to some long standing behavioral paradoxes, including overexpectation.

This thesis constitutes an evolution of ideas put forth by other researchers. The idea that Purkinje cells regulate the activity of IO cells projecting back to it, and that interactions in this feedback loop are critical for motor learning, is not new and it has recently received increased attention (Andersson et al., 1988; Bengtsson and Hesslow, 2006; Chaumont et al., 2013; Herreros and Verschure, 2013; Ito, 2008; Koziol et al., 2011; Lepora et al., 2010; Schweighofer et al., 2013). Other researchers have also suggested that the teaching signal from the IO, might not be an all-or-nothing signal and that this could have important implications for cerebellar learning (Lepora et al., 2010; Najafi and Medina, 2013). However, paper two in this thesis constitutes the first empirical evidence suggesting that this is in fact the case.

Interestingly other authors, who have also noted that the IO does not fire in an all-or-none fashion, have a different explanation of what determines the number of spikes in the IO signal. Specifically, it has been argued that the number of pulses in the climbing fibers reflects subthreshold IO oscillations (de Gruijl et al., 2012; Mathy et al., 2009). According to this view an IO discharge that occurs when the subthreshold IO oscillation is in the low phase, will have a smaller number of spikes compared to a discharge that occurs in the high phase. If it is the case that the oscillations in the IO is a kind of master clock in the nervous system, that organizes movements in time (Welsh et al., 1995), then the number of spikes in the discharge could provide information about the timing of the discharge relative to the IO clock.

Taken as a whole the results presented in this thesis answers a few important questions, however, it also raises a number of new questions. What kind of information is contained in IO bursts? Is the number of pulses in the climbing fibers essentially a time marker, or is it determined by the preceeding nucleo-olivary inhibition? Even though we know that the Purkinje cell CR inhibits the IO, resulting in a reduction in complex spikes, there is yet no direct evidence that the Purkinje cell CR can cause a reduction in the number of spikes in the climbing fiber signal. Our proposed explanation for overexpectation also makes the unproved assumption that a stronger pause response in the Purkinje cell will cause a weaker teaching signal. It is thus important to ascertain whether IO discharges reflect timing relative to subthreshold oscillations in the IO, or the state of learning, or perhaps both.

A different question concerns the relationship between the number of spikes in the climbing fiber signal and cortical plasticity. What molecular mechanisms can explain the fact that applying a burst of pulses to the climbing fibers results in acquisition whereas a single pulse results in extinction? One hint may come from studies on learning in the hippocampus, which have shown that opposite forms of plasticity can be induced by slight alterations of stimulus parameters (Malenka and Bear, 2004). Perhaps the induction of plasticity in the cerebellar cortex obeys similar principles? This would be consistent with the nonlinear relationship between cerebellar plasticity and calcium release in Purkinje cells (Vogt and Canepari, 2010). The task of finding answers to these important questions, whose answers will further enlighten our understanding of the neural basis of learning and memory, fall to future research, of which I hope to be apart.

Future directions

This thesis describes how feedback shapes learning within the context of eyeblink conditioning. Because of its relative simplicity this learning paradigm provides an optimal starting point for exploring how feedback affects learning. Nevertheless, feedback control of learning is a topic that has a much broader scope and there are many questions that loom on the horizon. For instance, is it possible to optimize feedback to the brain and thereby improve learning? Perhaps future technologies can provide instantaneous feedback on movements and performance and thereby enhance learning? Indeed in some cases it is difficult to understand how the brain can even know it is doing the right thing in the absence of such feedback. In the context of eyeblink conditioning it is reasonable to think that a puff of air on the cornea constitutes an error signal and that behavior that avoids this stimulus is adaptive. However, as a football player I often wonder how my brain knows that it was a good thing that the ball hit the back of the net, while hitting the post is bad. What type of feedback allows football players to get better, and is there any way we can enhance this learning through manipulating or adding feedback? A stroke patient who has lost part of his or her behavioral repertoire faces a similar, albeit much more serious challenge. How can we enhance learning through optimization of feedback?

Another interesting topic that I would personally like to explore further is the relationship between classical conditioning and operant conditioning. As mentioned briefly in the introduction there are some striking parallels between the feedback systems that govern these two types of learning. In fact, it may be asked whether this thesis is even about classical conditioning given that, at a cellular level, the CR affects the probability of the US. How are the neural systems governing these two types of learning interrelated? Are their shared features a result of shared neural mechanisms? Classical and operant conditioning can produce almost any type of behavior. Studying these two fundamental types of learning is certain to be an endeavor that not only tickles our curiosity but also leads to practical applications that, in turn, improve our lives.

Summary

The ability to anticipate future events and to modify erroneous anticipatory actions is crucial for the survival of any organism. Both theoretical and empirical lines of evidence implicate the cerebellum in this ability. It is often suggested that the cerebellum acquires "expectations" or "internal models". However, except in a metaphorical sense, the cerebellum, which consists of a set of interconnected nerve cells, cannot contain "internal models" or "have expectations".

The aim of this thesis is to untangle these metaphors by translating them back into neurophysiological cause and effect relationships. This task is approached from within the paradigm of classical conditioning, in which a subject, through repeated presentations of a conditional stimulus, followed by an unconditional stimulus, acquires a conditioned response. Importantly, the conditioned response is timed so that it anticipates the unconditioned response.

Available neurophysiological evidence suggests that Purkinje cells, in the cerebellar cortex, generate the conditioned response. In addition, Purkinje cells provide negative feedback to the IO, which is a relay for the unconditional stimulus, via the nucleo-olivary pathway. Purkinje cells can therefore regulate the intensity of the signal derived from the unconditional stimulus, which, in turn, decides subsequent plasticity. Hence, as learning progresses, the IO signal will become weaker and weaker due to increasing negative feedback from Purkinje cells. Thus, in an important sense, learning induced changes in Purkinje cell activity constitute an "expectation" or "anticipation" of a future event (the unconditional stimulus), and, consistent with theoretical models, future learning depends on the accuracy of this expectation.

Paper 1 in this thesis show that learned changes in Purkinje cells influences subsequent IO activity. The second paper show that, depending on the number of pulses it contains, the signal from the IO to the Purkinje cells can either cause acquisition or extinction. In the third paper we present evidence that can potentially help explain overexpectation, a behavioral phenomenon, which have for long been elusive. Collectively these papers advance our understanding of the feedback mechanisms that govern cerebellar learning and it proposes a potential solution to some long standing behavioral conundrums.

Sammanfattning på svenska

Inom vetenskapen gör vi experiment för att ta reda på om våra teorier beskriver verkligheten eller om de behöver ändras eller förkastas. När vi lär oss ett nytt beteende, som till exempel att cykla, använder vi oss av information eller "feedback", från våra sinnen för att utvärdera om vårt beteende är korrekt utfört eller om det behöver justeras. På samma sätt som vetenskap inte skulle leda någonvart utan experiment, så skulle vi inte kunna lära oss nya, eller justera redan existerande beteenden utan feedback från våra sinnen.

Syftet med denna avhandling är att utveckla vår förståelse av de feedback-mekanismer som styr klassisk betingning av blinkreflexen. Blinkbetingning går ut på att man presenterar ett neutralt "betingat" stimulus, till exempel en ton, följt av ett "obetingat stimulus", till exempel en luftpuff mot ögat, som utlöser en reflex. Om man presenterar det betingade stimulit och sedan det obetingade stimulit upprepade gånger så kommer till sist det betingade stimulit på egen hand att utlösa en "betingad" eller inlärd respons. Om man till exempel presenterar en ton följt av en luftpuff mot ögat kommer så småningom tonen att på egen hand utlösa en blinkrespons. Denna betingade blinkrespons är dessutom tajmad så att ögat stängs strax innan luftpuffen skulle ha kommit. Man skulle kunna säga att försökspersonen, med hjälp av tonen kan "förutsäga" luftpuffen.

För 30 år sedan visade man att denna form av inlärning är beroende av cerebellum (lillhjärnan). Forskning från bland annat vårt labb har sedan dess visat att den betingade responsen initieras av pauser i Purkinje celler, som sitter i cerebellums bark. Purkinje celler är spontanaktiva vilket betyder att om de inte påverkas utifrån så fyrar de aktionspotentialer med en frekvens som ligger på cirka 50 Hz, alltså 50 aktionspotentialer (elektriska impulser) i sekunden. Eftersom Purkinje celler är inhibitoriska så kommer en paus i deras fyrning att disinhibera de celler som de projicerar till, vilket i sin tur tillåter dem att initiera en blinkning. Man kan se det som att Purkinje cellerna hindrar andra celler från att sätta igång en blinkning och att bara när Purkinje cellen pausar kan de mottagande cellerna initiera en blinkrespons.

Förutom att initiera den betingade blinkningen så leder en paus i Purkinje cellen också till att signalen från det obetingade stimulit undertrycks. Det betyder att samtidigt som Purkinje cellen lär sig pausa och därmed utlösa en blinkning till det betingade stimulit, så blir också signalen från det obetingade stimulit gradvis svagare. Eftersom det är det obetingade stimulit som gör att Purkinje cellen lär sig att pausa så kommer man rimligen

till slut att nå en jämnviktspunkt där signalen från det obetingade stimulit inte leder till ytterligare förändringar i Purkinje cellen.

I de studier som denna avhandling baseras på har vi studerat hur signalen från det obetingade stimulit förändras under inlärning och hur signalens egenskaper i sin tur påverkar inlärningen i Purkinje cellerna. För att undersöka detta har vi analyserat fyrningsmönstret i enskilda Purkinje celler under klassisk betingning. När man avleder från Purkinje celler, det vill säga mäter deras elektriska aktivitet, ser man två typer av aktionspotentialer. Dels finns där så kallade "simple spikes" som genereras av Purkinje cellerna, och dels ser man så kallade "complex spikes" som uppstår till följd av det obetingade stimulit. Genom att studera frekvensen av dessa två typer av aktionpotentialer kan man få information om hur en inlärd paus i en Purkinje cell undertrycker signalen från det obetingade stimulit.

I den första delstudien tittade vi på hur den undertryckning som en paus i Purkinje cellen ger upphov till, påverkar signalen från det obetingade stimulit. Detta gjorde vi genom att titta på hur complex spike fyrningen förändras när det uppstår en paus i simple spike fyrningen. Med vissa undantag så bekräftade resultaten från denna studie vår hypotes om att en inlärd paus i Purkinje cellen undertrycker signalen från det obetingade stimulit.

I den andra delstudien manipulerade vi det obetingade stimulit för att undersöka hur signalen måste se ut för att generera en paus i Purkinje cellen. Istället för att ge en luftpuff mot ögat använde vi direkt elektrisk stimulering av de nervfibrer som förmedlar det obetingade stimulit till Purkinje cellerna i cerebellum. Resultaten från denna studie visar att om man stimulerar dessa nervfibrer med en enstaka puls, så får man inte inärning utan motsatsen, alltså utsläckning. För att få inlärning krävs en skur på cirka fem pulser. Detta är ett viktigt fynd eftersom de flesta forskare har antagit att det inte spelar någon roll hur stimuleringen man använder ser ut när man ersätter det obetingade stimulit med elektrisk stimulering av nervfibrerna.

I den tredje delstudien tog vi oss an "overexpectation", ett beteendefenomen som länge har varit svårt att förklara. Overexpectation uppstår när en försöksperson har lärt sig generera betingade svar till två betingade stimuli, till exempel en ton och ett ljus, och man sedan presentrar båda stimuli samtidigt, följt av det obetingade stimulit, till exempel en luftpuff. Trots att både tonen och ljuset hela tiden har varit följt av en luft puff, så kommer den betingade responsen, en blinkning, med tiden att försvagas när de presenteras samtidigt. Detta kan verka paradoxalt, men eftersom båda stimuli leder till en viss undertryckning av signalen från luftpuffen verkar det rimligt att det blir en ännu kraftigare undertryckning när dessa två stimuli kombineras. Om undertryckningen av signalen är tillräckligt kraftig kommer man inte att få inlärning utan motsatsen, nämligen utsläckning. I vår studie fann vi att pausen i Purkinje cellerna blev längre om man kombinerade två stycken betingade stimuli. Detta resultat stödjer vår hypotes eftersom en kraftigare paus i Purkinje cellen rimligen leder till en kraftigare undertryckning av det obetingade stimulit.

Denna avhandling bidrar till vår förståelse av de feedback-mekanismer som styr inlärning och med hjälp av denna typ av kunskap skulle man kunna utveckla metoder och hjälpmedel som påskyndar inlärning.

Acknowledgements

Team

This thesis is a result of the best teamwork I have experienced in my life. I count myself as extraordinarily fortunate to have been part of a research group characterized by co-operation, generosity, curiosity, open-mindedness, respect and friendship. I would like to thank all of those who made such an environment possible. Primarily this means the colleagues I have worked with during my studies, however, I should like to extend my thanks to those who have helped advance the research in our lab over the past six decades. Had it not been for this fine tradition, this thesis would never have materilized, so again, thank you.

Germund Hesslow

Ever since I first entered the lab you have provided me with first class tutorship. Your appetite for science in general, and cerebellar physiology in particular, is extremely contagious. You have influenced my thinking profoundly and through your tireless feedback on my texts you have helped me improve my writing to the extent that I almost feel embarrassed when I read texts I authored earlier on in my career. Your courage in letting evidence speak for itself, even when it flies in the face of current fashion, is another major inspiration that I take with me. Beyond this, I wish to thank you for the trust and support you have shown in more personal matters. You have always given me great personal freedom and you have always been understanding when family matters collided with work. If I had been religious I would have thanked God that I got you as a supervisor, but since I am not, I direct my gratitude to the person who truly deserves it, so thank you Germund, for everything.

Kersti Larsson

Kersti, you have taught me a great many things that have enhanced my productivity and for which i am very grateful. It must have been quite a challenge to turn a fumbling psychology graduate into someone with enough surgical skill to collect the data on which this thesis is based. You have taught me the importance of keeping the lab in order whilst being patient with my occasional… lack of tidiness. Whereas, all other lab members come and go on an almost random schedule, one can always count on the fact that you will be at work, with a welcoming smile, ready to have a chat about anything and everything.

Dan-Anders Jirenhed

I still remember the first time I walked into the lab and saw a Purkinje cell recording. It was a magical moment. You have taught me much of what I know today about experimental work and your work has been and remains an inspiration to me, just like your patient and methodical way of doing things is something I aspire to. (Though I doubt I will ever reach the same level as you.) Your 2007 paper is the foundation for this entire thesis. Without your work or without your feedback and encouragement this thesis would never have been what it is today.

Fredrik Johansson

Never before have I met a guy so high in the sky with ideas, projects and commitments, who is at the same time such a genuinely, down to earth, nice guy that you can also discuss sports with! Working with you, whether it is in the lab or in front of the computer doing data analysis or writing papers is simply a joy and I look forward to more collaborative efforts with you.

Riccardo Zucca

How many cells would I have lost while in wonderland, had it not been for Riccardo? Hard working, highly skilled, super nice and modest, it has been a true pleasure working with you. You have been instrumental in transforming our data acquisition and analysis in a way that contributed profoundly to the papers in this thesis as well as to my development as a scientist.

Fredrik Bengtsson

Thank you for being an all-around friendly and supportive colleague. Apart from Dan's 2007 paper, your prior work constitutes the most important foundation of this thesis. As a father it is also nice to have an authority on childhood medical issues, such as yourself, right next door.

Henrik Jörntell

Thank you for always making yourself available and for giving me invaluable guidance, particularly on technical issues, whenever I needed it.

Daniel Wetmore, Pär Svensson, Carolina Örtenblad and Hannes Carlson.

Though each of you were only in the lab for a relatively short time, you have all been very influential. **Daniel**, your visit from Stanford resulted in a period of unprecedented productivity and you also inspired me to start using matlab, which I have used in every analysis since. Thank you **Pär** for teaching me how to do intracellular recordings and for your interesting stories about life outside the laboratory. Thank you, **Caroline** for good collaboration on the Golgi cell article. Also thank you **Hannes** for your frequent visits

and fresh perspectives. I hope that you will take the torch and carry on the fine tradition in our lab.

My family

Last but not least I want to thank my beloved family. I wish to thank **my mother** for almost single handedly raising me and turning me into the person I am today. Knowing that you were always there for me, and later in life my family, has meant the world to us. Thank you **Marianne**, for being the best sister in the world. Your personality and your achievements are an inspiration and a source of pride. Thank you to my **father** for financial support and for serving as an example in your work ethics.

To my children, **Stella**, **Nanna**, **Viola**, **Lola** & **Aida**. Thank you for enriching my life in so many different ways. I feel enormously privileged to be a part your lives. Just thinking of you can light up even the most gloomy of days.

To **Laila**, my wife and my best friend. Thank you for all your support, your care, your compassion, and your love. Your way of simultaneously encouraging and challenging my more nerdy and skeptical sides, has had a humbling effect on me, and your feedback on my texts has improved my writing markedly. I want to thank you for being a wonderful mother to our children and for being an incredible wife. I feel tremendously lucky that out of all the people in the world, it is I who got the chance to share my life with you.

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